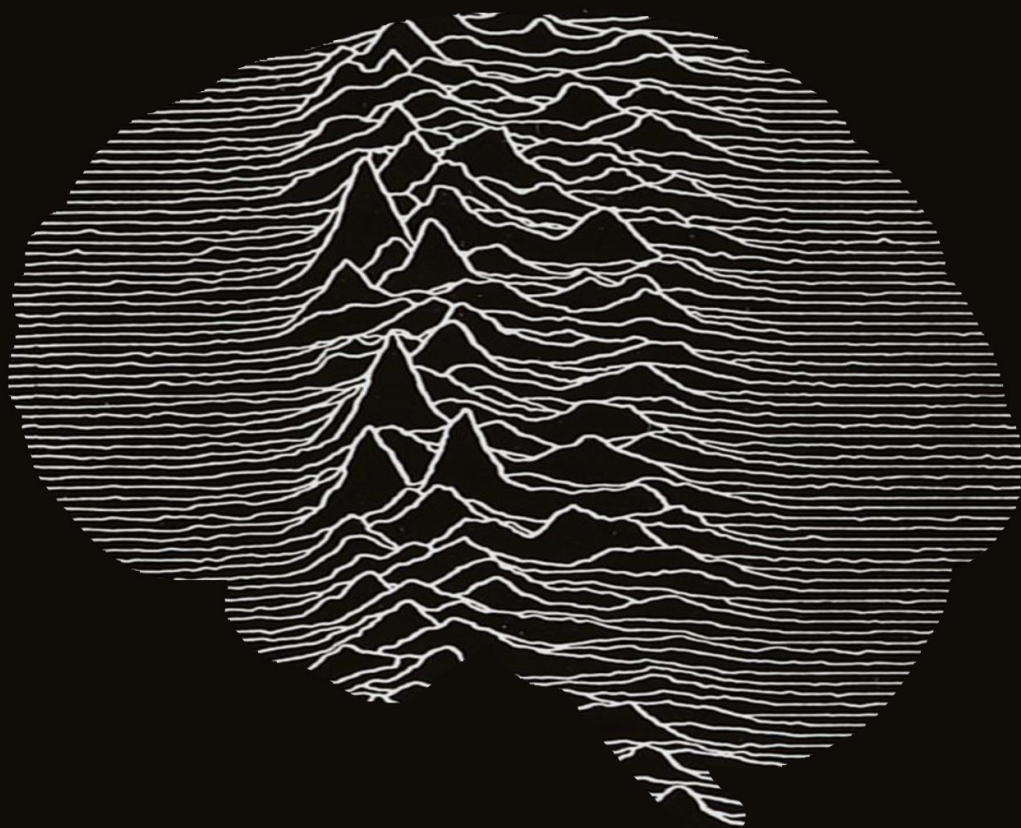


Event-related potentials in patients with refractory epilepsy



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*"Grab a calculator and fix yourself
Space is only noise if you can see"*

Nicolas Jaar

*"And she turned around and took me by the hand
And said I've lost control again
And how I'll never know just why or understand
She said I've lost control again
And she screamed out kicking on her side
And said I've lost control again
And seized up on the floor, I thought she'd die
She said I've lost control
She's lost control again
She's lost control"*

Ian Curtis was lead-singer and lyricist of the band Joy Division. He suffered from the comorbidity of epilepsy and depression. Age 23, he committed suicide on 18 May 1980. The figure on the cover of this thesis is based on the cover of Joy Division's album "Unknown pleasures" which contains the song "She's lost control".

TABLE OF CONTENTS

LIST OF ABBREVIATIONS	6
OUTLINE OF THE THESIS AND RESEARCH AIMS	7
GENERAL INTRODUCTION	9
PUBLICATIONS	39
CHAPTER 1 The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy	41
CHAPTER 2 Event-related potentials reveal preserved attention allocation but impaired emotion regulation in patients with epilepsy and comorbid negative affect	63
CHAPTER 3 Abnormal reappraisal during emotion regulation in refractory epilepsy: an ERP study	87
CHAPTER 4 Systematic gating of attention but versatile influence of emotion in the human amygdala revealed by intracranial event-related potential recordings	107
GENERAL DISCUSSION AND FUTURE PERSPECTIVES	133
SUMMARY	153
SAMENVATTING	155
DANKWOORD	157
CURRICULUM VITAE	160

LIST OF ABBREVIATIONS

ACC	anterior cingulated cortex
ADD	antidepressant drug
AED	antiepileptic drug
ANOVA	analysis of variance
BDI	Beck Depression Inventory
DBS	deep brain stimulation
DF	degrees of freedom
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4 th edition
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5 th edition
DLPFC	dorsolateral prefrontal cortex
ECG	electrocardiogram
EEG	electroencephalography
ERP	event-related potential
fMRI	functional magnetic resonance imaging
HEZ	hypothesized epileptogenic zone
IAPS	International Affective Picture System
ICA	independent component analysis
iERP	intracranial event-related potential
ILAE	International League Against Epilepsy
LC	locus coeruleus
LPP	late positive potential
MEG	magnetoencephalography
MRI	magnetic resonance imaging
NE	norepinephrine
NTS	nucleus tractus solitaries
OR	odds ratio
PET	positron emission tomography
PFC	prefrontal cortex
ROC	receiver operating characteristic
SPECT	single photon emission computed tomography
STAI	State-Trait Anxiety Inventory
tVNS	transcutaneous vagus nerve stimulation
VMPFC	ventromedial prefrontal cortex
VLPFC	ventrolateral prefrontal cortex
VPP	vertex positive potential
VNS	vagus nerve stimulation

OUTLINE OF THE THESIS AND RESEARCH AIMS

The **GENERAL INTRODUCTION** provides a concise overview of relevant literature and describes the key concepts about epilepsy, the comorbidity with depression, emotion regulation, vagus nerve stimulation (VNS) and event-related potentials (ERPs) that are studied in the four empirical chapters.

The aim of this doctoral thesis was to investigate the following research questions using event-related potentials in patients with refractory epilepsy:

1. Is the P3 a biomarker for the therapeutic response to VNS in epilepsy?

The first study discussed in **CHAPTER 1** is part of a translational research line of our research group LCEN3 about the role of the locus coeruleus noradrenergic (LC-NE) system in the mechanism of action of VNS. In animal models for epilepsy, our laboratory has found a strong positive correlation between the seizure-suppressing effect of VNS and the NE levels in the brain. In this study we evaluated the effect of VNS on NE signaling in the human brain through a non-invasive marker of LC-NE activity, i.e., the P3 component of the ERP. The aim of this study was to evaluate whether VNS-induced modulation of the P3 component could be used as a non-invasive biomarker for the efficacy of VNS in patients with epilepsy.

2. Is refractory epilepsy associated with dysfunction of emotion regulation?

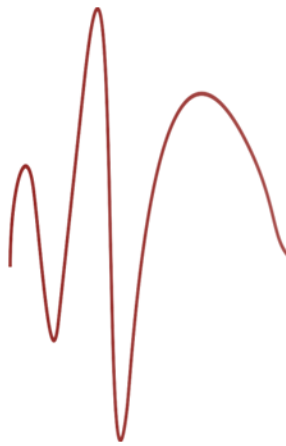
Patients with refractory epilepsy have a high prevalence of comorbid mood disorders. The second and third study aimed to evaluate whether refractory epilepsy is associated with dysfunction of emotion regulation. In the second study, discussed in **CHAPTER 2**, ERPs were used in order to investigate whether a possible dysfunction arises during early (attention) and/or late (regulation) stages of emotional stimuli processing. The aim of the third study, discussed in **CHAPTER 3**, was to investigate whether the ERP response to negatively valenced stimuli in patients with refractory epilepsy is sensitive to reappraisal, a cognitive emotion regulation strategy that can decrease negative affect.

3. Is visual processing in the amygdala modulated by attention and emotion in patients with refractory epilepsy?

How emotion and attention influence processing of visual stimuli in the amygdala remains a matter of intense debate in the literature. We hypothesized that attentional and emotional factors operate independently and in parallel on the sensory processing in the amygdala in order to increase the saliency and selection of behaviorally relevant information. To test this hypothesis local field potentials were recorded in the amygdala of ten patients with refractory epilepsy implanted with intracranial depth electrodes. In

this study we aimed to evaluate whether sensory processing of both attended and unattended emotional visual stimuli can be inferred from local field potentials recorded directly in amygdala and whether amygdala ERPs are modulated by attentional control and emotional factors. These results are discussed in **CHAPTER 4**.

The empirical chapters are followed by a **GENERAL DISCUSSION** in which the three central research questions are discussed in light of the new insights revealed by our studies and future research challenges and opportunities are marked out.



GENERAL INTRODUCTION

GENERAL INTRODUCTION

1 Introduction on epilepsy

1.1 Epidemiology, classification and diagnosis

The word “epilepsy” originates from the Greek verb “επιλαμβάνειν” (epilambanein) meaning “to seize, possess or afflict”, which is a consequence of the ancient belief that a patient having seizures was being possessed by gods, demons or evil spirits [Magiorkinis et al., 2010]. Nowadays, epilepsy is defined by the International League Against Epilepsy (ILAE) as a disease of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition [Fisher et al., 2005; Fisher et al., 2014]. An epileptic seizure is defined as a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity of the brain [Fisher et al., 2005]. Because the conceptual definition of epilepsy can be difficult to apply in everyday clinical practice, the ILAE has now proposed a practical definition for epilepsy which requires that any of the following conditions are met: (i) at least two unprovoked (or reflex) seizures occurring more than 24 hours apart, (ii) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures (at least 60 %) occurring over the next 10 years, and (iii) diagnosis of an epilepsy syndrome [Fisher et al., 2014].

Worldwide, the lifetime prevalence of epilepsy is estimated to be about 65 million people [Ngugi et al., 2010; Thurman et al., 2011]. In high-income countries, the annual incidence of epilepsy is 46 per 100,000, whereas the prevalence approaches 7 per 1000 people a year [Hirtz et al., 2007]. Higher incidence rates occur in low-income countries, rural areas and among infants and elderly people [Hirtz et al., 2007; Ngugi et al., 2010].

Epileptic seizures are classified into two subtypes according to the networks in which they originate: focal epileptic seizures originate within networks limited to one hemisphere, while generalized epileptic seizures arise within and rapidly engage, bilaterally distributed networks [Berg et al., 2010]. Generalized seizures can be further classified in absence, myoclonic, atonic, clonic, tonic and tonic-clonic seizures. Focal seizures used to be classified in simple or complex seizures on the basis of presumed changes in the level of consciousness [Bancaud et al., 1981]. However, the ILAE recommend abandoning these imprecise terms and instead describe focal seizures according to their clinical manifestations using the Glossary of Ictal Semiology (e.g., aura, focal motor, autonomic events, retained/altered responsiveness or awareness) [Blume et al., 2001; Berg et al., 2010].

Epilepsy is not a singular disease entity but encompasses a variety of disorders with different clinical manifestations reflecting underlying brain dysfunction that can result from many different causes [Fisher et al., 2005]. The etiology or underlying causes of epilepsies can be classified into three categories: genetic, structural-metabolic or unknown [Berg et al., 2010]. Genetic epilepsy designates that the epilepsy is, as best as understood, the direct result of known or presumed genetic defects in which seizures are the core symptom of the disorder and in which the causative or susceptibility genes are inherited (with Mendelian, mitochondrial, or complex patterns of inheritance) or result from de-novo mutations that might or might not be further inherited. In structural-metabolic epilepsy there is a distinct other structural or metabolic condition or disease (including traumatic brain injury, stroke, brain tumors, encephalitis, meningitis, malformations of cortical development) that has been demonstrated to be associated with substantially increase risk of developing epilepsy in appropriately designed studies. Epilepsy of unknown cause designates that the nature of the underlying cause is as yet unknown [Berg et al., 2010; Moshe et al., 2015].

The diagnosis of epilepsy is usually based on anamnesis of the clinical description of the seizure, family and personal medical history of the patient, neurological and cognitive status, electrocardiogram (ECG) to rule out cardiac abnormalities and the electroencephalogram (EEG) [Moshe et al., 2015]. The EEG in patients with epilepsy is characterized by the occurrence of epileptiform activity, i.e., spikes, polyspikes, sharp waves, and spike-wave discharges, that can be recorded during (ictal) and in between (interictal) seizures (Figure 1) [Duncan et al., 2006; Osorio et al., 2011]. Recording of habitual seizures during a video-EEG monitoring is the gold standard for confirmation of the diagnosis of epilepsy and classification of seizure type [Cascino, 2002]. A brain magnetic resonance imaging (MRI) to detect structural epileptogenic lesions is generally needed, except for patients presenting with typical syndromes such as juvenile absence epilepsy or juvenile myoclonic epilepsy [Moshe et al., 2015].

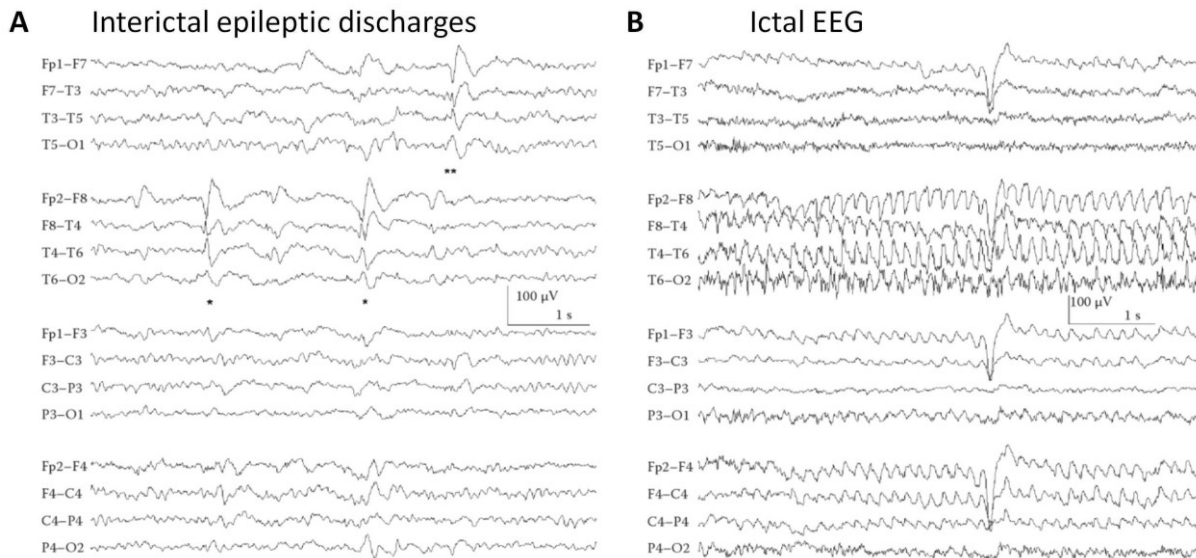


Figure 1. Electroencephalogram (EEG) in epilepsy. (A) Focal interictal epileptic discharges in bilateral temporal lobes with independent sharp and slow wave complexes over right (*) and left (***) anterior temporal regions. (B) Ictal EEG showing focal rhythmic seizure pattern localized to right temporal region. Figure adapted from [Osorio et al., 2011].

1.2 Treatment of epilepsy

Antiepileptic drugs (AEDs) are the first line treatment of epilepsy and approximately 70 % of patients achieve seizure freedom with appropriately used medication therapy [Moshe et al., 2015]. AEDs can induce a variety of adverse effects, including effects on metabolism, bone and connective tissue, the endocrine system and the central nervous system with changes in mood, cognition and behavior [Aldenkamp et al., 2003; Reijs et al., 2004]. The probability of becoming seizure free with drugs decreases proportionally with the number of AEDs unsuccessfully tried [Schiller and Najjar, 2008]. On the basis of this observation, the ILAE defined drug resistant or refractory epilepsy as a failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom [Kwan et al., 2010].

In patients with drug refractory epilepsy, resective surgery is the first treatment of choice. During the presurgical evaluation the results of different techniques, including video-EEG monitoring, structural and functional magnetic resonance imaging (MRI and fMRI), EEG source imaging (ESI), neuropsychological evaluation, interictal positron emission tomography (PET), ictal single photon emission computed tomography (SPECT) and magnetoencephalography (MEG), are combined in order to delineate the epileptogenic zone [Rosenow and Luders, 2001; Van Mierlo et al., 2014]. The

epileptogenic zone is the region that is necessary and sufficient to be totally removed or disconnected to achieve complete seizure-freedom [Rosenow and Luders, 2001]. Patients with non-localizing ictal scalp EEG, absence of a structural epileptogenic lesion on MRI, discongruent results between these techniques or potential overlap with eloquent cortex can require an invasive video-EEG monitoring with intracranial electrodes to identify the epileptogenic zone [Carrette et al., 2010]. About half of the patients undergoing surgical treatment will become long-term seizure-free [de Tisi et al., 2011].

Patients for whom resective surgery is not an option or in whom resection fails to control seizures alternative treatment options are available including neurostimulation of the peripheral nerve system with vagus nerve stimulation (VNS) [Vonck et al., 2009] or central nerve system with deep brain stimulation (DBS) [Sprengers et al., 2014]. Both types of neurostimulation involve direct application of electrical currents to the nervous system by means of implanted electrodes connected to a pulse generator. Neurostimulation represents an increasingly attractive treatment option that directly targets a specific neural region or network thereby avoiding the typical side effects associated with medications [Fisher and Velasco, 2014]. Various brain structures have been targeted with DBS including the anterior thalamic nucleus, the centromedian thalamic nucleus, the cerebellar cortex and the hippocampus [Sprengers et al., 2014]. Closed-loop or responsive neurostimulation systems are able to detect epileptiform EEG activity in real time and deliver in turn direct electrical stimulation to ictal onset zone in response [Morrell, 2011; Vonck and Boon, 2015]. Double-blind randomized controlled trials reported treatment effects of DBS similar or slightly superior to VNS [Sprengers et al., 2014]: the percentage seizure frequency reduction from baseline were higher for DBS of the anterior nucleus of the thalamus (40.4 % stimulated vs. 14.5 % control) [Fisher et al., 2010] and closed-loop ictal onset zone stimulation (37.9 % vs. 17.3 %) [Morrell, 2011] than two large randomized controlled trials of VNS (24.5 % vs. 6.1 % [George et al., 1995] and 27.9 % vs. 15.2 % [Handforth et al., 1998]). Both DBS and VNS trials showed further improvements in seizure reduction over a period of years during the non-blinded phases, with maximal effect seen typically 1 to 2 years after implantation [Fisher and Velasco, 2014]. The efficacy of neurostimulation therapies could be enhanced by improvement of our understanding of the mechanisms of action involved, which are currently incompletely understood. Further investigation of these mechanisms and identification of functional biomarkers, could help to identify the best candidates for specific types of neurostimulation and predict their responses to treatment [Vonck and Boon, 2015].

2 Comorbidity of epilepsy and depression

2.1 Epidemiology

Depression represents the most frequent comorbid psychiatric disorder in epilepsy. Epidemiological data on depression in epilepsy vary depending on diagnostic criteria, applied measures, surveyed populations, and time frames [Hoppe and Elger, 2011]. Large community-based studies of epilepsy patients report lifetime prevalence rates of major depression of about 20 %, but higher rates ranging from 30 to 65 % have been reported in specific populations, such as patients with refractory epilepsy or patients with focal seizures of temporal and frontal lobe origin [Grabowska-Grzyb et al., 2006; Kanner and Schachter, 2010]. These rates are much higher than the lifetime prevalence rates of major depressive disorder reported in the general population (12 to 16.6 %) [Alonso et al., 2004; Kessler et al., 2005]. In addition, more than 70 % of mood disorders in epilepsy are atypical and fail to meet any of the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) [Kanner and Schachter, 2010; Blumer et al., 2004]. Several studies suggest the existence of an epilepsy specific depressive disorder that has an unique manifestation which is poorly reflected by the criteria of conventional classification systems or taxonomies such as the DSM-5. This atypical mood disorder mostly resembles a dysthymic disorder, but with waxing and waning affective symptoms, and is therefore called “interictal dysphoric disorder” [Blumer et al., 2004] or “dysthymic-like disorder of epilepsy” [Kanner and Schachter, 2010]. In patients with epilepsy, presence of negative affect has a major impact on the quality of life, even more than seizure frequency and severity [Boylan et al., 2004; Johnson et al., 2004], and increases the risk of suicide up to tenfold [Jones et al., 2003]. Hence, a better understanding of the specific nature and pathogenic mechanisms underlying the comorbidity of mood disorders in epilepsy may have far-reaching diagnostic, therapeutic and preventive implications.

2.2 Bidirectional relation

Comorbid mood disorders in patients with epilepsy have often been considered to be a consequence or complication of the chronic seizure disorder. However, an intriguing bidirectional relationship between epilepsy and depression has recently been demonstrated [Kanner, 2011; Hesdorffer et al., 2012; Mula, 2012]: not only are patients with epilepsy at greater risk of developing a depressive disorder, conversely, patients with primary depressive disorders are also at greater risk of developing epilepsy [Hesdorffer et al., 2000; Hesdorffer et al., 2012; Adelow et al., 2012]. This suggests that the pathogenic mechanisms may be strongly intertwined and the structural and functional alterations from one disease are likely to trigger the other [Kondziella et al.,

2007; Kanner et al., 2012]. Epilepsy and depression are both heterogeneous diseases that are likely multifactorial, in which genetic vulnerability, developmental insults, traumas and environmental stressors can have important and synergistic contributions [Mayberg et al., 2005; Kanner and Schachter, 2010]. Research on the pathophysiological link between depression and epilepsy has generally focused on common neuroendocrine and neurotransmitter abnormalities. These studies have consistently reported a hyperactive hypothalamic–pituitary–adrenal axis and disturbances in serotonergic, noradrenergic, GABAergic and glutamatergic neurotransmitter systems, all of which may be interrelated (for reviews, see [Jobe, 2003; Pineda et al., 2010; Kanner, 2011]).

The role of decreased functioning of the monoamines serotonin and noradrenaline in depression is well recognized and the primary goal of antidepressant drugs is to enhance the noradrenergic and/or serotonergic neurotransmission [Elhwuegi, 2004]. It has been demonstrated that a decreased activity of the same monoaminergic neurotransmitters lowers the seizure threshold in several animal models of epilepsy [Jobe, 2003; Kanner and Schachter, 2010]. These observations are consistent with the possibility that monoaminergic abnormalities might be operant in the pathophysiology of both mood disorders and epilepsy. The occurrence of epileptic seizures in depressed patients is typically attributed by clinicians to the belief that antidepressant drugs can have proconvulsant effects. However, proconvulsant effects of antidepressants have only been associated with overdoses or high serum concentrations in patients with slow metabolism [Jobe, 2003; Kanner et al., 2012]. Doses within the therapeutic range of the antidepressant monoamine oxidase inhibitors and norepinephrine and serotonin reuptake inhibitors have long been known to produce anticonvulsant effects in animal models for epilepsy and in some human patients [Jobe, 2003; Jobe and Browning, 2005; Vermoesen et al., 2012]. Moreover, neurostimulation treatment with VNS enhances the concentration of noradrenaline and serotonin in the brain and is effective for both refractory epilepsy and mood disorders [Nahas, 2005; Ben-Menachem, 2002; Grimonprez et al., 2015].

In addition, converging evidence from neuropsychological, structural and functional neuroimaging studies has demonstrated that in both diseases abnormalities are not limited to a single brain region, but instead, there is widespread dysfunction in a broad corticolimbic network. The areas within this network that are most reproducibly found to be dysregulated include: (i) the temporal lobes, with hippocampus, amygdala, entorhinal and lateral neocortex, (ii) the frontal lobes, with anterior cingulate cortex (ACC), dorsolateral, ventrolateral and ventromedial prefrontal cortex (DLPFC, VLPFC and VMPFC, respectively), (iii) subcortical structures such as basal ganglia and thalamus, and (iv) the connecting pathways between them [Kondziella et al., 2007; Kanner and Schachter,

2010; Kanner et al., 2012]. Although the initial pathologic event from either depression or epilepsy can vary in its noxious effects, repeated (depressive or seizure) episodes may interact with the neurobiological processes that underpin the other disease to increase the extent of brain dysfunction and thereby the likelihood of developing the comorbidity. For example, in temporal lobe epilepsy, hyperexcitability and neuronal cell loss in the limbic system may evoke mood disturbances, whereas neurotransmitter disturbances and brain atrophy in depression may decrease the seizure threshold [Kondziella et al., 2007; Kanner and Schachter, 2010]. Several animal models of epilepsy, such as the genetically epilepsy-prone rat (GEPR) and the genetic absence epilepsy rats from Strasbourg (GAERS), show behaviors equivalent to depressive symptomatology [Jobe, 2003; Kanner, 2011]. The increased depressive-like behaviors in these animals were evident preceding and following epilepsy onset. These observations indicate that the depressive symptoms were not a consequence of seizures, but rather an expression of comorbid neural network dysfunction [Jobe, 2003; Kanner, 2011].

2.3 Dysfunction of the network for emotion regulation

Emotions are valenced responses to external stimuli and internal mental representations [Ochsner and Gross, 2005]. Considered from an evolutionary perspective emotions facilitate adaptive behavior, promoting survival and reproductive success [Lang, 1995]. The term "emotion regulation" refers to the initiation of new, or the alteration of ongoing emotional responses through the action of regulatory processes that can control the physiological, behavioral, and experiential components of affective responses [Ochsner and Gross, 2005; Hartley and Phelps, 2010; Gross, 2013]. The ability to modify or control the nature of our emotional responses upon changes of environmental circumstances is a critical component of normal adaptive human behavior and is often impaired in psychiatric disorders [Ochsner and Gross, 2005; Hartley and Phelps, 2010]. Emotion regulation involves both early automatic forms of regulation, like controlling attention to emotional arousing stimuli, as well as higher forms of cognitive control, such as the conscious reappraisal of the emotional valence or meaning of stimuli [Ochsner and Gross, 2005; Gross, 2013]. Recently, a new model for mood disorders was proposed, in which depression is hallmarked by dysfunction of both forms of emotion regulation [Holtzheimer and Mayberg, 2011]. This model emphasizes that it is not the negative affect or down state that is abnormal. Instead, it is the tendency to enter the negative affect state and the inability to disengage from this state because of the impaired emotion regulation that defines mood disorders (Figure 2). Thus, basic and clinical research on depression should further investigate the neurobiological bases for normal and abnormal mood reaction and regulation resulting in inadequate affective responses, rather than focusing on the negative mood state itself [Holtzheimer and Mayberg, 2011].

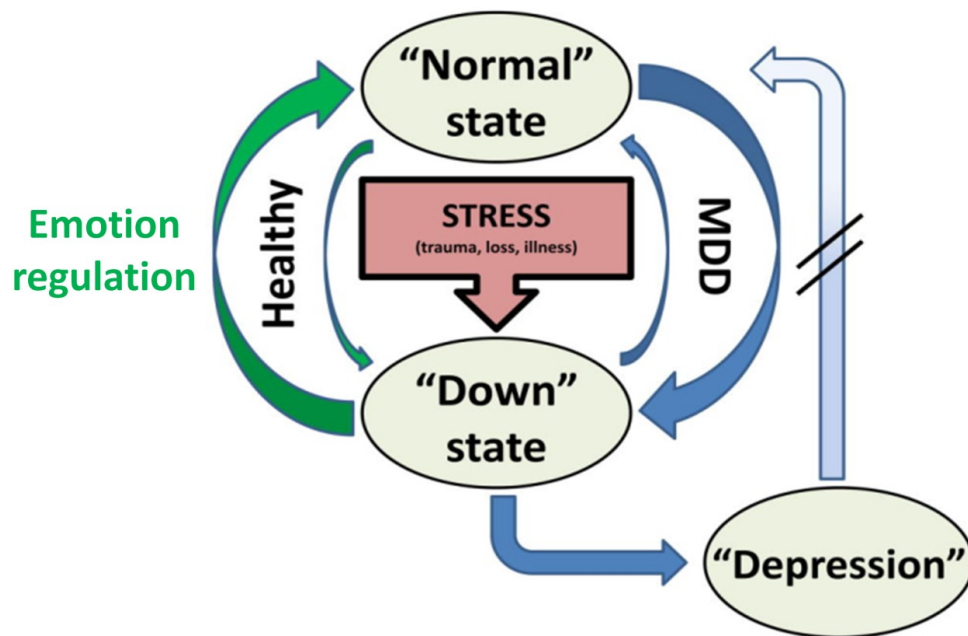


Figure 2. Model of depression defined by emotion regulation dysfunction.

This model emphasizes that the down state itself is not abnormal. Instead, it is the tendency to inappropriately (re-)enter the negative affect state and the inability to disengage from this down state because of the impaired emotion regulation that defines depression [Holtzheimer and Mayberg, 2011].

The neural correlates of the affective system are a group of cortical and subcortical structures that are intimately interconnected with each other in a corticolimbic network that enables a subject to generate and regulate emotional responses (Figure 3). Cortical structures of the affective system are the PFC, the ACC, the insular cortex, and the somatosensory cortical areas. Subcortical structures include the amygdala, hypothalamus, ventral striatum (with nucleus accumbens) and brainstem [Damasio et al., 2000; Price and Drevets, 2010]. The amygdala is an almond shaped group of nuclei in the anterior medial temporal lobe that has a central role in the processing and regulation of emotions [LeDoux, 2000; Phelps and LeDoux, 2005]. The amygdala sends output projections to the hypothalamus which mediates autonomic responses, to the ventral striatum that integrates emotional and motivational aspects of behavior and to the hippocampus reinforcing encoding and consolidation of emotional memories [Hartley and Phelps, 2010]. The amygdala sends bottom-up projections to the ACC, a region that integrates limbic feedback and relays it to the PFC regions that are involved in cognitive control. On the other hand the VLPFC and DLPFC exert top-down inhibitory control that down regulate activity in the amygdala, thereby reducing the influences of emotional stimuli on ongoing behavior [Ochsner and Gross, 2005; Price and Drevets, 2010; Disner et al., 2011].

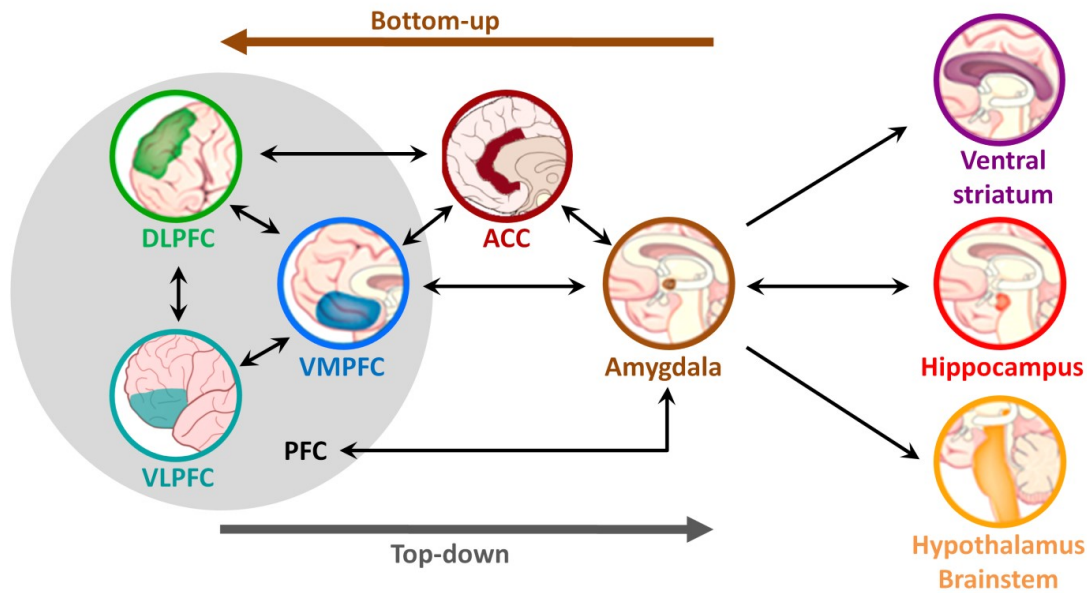


Figure 3. Neurocircuitry of emotion regulation.

Emotions are regulated in a broadly distributed network that involves both cortical structures, including the ventrolateral, dorsolateral and ventromedial prefrontal cortex (DLPFC, VLPFC and VMPFC, respectively) and anterior cingulate cortex (ACC), and subcortical structures, such as the amygdala, ventral striatum, hypothalamus and brainstem. The amygdala has a central function in the expression and regulation of emotions and sends output projections to the hypothalamus which mediates autonomic responses, to the ventral striatum that integrates emotional and motivational aspects of behavior and to the hippocampus reinforcing consolidation of emotional memories. The amygdala sends bottom-up projections to the ACC, a region that integrates limbic feedback and relays it to the PFC regions that are involved in cognitive control. On the other hand the VLPFC and DLPFC exert top-down inhibitory control that down regulate activity in the amygdala. Figure adapted from [Hartley and Phelps, 2010; Disner et al., 2011].

There are two main neuropsychological theories regarding hemispheric specialization for emotion: (i) the right hemisphere hypothesis, which proposes that the right hemisphere is dominant for emotion processing regardless of valence; and (ii) the valence hypothesis, which states that the right hemisphere is specialized for negative emotions and withdrawal, whereas the left hemisphere is dominant for positive emotions and approach behavior [Borod, 1992; Davidson et al., 2000]. These hypotheses are mainly based on observations in patients with unilateral brain damage (e.g., due to stroke). Stroke patients with right brain damage are significantly impaired relative to patients with left brain damage and matched controls with regards to facial, prosodic (emotional intonation) and lexical recognition of both positive and negative valenced emotions, supporting the right hemisphere hypothesis [Borod et al., 1998]. On the other hand, the valence hypothesis is supported by the observation that patients who have brain damage to the left frontal lobe have significantly greater frequency and severity of depression

than patients with lesions in other locations, whereas patients with damage to the right frontal lobe have significantly higher incidence of undue cheerfulness and mania [Starkstein et al., 1989]. However, such hemispheric asymmetries are not always reliably observed in healthy individuals. Meta-analysis of functional neuroimaging studies of emotion found no support for the hypothesis of overall right-lateralization of emotional processing, but provided partial support for the valence hypothesis with lateralization of emotional activity in frontal cortex, in which there was a trend toward left lateralization of positive and approach activations in the frontal cortex and a bilateral distribution of negative and withdrawal activations [Wager et al., 2003; Murphy et al., 2003].

The previously described regions with structural and functional abnormalities in patients with epilepsy and depression show an extensive regional overlap with the corticolimbic circuit involved in emotional control. Both disorders may arise due to alterations in structural and functional connectivity in this emotion regulating circuit leading to imbalanced regulatory top-down and bottom-up effects. Genetic factors, environmental stressors and brain traumas can affect the integrity of this network with aberrant neuronal plasticity and connectivity that might cause a predisposition to develop both depression and epilepsy. It is assumed that impaired emotion control in mood disorders is based on dysfunction of the PFC and the amygdala as well as of the connections between these structures, resulting in impaired top-down control of the PFC over amygdala function [Anand et al., 2005; Sheline et al., 2009; Disner et al., 2011].

3 Vagus nerve stimulation and the locus coeruleus noradrenergic system

VNS is a well-tolerated adjunctive therapy for patients with medically or surgically refractory epilepsy [Boon et al., 2001; Ben-Menachem, 2002]. VNS consists of two spiral nerve electrodes that are wound around the left vagus nerve at the cervical level and connected to a programmable pulse generator that delivers chronic, intermittent electrical stimulation (Figure 4). Since the first human VNS implantation in 1989, more than 100,000 patients with epilepsy worldwide have been treated with VNS. Two double-blind randomized controlled trials have shown a statistically significant decrease in seizure frequency by VNS [George et al., 1995; Handforth et al., 1998; Privitera et al., 2002]. Several long-term follow-up studies have further established the efficacy and safety of VNS [Vonck et al., 1999; DeGiorgio et al., 2000; De Herdt et al., 2007; Elliott et al., 2011]. A meta-analysis of VNS efficacy has shown that seizure reduction ranges from 0 to 100 % (mean 45 %), and varies considerably across patients [Englot et al., 2011]. In general, VNS reduces seizure frequency by at least 50 % in a third of patients, one-third experience a seizure frequency reduction of 30-50 %, and the remaining third show little to no response [Boon et al., 2001].

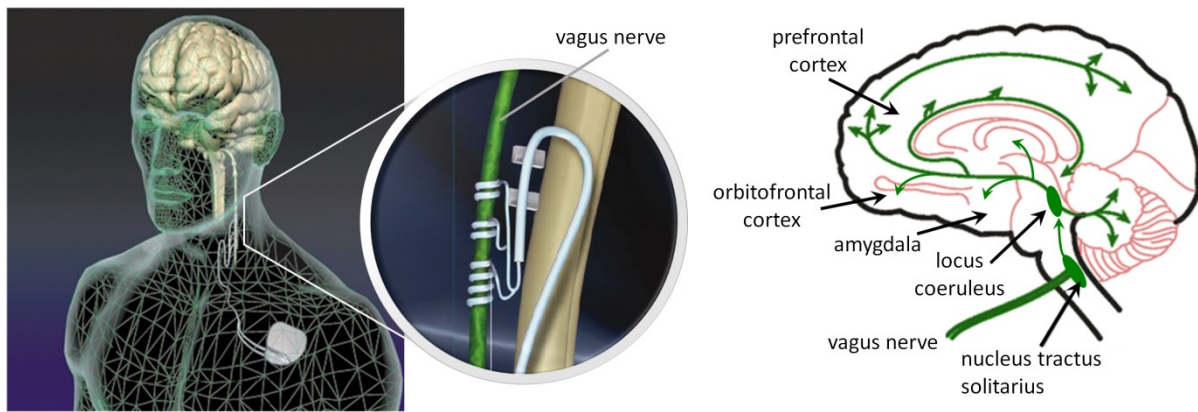


Figure 4. Vagus nerve stimulation and locus coeruleus noradrenergic system.

VNS consists of a programmable pulse generator, which is implanted subclavicularly and connected to two spiral electrodes. The electrodes are wound around the left vagus nerve at the cervical level and deliver chronic, intermittent electrical stimulation. The vagus nerve afferent fibers project to the nucleus tractus solitarius, which in turn projects both directly and indirectly to the locus coeruleus. The locus coeruleus is the main source of noradrenaline in the brain and has widespread noradrenergic projections to all cortical regions, as well as to thalamic nuclei, amygdala and hippocampus. Figure adapted from [George et al., 2000; Carter et al., 2014].

The exact mechanism by which VNS exerts its antiepileptic effect is not yet fully understood. Further elucidation of the mechanism of action is necessary for the identification of patients who will be the best candidates for VNS and a more rational selection of stimulation parameters. Over the last 20 years, there has been an increasing amount of evidence highlighting that electrical stimulation of the vagus nerve activates the locus coeruleus (LC) norepinephrine (NE) system and that this activation is critical for the antiepileptic effect of VNS [Fornai et al., 2011]. First, the vagus nerve afferent fibers project to the nucleus of the solitary tract (NTS), the NTS in turn projects both directly and indirectly to the LC [Van Bockstaele et al., 1999]. The LC is the main source of NE in the brain and has widespread noradrenergic projections to all cortical regions, as well as to thalamic nuclei, amygdala and hippocampus [Sara, 2009] (Figure 4). Acute electrical stimulation of the vagus nerve induces an increase in the discharge rate of LC noradrenergic neurons [Groves et al., 2005]. In addition, the basal firing rate in the LC is significantly increased after long-term treatment with VNS [Dorr and Debonnel, 2006]. In rats VNS induces an increase of NE concentration in the hippocampus [Roosevelt et al., 2006; Raedt et al., 2011; Manta et al., 2013], the amygdala [Hassert et al., 2004] and the prefrontal cortex [Follesa et al., 2007; Manta et al., 2013]. A recent PET study showed that VNS treatment markedly reduced the binding potential of the alpha-2 adrenoceptor antagonist [^{11}C]yohimbine in limbic, thalamic and cortical brain regions, suggesting increased noradrenaline release in these regions in response to VNS [Landau et al., 2015]. Moreover, lesions of the LC block the anticonvulsant effect of VNS [Krahl et

al., 1998]. A recent preclinical study carried out in our laboratory demonstrated a strong positive correlation between the VNS-induced increase in norepinephrine levels in the brain and the seizure-suppressing effects of VNS [Raedt et al., 2011]. Intrahippocampal application of an alpha-2 adrenoreceptor antagonist in a hippocampal seizure model blocked the anticonvulsant effect of VNS [Raedt et al., 2011]. Altogether, these results bolster the assumption that the degree of norepinephrine release in the brain can provide a useful biomarker for the therapeutic efficacy of VNS in patients with epilepsy.

Besides refractory epilepsy, VNS is also a safe and effective therapy for patients with treatment-resistant depression [Nahas et al., 2005; Daban et al., 2008; Bajbouj et al., 2010]. At the neurotransmitter level, reduced noradrenergic signaling has been proposed as one of the underlying mechanisms giving rise to both epilepsy and depression [Jobe, 2003]. In line with this assumption several antidepressive medications, such as the selective-noradrenaline reuptake inhibitors (e.g., reboxetine), work by increasing the norepinephrine levels in the brain [Cardamone et al., 2013]. Recently, a preclinical study conducted in our laboratory showed that selective lesioning of the noradrenergic LC neurons reduced the antidepressant-like effect of VNS [Grimonprez et al., 2015]. These results lend support to the theory that the antidepressant effects of VNS can also be caused by activation of the neuromodulatory LC-NE system [Jobe, 2003].

4 Electrophysiology

4.1 Electroencephalography

Electroencephalography (EEG) is an electrophysiological technique that measures electrical field potentials produced by the brain. These brain potentials can be recorded by placing electrodes on the scalp or into the brain tissue directly (i.e., intracranially). Scalp potentials mainly arise from summated excitatory and inhibitory postsynaptic potentials on the apical dendrites of the cortical pyramidal neurons that are orientated perpendicular to the surface of the head [Osorio et al., 2011]. This implies that scalp EEG recordings are the most sensitive to pick up activity from the cortical gyri near the surface of the head [Kropotov, 2009]. Postsynaptic potentials occur during neurotransmission when the binding of neurotransmitters to receptors changes the flow of ions across the cell membrane of the dendrite [Luck, 2014]. The postsynaptic potentials need to occur simultaneously in a large number of parallel oriented neurons, at least 6 cm² of cortex is considered necessary, for detection with scalp EEG [Osorio et al., 2011]. The signals recorded with EEG represent voltage fluctuations over space and time. The main advantage of the EEG is its very high temporal resolution in the order of milliseconds. In addition, EEG provides a direct measure of electrical neuronal activity during ongoing neurotransmission [Luck, 2014]. This makes it a powerful non-invasive

technique for investigating the electrophysiological time-course of brain activity.

4.2 Event-related potentials

Event-related potentials (ERPs) are electrical brain potentials embedded in the EEG that are time-locked to specific sensory, motor or cognitive events. The EEG reflects a variety of simultaneously ongoing brain processes and the ERP response to a single stimulus or event of interest is usually not visible on the scalp EEG recording during a single trial. This is because in most cases ERP changes are very small (microvolts) in relation to the EEG waveform (tens of microvolts) in which they are embedded [Rugg and Coles, 1996]. In order to see the ERP response to a specific stimulus or event, tens or hundreds of trials must be conducted and then averaged together to extract the time-locked ERP from the background EEG. Brain activity and noise that is unrelated to the event will vary randomly across epochs and this background EEG will tend to average to zero, while the brain response specific and time-locked to the event of interest will be constant and therefore be captured in this average [Rugg and Coles, 1996; Luck, 2014]. Accordingly, ERPs can provide an electrophysiological window during specific cognitive tasks that allows continuous analysis of the ongoing neuronal processes from stimulus onset until the response with a fine-grained temporal resolution. ERP studies usually focus on specific ERP components, which are characteristic parts of the ERP brain wave associated with specific cognitive processes and modulated by specific neurotransmitter systems [Luck, 2014].

ERP components have three main characteristics: (i) latency, which is the time in milliseconds between stimulus onset and when a component arises or reaches a maximum, (ii) amplitude, which is the height/strength of the component in microvolt, and (iii) topography, which is the distribution of the voltage over the scalp. The latency reflects the time-course of activation and can for example be used to investigate temporal differences in terms of neural processing between conditions. Amplitude differences suggest a quantitative instead of qualitative difference: conditions have engaged the same cognitive process, but to different degrees. Differences in topographic voltage maps imply that different anatomical sources or patterns of neural activity are associated with the two situations; however, the converse does not apply [Handy, 2005]. It should be noted that the high temporal resolution of the ERPs is accompanied by a relatively low spatial resolution and it is difficult to determine the neuroanatomical origins of a given ERP effect purely on the basis of the observed scalp distribution [Luck, 2014]. This is because of the inverse problem: any given voltage distribution at the scalp can be explained by an infinite number of intracranial source configurations [Rugg and Coles, 1996; Kropotov, 2009]. Therefore, intracranial recordings in patients with depth

electrodes for diagnostic purposes offer a unique opportunity to merge temporal and spatial precision into one single recording and measure local field potentials or even single unit activity directly from neurons deep inside the brain [Engel et al., 2005; Murray et al., 2014]. Because of its invasive nature, intracranial recordings in humans have been restricted to clinical circumstances and are primarily performed in patients with refractory epilepsy, in whom it can be indispensable for delineation of the epileptogenic zone [Lachaux et al., 2003; Engel et al., 2005; Lachaux et al., 2012]. In many patients with refractory epilepsy the hypothesized epileptogenic zone is located within the medial temporal lobe, and in these patients depth electrodes can record local field potentials from the hippocampus and the amygdala. These intracranial recordings can be used to address basic questions about neural encoding of memory and affective processing [Engel et al., 2005; Murray et al., 2014].

4.2.1 The P3 is an index for the locus coeruleus noradrenergic system

The P3 was first reported 50 years ago [Sutton et al. 1965] and is probably one of the most studied ERP components [Duncan et al., 2009; Luck, 2014]. The P3 is a broad positive component with centro-parietal scalp topography and with latency between 300 to 900 ms post stimulus onset. The P3 is usually measured using oddball paradigms in which a random sequence of stimuli is presented, with low-probability target stimuli (“oddballs”) embedded in a train of high-probability nontarget stimuli (“standards”). The task is to classify the stimuli and to respond only to the low-probability target stimuli. After presentation of the infrequent target stimuli a large P3 can be measured at parietal midline electrodes [Polich, 2007; Duncan et al., 2009; Luck and Kappenman, 2012]. The P3 component has been consistently associated with attention and memory processes, and is thought to index the allocation of attentional resources during information processing and the updating of working memory as incoming stimuli are evaluated [Donchin and Coles, 1988; Polich, 2007]. Given the nature of the P3 and its link to attention and memory-related operations it has been suggested to be generated by brain mechanisms engaged to inhibit concomitant task-irrelevant brain activity [Polich, 2007]. Since infrequent, low probability stimuli can be biologically important, it is adaptive to inhibit unrelated brain activity to promote the processing efficiency of the salient events thereby yielding large P3 amplitudes. In this regard, the P3 and its underlying sub-processes could reflect rapid neural inhibition of ongoing activity to enhance focal attention during stimulus detection. Reduction of irrelevant “neural chatter” would focus incoming stimulus information and sharpen memory encoding. Thus, the P3 may be an index of stimulus events important enough to inhibit extraneous brain activity [Polich, 2007; Luck and Kappenman, 2012].

The neural origins of the P3 component are imprecisely delineated, nevertheless there exists a broad consensus that the P3 has multiple neural generators [Soltani and Knight, 2000; Nieuwenhuis et al., 2005; Polich, 2007]. Evidence for the generators of the P3 component have been obtained from lesion studies, intracranial recordings in human patients and animals, source localization of scalp ERPs and fMRI. These studies have identified P3 generators in multiple cortical and subcortical regions, including frontal and parietal lobe regions, the temporo-parietal junction, the thalamus, and medial temporal lobe structures, such as the hippocampus and amygdala [Knight, 1989; Linden et al., 1999; Soltani and Knight, 2000; Mulert et al., 2003]. Taken together, converging evidence has demonstrated that the P3 component is not generated in a single brain region but instead represents distributed neural activity in a corticolimbic networks engaged during stimulus evaluation and salience detection [Soltani and Knight, 2000]. The fact that P3 can be recorded simultaneously from multiple scalp regions and within intracranial brain structures indicates that it reflects the activity of a central integrated system with widespread connections and a large-scale impact throughout different cortical and subcortical networks [Nieuwenhuis et al., 2005; Duncan et al., 2009]. A central system which is known to have a crucial modulatory effect on the P3 is the LC [Nieuwenhuis et al., 2005; Polich, 2007]. LC neurons display tonic and phasic discharge activity patterns: tonic activity involves its spontaneous (baseline) firing rate, whereas phasic activity refers to rapid increases in firing rate in response to salient sensory stimuli [Berridge and Waterhouse, 2003]. Behavioral performance is optimal during states of intermediate tonic activity which result in a maximal phasic response to sensory stimuli [Aston-Jones and Cohen, 2005]. The important role of phasic LC activity in the generation and modulation of the P3 is supported by several lines of evidence from animal, lesion, behavioral and pharmacological studies. (i) The LC-NE contribution to P3 generation is consistent with the LC-NE mediated engagement and allocation of attentional resources to optimize task performance [Polich, 2007; Corbetta et al., 2008; Sara, 2009; Nieuwenhuis et al., 2011]. In healthy human participants the largest P3 amplitudes are observed during optimal behavioral performance in a manner consistent with the LC phasic response [Murphy et al., 2011]. (ii) The timing and topographic distribution of intracranial and scalp-recorded P3 activity are consistent with the anatomical and physiological activation of these areas by the LC-NE system [Nieuwenhuis et al., 2005; Polich, 2007]. (iii) Lesions of the LC cell bodies reduce the auditory P3 amplitude in monkeys, indicating that the LC nucleus and its ascending fibers are important in the generation and modulation of the P3 [Pineda et al., 1989]. (iv) Monkey studies with the oddball paradigm have shown that the conditions for generating the P3 are highly similar to those for the LC phasic response: both are

preferentially elicited by attended, task-relevant, arousing, and salient stimuli that are important for goal-directed behavior [Nieuwenhuis et al., 2005; Aston-Jones and Cohen, 2005; Corbetta et al., 2008]. (v) Clonidine, a noradrenergic alpha-2 autoreceptor agonist, which inhibits LC activity and decreases NE release, decreases the amplitude of the P3 component in monkeys and healthy human participants [Joseph and Sitaram, 1989; Swick et al., 1994; Nieuwenhuis, 2005]. (vi) Simultaneous EEG-fMRI recordings during oddball tasks have found evidence of functional coupling between the brainstem LC-NE region and cortical attention networks thought to be involved in the generation of the P3 response [Walz et al., 2003]. Taken together, these findings support the notion that the P3 component of the scalp-recorded ERP can be used as an indirect index of the phasic activity of the neuromodulatory LC-NE system [Nieuwenhuis et al., 2005; Murphy et al., 2011].

4.2.2 The late positive potential is an index for emotion regulation

The ERP method is used extensively in affective neuroscience to investigate the temporal dynamics of emotion regulation processes, whereby an emotional response unfolds over time as a function of both how attention is allocated to the emotion-eliciting stimulus and how the meaning of the emotional stimulus or situation is interpreted [Gross, 2013]. A key component observed in ERPs evoked by emotionally engaging stimuli is the late positive potential (LPP). The LPP is a robust visual ERP component reaching a maximum amplitude over centro-parietal scalp sites and with onset latency around 300 ms after stimuli onset that can last up to several seconds [Hajcak et al., 2010; Luck and Kappenman, 2012; Gross, 2013]. The amplitude of the LPP is modulated by emotional content, with enhanced positive amplitudes following presentation of both positive and negative arousing emotional stimuli compared to neutral stimuli [Cuthbert et al., 2000; Schupp et al., 2000]. It has been argued that the LPP indexes sustained attentional engagement to emotional content [Robinson et al., 2013] and can be used as an indirect electrophysiological marker of the (covert) processing of the emotional intensity of the visual stimuli [Schupp et al., 2000; Hajcak et al., 2010]. Based on animal models, it has been suggested that the LPP might reflect re-entrant projections from the amygdala to the visual system that enhance the sensory processing of emotional stimuli [Lang and Bradley, 2010]. Combined EEG-fMRI research indicated that the LPP reflects modulation of a broad network of interconnected cortical and subcortical areas, including lateral occipital, inferior temporal and parietal extrastriate visual areas, the prefrontal cortex, the anterior cingulate cortex and the amygdala [Sabatinelli et al., 2007; Liu et al., 2012; Sabatinelli et al., 2013]. In addition, it has been shown that the LPP is associated with increased bidirectional functional connectivity between prefrontal and parietal-occipital cortex [Moratti et al., 2011]. These results suggest that the LPP response to emotional

stimuli is a product of automatic facilitation of visual processing, as well as post-perceptual processing under cognitive control. Thus, both bottom-up and top-down mechanisms account for the modulation of the parietal LPP response [Ferrari et al., 2008; Moratti et al., 2011; Gross, 2013]. In this regard, it has consistently been shown in human ERP studies that the magnitude of the LPP is sensitive to cognitive emotion regulation strategies, including suppression, distraction or reappraisal of the emotional significance of stimuli [Hajcak and Nieuwenhuis, 2006; Moser et al., 2006; Foti and Hajcak, 2008; Dennis and Hajcak, 2009; MacNamara and Hajcak, 2009; Thiruchselvam et al. 2011; Moran et al., 2013; Schonfelder et al., 2014]. Moreover, patients with general anxiety disorder [Weinberg and Hajcak, 2010], major depressive disorder [Foti et al., 2010], obsessive compulsive disorder [Paul et al., 2015] and schizophrenia [Horan et al., 2012] show abnormal modulation of the LPP response, indicating impairment of emotion regulation in these psychiatric disorders. Taken together, the LPP has proven to be a sensitive, valid and effective neurophysiological marker for investigating normal emotion regulation processes in healthy individuals, but also abnormal emotion regulation in psychiatric patients [Hajcak et al., 2010; Gross, 2013; Moran et al., 2013].

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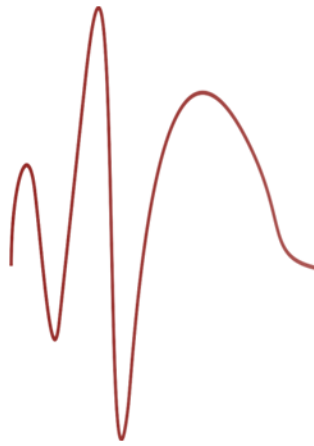
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PUBLICATIONS

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- CHAPTER 1: De Taeye L, Vonck K, van Bochove M, Boon P, Van Roost D, Mollet L, Meurs A, De Herdt V, Carrette E, Dauwe I, Gadeyne S, van Mierlo P, Verguts T, Raedt R (2014) The P3 Event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy. *Neurotherapeutics* 11: 612-622.
- CHAPTER 2: De Taeye L, Meurs A, Boon P, Vonck K, Carrette E, Raedt R (2015) Event-related potentials reveal preserved attention allocation but impaired emotion regulation in patients with epilepsy and comorbid negative affect. *Plos One* 10: e0116817.
- CHAPTER 3: De Taeye L, Pourtois G, Meurs A, Boon P, Vonck K, Carrette E, Raedt R (Under review) Abnormal reappraisal during emotion regulation in refractory epilepsy: an ERP study. *Epilepsy & Behavior*.
- CHAPTER 4: De Taeye L, Pourtois G, Meurs A, Van Roost D, Achten R, Boon P, Vonck K, Carrette E, Raedt R (Under review) Systematic gating of attention but versatile influence of emotion in the human amygdala revealed by intracranial event-related potential recordings. *Human Brain mapping*.

CHAPTER 1

The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy

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ABSTRACT

Currently, the mechanism of action of vagus nerve stimulation (VNS) is not fully understood, and it is unclear which factors determine a patient's response to treatment. Recent preclinical experiments indicate that activation of the locus coeruleus noradrenergic system is critical for the antiepileptic effect of VNS. This study aims to evaluate the effect of VNS on noradrenergic signaling in the human brain through a non-invasive marker of locus coeruleus noradrenergic activity: the P3 component of the event-related potential. We investigated whether VNS differentially modulates the P3 component in VNS responders versus VNS non-responders. For this purpose, we recruited 20 patients with refractory epilepsy who had been treated with VNS for at least 18 months. Patients were divided into 2 groups with regard to their reduction in mean monthly seizure frequency: 10 responders (>50 %) and 10 non-responders (≤50 %). Two stimulation conditions were compared: VNS OFF and VNS ON. In each condition, the P3 component was measured during an auditory oddball paradigm. VNS induced a significant increase of the P3 amplitude at the parietal midline electrode, in VNS responders only. In addition, logistic regression analysis showed that the increase of P3 amplitude can be used as a non-invasive indicator for VNS responders. These results support the hypothesis that activation of the locus coeruleus noradrenergic system is associated with the antiepileptic effect of VNS. Modulation of the P3 amplitude should be further investigated as a non-invasive biomarker for the therapeutic efficacy of VNS in patients with refractory epilepsy.

INTRODUCTION

Vagus nerve stimulation (VNS) is a well-tolerated adjunctive therapy for patients with medically or surgically refractory epilepsy [Boon et al., 2001; Ben-Menachem, 2002]. VNS consists of a programmable pulse generator, which is implanted subclavicularly and connected to 2 spiral electrodes. The electrodes are wound around the left vagus nerve at the cervical level, and deliver chronic, intermittent electrical stimulation. Since the first human VNS implantation in 1989, more than 100,000 patients with epilepsy worldwide have been treated with VNS. Two randomized, double-blind clinical trials have shown a statistically significant decrease in seizure frequency by VNS [George et al., 1995; Handforth et al., 1998; Privitera et al., 2002]. Several long-term follow-up studies have further established the efficacy and safety of VNS [Vonck et al., 1999; DeGiorgio et al., 2000; De Herdt et al., 2007; Elliott, 2011]. A meta-analysis of VNS efficacy has shown that seizure reduction ranges from 0 to 100 % (mean 45 %), and varies considerably across patients [Englot et al., 2011]. In general, VNS reduces seizure frequency by at least 50 % in a third of patients; one-third experience a seizure frequency reduction of 30–50 %; and the remaining third show little to no response [Boon et al., 2001]. Therefore, at this moment, 1 of 3 patients undergoing the invasive and costly VNS surgical procedure does not experience any benefit from it.

The exact mechanism by which VNS exerts its antiepileptic effect is not yet fully understood. Therefore, prior to implantation of the VNS device, we are currently unable to predict which patients will be responders or non-responders. Further elucidation of the mechanism of action is necessary for the identification of predictive biomarkers for positive therapeutic response and a more rational setting of stimulation parameters. Over the last 20 years, there has been an increasing amount of evidence indicating that activation of the locus coeruleus (LC) norepinephrine (NE) system is critical for the antiepileptic effect of VNS [Fornai et al., 2011]. In addition, a recent preclinical study carried out in our laboratory has demonstrated a strong positive correlation between the VNS-induced increase in NE levels in the brain and the seizure-suppressing effect of VNS [Raedt et al., 2011]. These results suggest that the degree of NE release in the brain can be a useful biomarker for the therapeutic efficacy of VNS in epileptic patients.

Currently, there are no techniques available for direct measurement of NE levels in the human brain. Fortunately, changes in NE levels can be indirectly inferred from parameters that are modulated by the amount of noradrenergic signaling in the brain. One of these parameters is the P3 component of the event-related potential (ERP). The P3 is a broad positive component with an onset latency of 300–900 ms that can be measured using the auditory oddball paradigm. In this cognitive paradigm, low-

probability target stimuli (“oddballs”, e.g., low frequency tones) are embedded in a train of high-probability nontarget stimuli (“standards”, e.g., high-frequency tones). After the presentation of the infrequent target stimuli a large P3 can be measured at parietal midline electrodes [Polich, 2007; Duncan et al., 2009].

Converging evidence from animal, genetic, and pharmacological studies suggests that the P3 component of the scalp-recorded ERP reflects the phasic activity of the neuromodulatory LC–NE system [Nieuwenhuis et al., 2005; Murphy et al., 2011]. The hypothesis that the LC–NE system contributes to P3 generation during a target detection task is consistent with the allocation of attentional resources and arousal effects in humans [Berridge and Waterhouse, 2003; Polich, 2007; Sara, 2009; Nieuwenhuis et al., 2011]. Monkey studies with the oddball paradigm have shown that the conditions for generating the P3 are highly similar to those for the LC phasic response: both are preferentially elicited by attended, task-relevant, arousing, and salient stimuli that are important for goal-directed behavior [Nieuwenhuis et al., 2005; Aston-Jones and Cohen, 2005]. Furthermore, the timing and topographic distribution of intracranial and scalp-recorded P3 activity are consistent with the anatomical and physiological activation of temporo-parietal areas by the LC–NE system [Nieuwenhuis et al., 2005; Polich, 2007]. Finally, lesions of the LC cell bodies reduce the auditory P3 amplitude in monkeys, indicating that the LC nucleus and its ascending fibers are important in the generation and modulation of the P3 [Pineda et al., 1989].

In this study, we assessed the effect of VNS on the NE signaling in patients with epilepsy by investigating VNS-induced modulation of the P3 ERP component recorded during a standard auditory oddball paradigm. In light of the important role of the noradrenergic system in the therapeutic effect of VNS, and given the association between the P3 component and this specific neurotransmitter system, we hypothesized that we would find a different modulation of the P3 component in VNS responders versus VNS non-responders. The goal of this study was to investigate whether VNS-induced modulation of the P3 ERP component could be used as a non-invasive biomarker for the treatment response to VNS.

METHODS

Patients

Twenty patients with epilepsy were included (8 men and 12 women, mean age 44 years). The study took place during a video-electroencephalogram (EEG) monitoring session in the Reference Center for Refractory Epilepsy, Ghent University Hospital, Ghent, Belgium. Patients were included in the study if they met the following criteria: 1) at least 18 months of treatment with VNS for refractory epilepsy; 2) older than 18 years; 3) full-scale IQ score ≥ 70 on the Wechsler Adult Intelligence Scale, Third Edition. Only patients who were treated with VNS for at least 18 months were included because current reports suggest that VNS efficacy has a tendency to improve up to 18 months after surgery, after which a plateau is usually reached [Boon et al., 2007; Shahwan et al., 2009]. Patients were divided into 2 groups depending on their reduction in mean monthly seizure frequency: 10 responders ($>50\%$ reduction) and 10 non-responders ($\leq 50\%$ reduction). Mean monthly seizure frequency was defined as the mean seizure frequency during the 3 consecutive months before implantation and before testing. The mean monthly seizure frequency before VNS was not significantly different between both groups [non-responders: 53.2 ± 54.4 seizures/month; responders: 39.3 ± 48.4 seizures/month; $t(18) = 0.61$, $p = 0.55$]. Conversely, the mean monthly seizure frequency reduction post-VNS was significantly higher in the group of responders (86.7 %) than in the group of non-responders (14.2 %) [$t(18) = 8.53$, $p < 0.001$]. The main clinical characteristics of patients and habitual VNS parameters are summarized in Table 1. The study was approved by the ethics committee of Ghent University Hospital. After a full description of the procedure was provided and explained, all patients gave written informed consent.

VNS Parameters and Procedure

All patients had a chronically implanted VNS device (Cyberonics, Houston, TX, USA), comprising a programmable pulse generator placed subcutaneously under the left clavicle and 2 helical electrodes wound around the left vagus nerve in the neck. Time since start of VNS treatment varied between 1.5 and 16.2 years. During this period, the stimulation parameters were individually adjusted by a previously described standard ramping-up scheme [Vonck et al., 1999; De Herdt et al., 2007]. Stimulation parameters were gradually changed in order to achieve maximal therapeutic effect with minimal side effects. At the start of the study VNS parameters, battery voltage and lead impedance were checked with a handheld computer and programmable wand.

Patients performed the task during 2 stimulation conditions—VNS OFF and VNS ON—in a randomized, counterbalanced order. During the VNS ON condition the duty cycle was 7 s

ON/18 s OFF. Other stimulation parameters during the VNS ON condition were patient-specific (see Table 1), with output current ranging between 0.75 and 3.00 mA, a frequency of 20 or 30 Hz, and a pulse width of 250 or 500 μ s. These values were the habitual therapeutic parameters of each patient that had optimal clinical efficacy. There were no significant differences in stimulation parameters between the group of responders and non-responders. After switching the VNS device ON or OFF, there was a pause of 20 min in order to allow habituation and achieve a stable baseline condition before the oddball task was initiated.

Auditory Oddball Paradigm

Patients performed a standard auditory oddball task [Duncan et al., 2009]. This task requires participants to press a predefined button with the index finger of the dominant hand in response to "target" tones (low frequency), but not to respond to "nontarget" tones (high frequency). Participants were given a practice session in order to become familiar with the target and nontarget tones. The target tones were presented with a probability of 10 %. During 4 blocks of 140 trials (total = 560 trials), participants listened to a series of tones consisting of 504 nontarget and 56 target tones that were presented in a random order with an inter stimulus interval of 1 s. Both speed and accuracy of the response to the infrequent target tone were emphasized. To reduce ocular artifacts, participants were instructed to fixate their gaze on a cross on the monitor while listening to the stimuli. Stimulus presentation and response time recording were controlled using E-Prime software 2.0 (Psychology Software Tools, Pittsburgh, PA, USA) on a Dell (Round Rock, TX, USA) desktop computer.

Table 1 Patient characteristics

Patient ID	Sex	Age (years)	Seizure reduction (%)	VNS Impl Year	VNS Parameters			HEZ		AED	P3 absolute amplitude (μ V)			P3 relative amplitude (%)
					Output (mA)	Frequency (Hz)	Pulsewidth (μ s)	Lobe	Side		OFF	ON	ON-OFF	(ON-OFF)/OFF
Responders														
R_1	M	52	100.0	1995	2.00	30	500	TL	Bilateral	VPA, VGB, CBZ	7.41	11.77	4.36	58.81
R_2	F	57	100.0	1997	1.50	30	500	FL+TL+PL	Right	LTG	3.94	5.58	1.64	41.78
R_3	F	52	100.0	2003	2.50	30	500	TL	right	LEV, CBZ	3.77	3.63	-0.14	-3.61
R_4	M	22	100.0	2007	0.75	20	500	General	bilateral	VPA, LTG	3.96	6.21	2.26	57.09
R_5	M	36	95.6	2010	2.25	20	250	FL	right	LEV, PGB, CZP	2.37	2.04	-0.34	-14.15
R_6	F	66	95.0	2003	2.50	20	500	General	bilateral	LEV, LTG, CZP	3.15	4.48	1.32	41.92
R_7	F	55	85.0	2002	3.00	20	500	TL	right	LEV, LTG, CBZ	7.30	8.88	1.58	21.65
R_8	F	45	73.3	1997	2.75	20	250	TL	right	LEV, LTG, CZP	4.34	7.44	3.10	71.48
R_9	F	30	63.3	2005	2.50	25	500	General	bilateral	VPA, LEV, PGB, CZP	4.99	7.06	2.07	41.45
R_10	F	21	54.5	2009	3.00	30	500	General	bilateral	VPA, LTG, PGB, LCZ	2.45	2.68	0.23	9.55
Mean		44	86.7	2003	2.28	25	450				4.37	5.98	1.61	32.60
Non-responders														
NR_1	F	62	50.0	2008	3	30	130	General	bilateral	PHT, LEV, LTG, CZP, RG	5.30	5.94	0.64	11.98
NR_2	M	55	46.9	2003	2.75	30	500	FL+TL	bilateral	PHT, LCZ	16.45	16.29	-0.16	-0.98
NR_3	M	61	33.3	1999	2.5	20	500	FL	bilateral	CBZ, PB, LEV, PGB, CZP	6.50	5.56	-0.93	-14.38
NR_4	M	53	9.1	2007	2.75	30	500	FL+PL	bilateral	VPA, LTG	2.32	1.77	-0.55	-23.75
NR_5	M	23	3.1	2007	2.75	30	500	FL+PL	bilateral	LTG, CZP, OXC, LCZ	3.35	3.82	0.46	13.83
NR_6	M	25	0.0	2008	2	30	500	OL	left	LEV, CZP, CBZ	5.90	7.80	1.89	32.08
NR_7	F	32	0.0	2007	2	20	250	FL+OL	right	LEV, CZP, CBZ	10.06	5.95	-4.11	-40.89
NR_8	F	30	0.0	2011	0.75	20	250	FL+TL+PL	left	CLB, CZP, OXC	6.61	6.09	-0.52	-7.94
NR_9	F	54	0.0	2010	2.75	20	500	FL	left	CZP, CBZ, RG, LCZ	5.74	6.71	0.98	17.06
NR_10	F	48	0.0	2010	1.75	30	500	FL	left	VPA, LEV, PB, LCZ	4.14	4.46	0.32	7.82
Mean		44	14.2	2007	2.30	26	413				6.64	6.44	-0.20	-0.52

VNS vagus nerve stimulation; HEZ hypothesized epileptogenic zone; AEDs antiepileptic drugs; R responder; NR non-responder; M male; F female; TL temporal lobe; FL frontal lobe; PL parietal lobe; OL occipital lobe; VPA valproic acid; VGB vigabatrin; CBZ carbamazepine; LTG lamotrigine; LEV levetiracetam; PGB pregabalin; CZP clonazepam; PHT phenytoin; RG retigabine; LCZ lacosamide; PB phenobarbital; OXC oxcarbazepine; CLB clobazam

Electrophysiological Recordings

The EEG was recorded with a Micromed System Plus (Micromed, Mogliano, Italy) using Ag/AgCl electrodes placed at 59 standard locations according to the extended International 10-20 System using a precabled electrode cap (WaveGuard EEG cap system; Eemagine, Berlin, Germany). The online reference electrode was placed on CPz and the ground electrode on AFz. The vertical electro-oculogram was monitored using 2 facial electrodes placed on inferior and superior areas of the left orbit. The electrocardiogram (ECG) was recorded with 2 ECG electrodes placed above the heart. Two additional electrodes were placed in the neck cranial and caudal to the vagus nerve electrode to monitor the VNS artifact. The EEG, electro-oculogram, ECG, and VNS signals were digitized online with a sampling frequency rate of 1024 Hz, antialiasing filter of 250 Hz, gain of 50 dB, and a resolution of 16 bits. Electrode impedance was maintained below 10 k Ω . ERPs of interest were computed offline following a standard sequence of data transformations [Picton et al., 2000]. Using an independent component analysis that subtracts artifact components from each electrode, the EEG was corrected for vertical and horizontal eye movements, blinks, heartbeat, and VNS artifacts. The EEG signal was then re-referenced to the average of all 59 recorded channels. The continuous EEG was first digitally filtered with a 50-Hz notch filter and a half-power band-pass filter between 0.1 and 30 Hz, and a roll-off of 12 dB/octave and then down-sampled to 256 Hz. The EEG was segmented into epochs from -200 ms to +1000 ms relative to the onset of the target and standard stimuli. Baseline correction was performed on the 200-ms prestimulus interval, and epochs with a voltage exceeding $\pm 75 \mu\text{V}$ were excluded from averaging. Artifact-free epochs were averaged separately for standard and target stimuli, for each condition and each individual. To isolate the P3 component, we created a classical target-standard difference waveform; P3 amplitude and latency were measured from the resulting difference waves. For each patient, the P3 peak was determined using automatic local peak detection in the 300–900 ms interval poststimulus onset at the parietal midline electrode Pz. The latency of the P3 was measured as the time point at which the voltage reached 50 % of the peak amplitude. This is because in averaged waveforms the absolute onset latency will reflect the trials with the earliest onsets rather than the average of the single trial onset latencies. Therefore, the 50 % peak latency measure is a much more accurate and sensitive measure of the relative onset time of an ERP component [Luck, 2005; Kappenman et al., 2012]. The P3 amplitude was calculated as the mean amplitude during the 100-ms interval round the peak detected by the automatic peak detection relative to baseline. Mean amplitude measurements capture more of a component than just the extreme value, and are less sensitive to noise than peak amplitude measurements [Luck, 2005]. ERP amplitude measurements can be

influenced by individual differences in nuisance variables [Luck, 2011], such as skull thickness [Frodl et al., 2001] and cortical folding patterns [Ahlfors et al., 2010]. To minimize the impact of these nuisance factors, we calculated the percentage difference in P3 amplitude of the ON condition relative to the OFF condition with following formula: $P3 \text{ amplitude (ON-OFF)/OFF}$.

Statistical Analysis

All statistical analyses were conducted with SPSS version 20.0 (SPSS, Chicago, IL, USA). The level of statistical significance was set at 0.05. The behavioral and electrophysiological data were analyzed using a mixed model analysis of variance (ANOVA) with between-subject factor group (responders vs.non-responders) and within-subject factor condition (ON vs.OFF). Two-tailed paired t tests were also computed as post hoc analyses. Logistic regression analysis was used to test whether VNS-induced modulation of the P3 amplitude is a good indicator to differentiate between responders and non-responders. Odds ratios (OR) were calculated with 95 % confidence intervals (CI). Receiver operating characteristic (ROC) curve analysis was used to assess the optimal cut-off value with maximal sensitivity and specificity. Correlation between the relative P3 amplitude and behavioral responses (reaction time and accuracy) were tested using Pearson's correlation coefficient.

RESULTS

Behavioral Results

The behavioral results are summarized in Table 2: mean accuracy (percentage correct responses) and reaction times are shown, along with F- and p-values of the statistical analyses. During the auditory oddball task patients detected the target stimuli with a very high accuracy: mean performance was 97 % correct. The high accuracy confirms that patients were paying attention to the stimuli and could easily discriminate between target and nontarget stimuli. Patients had significantly greater accuracy and faster reaction times during VNS ON than during VNS OFF condition. The accuracy and reaction time measures indicate that both groups of patients were generally better in detecting the targets during VNS ON condition, leading to a significant main effect of condition, but no group with condition interaction.

Table 2 Behavioral results and P3 measures (mean \pm SD), with F- and p-values for the statistical analyses

Dependent variable	Responders		Non-responders		Statistics		
	OFF	ON	OFF	ON	Group df=1,18	Condition df=1,18	Group \times Condition df=1,18
Accuracy (%)	95.71 (± 2.82)	98.21 (± 2.06)	96.61 (± 5.29)	98.21 (± 2.66)	$F=0.12$ $p=0.729$	$F=5.58$ $p=0.030$	$F=0.26$ $p=0.615$
Reaction time (ms)	417.52 (± 59.85)	408.98 (± 78.40)	419.34 (± 68.17)	394.54 (± 62.24)	$F=0.05$ $p=0.831$	$F=4.53$ $p=0.047$	$F=1.07$ $p=0.313$
Amplitude (μV)	4.37 (± 1.77)	5.98 (± 2.97)	6.64 (± 4.04)	6.44 (± 3.84)	$F=0.92$ $p=0.351$	$F=4.22$ $p=0.055$	$F=5.39$ $p=0.017$
Latency (ms)	439.84 (± 107.14)	437.11 (± 149.59)	420.32 (± 82.99)	400.39 (± 80.08)	$F=0.35$ $p=0.561$	$F=1.26$ $p=0.276$	$F=0.73$ $p=0.405$

df degrees of freedom

Electrophysiological Results

Consistent with many previous ERP studies [16, 30–36], the P3 component of the auditory ERP was recorded at the parietal midline electrode Pz. During the auditory oddball task, the processing of deviant auditory stimuli was associated with the generation of this well-characterized P3 component. Table 1 summarizes the P3 amplitudes of each individual patient in the responder and non-responder groups. Owing to the large interindividual variability in P3 latency and amplitude in the heterogeneous patient population, visualization of the effect of VNS on the P3 was optimized by plotting the separate difference waves of each individual patient (Figure 1).

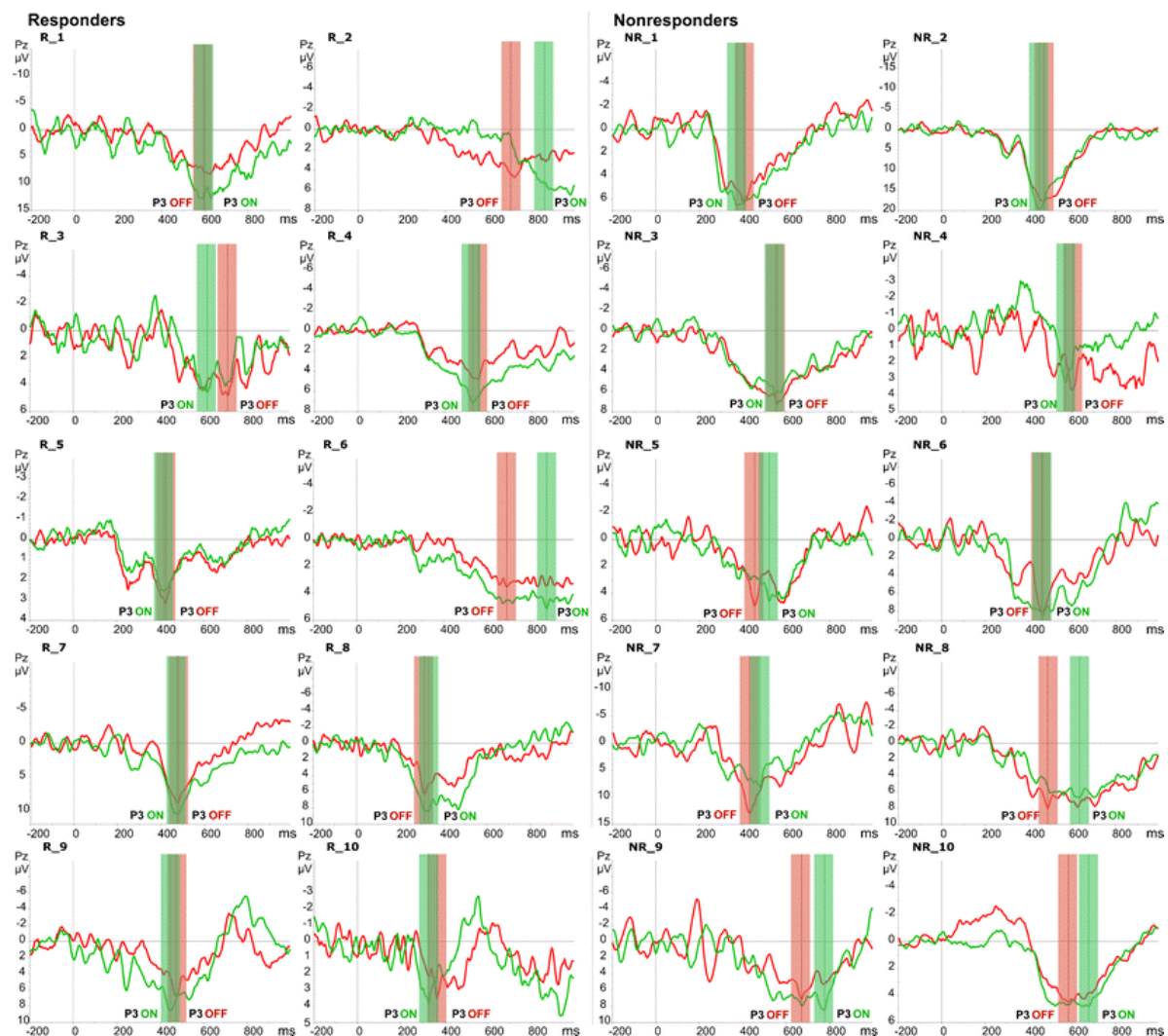


Figure 1. Target-standard event-related potential difference waveforms at the parietal midline electrode Pz displayed separately for each patient: R_1–10 responders and NR_1–10 non-responders. P3 amplitude was measured as the mean of the 100-ms marked interval round the peak in the OFF condition (red) and ON condition (green).

Table 2 summarizes the means of the P3 latency and amplitude and F and p-values of the statistical analysis. Mixed-model ANOVA of the latency of the P3 component revealed no significant effects of condition, group or the group \times condition interaction. Post hoc tests confirmed that there were no significant differences in the latency of the P3 component between the ON and OFF conditions in both groups: responders [$t(9) = 0.16$, $p = 0.875$] and non-responders [$t(9) = 1.81$, $p = 0.105$]. However, the mixed-model ANOVA of the amplitude of the P3 component revealed a significant interaction between group and condition [$F(1,18) = 5.39$, $p = 0.017$]. The main effects of group and condition were not significant. Post hoc analysis comparing the VNS ON and OFF conditions revealed that the amplitude of the P3 was significantly increased in responders: amplitude OFF $4.4 \pm 1.8 \mu\text{V}$ and ON $6.0 \pm 3.0 \mu\text{V}$ [$t(9) = 3.48$, $p = 0.007$], while in non-responders this increase was not observed: amplitude OFF $6.6 \pm 4.0 \mu\text{V}$ and ON $6.4 \pm 3.8 \mu\text{V}$ [$t(9) = 0.39$, $p = 0.706$] (Figure 2). VNS induced an average P3 amplitude increase of 32.6 % in responders, while in non-responders there was an average decrease of 0.5 % when the ON condition was compared with the OFF condition (see Table 1). In conclusion, VNS induces a significant increase of the oddball P3 amplitude at the parietal midline electrode in VNS responders only.

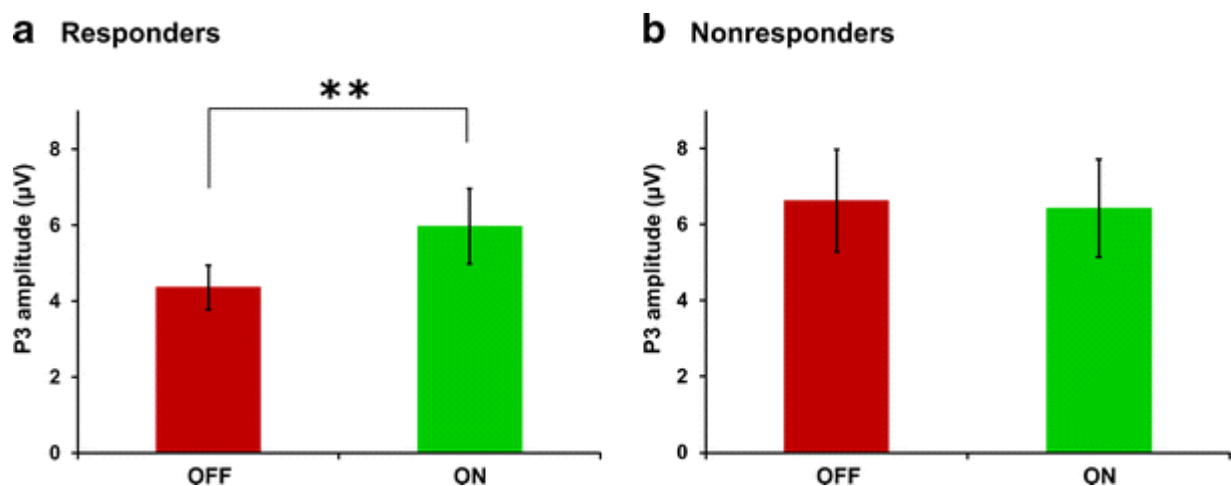


Figure 2. Bar plots showing the average and standard error of the P3 peak amplitude, displayed separately for each group: (a) responders and (b) non-responders. Only in the responder group was the amplitude of the P3 significantly larger for the vagus nerve stimulation (VNS) ON condition compared with the VNS OFF condition. $**p < 0.01$.

Logistic regression analysis with VNS responder as the dependent variable showed that a 1 % VNS-induced increase of the P3 amplitude has an OR of 1.056 (95 % CI 1.005–1.109; $p = 0.030$). This OR means that with each percentage of increase of the P3 amplitude the odds of being a VNS responder will be 5.6 % higher. The ROC analysis revealed an area under the curve of 0.82 (95 % CI 0.63–1.00; $p = 0.016$) (Table 3, Figure 3). This indicates that the measured variable has a very good predictability for

VNS responders vs. non-responders. ROC analysis of the VNS-induced P3 amplitude increase shows that a cut-off score of >20 % has the optimal trade-off between sensitivity and specificity, and the highest predictive values. Sensitivity and specificity for a P3 amplitude cut-off score of >1.0 μV were 70 % and 90 %, respectively. The positive predictive value for this cut-off score was 88 %; the negative predictive value was 75 %.

Table 3. Receiver operating characteristic and predictive values of P3 amplitude for vagus nerve stimulation responders

Cut-off score	NPV	PPV	Specificity	Sensitivity	AUC	SE	95 % CI	p-value
> 15 %	0.73	0.78	0.80	0.70				
> 20 %	0.75	0.88	0.90	0.70	0.82	0.097	0.63 - 1	0.016
> 25 %	0.69	0.86	0.90	0.60				
> 35 %	0.71	1.00	1.00	0.60				

NPV negative predictive value; PPV positive predictive value; AUC area under curve; SE standard error; CI confidence interval

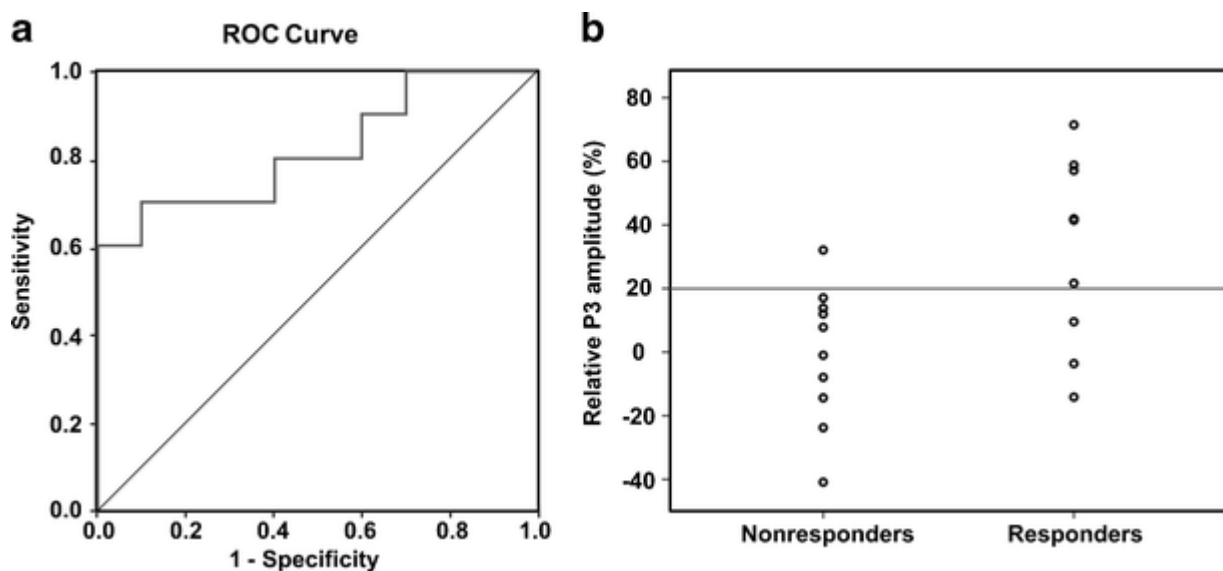


Figure 3. (a) Receiver operating characteristic (ROC) curve of the relative P3 amplitude for vagus nerve stimulation (VNS) responders/non-responders. (b) Scatter plot of the group of responders and non-responders. Logistic regression analysis with VNS responder as the dependent variable and cut-off score of >20 % (horizontal line) has a sensitivity of 70 % and specificity of 90 %.

DISCUSSION

This study provides novel evidence that supports the hypothesis that VNS-induced activation of the LC-NE system is associated with the therapeutic response to VNS in patients with epilepsy. Our ERP results show that VNS induces a significant increase of the oddball P3 amplitude at the parietal midline electrode in VNS responders only. In addition, logistic regression analysis revealed that the increase of the P3 amplitude can be used as a non-invasive indicator for VNS responders and non-responders with high sensitivity and specificity when a cut-off value of >20 % amplitude increase is used.

Our new ERP results are consistent with the research of Neuhaus et al., who examined the effect of VNS on the P3 component of the auditory ERP in patients with major depressive disorder [Neuhaus et al., 2007]. Their study reported that after 10 weeks of VNS the amplitude of the P3 was significantly increased, but only in VNS responders, that is in patients with a significant reduction of the depressive symptoms, as measured using the Hamilton Depression Rating Scale. So far, only 2 ERP studies focusing on the effect of VNS on the P3 have been conducted in patients with refractory epilepsy [Hammond et al., 1992; Brázdil et al., 2001]. Hammond et al. found no effect of either acute or chronic VNS on the latency and amplitude of the auditory P3 component [Hammond et al., 1992]. Brázdil et al. showed that VNS had no effect on auditory ERP components [Brázdil et al., 2001]. In contrast, VNS induced higher visual N2/P3 peak-to-peak amplitude on visual ERPs [Brázdil et al., 2001]. However, both studies had rather small sample sizes (9 and 10 patients, respectively) and did not distinguish between VNS responders and non-responders, which might explain these discrepant results. The results of the previous ERP studies of VNS in epilepsy patients are not inconsistent with our study or the study by Neuhaus et al. [Neuhaus et al., 2007], as in both the latter studies clear effects at the level of the P3 were evidenced only when separating responders and non-responders to VNS.

Early observations in patients with epilepsy have shown that VNS has mood-enhancing effects [Handforth et al., 1998]. Prospective studies using standard depression rating scales confirmed that VNS is associated with statistically significant mood improvements [Elger et al., 2000; Harden et al., 2000]. Today, VNS is used as a safe and effective therapy for treatment-resistant depression [Nahas et al., 2005; Daban et al., 2008]. Depression is the most frequent psychiatric comorbidity in epilepsy [Kanner and Schachter, 2010]. Converging evidence indicates that mood disorders and epilepsy have a complex bidirectional relationship with the existence of common pathogenic mechanisms that are operant in both conditions [Kanner, 2011]. At the neurotransmitter level, reduced noradrenergic signaling has been proposed as one of the underlying

mechanisms giving rise to epilepsy and depression [Jobe, 2003]. In line with this assumption several antidepressive medications, such as selective noradrenaline reuptake inhibitors, work by increasing the NE levels in the brain. Therefore, the antidepressive effects of VNS can also be caused by activation of the neuromodulatory LC–NE system [Jobe, 2003]. This hypothesis is supported by the study of Neuhaus et al. [Neuhaus et al., 2007], which demonstrated that the P3 is enhanced in responders to VNS for treatment of major depressive disorder. This suggests that the P3 can be used as an indirect measure for VNS-induced activation of the LC–NE system to detect VNS responders in both refractory epilepsy and depression. As such, our new results confirm that ERPs may provide a valuable tool as potential biomarkers for several psychiatric and neurologic disorders (for a review, see [Luck et al., 2011]).

In this study, we found that in VNS responders, the ON condition led to an increase of the P3 amplitude compared with the OFF condition, which suggests, though indirectly, a mediation of this antiepileptic effect by the LC–NE system. Our new ERP results add to the existing literature showing that electrical stimulation of the vagus nerve activates the LC–NE system and that this activation is critical for the antiepileptic effect of VNS [Fornai et al., 2011]. This hypothesis is supported by several lines of converging evidence in the literature. First, the vagus nerve afferent fibers project to the nucleus of the solitary tract; in turn, the nucleus of the solitary tract projects both directly and indirectly to the LC [Van Bockstaele et al., 1999]. Acute electrical stimulation of the vagus nerve induces an increase in the discharge rate of LC noradrenergic neurons [Groves et al., 2005]. In addition, the basal firing rate in the LC is significantly increased after long-term treatment with VNS [Dorr and Debonnel, 2006]. In rats, VNS induces an increase of NE concentration in the hippocampus [Roosevelt et al., 2006], the amygdala [Hassert et al., 2004], and the cerebral cortex [Roosevelt et al., 2006; Follesa et al., 2007]. Moreover, lesions of the LC block the anticonvulsant effect of VNS [Krahl et al., 1998]. Furthermore, a strong positive correlation was found between the VNS-induced increase in NE levels and the seizure-suppressing effects of VNS [Raedt et al., 2011]. Intrahippocampal application of an alpha-2 adrenoceptor antagonist in a hippocampal seizure model blocked the anticonvulsant effect of VNS [Raedt et al., 2011]. Together, these results bolster the assumption that the degree of NE release in the brain can be a useful biomarker for the therapeutic efficacy of VNS in epileptic patients.

To our surprise, we found a general improvement in behavioral response (i.e., faster reaction times and greater accuracy) during VNS in all patients, regardless of the therapeutic efficacy of VNS. These results are consistent with previous evidence demonstrating VNS-induced cognitive and behavioral improvements that were not related to the changes in seizure frequency [Clark et al., 1999; Sackeim et al., 2001; Aldenkamp

et al., 2002; Sjogren et al., 2002]. It seems contradictory that VNS improves the behavioral performance in all patients, while it increases the P3 amplitude only in responders. However, it is possible that the threshold for “functional” activation of the LC–NE system (reflected by the behavioral improvement) is much lower than the threshold for the antiepileptic effect of the LC–NE system (reflected by the P3). The P3 component is thought to originate from neuronal inhibitory signals that inhibit unrelated neural activity to promote processing efficiency of task-relevant stimuli, thereby yielding large P3 amplitudes [Polich, 2007]. Correspondingly, activation of the LC–NE system in response to an important stimulus sends a “network reset” signal to the brain that interrupts the activity of the ongoing functional networks and facilitates their reorganization to promote rapid behavioral adaptation [Sara, 2009; Sara et al., 2012]. It is probable that only in the group of responders, because of differences in network connectivity and interactions with other neuromodulators, the VNS-induced activation of the LC–NE system results in a stronger inhibition and network reorganization that causes the antiepileptic effect. The P3 amplitude is proposed to reflect the LC–NE inhibition [Polich, 2007] and this could explain why the amplitude is increased only in responders. Nonetheless, this remains a hypothetical explanation and further research is necessary to clarify the different effects of VNS on cognition and seizure control.

The current results should be interpreted with caution because epilepsy is a complex, heterogeneous, and variable neurological condition, and various potential confounding factors need to be taken into account, such as type of epilepsy, type and frequency of seizures, age of onset and duration of epilepsy, brain lesions, side and localization of the hypothesized epileptogenic zone, and antiepileptic drugs (AEDs) [Kubota et al., 1998; Caravaglios et al., 2001]. The strength of our approach was to compare responder and non-responder patients with epilepsy while controlling as much as possible for potential confounding factors stemming from the heterogeneous clinical parameters and the AEDs. It is conceivable that the differential P3 modulation in responders versus non-responders could be a consequence of different AEDs. However, this seems unlikely given the extensive evidence that AED can increase the latency of P3 components, but are not responsible for P3 amplitude modulation in both healthy adults [Meador et al., 1995] and epilepsy patients [Enoki et al., 1995; Kubota et al., 1998; Caravaglios et al., 2001]. In our study, both groups took a comparable range of AEDs, and no reliable modulation of the latency of the P3 component was found depending on the experimental condition. Only the P3 amplitude varied systematically with the protocol in the responder group exclusively. None of the patients’ AEDs were tapered after they became VNS responders, which excludes that the VNS-induced effects on P3 amplitude are caused by reduced AED-related side effects after drug tapering.

Our analysis has several other limitations. First, there is considerable overlap between the P3 amplitude values of the responder and non-responder groups. Although a cut-off of P3 amplitude increase of >20 % has reasonably good sensitivity (70 %) and specificity (90 %), this biomarker is definitely not perfect, because 30 % of responders and 10 % of non-responders are misclassified with this cut-off. The patients in our study had very heterogeneous ERP waveshapes and clinical characteristics. It is highly probable that because of these interindividual differences VNS will not have the same effect on the P3 in all patients. The P3 component of the ERP is only an indirect measure of LC-NE activity, and other confounding factors could influence the amplitude of the P3. We have tried to control for these factors by comparing the P3 amplitude between the ON and OFF condition within the same subject and by calculating the relative amplitude differences. Second, the vagus nerve has widespread projections to nuclei in the brainstem and to all cortical regions, as well as to the thalamus, hippocampus, and amygdala, where they modulate the activity of target cells and networks [Boon et al., 2001; Ben-Menachem, 2002]. In addition, there are strong reciprocal connections between the different neuromodulatory systems (noradrenaline, dopamine, serotonin, and acetylcholine), which makes it very hard to delineate the role of one single system [Sara, 2009]. Consequently, the LC-NE system does not act alone, but interacts with other modulatory neurotransmitter pathways that also play an important contributive role in the antiepileptic effect of VNS. A third limitation is that we were unable to determine causality in our study. We found a significant increase of the P3 in the group of responders, but we cannot be certain what caused the seizure reduction. The P3 is probably an epiphenomenon of the LC-NE activation and subsequent inhibition. These limitations notwithstanding, we believe that the significant difference that was found in our analysis indicates that the P3 has potential as a non-invasive biomarker for VNS responders.

In the present study, we have recruited patients who received VNS therapy for at least 18 months. Presumably, this chronic stimulation has led to long-term changes in the neuronal networks and neurotransmitter systems [Boon et al., 2001]. Therefore, it remains unclear whether these P3-effects will also be observed in epilepsy patients prior to initiation of VNS therapy. Future longitudinal prospective studies are needed to resolve this issue. Patients with epilepsy should be tested prior to implantation of the VNS device as well as 12–18 months afterwards in order to compare the acute versus chronic effects of VNS. These longitudinal studies are required to assess whether we can predict, based on the baseline measurement (phasic effects), which patients will eventually become responders to the VNS therapy (chronic effects) and whether modulation of P3 amplitude can be used as a prospective measure to predict the therapeutic efficacy of VNS.

In conclusion, our novel ERP results support the hypothesis that VNS-induced activation of the LC noradrenergic signaling is associated with the antiepileptic effect of VNS. Amplitude modulations of the P3 should be further investigated as a non-invasive biomarker to predict the treatment response to VNS in patients with refractory epilepsy. A biomarker for the efficacy of VNS could help neurologists to choose the optimal stimulation parameters in a more objective way. In combination with a non-invasive technique to deliver VNS, such as transcutaneous VNS (tVNS) [Stefan et al., 2012], responders could be identified prior to surgery. Hence, the biomarker could avoid unnecessary implantations of a VNS device in non-responders and consequently improve the clinical efficacy of VNS.

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CHAPTER 2

Event-related potentials reveal preserved attention allocation but impaired emotion regulation in patients with epilepsy and comorbid negative affect

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ABSTRACT

Patients with epilepsy have a high prevalence of comorbid mood disorders. This study aims to evaluate whether negative affect in epilepsy is associated with dysfunction of emotion regulation. Event-related potentials (ERPs) are used in order to unravel the exact electrophysiological time course and investigate whether a possible dysfunction arises during early (attention) and/or late (regulation) stages of emotion control. Fifty epileptic patients with (n=25) versus without (n=25) comorbid negative affect plus twenty-five matched controls were recruited. ERPs were recorded while subjects performed a face- or house-matching task in which fearful, sad or neutral faces were presented either at attended or unattended spatial locations. Two ERP components were analyzed: the early vertex positive potential (VPP) which is normally enhanced for faces, and the late positive potential (LPP) that is typically larger for emotional stimuli. All participants had larger amplitude of the early face-sensitive VPP for attended faces compared to houses, regardless of their emotional content. By contrast, in patients with negative affect only, the amplitude of the LPP was significantly increased for unattended negative emotional expressions. These VPP results indicate that epilepsy with or without negative affect does not interfere with the early structural encoding and attention selection of faces. However, the LPP results suggest abnormal regulation processes during the processing of unattended emotional faces in patients with epilepsy and comorbid negative affect. In conclusion, this ERP study reveals that early object-based attention processes are not compromised by epilepsy, but instead, when combined with negative affect, this neurological disease is associated with dysfunction during the later stages of emotion regulation. As such, these new neurophysiological findings shed light on the complex interplay of epilepsy with negative affect during the processing of emotional visual stimuli and in turn might help to better understand the etiology and maintenance of mood disorders in epilepsy.

INTRODUCTION

Patients with epilepsy have a very high prevalence of comorbid psychiatric disorders [Kanner and Schachter, 2010]. Negative affect occurs in up to 80 % of patients with epilepsy [Miller et al., 2008] and may manifest as major depressive disorder (MDD) meeting the diagnostic and statistical manual IV (DSM-IV) criteria, or atypical mood disorders with waxing and waning affective symptoms called “interictal dysphoric disorder” [Blumer et al., 2004] or “dysthymic-like disorder of epilepsy” [Kanner and Schachter, 2010]. In patients with epilepsy, depressive symptoms have a major negative impact on the quality of life [Boylan et al., 2004; Johnson et al., 2004] and increase the risk of suicide up to 10-fold [Jones et al., 2003]. Given the high impact on the quality of life and the associated elevated mortality due to suicide a better understanding of the pathogenic mechanisms of negative affect in epilepsy is important [Jones et al., 2005].

Negative affect in epilepsy has been attributed to several causes, including the psychological reaction to the chronic seizure disorder, endocrine or metabolic effects of seizures, adverse effects of antiepileptic drugs (AEDs) and common pathophysiological mechanisms between depression and epilepsy, such as neurotransmitter disturbances and abnormal frontotemporal networks [Kondziella et al., 2007; Miller et al., 2008; Kanner and Schachter, 2010; Hoppe and Elger, 2011; Kanner, 2011]. The common pathological changes can compromise the integrity of a functional neuronal network that is implicated in emotion control [Ochsner and Gross, 2005; Price and Drevets, 2010; Disner et al., 2011]. Emotion control refers to both early automatic forms of regulation, like controlling attention to emotional arousing stimuli, as well as higher forms of cognitive control, such as the conscious reappraisal of the emotional valence of stimuli [Ochsner and Gross, 2005]. Recently, Holtzheimer and Mayberg proposed a model for negative affect that is hallmarked by dysfunction of both forms of emotion control [Holtzheimer and Mayberg, 2011]. This model emphasizes that it is not the negative affect state that is abnormal. Instead, it is the tendency to enter the negative affect state and the inability to disengage from this state because of the impaired emotion regulation that defines mood disorders. Therefore, this study focuses on emotion control and more specifically investigates whether negative affect in patients with epilepsy is associated with dysfunction during early attention processes and/or later stages of emotion regulation.

To address this question, we used a variant of the face- or house-matching task [Wojciulik et al., 1998], a standard task for measurement of attention and emotion regulation [Vuilleumier et al., 2001; Holmes et al., 2003; Bentley et al., 2003; Vuilleumier et al., 2004; Bishop et al., 2004a; Bishop et al., 2004b; Fales et al., 2008].

In this procedure, participants are shown a display with two houses and two faces presented in vertical and horizontal pairs. They have to attend only one pair and have to make a demanding same/different judgment on the attended pair of stimuli. The faces have either a neutral or emotional expression and are positioned either in attended or unattended spatial locations. This paradigm provides an ideal situation in which both attention and emotion can be manipulated independently [Vuilleumier et al., 2001].

Event-related potentials (ERP) are recorded during this paradigm in order to disentangle effect of attention and emotion during early (attention) and late (regulation) stages of emotion processing. One previous ERP study has investigated spatial attention during the face- or house matching task in healthy participants [Holmes et al., 2003]. This study has demonstrated that the early face-sensitive N170 component amplitudes were significantly enhanced when faces were at attended spatial locations. The N170 is a negative component with latency around 170 ms that has a larger amplitude for faces than houses or other objects at occipitotemporal electrodes. The N170 has remarkable temporal and functional similarity with the vertex positive potential (VPP) that is recorded at the central midline electrode and is also typically enhanced in response to face stimuli [Botzel and Grusser, 1989; Botzel et al., 1995; Jeffreys, 1989; George et al., 1996]. Hence, it has been suggested that both N170 and VPP components are part of the same neural dipole located in or near the fusiform gyrus [Rossion et al., 2003; Luo et al., 2010]. The temporally coincident N170 and VPP are the earliest markers of a reliable processing difference between faces and objects and are linked with the structural encoding of faces [Rossion et al., 2003; Holmes et al., 2003]. Therefore, we measured the early face-sensitive N170/VPP components to examine whether epilepsy and negative affect have an influence on object-based attention.

Many ERP studies that study emotion have focused on a broad parietal positive component that occurs roughly 300 ms after emotional stimuli, called the late positive potential (LPP). The LPP is a robust visual ERP component that is known to have an enhanced amplitude for both positive and negative arousing emotional stimuli compared to neutral stimuli [Schupp et al., 2000; Schupp et al., 2006; Sabatinelli et al., 2007; Olofsson et al., 2008; Hajcak et al., 2010]. The magnitude of the LPP is sensitive to emotion regulation strategies and can be reduced by reappraisal of the emotional significance of stimuli, e.g., reappraising unpleasant stimuli as less negative decreases the LPP amplitude [Moser et al., 2006; Foti and Hajcak, 2008; Hajcak et al., 2010]. Hence, the LPP can be used indirectly as an electrophysiological marker of the covert processing of the emotional intensity of the visual stimuli.

In the present study, attention and emotion effects during the face- or house-matching task were compared between epileptic patients with vs. without comorbid negative affect and matched healthy controls. ERPs were used in order to explore the exact electrophysiological time course and investigate whether a possible dysfunction arises during early (attention, VPP/N170) and/or late (regulation, LPP) stages of emotion control.

METHODS

Ethics statement

The study was approved by the ethics committee of Ghent University Hospital and conducted in accordance with the declaration of Helsinki. After a full description of the procedure was provided and explained, all participants gave written informed consent prior to participation.

Participants

A total of fifty patients with refractory epilepsy were included (M/F: 26/24, mean age 34.7 years). The study took place during presurgical video-EEG monitoring in the Reference Center for Refractory Epilepsy (Ghent University Hospital, Belgium). Patients were included in the study if they met the following inclusion criteria: (i) confirmed epilepsy based on continuous video/EEG monitoring, (ii) age 18–65, (iii) no arguments for mental retardation. Twenty-five healthy volunteers free from neurological or psychiatric symptoms were matched as closely as possible to the patients with respect to age, sex, and education (M/F: 14/11, mean age 37.0 years). The main clinical characteristics of participants are summarized in Table 1.

Presence of negative affect was assessed by using the validated Dutch version of the Beck Depression Inventory II (BDI-II) [Beck et al., 1996; Van der Does, 2002]. The BDI-II is a 21-item self-report questionnaire that assesses the severity of depressive symptomatology, including affective, cognitive, behavioral, somatic and motivational symptoms of depression. Individuals rate each symptom on a scale ranging from 0 to 3. Higher scores on the BDI reflect more negative affect with scores ranging from 0 to 63. Using the criteria proposed by Beck et al. (0–13 minimal, 14–19 mild, 20–28 moderate, 29–63 severe depressive symptoms) [Beck et al., 1996], a cut-off score of >14 was used to subdivide the patients in two groups: 25 patients with negative affect (mean BDI: 23.6 ± 9.4), 25 patients without negative affect (mean BDI: 5.0 ± 3.5) and 25 control participants (mean BDI: 3.6 ± 3.0). In addition, state and trait anxiety levels of all participants were measured, following standard practice, using the State-Trait Anxiety Inventory (STAI) [Spielberger et al., 1983], translated in Dutch [Van der Ploeg et al., 2000].

Table 1. Demographic data for each group of participants

	Patients with negative affect (n=25)	Patients without negative affect (n=25)	Controls (n=25)	Statistics
BDI	23.6 (\pm 9.4)	5.0 (\pm 3.5)	3.6 (\pm 3.0)	p<0.001
Age (years)	33.7 (\pm 10.0)	35.7 (\pm 10.9)	37.0 (\pm 11.9)	p=0.502
Sex (M/F)	16 / 9	10 / 15	14 / 11	p=0.156
Education (years)	13.1 (\pm 1.5)	13.6 (\pm 1.7)	14.6 (\pm 1.5)	p=0.292
HEZ: side			NA	p=0.683
Right	9	12		
Left	15	12		
Bilateral	1	1		
HEZ: lobe			NA	p=0.232
Frontal	10	5		
Temporal	11	17		
Fronto-temporal	1	2		
Parietal/Occipital	3	1		
3T MRI			NA	p=0.733
Normal	6	5		
Abnormalities	19	20		
• Frontal	7	8		
• Temporal	10	12		
○ Medial temporal	9	10		
• Parietal/Occipital	3	2		
Epilepsy duration (years)	11.7 (\pm 9.5)	18.9 (\pm 10.8)	0	p=0.016
Seizure frequency (/month)	11.9 (\pm 12.2)	11.1 (\pm 13.0)	0	p=0.825
Number of AEDs (/day)	2.4 (\pm 0.8)	2.9 (\pm 0.9)	0	p=0.074
AEDs total dose (mg/day)	2474.8 (\pm 1416.1)	2631.6 (\pm 1773.7)	0	p=0.731
ADDs total dose (mg/day)	27.7 (\pm 67.2)	0	0	p=0.045
STAI State	46.3 (\pm 10.4)	34.8 (\pm 8.1)	28.0 (\pm 4.9)	p<0.001
STAI Trait	52.3 (\pm 8.3)	35.8 (\pm 7.9)	34.6 (\pm 7.5)	p<0.001

Values represent means (\pm 1 standard deviation) or numbers.

Abbreviations: *BDI* Beck Depression Inventory, *M* male, *F* female, *HEZ* hypothesized epileptogenic zone; *3T MRI* 3 tesla magnetic resonance imaging, *AEDs* antiepileptic drugs; *ADDs* antidepressant drugs, *STAI* state-trait anxiety inventory

Stimuli

All stimuli comprised displays of four pictures, with two faces and two houses arranged in vertical and horizontal pairs around a central black fixation cross (Figure 1). All pictures were black and white photographs presented on a gray background and had the same size across all experiments (108 [width] × 154 [height] pixels on a 1024 * 768 resolution screen) subtending 4.0 × 5.7° of visual angle at a 50 cm viewing distance. The stimuli included 10 fearful faces, 10 neutral faces, 10 sad faces and 20 houses, with pictures from each category repeated equally across all trials. The neutral, fearful and sad facial expression photographs were drawn from the set of Ekman and Friesen [Ekman and Friesen, 1976].



Figure 1. Sample visual stimuli of the face- or house-matching task.

Each trial comprised a display of four pictures, with two houses and two faces arranged in vertical and horizontal pairs around a central black fixation cross. Before each block, a visual cue (i.e., thickening of two frames) instructed participants to attend either to the vertical pair or the horizontal pair of stimuli, while ignoring the other pair. Subjects had to indicate quickly and accurately whether the two stimuli at the task-relevant locations were the same or different (i.e., matching task). On any given trial, both faces had either a fearful, sad or neutral expression and were shown at task-relevant or task-irrelevant locations. The neutral, fearful and sad facial expression photographs were drawn from the set of Ekman and Friesen [Ekman and Friesen, 1976].

Procedure

The face- or house-matching task was adapted from previous studies [Vuilleumier et al., 2004]. Stimulus presentation and response time recording were controlled using E-Prime software 2.0 (Psychology Software Tools Inc., Pittsburgh, USA). Before each block, a 3 s display instructed subjects to attend to horizontal or vertical stimulus pairs, while ignoring the other stimulus pairs. The instruction display consisted of four empty frames placed at the location of the stimuli, with either the two horizontal or the two vertical frames being thickened. Trials began with a central fixation cross for 1 s, followed by the four-picture display for 300 ms. Subjects were asked to maintain fixation centrally throughout the trials and attend covertly to the stimulus pair at the relevant locations, in

order to judge whether these two stimuli were the same or different by pressing one out of two keys. The inter-trial interval (ITI) varied randomly between 1 and 3 s. All participants completed 10 practice trials and 4 blocks of 48 trials, with two blocks where the attention was directed to horizontal positions and two blocks where the attention was directed to vertical positions. In each block, all possible combinations of two object categories (faces vs. houses), their locations, same/different identity, and facial expression were fully randomized and counterbalanced across trials, resulting in a total of 32 neutral, 32 sad and 32 fearful faces at task-relevant locations, and the same number for each expression at task-irrelevant locations (total 192 trials). Instructions emphasized both accuracy and speed. Response times were recorded from stimulus onset. Trials were excluded when there was no response within 2 seconds.

EEG recording

The electroencephalogram (EEG) was recorded with Micromed System Plus (Micromed, Mogliano Veneto, Italy) using gold electrodes placed at 27 standard locations from the extended international 10–20 system (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, O2, T9, TP9, FT9, T10, TP10 and FT10). The online reference electrode was placed on the right mastoid and ground electrode on the left mastoid. The electrocardiogram (ECG) was recorded with two ECG electrodes placed above the heart. The EEG and ECG signals were digitized online with a sampling frequency rate of 1024 Hz, anti-aliasing filter of 250 Hz, gain of 50 dB and 16 bits resolution. Electrode impedance was maintained below 10 k Ω .

ERP analysis

ERPs of interest were computed offline following a standard sequence of data transformations [Picton et al., 2000]. All offline ERP analyses were performed using BrainVision Analyzer 2 software (Brain Products GmbH, Gilching, Germany). The EEG was corrected for vertical and horizontal eye movements, blinks and ECG artifacts with an independent component analysis (ICA) that subtracts these artifact components from each electrode. The raw EEG was first decomposed into ICA components using the restricted infomax algorithm. Then the three components related to eye-movements, blinks and ECG artifacts were selected by visual inspection, relying on both the time course and the spatial maps of the components herewith generated. These components were removed and the remaining ICA components were projected back using an inverse ICA to reconstruct the artifact-free EEG. The EEG signal was then re-referenced to the average of all 27 recorded channels. The continuous EEG was first digitally filtered with a 50 Hz notch filter and a half-power band-pass filter between 0.1–30 Hz with a roll-off of 12 dB/octave. The EEG was segmented into epochs from –200 ms to +1000 ms relative

to the onset of the stimuli. Baseline correction was performed on the 200 ms pre-stimulus interval and epochs with voltage exceeding $\pm 75 \mu\text{V}$ were excluded from averaging. The average fraction of rejected epochs was 4.7 % in the group of patients with negative affect, 4.1 % in the group of patients without negative affect and 5.4 % in the control group (one-way ANOVA $F=0.2$, $p=0.8$). Artifact free epochs were averaged separately for each condition and each individual. The grand average ERPs were generated by computing mean ERPs across subjects, for each condition separately. The effects of attention and emotion on sensory processing were analyzed by focusing on two well-documented ERP components: the vertex positive potential (VPP) and the late positive potential (LPP). The VPP was detected automatically as the maximum positive amplitude in the 140–210 ms interval post-stimulus onset at the central midline Cz [Rossion et al., 2003]. The VPP amplitude was calculated as the mean amplitude of the 20 ms interval around this peak. The N170 measurements were made on lateral temporal-occipital electrode sites T5 and T6 using the same time window [Rossion et al., 2003; Holmes et al., 2003]. The LPP amplitude was measured as the average amplitude of the 350–600 ms interval post-stimulus onset at parietal midline electrode Pz [Schupp et al., 2000; Moser et al., 2006]. Repeated measures analysis of variance (ANOVA) was used with a 2-tailed alpha level of 0.05 for all statistical tests. When assumptions of sphericity were violated (Mauchly's sphericity test, $p<0.05$), Greenhouse–Geisser epsilon correction was applied. The analyses of the ERP measures included a between-subjects factor of group (with negative affect vs. without negative affect vs. control) and a within-subjects factor of attention (task-relevant vs. task-irrelevant) and emotion (neutral vs. sad vs. fear). Post-hoc tests of simple effects were adjusted with the Bonferroni correction for multiple comparisons. In order to control for possible confounding factors of damage to the medial temporal lobe, frontal lobe and antidepressant dose, separate ANOVA's were performed with these factors entered as covariates. Correlation between the LPP amplitude differences and antidepressant dosing were tested using 2-tailed Pearson's correlation coefficient.

RESULTS

Clinical data

Differences in clinical parameters between epileptic patients with negative affect and without negative affect were assessed with unpaired t-test for continuous variables and Pearson chi-square test for categorical variables. There were no significant differences in age [$t(48)=0.7$, $p=0.5$], sex [$X^2=2.9$, $p=0.1$], years of education [$t(48)=1.1$, $p=0.3$], side of the hypothesized epileptogenic zone (HEZ) [$X^2=1.1$, $p=0.3$], lobe of the HEZ [$X^2=4.3$, $p=0.2$], 3T MRI abnormalities [$X^2=0.1$, $p=0.7$], damage to medial temporal lobe [$X^2=0.1$, $p=0.8$], seizure frequency [$t(48)=0.2$, $p=0.8$], number AEDs [$t(48)=1.8$, $p=0.1$] and AEDs dose [$t(48)=0.3$, $p=0.7$]. Duration of epilepsy was significantly higher in the group of patients without than with negative affect [$t(48)=2.5$, $p=0.02$]. Accordingly, longer duration of epilepsy was not associated with increased negative affect. As expected, the mean BDI score was significantly higher in the group with negative affect (23.6 ± 9.4) than the group without negative affect (5.0 ± 3.5) [$t(48)=9.2$, $p<0.001$]. The STAI-S and STAI-T scores were also significantly higher in the group with than without negative affect [STAI-S: $t(48)=4.3$, $p<0.001$, STAI-T: $t(48)=7.2$, $p<0.001$]. Correlation analysis using a 2-tailed Pearson coefficient showed significant positive correlations of the BDI scores with the STAI-S ($r=0.73$, $p<0.001$) and the STAI-T ($r=0.81$, $p<0.001$) scores.

Behavioral results

Mean response times and accuracy in same/different judgments, performed during continuous video-EEG monitoring, were computed for each subject in each of the six conditions. Behavioral results are summarized in Figure 2. To examine data, mixed model ANOVA was performed with group as between-subject factor (control vs. patients with negative affect vs. patients without negative affect) and two within-subject factors: attention (faces vs. houses) and emotional expression (fearful vs. neutral vs. sad).

Mean error rate was 25 % indicating that the matching task was relatively demanding. A significant group-related effect was found for accuracy: epileptic patients with negative affect made most errors (35 %), epileptic patients without negative affect made 25 % errors, while control subject had an error-rate of 18 % [main effect of group $F(2,72)=10.4$, $p<0.001$]. Subjects made more errors overall when judging faces (30 %) than houses (19 %) [main effect of attention $F(1,72)=103.2$, $p<0.001$]. Participants had lower accuracy when the faces carried a fearful or sad emotional expression [main effect of emotion $F(2,72)=4.2$, $p=0.017$]. Subjects made significantly more errors when the negative emotional faces were task-relevant [interaction attention*emotion $F(2,72)=7.2$, $p=0.001$]. Post-hoc analysis with Bonferroni correction revealed higher error rates for

relevant sad [$p < 0.001$] and fearful faces [$p = 0.031$] relative to neutral faces. All other comparisons were not significant. There was no significant interaction of attention or emotion with group [interaction attention*group $F(2,72) = 0.3$, $p = 0.8$, interaction emotion*group $F(2,72) = 1.3$, $p = 0.3$, interaction emotion*attention*group $F(4,72) = 0.3$, $p = 0.9$].

Analysis of reaction times (RTs) showed a mean RT of 639 ms, that was not significantly different between groups [main effect of group $F(2,72) = 0.8$, $p = 0.4$]. Participants were significantly slower to make same/different judgments with faces (672 ms) compared to houses (606 ms) [main effect of attention $F(1,72) = 44.2$, $p < 0.001$]. In addition, we found a significant interaction of group with attention [$F(2,72) = 4.1$, $p = 0.02$]. In the control group there was a larger difference in RT when comparing houses (mean 554 ms) than faces (mean 659 ms) [$p < 0.001$] than in the other two groups. RTs were significantly slower for emotional compared to neutral faces [main effect of emotion $F(2,72) = 4.3$, $p = 0.019$]. RT analysis revealed a significant interaction of attention and emotion: all subjects showed significantly slower reaction times in displays in which task-relevant faces had a fearful (681 ms) or sad (683 ms) compared to neutral (651 ms) expression [interaction attention*emotion $F(2,72) = 6.7$, $p = 0.002$]. Post-hoc analysis with Bonferroni correction revealed slower reaction times for task-relevant sad [$p = 0.002$] and fearful faces [$p = 0.004$] relative to neutral faces. All other comparisons were not significant. However, there was no significant interaction of group with emotion [interaction emotion*group $F(2,72) = 0.6$, $p = 0.6$; interaction emotion*attention*group $F(4,72) = 0.6$, $p = 0.7$].

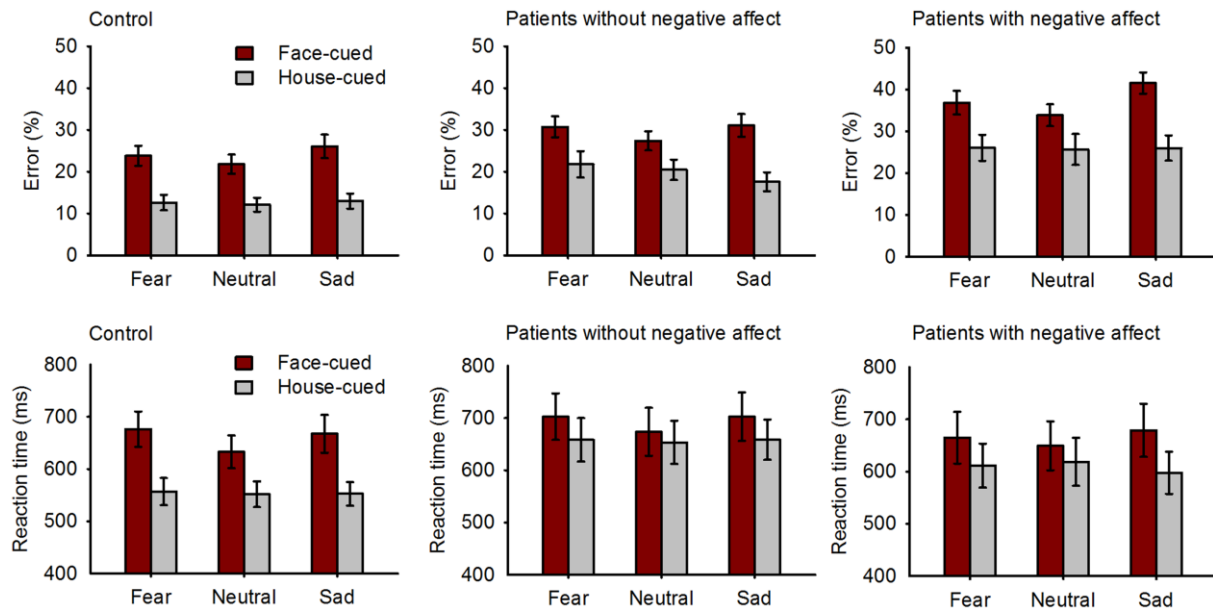


Figure 2. Behavioral results.

Percentage errors (upper panels) and reaction time in ms (lower panels) in response to faces (red bars) and houses (grey bars), displayed separately for each group: control (left panels), patients with epilepsy without negative affect (middle) and with comorbid negative affect (right). All three groups made more errors and had slower reaction times when attended faces carried a fearful or sad emotional expression, relative to a neutral expression.

Electrophysiological results

VPP/N170. All groups had a large face-sensitive VPP component at central midline electrode Cz (Figure 3). The amplitude of the VPP was significantly larger on trials in which attention was focused on the face pairs relative to trials during which faces were presented outside the attention focus [main effect of attention $F(1,72)=13.0$, $p<0.001$]. Noteworthy, there was no significant effect of group or interaction group*attention on the amplitude of the face-sensitive VPP component, indicating that the processing of the face stimuli was normal and preserved in all groups [main effect of group $F(2,72)=2.2$, $p=0.1$, interaction group*attention $F(2,72)=0.8$, $p=0.4$]. The effect of emotion or any interaction with this factor did not reach significance [$F(2,72)\leq 1.6$, $p\geq 0.2$].

The N170 is suggested to be the negative counterpart of the positive VPP, because both components are temporally coincident and have a high functional sensitivity for faces [Rossion et al., 2003]. This is confirmed by our ERP results showing very similar effects of attention for the VPP and N170 components. The amplitude of the N170 at the T5 and T6 electrodes was significantly larger when faces were attended compared to when houses were attended [main effect of attention $F(1,72)=5.4$, $p=0.023$ at T5 electrode and $F(1,72)=22.1$, $p<0.001$ at T6 electrode]. However, not all subjects showed a clear N170 component, because there were more artifacts and noise at the lateral T5 and T6

electrodes than at the central midline electrode Cz. Therefore, we focus on the VPP component in this study.

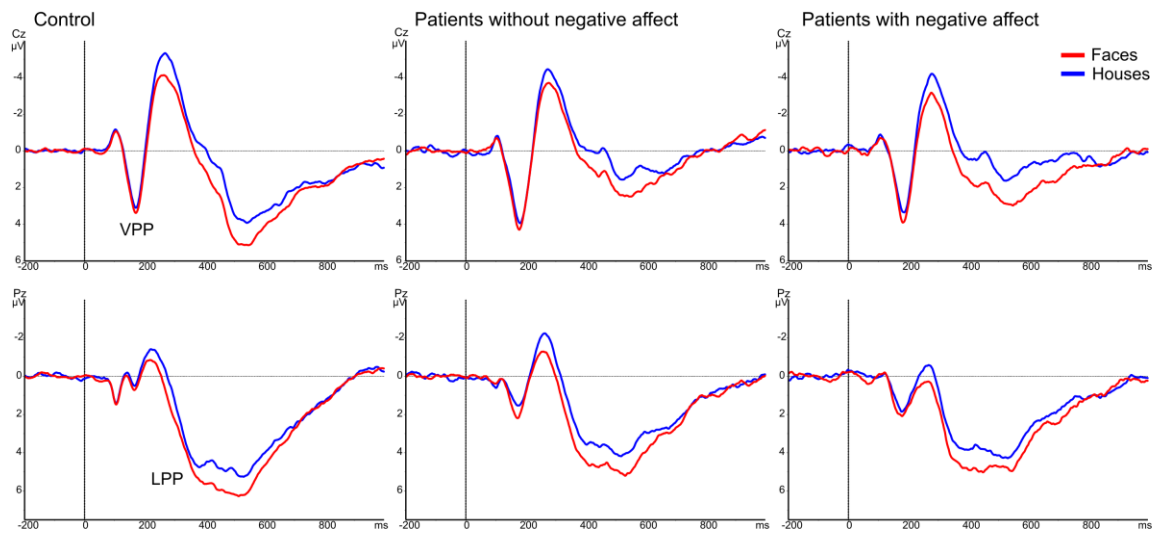


Figure 3. Stimulus-locked grand average ERP waveforms.

Grand average ERP waveforms recorded from central midline electrode Cz (upper panels) and parietal midline electrode Pz (lower panels) in response to faces (red lines) and houses (blue lines), displayed separately for each group: control (left panel), patients with epilepsy without negative affect (middle) and with comorbid negative affect (right). In the three groups alike, the amplitude of the VPP and LPP components was significantly larger for face-cued relative to house-cued trials.

LPP. The emotion-sensitive LPP component was analyzed at the parietal midline electrode Pz from 350 to 600 ms post stimulus (Figure 4). There were no significant differences between groups on the mean amplitude of the LPP [main effect of group $F(2,72)=1.7$, $p=0.2$]. In all subjects the amplitude of the LPP component was enhanced for displays in which faces were task-relevant compared to displays in which houses were task-relevant [main effect of attention $F(1,72)=49.7$, $p<0.001$] (Figure 3). In addition, we found a significant three-way interaction of group with attention and emotion [$F(4,72)=3.0$, $p=0.021$]. For post-hoc analyses we made pairwise comparisons with Bonferroni correction between the 3 emotional conditions (fear vs. neutral vs. sad) within each attention condition (house vs. face) and within each group. In total 18 comparisons were made (3 emotion * 2 attention conditions * 3 groups). These analyses revealed that only in patients with negative affect the LPP component was significantly increased in response to task-irrelevant sad [$p=0.026$] and fearful faces [$p=0.003$] relative to task-irrelevant neutral faces. All other comparisons were not significant.

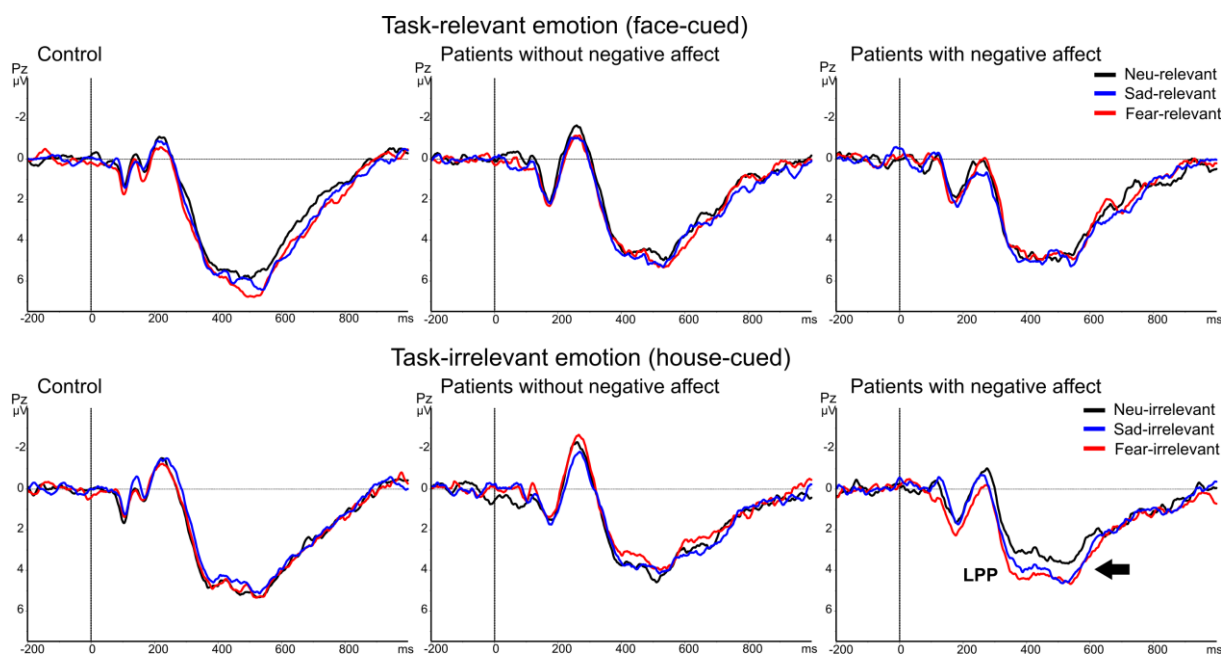


Figure 4. LPP results.

Grand average ERP waveforms recorded from parietal midline electrode Pz in responses to task-relevant faces (upper panels) and houses (lower panels), displayed separately for each group: control (left), patients with epilepsy without negative affect (middle) and with comorbid negative affect (right). Note that only in the group of epileptic patients with negative affect, when the emotion was task-irrelevant, the amplitude of the LPP was significantly larger for sad and fearful expressions compared to neutral faces.

Correlation between the differences of the LPP amplitudes between unattended emotional and neutral faces and BDI and STAI scores were tested using 2-tailed Pearson's correlation coefficients. There were significant positive correlations found between the LPP amplitude differences between unattended fearful and neutral faces and the BDI ($r=0.34$, $p=0.014$) as well as the STAI ($r=0.29$, $p=0.038$). No such significant correlations were found between the LPP amplitude differences between unattended sad and neutral faces and either the BDI ($r=0.20$, $p=0.155$) or the STAI ($r=0.14$, $p=0.335$).

The type and location of the lesions in each group turned out to be very heterogeneous (Supplementary Table 1). Most common lesions were hippocampal sclerosis, focal cortical dysplasia, cysts and cavernomas. Only one patient had a brain tumor, which was located in the left posterior hippocampus and suspected to be a low-grade glioma. This patient was part of the group of patients with epilepsy without negative affect. In order to examine whether damage to the medial temporal lobe has an influence on the emotion modulation of the LPP, all patients with epilepsy, regardless of negative affect, were subdivided into two groups: one group where the 3T MRI showed clear damage to the medial temporal lobe ($n=19$) and one group without damage to the medial temporal lobe ($n=31$). Noteworthy, repeated-measures ANOVA showed no significant main effect of

medial temporal lobe damage on the LPP amplitude [$F(1,48)=1.3, p=0.3$] and there were no significant interactions with other factors. In addition, a separate analysis that examined the effect of damage to the frontal lobe showed neither significant main effect of frontal lobe damage on LPP amplitude [$F(1,48)=0.4, p=0.5$] nor significant interactions with other factors. These results suggest that damage to the medial temporal lobe or frontal lobe did not account for the amplitude modulation of the LPP.

Another possible confounding factor is that some patients of the group with negative affect took antidepressant drugs (ADDs) while none of the participants in the other two groups took ADDs. However, there was no significant main effect of ADD dose on the LPP [$F(1,23)=0.02, p=0.9$] and no significant interactions of the ADD dose with the other experimental factors [attention*ADD dose $F(1,23)=3.1, p=0.1$; emotion*ADD dose $F(2,23)=1.8, p=0.2$; attention*emotion*ADD dose $F(2,23)=0.4, p=0.7$]. Moreover, a correlation analysis using a 2-tailed Pearson coefficient failed to show a significant association between the ADD dose and the amplitude difference between unattended fear faces compared to unattended neutral faces [$r=0.10, p=0.62$] or unattended sad faces compared to unattended neutral faces [$r=0.14, p=0.50$].

DISCUSSION

This study provides novel neurophysiological findings on the processing of emotional stimuli in patients with epilepsy with and without comorbid negative affect, when compared to a group of matched healthy controls. At the behavioral level, all subjects made more errors and had slower reaction times when attended faces carried a fearful or sad emotional expression, relative to a neutral expression. These results suggest that negative emotional face expressions, when attended, interfered with the matching task requiring the processing of the identity (as opposed to emotional content) of the face stimuli. At the electrophysiological level, the face-sensitive VPP had enhanced amplitude for attended faces compared to houses, equally so in all three groups and regardless of the emotional content of the face stimulus. These ERP results indicate that attention was directed to the correct stimulus category independently of the emotional content of the face, and that this early structural encoding of faces was normal and preserved in patients with epilepsy, regardless of negative affect. By contrast, the amplitude of the LPP was significantly enhanced for negative emotional expressions when faces were unattended, but only in patients with comorbid negative affect. The modulation of the LPP component by unattended emotional stimuli during the late stages of stimulus processing suggests that emotion regulation is disturbed in patients with epilepsy and comorbid negative affect.

Our behavioral results show that in all groups the attended negative emotional face expressions decreased task performance, which resulted in lower accuracy and slower reaction times, compared to attended neutral faces. These findings are in line with previous studies that have reported interference effects created by negative emotional stimuli [Vuilleumier et al., 2001; Bishop et al., 2004a; Pessoa, 2009]. From an evolutionary perspective, priority processing of emotional information facilitates adaptive behavior, promoting survival and reproductive success [Lang, 1995; Lang and Bradley, 2010]. The enhanced processing demands associated with emotional stimuli leave limited resource capacities for performance during the task, that requires to match the identity of the two attended visual stimuli [Vuilleumier, 2005; Pessoa, 2009; Pourtois et al., 2013]. Accordingly, these behavioral findings confirm that in all three groups, emotion interfered with task performance when it was attended, although not explicitly task-relevant.

The results obtained for the VPP/N170 component reveal a clear gating effect, in the expected direction, of object-based attention mechanisms. All subjects showed enhanced amplitudes of the VPP/N170 components for attended faces (regardless of their emotional content) compared to attended houses. Our findings are in agreement with a

previous ERP study using the same task in healthy adult participants that reported similar increased amplitudes of the N170 in response to attended faces [Holmes et al., 2003]. Importantly, in our study, this object-based attention effect was evidenced in all three groups alike; suggesting that neither epilepsy alone, nor epilepsy combined with negative affect actually impaired the normal and early structural encoding of faces. According to previous ERP studies, [Botzel et al., 1995; Jeffreys, 1989; George et al., 1996; Rössion et al., 2003], the VPP is the counterpart at the vertex of the occipito-temporal N170 component and this dipolar activity reflects the earliest markers of a reliable processing difference between faces and objects. Therefore, our new ERP findings clearly show that this early categorization process is spared in epilepsy with or without negative affect.

By contrast, at a later time point following stimulus onset than the VPP, we found evidence for a modulatory effect of epilepsy with comorbid negative affect on the processing of these complex stimuli. We found that in patients with epilepsy and comorbid negative affect the amplitude of the LPP was significantly modulated when the emotional faces were unattended. It seems contradictory that emotional stimuli presented outside the focus of attention have a stronger influence on the LPP than when the same stimuli are attended. This is in contrast with many ERP studies that have shown that the LPP component has enhanced amplitudes for attended negative emotional stimuli compared to neutral stimuli in healthy participants [Schupp et al., 2000; Moser et al., 2006; Schupp et al., 2006; Sabatinelli et al., 2007; Foti and Hajcak, 2008; Olofsson et al., 2008; Hajcak et al., 2010]. However, these studies had longer picture presentation time (≥ 1 second) and the emotional expression of the face was task relevant because participants had to rate pictures for arousal and valence. Therefore, the task-relevant emotional content was probably much more strongly processed, reflected by increased LPP amplitudes. In our study, the stimuli were presented very briefly (300 ms) and the emotional expression was not explicitly relevant for the matching task. This might explain why in healthy control group and the group of patients without negative affect there was no significant modulation of the LPP by the emotional expression of the faces. By contrast, the LPP was enhanced in patients with epilepsy and comorbid negative affect when the emotional faces were unattended. It is probable that the negative affect triggers an automatic emotional processing or vigilance effect (reflected by increased amplitude of the LPP) when negative stimuli are distracters. This could point to a deficit to inhibit distracting negative emotional information, or conversely, to a better sensitivity to process them “covertly” outside the focus of attention. Accordingly, our LPP results show that negative emotional distracters have an influence on the late stages of stimulus processing in epilepsy patients with negative affect. Hence, the deficit in these patients is a deficit during the late stages of emotion control, during which they

fail to ignore distracting emotional information, unlike the two other groups where the late processing of visual stimuli is not influenced by emotional distracters at unattended locations.

A few limitations have to be pointed out. Firstly, we have not included an additional control group of patients with negative affect but without epilepsy. More than 70 % of mood disorders in epilepsy are atypical and fail to meet any of the diagnostic criteria of the DSM-IV [Blumer et al., 2004; Kanner and Schachter, 2010]. Therefore, a group of patients with mood disorders would not fully control for the type of negative affect in epilepsy. Patients with major depressive disorder, for example, would have more severe depression than the patients included in this study. As expected, epileptic patients with negative affect were not only showing higher levels of depressive symptoms (BDI) than patients without negative affect, but also higher levels of anxiety (STAI). This multicollinearity is not surprising, but instead in line with previous studies that have identified comorbid anxiety symptoms in 73 % of patients with epilepsy and depression [Jones et al., 2005]. There is a substantial symptom overlap and comorbidity between depression and anxiety and both disorders are characterized by high levels of negative affect [Clark and Watson, 1991]. Accordingly, future ERP studies are needed in order to establish whether depression or rather anxiety lies at the root of the emotion regulation disorder observed in our study at the level of the LPP. To the best of our knowledge, until now no ERP studies have been published on the face- or house-matching task in patients with negative affect. Studies on the VPP and LPP in mood and anxiety disorders during other tasks have yielded mixed results, depending on the used paradigm, type of stimuli, stimulus presentation time and study population. There is evidence for increased processing of negative emotional stimuli soon after stimulus presentation, reflected by enhanced early ERPs like the VPP, followed by avoidance of unpleasant stimuli at later processing stages, reflected by a reduced LPP for aversive stimuli, both in patients with general anxiety disorder [Weinberg and Hajcak, 2011] and patients with major depressive disorder [Foti et al., 2010]. In contrast, other studies have reported increased LPP for aversive compared to neutral pictures among subjects with high negative affect [Dennis and Hajcak, 2009; MacNamara and Hajcak, 2009]. Therefore, it would be very interesting in future studies to compare ERP results during the face-or house-matching task in different control groups of patients without epilepsy but with other types of negative affect disorders, such as major depressive disorder, dysthymic disorder, bipolar disorder, general anxiety disorder, in order to evaluate whether this is a general effect found across these negative affect disorders, or instead, whether it is specific for negative affect in epilepsy.

Secondly, the administration of antidepressant drugs (ADDs) in one group selectively but not in the two others may have obscured our new ERP findings. However, if ADDs would influence the amplitude of the LPP, then we would expect to see a main effect, and not a complex three-way interaction effect, as we report here. Moreover, when the ADD dose was added as a covariate in the statistical analysis for the LPP, no significant contribution of this factor was found. Furthermore, there was no significant correlation between the ADD dose on the one hand and the LPP amplitude differences between unattended sad or fearful compared to unattended neutral faces on the other hand. Taken together, it therefore appears very unlikely that the condition-specific modulation of the LPP might be explained by exposure to antidepressant medication.

Thirdly, we did not use eye tracking during the face- or house-matching task. However, participants were asked to maintain central fixation and eye movements were discouraged and unlikely with this specific demanding matching task, given the brief stimulus duration and task requirements used. This was formally confirmed by previous studies with eye tracking during the same task that demonstrated that saccades were very rare, with no major differences in eye position associated with the experimental factors [Wojciulik et al., 1998; Vuilleumier et al., 2001; Bentley et al., 2003; Vuilleumier et al., 2004]. In addition, an ICA was used to correct for horizontal and vertical eye movements.

Fourthly, it is important to consider the type of lesions because specific lesions can cause widespread reorganization of neural networks. For example, patients with left sided tumors show signs of functional reorganization and employ a much broader bilateral network during language processing than healthy controls [Rosler et al., 2014]. However, in our study, only one patient had a brain tumor in the group of patients without negative affect. The type, size and location of lesions of the patients were very heterogeneous and therefore the groups for each lesion were too small in size to compare at the statistical level the possible differential effect of each specific type of underlying lesion on negative affect and ERP results. Notwithstanding this limitation, we note that it may be possible that some of these lesions could have had a greater influence on our ERP results than other ones, especially lesions located in regions that are presumably important for emotion control processes, like the frontal and medial temporal regions. Therefore, we performed additional data analyses and grouped the patients based on the presence of either frontal or medial temporal lobe structures lesions, but these analyses failed to show any significant effect on the LPP amplitude. Hence, the abnormalities arising during the later stages of emotion stimulus processing in patients with epilepsy and comorbid negative affect could not be linked to damage in one specific lobe but are more likely the result from dysfunction in a broad network for emotion control in which both cortical and

subcortical structures interact with each other [Ochsner and Gross, 2005; Price and Drevets, 2010].

Comorbid negative affect in patients with epilepsy has often been considered to be a consequence or complication of the chronic seizure disorder. However, a fascinating bidirectional relationship between epilepsy and depression has recently been demonstrated [Kanner, 2011; Hesdorffer et al., 2012]: not only are patients with epilepsy at greater risk of developing a depressive disorder, but patients with primary depressive disorders are at greater risk of developing epilepsy [Hesdorffer et al., 2000; Hesdorffer et al., 2012]. This suggests that the pathogenic mechanisms may be strongly intertwined and the structural and functional alterations from one disease are likely to trigger the other [Kondziella et al., 2007; Kanner et al., 2012]. Identification of these common underlying pathogenic mechanisms may shed new light on the neurobiological bases of mood disorders and epilepsy. Our findings suggest comorbid negative affect in patients with epilepsy may be due to impaired emotion regulation.

In conclusion, the face-sensitive VPP results indicate that early attention was allocated to the correct stimulus category and that early stimulus processing was preserved in all patients with epilepsy, regardless of negative affect. Conversely, the LPP results suggest that during later stages of stimulus processing the emotion regulation is disturbed, but only in patients with epilepsy and comorbid negative affect. These new neurophysiological findings shed light on the complex interplay of epilepsy with negative affect during the processing of emotional visual stimuli and in turn might help to better understand the etiology and maintenance of mood disorders in epilepsy.

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CHAPTER 3

Abnormal reappraisal during emotion regulation in refractory epilepsy: an ERP study

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ABSTRACT

Patients with refractory epilepsy have a very high comorbidity of negative affect disorders. Reappraisal is a cognitive emotion regulation strategy that is successful in decreasing negative affect. Cognitive reappraisal involves reinterpreting the meaning of emotional situations in a way that alters its emotional impact. The late positive potential (LPP) component of the visual event-related potential is larger following the presentation of emotional compared to neutral stimuli. The amplitude of the LPP can be reduced by cognitive reappraisal of emotional stimuli. Therefore, the LPP is a sensitive neurophysiological marker for cognitive emotion regulation. The aim of this study was to investigate whether the electrophysiological response to negatively valenced stimuli in patients with refractory epilepsy is sensitive to reappraisal.

Fifty-two patients with refractory epilepsy participated in the study, of whom 25 with negative affect (BDI-score >14) and 27 without negative affect (BDI-score ≤14). Event-related potentials were recorded while subjects viewed negatively valenced and neutral pictures that were preceded by either more neutral or more negative descriptions.

In all patients, the valence-ratings, arousal-ratings and LPP amplitude were significantly increased in response to unpleasant compared to neutral pictures. In addition, unpleasant pictures following the more neutral description (reappraisal condition) had significantly reduced ratings for negative valence and arousal. However, there was no significant modulation of the LPP amplitude of unpleasant pictures during the reappraisal condition. Deficient reappraisal of the LPP activity in response to unpleasant stimuli was independent of negative affect and not linked to localization of the epileptogenic zone in frontal or medial temporal lobe.

This study shows that modulation of LPP amplitude as electrophysiological correlate of cognitive emotion regulation is absent in patients with epilepsy. This suggests abnormal functioning of the brain during cognitive emotion regulation in patients with epilepsy.

INTRODUCTION

The ability to cognitively regulate our emotional responses to negative emotional events is a critical component for good mental health [Ochsner and Gross, 2005]. Disturbance of our emotion control capacity may lead to an increased vulnerability to negative affect [Mathews et al., 2005; Disner et al., 2011]. Patients with epilepsy have a very high comorbidity of negative affect disorders [Kanner and Schachter, 2010]. Negative affect occurs in up to 80 % of patients with refractory epilepsy [Miller et al., 2008], with a major negative impact on the quality of life [Boylan et al., 2004] and an elevated mortality due to suicide [Jones et al., 2003]. Negative affect comorbidity has been attributed to several causes, including the psychological burden of having a chronic seizure disorder, adverse effects of antiepileptic drugs (AEDs), endocrine or metabolic effects of seizures, neurotransmitter disturbances and network reorganization associated with chronic epilepsy [Miller et al., 2008; Kanner and Schachter, 2010; Hoppe and Elger, 2011; Kanner, 2011]. These pathological changes can compromise the integrity of a functional neuronal network that is implicated in emotion control [Ochsner and Gross, 2005; Price and Drevets, 2010; Disner et al., 2011].

Reappraisal is a commonly used cognitive emotion regulation strategy that is successful in decreasing negative affect [McRae et al., 2010]. Cognitive reappraisal involves reinterpreting the meaning of emotional situations in a way that alters its emotional impact [Ochsner and Gross, 2005; Todd et al., 2012]. Functional neuroimaging studies have shown that successful reappraisal depends upon interactions between prefrontal cortex that implements cognitive control, and amygdala that mediates the emotional responses [Ochsner and Gross, 2005; McRae et al., 2010].

Event-related potentials (ERPs) have been used to investigate the time course of cognitive emotion regulation processes. These studies have focused on the late positive potential (LPP) component of ERP that has proven to be a sensitive and effective neurophysiological marker for investigating the time course of emotional processing in both healthy individuals and patients [Hajcak et al., 2010; Moran et al., 2013]. The LPP is a robust visual ERP component known to have an enhanced amplitude for both positive and negative arousing emotional stimuli compared to neutral stimuli [Schupp et al., 2000; Sabatinelli et al., 2007; Hajcak et al., 2010]. Moreover, the magnitude of the LPP is sensitive to emotion regulation strategies and can be reduced by reappraisal of the emotional significance of stimuli [Hajcak and Nieuwenhuis, 2006; Moser et al., 2006; Foti and Hajcak, 2008; Dennis and Hajcak, 2009; MacNamara and Hajcak, 2009]. Hence, the LPP is a useful tool for studying emotion regulation during a reappraisal task [Foti and Hajcak, 2008; Dennis and Hajcak, 2009; Hajcak et al., 2010].

Our previous study in patients with refractory epilepsy [De Taeye et al., 2015] provided neurophysiological evidence that early attention processes were not compromised by epilepsy. By contrast, patients with epilepsy and comorbid negative affect showed an abnormal enhanced LPP in response to unattended negative stimuli, suggesting dysfunction during the later stage of emotion regulation [De Taeye et al., 2015]. Whether the LPP response in patients with epilepsy and comorbid negative affect can be reduced by cognitive reappraisal remains an intriguing question.

The aim of the present study was quadruple: (i) to confirm that negative emotional stimuli induce a stronger LPP response compared to neutral stimuli in patients with refractory epilepsy, (ii) to examine whether this emotional LPP response is sensitive to reappraisal, a cognitive emotion regulation strategy, (iii) to investigate the hypothesis that epilepsy with comorbid negative affect is associated with insufficient reappraisal, and (iv) to determine whether efficacy of reappraisal is affected by localization of the epileptogenic zone or brain lesions in crucial regions within the network for emotion control such as the medial temporal lobe and the frontal lobe.

METHODS

1 Ethics statement

The study was approved by the ethics committee of Ghent University Hospital and conducted in accordance with the declaration of Helsinki. After a full description of the procedure was provided and explained, all patients gave written informed consent prior to participation.

2 Participants

A total of 52 patients with refractory epilepsy were included (M/F: 24/28, mean age 36.3 years). The study took place during presurgical video-EEG monitoring at the Reference Center for Refractory Epilepsy (Ghent University Hospital, Belgium). Patients were included in the study when they met following inclusion criteria: (i) confirmed epilepsy based on continuous video-EEG monitoring, (ii) age 18-65, (iii) no arguments for mental retardation. The main clinical patient characteristics are summarized in Table 1.

Presence of negative affect was assessed using the validated Dutch version of the Beck Depression Inventory II (BDI-II) [Beck et al., 1996; Van der Does, 2002]. The BDI-II is a 21-item self-report questionnaire that assesses the severity of depressive symptomatology, including affective, cognitive, behavioral, somatic and motivational symptoms of depression. Higher scores on the BDI reflect more negative affect with scores ranging from 0 to 63. Using the criteria proposed by Beck *et al.* (0-13 minimal, 14-19 mild, 20-28 moderate, 29-63 severe depressive symptoms) [Beck et al., 1996], a cut-off score of >14 was used to subdivide the patients in two groups: 25 patients with negative affect (mean BDI: 26.1 ± 10.9) and 27 patients without negative affect (mean BDI: 7.4 ± 4.4). In addition, state and trait anxiety levels of all participants were measured, following standard practice, using a validated Dutch version of the State-Trait Anxiety Inventory (STAI) [Spielberger et al., 1983; Van der Ploeg et al., 2000].

Ten healthy volunteers (M/F: 3/7, mean age 26.5 years) were recruited to validate our Dutch version of the reappraisal paradigm [Foti and Hajcak, 2008].

Table 1. Clinical characteristics of patients

	Patients with negative affect (n=25)	Patients without negative affect (n=27)	Statistics
BDI	26.1 (\pm 10.9)	7.4 (\pm 4.4)	p<0.001
Age (years)	38.6 (\pm 10.9)	34.1 (\pm 12.5)	p=0.174
Sex (M/F)	9/16	15/12	p=0.177
Education (years)	12.4 (\pm 2.0)	13.3 (\pm 2.0)	p=0.127
HEZ: side			p=0.712
Right	13	17	
Left	10	8	
Bilateral	2	2	
HEZ: lobe			p=0.343
Frontal	7	6	
Temporal	11	18	
Fronto-temporal	1	0	
Parietal/Occipital	4	1	
General	2	2	
3T MRI			p=0.687
Normal	7	6	
Abnormalities	18	20	
• Frontal	5	4	
• Temporal	10	13	
○ Medial temporal	9	8	
• Parietal/Occipital	5	8	
Epilepsy duration (years)	17.9 (\pm 11.8)	17.2 (\pm 13.3)	p=0.852
Seizure frequency (/month)	7.8 (\pm 9.4)	11.2 (\pm 15.4)	p=0.350
Number of AEDs (/day)	2.6 (\pm 1.1)	2.6 (\pm 1.2)	p=0.925
AEDs total dose (mg/day)	2612.2 (\pm 1604.1)	2605.4 (\pm 1383.5)	p=0.987
ADDs total dose (mg/day)	36.5 (\pm 149.24)	0	
STAI State	50.2 (\pm 10.1)	39.3 (\pm 10.7)	p<0.001
STAI Trait	53.0 (\pm 8.4)	39.9 (\pm 9.7)	p<0.001

Values represent means (\pm 1 standard deviation) or numbers. *BDI* Beck Depression Inventory, *M* male, *F* female, *HEZ* hypothesized epileptogenic zone; *3T MRI* 3 tesla magnetic resonance imaging, *AEDs* antiepileptic drugs; *ADDs* antidepressant drugs, *STAI* state-trait anxiety inventory

3 Stimuli

The stimuli consisted of 75 color photographs taken from the International Affective Picture System (IAPS) [Lang et al., 2008]: 25 neutral pictures (mean normative valence= 5.05 ± 1.21 ; mean normative arousal= 2.91 ± 1.93) and 50 negative pictures (mean normative valence= 2.82 ± 1.64 ; mean normative arousal= 5.71 ± 2.16). Each neutral picture was preceded by a corresponding neutral description and each negative picture was preceded by either a negative description that highlighted the negative aspects of the upcoming image, or a more neutral description that described the image in more neutral terms. The paradigm was adapted from a previous study conducted in healthy participants [Foti and Hajcak, 2008]. Exactly the same pictures were used and the corresponding descriptions were translated to Dutch. A complete list of the used IAPS picture numbers and corresponding neutral and negative descriptions can be found in [Foti and Hajcak, 2008].

4 Procedure

Each trial started with a white fixation cross presented on a black screen for 6 s during which participants heard a short (3–5 s) description through headphones. Following the description, the unpleasant or neutral IAPS picture was presented against a homogenous dark background for 3 s. After the offset of each picture, participants rated the picture on the dimensions of valence and arousal. After a delay that varied randomly between 1 and 2 s, the next trial began (Figure 1).

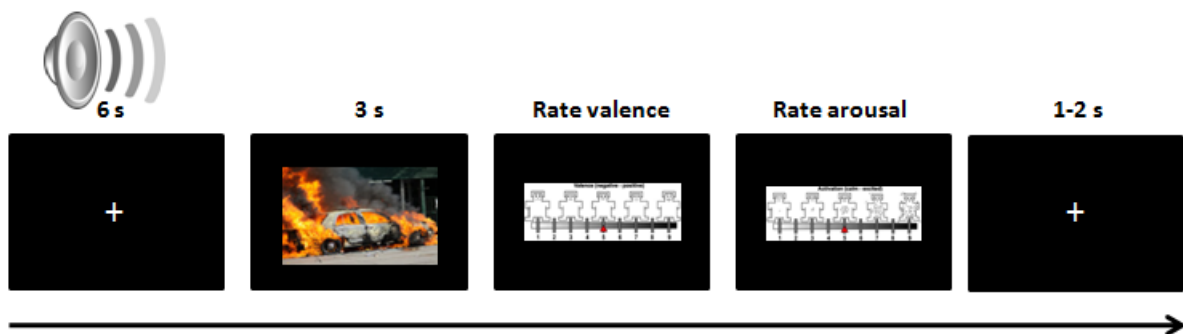


Figure 1. Sequence of events in the reappraisal task.

Prior to each picture, a white fixation cross was presented on a black screen for 6 s. During this period, a brief (3–5 s) description of the upcoming picture was played through headphones. The description was neutral for neutral pictures. For negative pictures, this description could either be negative or neutral (reappraisal condition). Following the description, the negatively valenced or neutral IAPS picture was presented for 3 s. After the picture the patients had to give separate ratings for valence and arousal on two visual analog scales. The inter-trial interval varied randomly between 1 to 2 s.

Participants were told that they would be viewing emotional pictures and that they would hear a short description before each picture about the content of the upcoming picture. After presentation of each picture, subjects were instructed to rate the picture for valence and arousal on two separate scales, i.e., the Self Assessment Manikin (SAM) arousal and valence scales [Bradley and Lang, 1994]. The SAM valence scale shows five cartoon figures ranging from unhappy to happy; below this scale were the numbers "1" through "9". Participants were told to use this scale to indicate how unpleasant or pleasant they felt about the preceding picture. The SAM arousal scale shows five cartoon figures with expressions ranging from calm and relaxed to excited and wide-eyed; again, the numbers "1" through "9" were presented below this scale, and participants were told to rate the picture, but this time based on the strength of their emotional activation by the picture. On both the valence and arousal dimension, a score of 5 represented the midpoint between the two extreme ratings. Valence and arousal ratings were reverse-scored so that a score of 9 represented pleasant valence and high arousal.

All participants completed one practice block with three trials to ensure that the sequence of events was clear (Figure 1), and that they understood the rating scales. After the practice block, each participant performed 75 trials subdivided in 5 blocks of 15 trials with short (self-paced) breaks in between. In each block, the three conditions were randomized, resulting in a total of 25 neutral trials, 25 negative trials with a preceding negative description and 25 negative trials with a preceding more neutral description (reappraisal condition). Each picture was presented only once and the order of trials and the description that preceded each negative picture were chosen randomly for each subject [Foti and Hajcak, 2008]. Stimulus presentation and response recording were controlled using E-Prime software 2.0 (Psychology Software Tools Inc., Pittsburgh, USA) on a Dell laptop computer.

5 EEG recording

The electroencephalogram (EEG) was recorded with Micromed System Plus (Micromed, Mogliano Veneto, Italy) using gold electrodes placed at 27 standard locations from the extended international 10–20 system (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, O2, T9, TP9, FT9, T10, TP10 and FT10). The online reference electrode was placed on the right mastoid and ground electrode on the left mastoid. The electrocardiogram (ECG) was recorded with two ECG electrodes placed above the heart. The EEG and ECG signals were filtered using an anti-aliasing filter of 250 Hz and digitized at a sampling frequency of 1024 Hz, gain of 50 dB and 16 bits resolution. Electrode impedance was maintained below 10 k Ω .

6 ERP analysis

ERPs of interest were computed offline following a standard sequence of data transformations [Picton et al., 2000]. All offline ERP analyses were performed using BrainVision Analyzer 2 software (Brain Products GmbH, Gilching, Germany). The EEG was corrected for vertical and horizontal eye movements, blinks and ECG artifacts with an independent component analysis (ICA). The raw EEG was first decomposed into ICA components using the restricted infomax algorithm. Then the three components related to eye-movements, blinks and ECG artifacts were selected by visual inspection, relying on both the time course and the spatial maps of the components herewith generated. These artifact components were removed and the remaining ICA components were projected back using an inverse ICA to reconstruct the artifact-free EEG. The EEG signal was then re-referenced to the average of all 27 recorded channels. The continuous EEG was first digitally filtered with a 50 Hz notch filter and a half-power band-pass filter between 0.1–30 Hz with a roll-off of 12 dB/octave. The EEG was segmented into epochs from –200 ms to +1000 ms relative to the onset of the stimuli. Baseline correction was performed on the 200 ms pre-stimulus interval and epochs with voltage exceeding ± 75 μV were excluded from averaging. The average fraction of rejected epochs was balanced across conditions and groups: 23.7 % in the group of patients with negative affect and 23.6 % in the group of patients without negative affect (unpaired t-test $t(50)=0.2$, $p=0.9$); and 23.5 % in the neutral condition, 23.7 % in the negative condition and 23.8 % in the reappraisal condition (one-way ANOVA $F=0.3$, $p=0.8$). Artifact free epochs were averaged separately for each condition and each individual. The grand average ERPs were generated by computing mean ERPs across subjects, for each condition separately. The LPP amplitude was measured as the average potential of the 300 to 1000 ms interval post-stimulus onset at the parietal midline electrode Pz [Schupp, 2000; Moser, 2006; Foti, 2008; Dennis, 2009].

Repeated measures analysis of variance (ANOVA) was used with a 2-tailed alpha level of 0.05 for all statistical tests. When assumptions of sphericity were violated (Mauchly's sphericity test, $p<0.05$), Greenhouse-Geisser epsilon correction was applied. The analyses of the valence and arousal ratings and LPP measures included the between-subjects factor group (with negative affect vs. without negative affect) and the within-subjects factor condition (neutral vs. negative vs. reappraisal). Post-hoc tests of simple effects were adjusted with the Bonferroni correction for multiple comparisons. Correlations of the LPP emotion effect (LPP amplitude difference between neutral pictures and unpleasant pictures with preceding negative description) and LPP reappraisal effect (LPP amplitude difference between unpleasant pictures with preceding negative and neutral description) with BDI and STAI were tested using 2-tailed Pearson's correlation

test. To examine the influence of hypothesized epileptogenic zone (HEZ) lobe and side, and damage to the medial temporal lobe and frontal lobe, separate ANOVA's were performed with these factors entered as covariates.

RESULTS

1 Clinical data

Differences in clinical parameters between epilepsy patients with negative affect and without negative affect were assessed with unpaired t-test for continuous variables and Pearson chi-square test for categorical variables (Table 1). There were no significant differences in age [$t(50)=1.38$, $p=0.174$], sex [$X^2=2.00$, $p=0.177$], years of education [$t(50)=1.55$, $p=0.127$], side of the hypothesized epileptogenic zone (HEZ) [$X^2=0.68$, $p=0.712$], lobe of the HEZ [$X^2=4.50$, $p=0.343$], 3T MRI abnormalities [$X^2=0.17$, $p=0.687$], damage to medial temporal lobe [$X^2=0.24$, $p=0.63$], epilepsy duration [$t(50)=0.19$, $p=0.852$], seizure frequency [$t(50)=0.94$, $p=0.350$], number of AEDs [$t(50)=0.09$, $p=0.925$] and AEDs dose [$t(50)=0.02$, $p=0.987$]. As expected, the mean BDI score was significantly higher in the group with negative affect (26.1 ± 10.9) compared to the group without negative affect (7.4 ± 4.4) [$t(50)=8.25$, $p<0.001$]. The STAI-S and STAI-T scores were also significantly higher in the group with than without negative affect [STAI-S: $t(50)=3.79$, $p<0.001$, STAI-T: $t(50)=5.18$, $p<0.001$]. Correlation analysis using a 2-tailed Pearson coefficient showed significant positive correlations of the BDI scores with the STAI-S ($r=0.56$, $p<0.001$) and the STAI-T ($r=0.72$, $p<0.001$) scores.

2 Behavioral results

Mean rating scores for valence and arousal are summarized in Figure 2. Unpleasant pictures were rated as more negative and more arousing compared to neutral pictures. In addition, the description that preceded the unpleasant picture significantly influenced both valence and arousal ratings: during the reappraisal condition, the neutral descriptions increased valence (towards the pleasant extreme) and decreased arousal ratings compared to the negative descriptions. Condition (neutral vs. negative vs. reappraisal) had a significant effect on both valence [main effect of condition $F(2,49)=179.59$, $p<0.001$] and arousal [main effect of condition $F(2,49)=120.63$, $p<0.001$]. Post-hoc comparisons with Bonferroni correction revealed that relative to neutral pictures (5.62 ± 0.87) patients gave more negative valence-ratings for unpleasant pictures following both negative (2.58 ± 0.86) [$p<0.001$] and neutral descriptions (4.22 ± 0.95) [$p<0.001$]. In addition, unpleasant images that followed negative descriptions were rated as less pleasant than unpleasant images that followed neutral descriptions [$p<0.001$]. Post-hoc comparisons with Bonferroni correction of arousal ratings revealed that unpleasant images following both negative (6.48 ± 1.69) and neutral (5.40 ± 1.58) descriptions were rated as more emotionally arousing than neutral pictures (2.84 ± 1.76) [both comparisons $p<0.001$]. Unpleasant pictures

following negative descriptions were rated as more arousing compared to unpleasant pictures that followed neutral descriptions [$p < 0.001$]. There was no group (patients with negative affect vs. patients without negative affect) difference for valence [main effect of group $F(1,50) = 0.08$, $p = 0.778$]. Arousal ratings were significantly higher in patients with comorbid negative affect (5.36 ± 2.23) compared to patients without negative affect (4.49 ± 2.22) [main effect of group $F(1,50) = 5.91$, $p = 0.019$]. However, there was no significant interaction of condition with group for valence or arousal ratings [interaction group*condition $F(2,49) = 2.98$, $p = 0.063$ and $F(2,49) = 0.12$, $p = 0.939$, respectively].

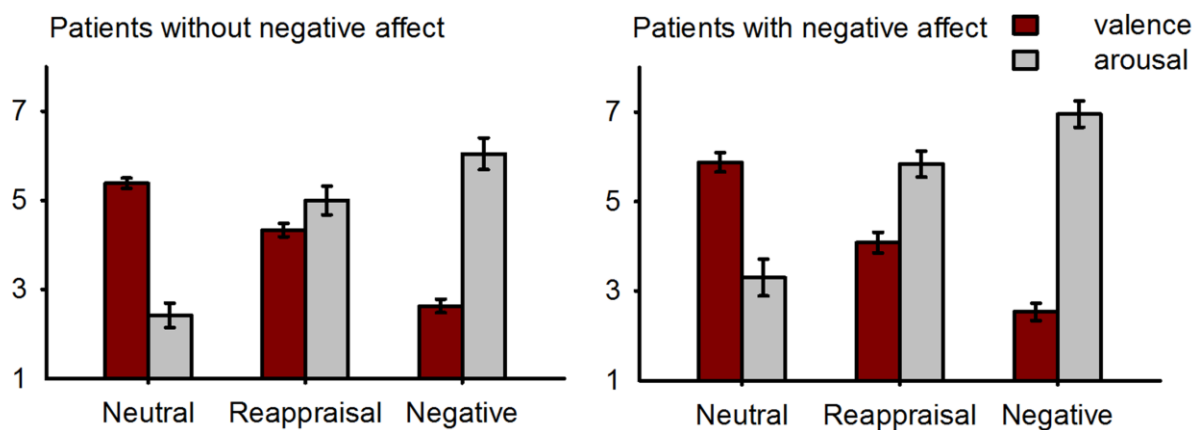


Figure 2. Behavioral results.

Valence and arousal ratings on the SAM-rating scales, displayed separately for each group: patients with epilepsy without negative affect (left panel) and with comorbid negative affect (right panel). Higher scores reflect more positive and higher arousal ratings. Both groups rated unpleasant pictures as more negative and higher arousing compared to neutral pictures. In addition, the description that preceded the unpleasant picture also significantly influenced both valence and arousal ratings: during the reappraisal condition the neutral descriptions increased valence and decreased arousal ratings compared to the negative descriptions.

3 Electrophysiological results

A pilot study was performed in 10 healthy participants to validate the paradigm [Foti and Hajcak, 2008] translated to Dutch with our EEG-recording set-up. The LPP component was analyzed at the parietal midline electrode Pz from 300 to 1000 ms post stimulus (Figure 3). There was a significant effect of condition on the LPP amplitude [main effect of condition $F(2,8) = 3.64$, $p = 0.047$], with larger amplitudes for unpleasant pictures compared to neutral pictures [$p = 0.029$]. In addition, the description that preceded the unpleasant picture also modulated the LPP amplitude: during the reappraisal condition the neutral descriptions induced trend significant smaller LPP amplitudes compared to negative descriptions [$p = 0.078$].

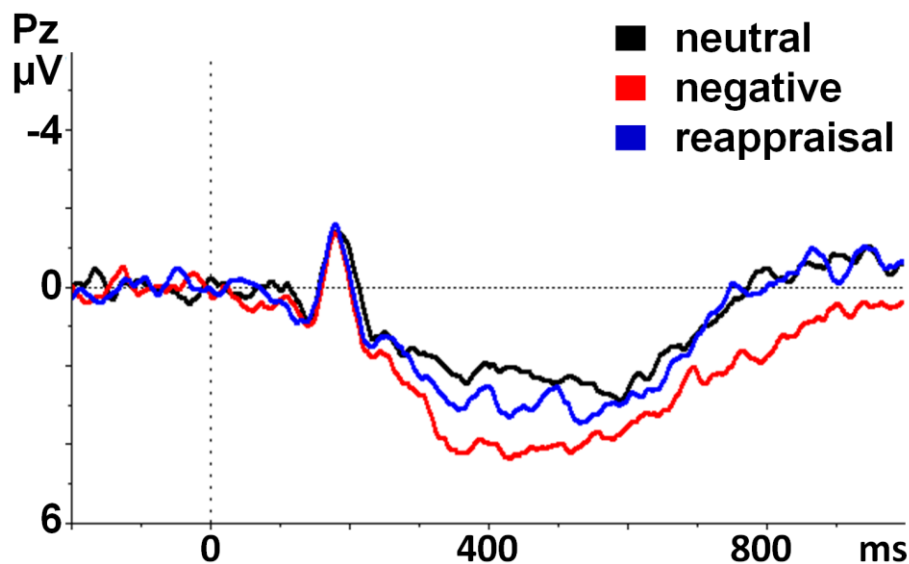


Figure 3. LPP results of pilot study performed in 10 healthy participants.

Grand average ERP waveforms recorded from parietal midline electrode Pz in responses to neutral pictures (black line), unpleasant pictures with preceding negative descriptions (red line) and unpleasant pictures with preceding neutral descriptions, i.e., reappraisal condition (blue line). Note that the amplitude of the LPP was significantly larger for unpleasant pictures compared to neutral pictures. In addition, the description that preceded the unpleasant picture also modulated the LPP amplitude: during the reappraisal condition the neutral descriptions induced smaller LPP amplitudes compared to negative descriptions.

After validation of the reappraisal paradigm, the LPP was measured in 52 patients with refractory epilepsy, of whom 25 with negative affect and 27 without negative affect (Figure 4). There were no significant differences between groups on the mean amplitude of the LPP [main effect of group $F(1,50)=0.75$, $p=0.389$]. In all patients, the amplitude of the LPP component was enhanced for negative stimuli/scenes with preceding negative or neutral descriptions compared to neutral ones [main effect of condition $F(2,49)=30.06$, $p<0.001$]. There was no interaction of group with condition [interaction group*condition $F(2,49)=0.08$, $p=0.922$]. For post-hoc analyses, we made pairwise comparisons with Bonferroni correction between the three conditions (neutral vs. negative vs. reappraisal) within each group. In total six comparisons were made (3 conditions * 2 groups). These analyses revealed that in all patients, neutral pictures induced smaller LPP amplitudes compared to unpleasant pictures with a preceding more negative description [$p<0.001$ in both groups] and unpleasant pictures with a preceding more neutral description [$p=0.002$ in the group with negative affect; $p<0.001$ in the group without negative affect]. However, in both groups, the amplitude of the LPP did not significantly differ between unpleasant pictures described neutrally and negatively [$p=1.000$, in both groups]. A possible confounding factor is that some patients of the

group with negative affect took antidepressant drugs (ADDs) while none of the patients in the group without negative affect took ADDs. However, there was no significant effect of ADD dose on the LPP [$F(1,23)=1.41$, $p=0.248$].

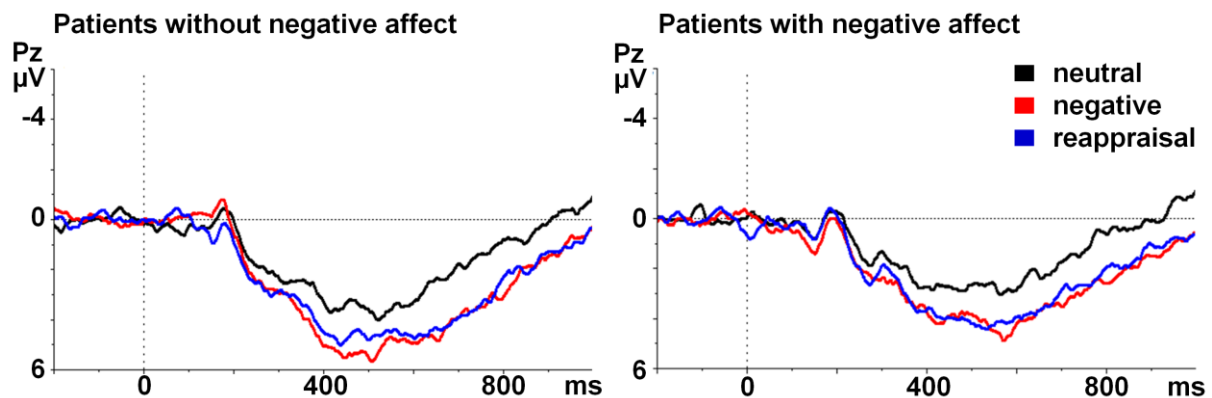


Figure 4. LPP results of patients with epilepsy.

Grand average ERP waveforms recorded from parietal midline electrode Pz in responses to neutral pictures (black line), unpleasant pictures with preceding negative descriptions (red line) and unpleasant pictures with preceding neutral descriptions, i.e., reappraisal condition (blue line), displayed separately for each group: patients with epilepsy without negative affect (left) and with comorbid negative affect (right). Note that the amplitude of the LPP was significantly larger for unpleasant pictures compared to neutral pictures. However, the amplitude of the LPP did not significantly differ between unpleasant pictures described neutrally and negatively.

Correlation of the LPP emotion effect (LPP amplitude difference between neutral pictures and unpleasant pictures with preceding negative description) and LPP reappraisal effect (LPP amplitude difference between unpleasant pictures with preceding negative and neutral description) with BDI and STAI scores were tested using 2-tailed Pearson's correlation coefficients. No significant correlations were found between the LPP emotion effect with either the BDI ($r=0.15$, $p=0.302$), the STAI-S ($r=0.11$, $p=0.430$) and the STAI-T ($r=0.15$, $p=0.278$). In addition, no significant correlations were found between the LPP reappraisal effect with either the BDI ($r=0.16$, $p=0.246$), the STAI-S ($r=0.22$, $p=0.111$) or the STAI-T ($r=0.02$, $p=0.881$).

To evaluate whether modulation of LPP was influenced by the localization of the HEZ, repeated-measures ANOVAs with HEZ side (left vs. right vs. bilateral) and HEZ lobe (frontal vs. temporal vs. frontotemporal vs. parietal/occipital vs. general) as between-subject factors were performed. There were no significant main effects of HEZ lobe [$F(4,47)=0.22$, $p=0.927$] and HEZ side [$F(2,49)=0.04$, $p=0.962$] on the LPP and there were no significant interactions of both factors with condition. To reduce the number of comparisons, a separate ANOVA compared only the patients with HEZ in the frontal lobe ($n=13$) with patients with HEZ in the temporal lobe ($n=29$), but again this analysis

showed no significant effect of HEZ in frontal or temporal lobe [$F(1,40)=0.64$, $p=0.427$], nor significant interaction with condition [$F(2,39)=1.42$, $p=0.247$]. These results suggest that localization of the HEZ in a specific lobe or side could not explain the modulation of the LPP.

In order to examine whether structural damage to the medial temporal lobe has an influence on the emotion modulation of the LPP, all patients with epilepsy, regardless of negative affect, were subdivided into two groups: one group where the 3T MRI showed damage to the medial temporal lobe ($n=17$) and one group without damage to the medial temporal lobe ($n=35$). Repeated-measures ANOVA showed no significant main effect of medial temporal lobe damage on the LPP amplitude [$F(1,50)=1.92$, $p=0.173$] and there was no significant interaction with condition [$F(2,49)=0.71$, $p=0.496$]. In addition, a separate analysis that examined the effect of damage to the frontal lobe showed neither significant main effect of frontal lobe damage on LPP amplitude [$F(1,50)=1.34$, $p=0.252$] nor significant interaction with condition [$F(2,49)=0.31$, $p=0.737$]. These results suggest that damage to the medial temporal lobe or frontal lobe did not account for the modulation of the LPP.

DISCUSSION

In the present study, emotional (self-reports) and reappraisal effects during a cognitive emotion regulation task were examined in patients with refractory epilepsy. The main findings are that: (i) negative emotional stimuli induce a stronger LPP response than neutral stimuli, (ii) this emotional LPP response is not modulated by reappraisal, a cognitive emotion regulation strategy, (iii) deficient reappraisal of the LPP does not correlate with comorbid negative affect, and (iv) there is no relation between the efficacy of reappraisal and location of the epileptogenic zone or brain lesions in medial temporal and frontal lobe. Taken together, our results provide novel electrophysiological evidence for deficient reappraisal of unpleasant pictures in patients with refractory epilepsy at the level of the LPP component.

Reappraisal tasks often test the capacity to generate a personal alternative interpretation of emotional stimuli in a less negative way in order to reduce their emotional impact. The advantage of these tasks is that the cognitive reinterpretation is individualized and the alternate context is optimal for each subject. The downside is that it remains unclear whether reduction in LPP amplitude associated with reappraisal reflects reduced emotional response or increased cognitive demands [Foti and Hajcak, 2008]. By reframing the pictures in a more neutral or negative context with a description preceding their presentation, as is done in the reappraisal paradigm used in this study, the cognitive demands during processing of the picture are balanced across conditions. Moreover, this reappraisal paradigm is particularly effective because it changes the emotional meaning of a picture 'proactively', that is prior to the emotional response being elicited [Foti and Hajcak, 2008; MacNamara and Hajcak, 2009; Dennis and Hajcak, 2009].

The valence and arousal ratings are consistent with previous studies using the same paradigm in healthy controls [Foti and Hajcak, 2008], with unpleasant IAPS pictures rated as more negative and higher arousing compared to neutral IAPS pictures. In addition, the description that preceded the unpleasant picture also significantly influenced both valence and arousal ratings: during the reappraisal condition, the neutral descriptions increased valence and decreased arousal ratings compared to the negative descriptions [Foti and Hajcak, 2008]. In our population of patients with refractory epilepsy reappraisal did not modulate the amplitude of the LPP while it did influence subjective ratings. This dissociation is not odd as similar evidence was found in patients with bipolar disorder [Kanske et al., 2015], which suggests that neurophysiological correlates of reappraisal (such as the LPP) may capture alterations that are not visible in self-reports.

Negative emotional stimuli induced a stronger LPP response relative to neutral stimuli, across all patients with refractory epilepsy. The observed enhanced LPP amplitude in response to emotional arousing pictures is similar to the LPP findings obtained in healthy subjects [Schupp et al., 2000; Moser et al., 2006; Hajcak and Nieuwenhuis, 2006; Sabatinelli et al., 2007; Foti and Hajcak, 2008; MacNamara and Hajcak, 2009; Moran et al., 2013]. The significant LPP enhancement by unpleasant pictures indicates that the observed reappraisal deficiency was not caused by a reduced processing of emotional arousing stimuli. The emotional pictures modulate the LPP in a normal way but this response seems insensitive to the emotion regulation strategy.

Our previous study [De Taeye et al., 2015] provided electrophysiological evidence that early attention processes were not compromised by epilepsy. By contrast, the amplitude of the LPP was significantly enhanced for unattended negative pictures, but only in patients with comorbid negative affect. The modulation of the LPP component by unattended emotional stimuli during the late stages of stimulus processing suggested that emotion regulation is disturbed in patients with epilepsy and comorbid negative affect [De Taeye et al., 2015]. However, in the present study, we were unable to find a relation between reappraisal of the electrocortical response and negative affect. BDI and STAI scores are subjective, self-reported ratings for depressive and anxiety symptoms, respectively. Subdividing patients with refractory epilepsy in two groups based on these simple measures is likely an oversimplification, because refractory epilepsy is a complex, heterogeneous and variable neurological condition and various potential confounding factors need to be taken into account, such as type of epilepsy, type and frequency of seizures, age of onset and duration of epilepsy, brain lesions, side and localization of the HEZ and antiepileptic drugs. Our approach was to compare patients with epilepsy with and without negative affect while controlling as much as possible for potential confounding factors stemming from the heterogeneous clinical parameters and the AEDs.

It is important to consider the localization of epileptogenic zone and brain lesions because it is possible that some of these lesions could have influenced our ERP results, especially lesions located in regions that are presumably important for emotion control processes, like the frontal and medial temporal regions. Neuroimaging studies found that reappraisal of unpleasant stimuli was associated with increased activation of the prefrontal cortex and decreased activation of the amygdala [Ochsner and Gross, 2005; McRae et al., 2010]. Therefore, we performed additional data analyses and grouped the patients based on location of the HEZ and the presence of either frontal or medial temporal lobe structures lesions, but these analyses failed to show any significant effect on the LPP amplitude. Hence, the deficient reappraisal in patients with refractory epilepsy could not be easily linked to damage in one specific lobe but is more likely the result from

dysfunction in a broad network underlying emotion control in which both cortical and subcortical structures presumably interact with each other [Ochsner and Gross, 2005; Price and Drevets, 2010]. Recurrent seizures in refractory epilepsy can cause widespread reorganization of overlapping neuronal networks [Spencer, 2002; Richardson, 2010]. Emerging neuropsychological and neuroimaging evidence indicates that temporal lobe epilepsy is associated with frontal lobe dysfunction, in which both executive function and working memory are compromised [Bell et al., 2011; Stretton and Thompson, 2012]. It has been speculated that surround inhibition in the frontal lobe is a dynamic defense mechanism against seizure propagation and could be responsible for functional deficits observed in temporal lobe epilepsy [Nelissen et al., 2006]. Resection of the epileptogenic zone often results in a gain in frontal lobe functions, with cognitive improvement and normalization of the neuroimaging abnormalities [Stretton and Thompson, 2012]. These findings highlight the importance of looking at broader network function instead of specific lesions or lobes when examining psychophysiological measures of emotional regulation in epilepsy.

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CHAPTER 4

Systematic gating of attention but versatile influence of emotion in the human amygdala revealed by intracranial event-related potential recordings in patients with refractory epilepsy

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Aim: Whether unattended negative emotional stimuli activate the amygdala or not remains a matter of controversy in the literature. This study investigated whether the sensory processing of negative emotional visual stimuli can be evidenced in the amygdala when these stimuli are shown either inside or outside the focus of attention, using a time-resolved neurophysiological technique with enhanced spatial resolution.

Methods: Ten patients with refractory epilepsy, implanted with depth electrodes in mesial temporal lobe regions for presurgical evaluation, were included in this study. Local field potentials were unilaterally or bilaterally recorded in the amygdala while the patients performed a face- or house-matching task in which fearful, sad or neutral faces were presented either at attended or unattended spatial locations. This paradigm enables to tease apart effects of attention versus emotion on stimulus processing in the amygdala.

Results: Event-related potentials (ERPs) in the amygdala showed a very reproducible wave morphology. Attending houses led to significantly higher negative amplitudes (300-600 ms post stimulus onset) compared to attending faces across the entire sample. However, at the group level, amygdala ERPs were not systematically modulated by either emotional valence (fear and sad vs. neutral) or arousal (fear vs. sad and neutral) of the face stimuli. Analysis of single trial amygdala ERPs from individual subjects did show significant effects of arousal or valence in six out of ten patients, occurring at different latencies from 110 to 920 ms following stimulus onset.

Conclusions: This study provides direct evidence for asymmetric effects of attention and emotion in the human amygdala during visual face stimulus processing. While object-based attention exerts a systematic effect on amygdala ERPs at group-level, only a subset of the patients shows amplitude modulations of amygdala ERPs by emotion at variable delays after stimulus presentation. These results suggest that attention and emotion can differentially influence sensory processing in the amygdala, likely via different pathways, with stable gating by attention but versatile modulation by emotion.

INTRODUCTION

Emotional facial expressions form an essential part of social communication and are processed by a broad neuronal network in which the amygdala plays a central role [Davis and Whalen, 2001; Adolphs, 2002]. Functional imaging studies have shown that the amygdala responds preferentially to both positive and negative emotionally valenced faces [Breiter et al., 1996]. Moreover, converging evidence indicates that the amygdala is not only responsible for recognition of emotional expressions but has a much broader role in visual processing and serves to coordinate the function of cortical networks to allocate processing resources to (salient) stimuli that have biological significance [Pessoa and Adolphs, 2010]. This explains why the amygdala is activated not only during processing of emotional stimuli, but also shows robust selectivity for faces in the absence of emotional expressions [Mende-Siedlecki et al., 2013], and is implicated in stimulus evaluation during attention tasks where no emotional stimuli are involved [Halgren et al., 1995; Pourtois et al., 2010a; Davis and Whalen, 2001].

How emotion and attention interact in the amygdala remains a matter of intense debate in the literature. Although some studies have found evidence that unattended emotional facial expressions “automatically” activate the amygdala [Vuilleumier et al., 2001; Vuilleumier et al., 2004; Pourtois et al., 2010b], other studies indicate that the activity in the amygdala can be regulated by top-down attention control and specific task demands [Pessoa et al., 2002; Krolak-Salmon et al., 2004]. We hypothesized that attention and emotional factors operate independently and in parallel on the sensory processing in the amygdala in order to increase the saliency and selection of behaviorally relevant information.

To address this question, we used a variant of the face- or house-matching task [Wojciulik et al., 1998], a standard task for measurement of attention and emotion effects [Vuilleumier et al., 2001; Holmes et al., 2003; Bentley et al., 2003; Vuilleumier et al., 2004; Bishop et al., 2004a; Bishop et al., 2004b; Fales et al., 2008; Pourtois et al., 2010b; De Taeye et al., 2015]. In this procedure, participants are shown a display with two houses and two faces presented in vertical and horizontal pairs. They have to attend only one pair and have to make a demanding same/different judgment on the attended pair of stimuli. The faces have either a neutral or emotional expression and are positioned either in attended or unattended spatial locations. This paradigm provides an ideal situation in which both attention and emotion can be manipulated independently [Vuilleumier et al., 2001]. Previous fMRI studies of the face- or house-matching task in healthy subjects reported that emotionally negative stimuli (fearful faces) induced a strong activation of the amygdala, even when the faces were unattended [Vuilleumier et

al., 2001]. Moreover, emotional visual stimuli also induced stronger activation in remote but interconnected visual areas such as the fusiform face area and occipital cortex [Vuilleumier et al., 2001; Vuilleumier et al., 2004]. Subsequent fMRI studies demonstrated that amygdala lesions abolished this enhanced neural response to emotional fearful faces in the distant visual areas, indicating that these visual areas are modulated by the amygdala [Vuilleumier et al., 2004].

The small size and its location deep inside the brain, in combination with a relatively closed field electrical configuration, make that local field potentials from the amygdala cannot be measured with scalp electrodes [Hudry et al., 2001]. Therefore, patients with refractory epilepsy implanted with intracranial depth electrodes in the amygdala for diagnostic purposes offer a unique opportunity to directly record neural activity within the amygdala with a very high temporal resolution (ms). Intracranial event-related potentials (iERPs) in the amygdala of patients with refractory epilepsy were reproducibly recorded during varying tasks with different types of emotional and non-emotional stimuli (for a recent review see [Murray et al., 2014]).

In the present study, local field potentials were recorded in the amygdala of ten patients with refractory epilepsy implanted with depth electrodes in mesial temporal lobe regions for presurgical evaluation. This study investigated whether sensory processing of both attended and unattended emotional visual stimuli can be inferred from local field potentials recorded in amygdala and whether amygdala iERPs are modulated by attentional control and emotional factors. Previous fMRI and iERP studies of the face- or house-matching task [Vuilleumier et al., 2001; Vuilleumier et al., 2004; Pourtois et al., 2010b] only examined the effect of high arousing negative stimuli (fearful faces), while our study also included faces with sad emotional expressions in order to disentangle the effects of emotional valence versus arousal on the sensory processing in the amygdala.

METHODS

1 Ethics statement

The study was approved by the ethics committee of Ghent University Hospital and conducted in accordance with the declaration of Helsinki. After a full description of the procedure was provided and explained, all participants gave written informed consent prior to participation.

2 Participants

Local field potentials were recorded in ten patients with refractory partial epilepsy (M/F: 5/5, mean age 38.7 years). The study took place during presurgical invasive video-EEG monitoring in the Reference Center for Refractory Epilepsy (Ghent University Hospital, Belgium). Patients were implanted with intracranial depth electrodes in mesial temporal lobe regions for diagnostic purposes in order to delineate the epileptogenic zone for potential surgical resection. Presence of negative affect (as a predisposition) was assessed by using the validated Dutch version of the Beck Depression Inventory II (BDI-II) [Beck et al., 1996; Van der Does, 2002]. Patients' main characteristics are summarized in Table 1.

Table 1. Patients' characteristics and anatomical coordinates of the electrode contacts in the amygdala

ID	Age	Sex	BDI	HEZ side	Amygdala contacts	MNI coordinates			Talairach coordinates			Artifact rejection	AEDs
						x	y	z	x	y	z		
1	47	male	7	left	left	29	-4	-26	28	-7	-18	12 %	LEV, OXC
					right	-24	-3	-31	-24	-6	-23		
2	54	male	8	right	left	26	-4	-23	25	-7	-16	22 %	LEV, LTG
					right	-27	-4	-26	-26	-7	-19		
3	38	female	4	right	left	25	-5	-24	24	-7	-17	19 %	LCZ, LEV, VPA
					right	-27	-5	-26	-26	-8	-17		
4	47	male	11	right	left	No post-implantation MRI available						13 %	CBZ, LEV, PB
					right								
5	29	female	21	left	left	25	-2	-28	24	-5	-20	10 %	LEV, PGB
					right	-24	0	-27	-24	-3	-20		
6	53	female	5	left	left	-26	-4	-23	-25	-7	-16	10 %	CZP, CBZ, PGB
7	27	male	17	left	left	-23	-3	-28	-23	-6	-21	8 %	CBZ, LEV, TPM
8	29	female	1	right	right	22	-5	-28	21	-8	-20	7 %	CBZ, LCZ, LEV, TGB
9	33	female	14	right	right	31	-5	-26	30	-8	-18	2 %	CBZ, LCZ, LEV
10	30	male	36	right	right	23	-3	-28	22	-6	-20	4 %	CBZ, CLB, LCZ

Abbreviations: *ID* identification number, *BDI* Beck Depression Inventory, *HEZ* hypothesized epileptogenic zone, *MNI* Montreal Neurological Institute, *AED* anti-epileptic drugs: *CBZ* carbamazepine, *CLB* clobazam, *CZP* clonazepam, *OXC* oxcarbazepine, *LCZ* lacosamide, *LTG* lamotrigine, *LEV* levetiracetam, *PB* phenobarbital, *PGB* pregabalin, *TGB* tiagabine, *TPM* topiramate, *VPA* valproic acid

3 Stimuli

All stimuli comprised four pictures, two faces and two houses arranged in vertical and horizontal pairs around a central black fixation cross (Figure 1). All pictures were black and white photographs presented on a gray background and had the same size across all experiments (108 [width] x 154 [height] pixels on a 1024 * 768 pixel resolution screen) subtending 4.0 x 5.7 ° of visual angle at a 50 cm viewing distance. The stimuli included 10 fearful faces, 10 neutral faces, 10 sad faces and 20 houses, with pictures from each category repeated equally across all trials. The neutral, fearful and sad facial expression photographs were drawn from the set of Ekman and Friesen [Ekman and Friesen, 1976].



Figure 1. Sample visual stimuli of the face- or house-matching task.

Each trial comprised a display of four pictures, with two houses and two faces arranged in vertical and horizontal pairs around a central black fixation cross. Before each block, a visual cue (i.e., thickening of two frames) instructed participants to attend either to the vertical pair or the horizontal pair of stimuli, while ignoring the other pair. Subsequently, subjects had to indicate quickly and accurately whether the two stimuli at the task-relevant locations were the same or different (i.e., matching task). On any given trial, both faces had either a fearful, sad or neutral expression and were shown at task-relevant or task-irrelevant locations.

4 Procedure

The face- or house-matching task was the same as in our previous study with scalp EEG in 50 patients with epilepsy and 25 healthy controls [De Taeye et al., 2015]. Stimulus presentation and response time recording were controlled using E-Prime software 2.0 (Psychology Software Tools Inc., Pittsburgh, USA). Before each block, a 3 s display instructed subjects to attend to horizontal or vertical stimulus pairs, while ignoring the other stimulus pairs. The instruction display consisted of four empty frames placed at the location of the stimuli, with either the two horizontal or the two vertical frames being thickened. Trials began with a central fixation cross for 1 s, followed by the four-picture display for 300 ms. Subjects were asked to maintain gaze fixation centrally throughout the trials and attend covertly to the stimulus pair at the relevant locations, in order to judge whether these two stimuli were the same or different by pressing one out of two

keys. Hence, on each and every trial, a two-alternative forced choice task based in stimulus identity was required. The inter-trial interval (ITI) varied randomly between 1 and 3 s. To ensure that patients understood the paradigm, they completed 2 practice blocks with 5 trials, with one block where the attention was directed to horizontal positions and one block where the attention was directed to vertical positions. After the practice block, patients 1 to 4 completed 4 blocks of 48 trials, with two blocks where attention was directed to horizontal positions and two blocks where attention was directed to vertical positions. In each block, all possible combinations of two object categories (pairs of faces vs. pairs of houses), their locations, same/different identity, and facial expression were fully randomized and counterbalanced across trials, resulting in a total of 32 neutral, 32 sad and 32 fearful faces at task-relevant (attended) locations, and the same number for each expression at task-irrelevant (unattended) locations (total 192 trials). Patients 5 to 10 completed 6 blocks of 48 trials, with three blocks where the attention was directed to horizontal positions and three blocks where the attention was directed to vertical positions, resulting in a total of 288 trials. Task instructions emphasized both accuracy and speed. Response times were recorded from stimulus onset using a response box (RB-730, Cedrus).

Patients with mesial temporal lobe epilepsy can experience difficulties in recognizing emotional expressions [Meletti et al., 2009]. Therefore, after the matching task, patient 5 to 10 (n=6) were instructed to rate each picture for emotional valence and arousal on two separate scales, i.e., the Self Assessment Manikin (SAM) arousal and valence scales [Bradley et al., 1994]. The SAM valence scale shows five cartoon figures ranging from unhappy to happy ; below this scale were the numbers "1" through "5". Participants were told to use this scale to indicate how pleasant or unpleasant they felt about the preceding picture. The SAM arousal scale shows five cartoon figures with expressions ranging from calm and relaxed to excited and wide-eyed; again, the numbers "1" through "5" were presented below this scale, and participants were told to rate the picture, but this time based on the strength of their emotional activation by the picture. Valence ratings were reverse-scored to arousal ratings in such a manner that a score of 5 represented pleasant valence and high arousal.

5 Intracranial EEG recording

Intracranial local field potentials were continuously recorded with Micromed System Plus (Micromed, Mogliano Veneto, Italy) with a sampling frequency rate of 1024 Hz, anti-aliasing filter of 250 Hz, gain of 50 dB and 16 bits resolution, using depth electrodes containing four to ten evenly spaced platinum contacts. In patients 1, 2, 6 and 7 depth electrodes (model T08-08BM, DIXI) had 8 recording contacts with 5 mm contact length

and 2 mm inter-contact spacing. In patient 3, depth electrodes (model T08-10AM, DIXI) had 10 recording contacts with 3.5 mm contact length and 2 mm inter-contact spacing. In patient 4, depth electrodes (model 3387, Medtronic) had 4 recording contacts with 1.5 mm contact length and 1.5 mm inter-contact spacing. In patients 5 and 10, depth electrodes (model SD08R-SP05X-000, Ad-Tech Medical Instruments) had 8 recording contacts with 2 mm contact length and 3.5 mm inter-contact spacing. The position of the electrode contacts in the amygdala was verified by a neuroradiologist (E.A.) based on post-implantation MRI. If more than one contact was in amygdala, the one with the clearest (neurophysiological) response and most consistent signal was chosen for subsequent analysis. The anatomical coordinates of each electrode contact in the amygdala (Table 1) were determined with spm12 [<http://www.fil.ion.ucl.ac.uk/spm/>] by coregistration of the individual pre- to post-implantation T1 MRI images and normalization of the pre-implantation MRI to the Montreal Neurological Institute (MNI) template. Additionally, MNI coordinates were translated to Talairach space according to the Nonlinear Yale MNI to Talairach Conversion Algorithm [Lacadie et al., 2008; <http://sprout022.sprout.yale.edu/mni2tal/mni2tal.html>]. The estimated Talairach coordinates of the electrodes (Table 1) overlapped with the basal and lateral amygdala, as according to the Talairach atlas, the amygdala occupies a region that runs from 17 to 30 mm lateral to the midline, 1 mm anterior to 11 mm posterior to the anterior commissure and 7 to 21 mm below the intercommissural line [Zald, 2003]. Patient 4 had no post-implantation MRI and the exact coordinates could not be determined in this patient, however the electrodes were stereotactic implanted with the bilateral amygdala as targets. In all patients, the online reference electrode was placed on the right mastoid and ground electrode on the left mastoid.

6 Data analysis

Behavioral results were analyzed with mixed-model analysis of variance (ANOVA) with within-subjects factor of attention (house-cued vs. face-cued trials), valence [negative (fearful or sad) vs. neutral] and arousal [high (fearful) vs. low (sad or neutral)].

Offline ERP analyses were performed using BrainVision Analyzer 2 software (Brain Products GmbH, Gilching, Germany). The continuous EEG was first visually inspected to mark interictal epileptic discharges. Subsequently, it was digitally filtered with a 50 Hz notch filter and a half-power band-pass filter between 0.1–30 Hz with a roll-off of 12 dB/octave, and then down-sampled to 512 Hz. The EEG was segmented into epochs from –200 ms to +1000 ms relative to the onset of the stimuli and submitted to automatic artifact rejection to exclude epochs with voltage exceeding ± 200 μ V from averaging. The average fraction of rejected epochs with artifacts and interictal epileptic discharges was

11 % (ranging from 2 to 22 %, see Table 1). The frequencies of artifact-contaminated epochs showed no significant systematic differences across experimental conditions with 10.7 % fearful face-cued, 10.4 % neutral face-cued, 11.4 % sad face-cued, 11.7 % fearful house-cued, 10.5 % neutral house-cued and 9.2 % sad house-cued epochs (one-way ANOVA $F=0.1$, $p=0.986$). Baseline correction was applied on the 200 ms pre-stimulus interval and potentials were averaged separately for each condition and each individual.

Two different types of statistical analyses were performed on the amygdala iERPs. First, a group-level analysis compared the mean voltages of successive contiguous 100 ms time windows from -200 ms to 1000 ms latency around stimulus onset on the averaged potentials for each condition [Krolak-Salmon et al., 2004; Meletti et al., 2012]. The 100 ms segments were analyzed with mixed-model ANOVA with within-subjects factor of side (left vs. right amygdala), attention (house-cued vs. face-cued trials), valence (negative vs. neutral), arousal (high vs. low) and BDI as covariate. Post-hoc tests were Bonferroni corrected. Second, individual analyses were performed on the single trial responses of each of the ten individual patients separately using Cartool software (developed by Denis Brunet, Functional Brain Mapping Laboratory, Geneva, Switzerland). Experimental conditions were compared using sample-by-sample unpaired t-tests with a significant alpha cutoff set to $p<0.05$ and with an additional criterion of temporal stability for at least five consecutive samples (=10 ms at 512 Hz sampling rate) [Naccache et al., 2005; Gaillard et al., 2009; Pourtois et al., 2010b].

RESULTS

1 Behavioral results

Mean response times and accuracy in same/different judgments were computed for each subject in each condition. Behavioral results during the face- or house-matching task are summarized in Figure 2A. Mean error rate was 25.5 ± 8.0 % indicating that the matching task was relatively demanding but could be performed well above chance level. Patients made significantly more errors when judging faces (31.3 %) than houses (20.0 %) [main effect of attention $F(1,8)=20.5$, $p<0.001$]. Judging faces carrying a negative emotional expression (26.4 %) was trend significant worse than judging neutral faces (22.1 %) [main effect of valence $F(1,8)=2.8$, $p=0.099$]. There was no significant effect of arousal [main effect of arousal $F(1,8)=2.4$, $p=0.126$], nor significant interaction of attention with either valence or arousal [interaction attention*valence $F(2,8)=0.0$, $p=0.976$, interaction attention*arousal $F(2,8)=0.0$, $p=0.968$].

Analysis of reaction times (RTs) showed that participants were significantly slower to make same/different judgments with faces (630 ms) compared to houses (568 ms) [main effect of attention $F(1,8)=9.8$, $p=0.003$]. RTs were not significantly influenced by valence [main effect of valence $F(1,8)=0.3$, $p=0.616$], nor by arousal [main effect of arousal $F(1,8)=1.0$, $p=0.326$], nor was there a significant interaction of these factors with attention [interaction attention*valence $F(2,8)=2.3$, $p=0.140$, interaction attention*arousal $F(2,8)=0.1$, $p=0.728$]. This pattern of results was similar to the behavioral performance of a group of 25 healthy participants from our previous study using the same paradigm [De Taeye et al., 2015] with more errors and slower reaction times observed when faces had to be compared relative to houses (Figure 2).

After the matching task, 6 patients were asked to rate the faces for valence and arousal using the SAM-rating scales (Figure 2B). Emotional expression (neutral vs. sad vs. fearful) had a significant effect on valence ratings [$F(2,4)=13.4$, $p=0.003$]. Post-hoc comparisons (Bonferroni corrected) revealed that relative to neutral faces (2.98), patients gave significantly more negative valence ratings for both sad faces (1.67) [$p=0.023$] and fearful faces (1.70) [$p=0.004$]. There was a trend of patients giving slightly higher arousal scores for fearful (3.38) compared to sad (3.00) and neutral (2.50) faces [$F(2,4)=1.9$, $p=0.199$].

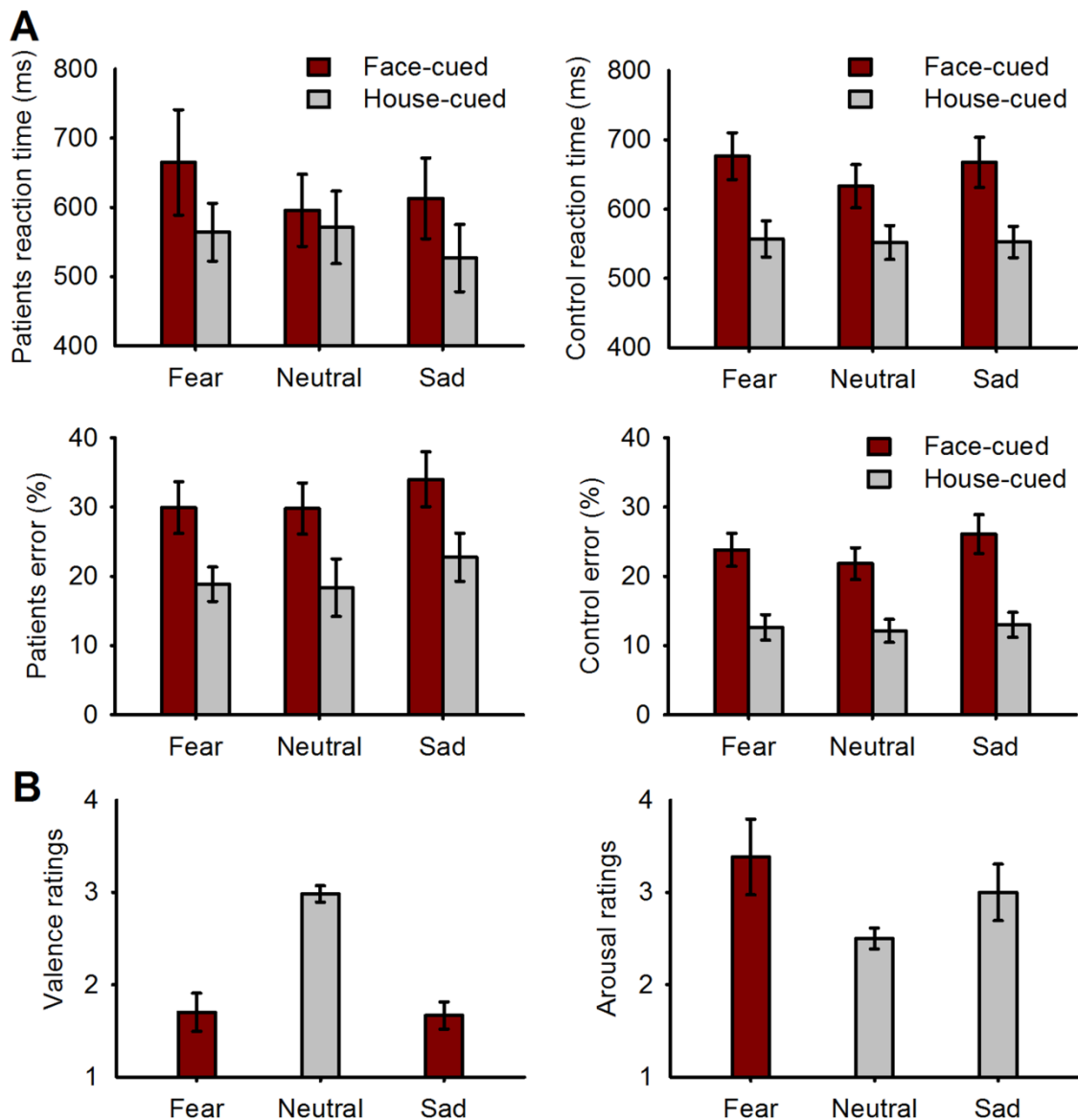


Figure 2. Behavioral results.

(A) Reaction time in ms (upper panels) and percentage errors (lower panels) shown separately for the face-cued trials (red bars) and the house-cued trials (grey bars) of the ten patients (left panels), and in comparison with a group of 25 healthy controls (right panels) [De Taeye et al., 2015]. The ten patients showed a response pattern similar to these healthy participants with more errors and slower reaction times when they had to make same/different judgments for faces compared to houses. (B) Valence and arousal ratings on the SAM-rating scales. Higher scores reflect more positive and higher arousal ratings. Patients gave significantly more negative ratings for fearful and sad faces (red bars) compared to neutral faces (grey bars). In addition, patients rated fearful faces (red bars) as slightly more arousing than either sad or neutral faces (grey bars).

2 Electrophysiological results

Clear stimulus-locked iERP in the amygdala were evident in each of the ten patients. The amygdala iERPs showed a reproducible wave morphology across them (Figure 3), sharing many similarities with previous and independent results obtained for this mesial temporal lobe structure [Krolak-Salmon et al., 2004; Meletti et al., 2012]. In all patients, a first negative component was found arising between 200 to 600 ms post stimulus onset, followed by a positive component between 600 to 1000 ms. The wave morphology of the local field potentials recorded in the contacts in the amygdala was clearly different from the morphology of the local field potentials recorded on the adjacent contacts in the anterior hippocampus (data not shown).

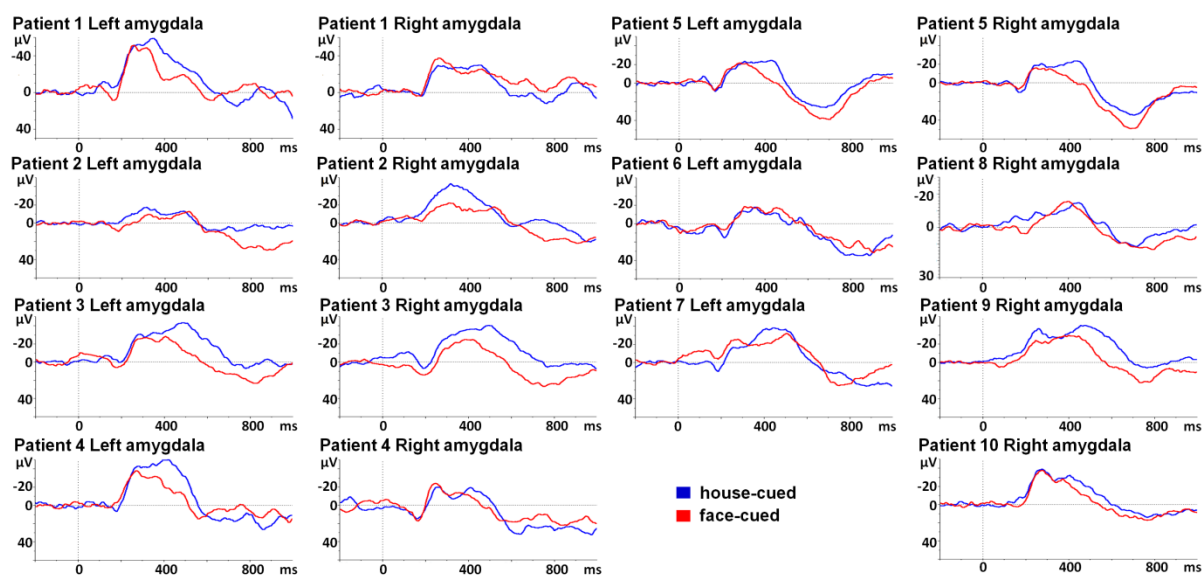


Figure 3. Wave morphology of the amygdala iERPs.

Averaged intracranial ERPs recorded from the amygdala displayed separately for each of the 10 patients. In all insets, blue lines represent iERPs to house-cued trials and red lines to face-cued trials. Results show that the stimulus-locked iERP wave morphology was very reproducible across patients with a negative component arising from 200 to 600 ms followed by a positive component between 600 to 1000 ms.

2.1 Group-level analysis

Statistical analyses were performed on grand average iERPs by comparing the mean voltages of successive contiguous 100 ms time windows from -200 ms to 1000 ms latency around stimulus onset for each condition. We found significant effects of attention with larger amplitudes of the negative component when judging houses compared to faces in three consecutive time windows: from 300 to 400 ms [$F(1,74)=5.9$, $p=0.018$], 400 to 500 ms [$F(1,74)=29.5$, $p<0.001$] and 500 to 600 ms [$F(1,74)=12.5$, $p<0.001$]. The mean amplitude of the 300 to 600 ms time window was significantly higher in

amplitude for attending to houses compared to attending to faces in both left amygdala [$p < 0.001$] and right amygdala [$p < 0.001$] (Figure 4). By comparison, at group-level, there was no significant systematic modulation of the amygdala ERPs by either emotional arousal (high vs. low) or emotional valence (negative vs. neutral) of the face stimuli (Figure 5). In addition, there were no significant interactions of valence and arousal factors with attention or side (left vs. right amygdala). Modulation of the amygdala ERPs was also not associated with BDI-scores of self-reported depressive symptoms.

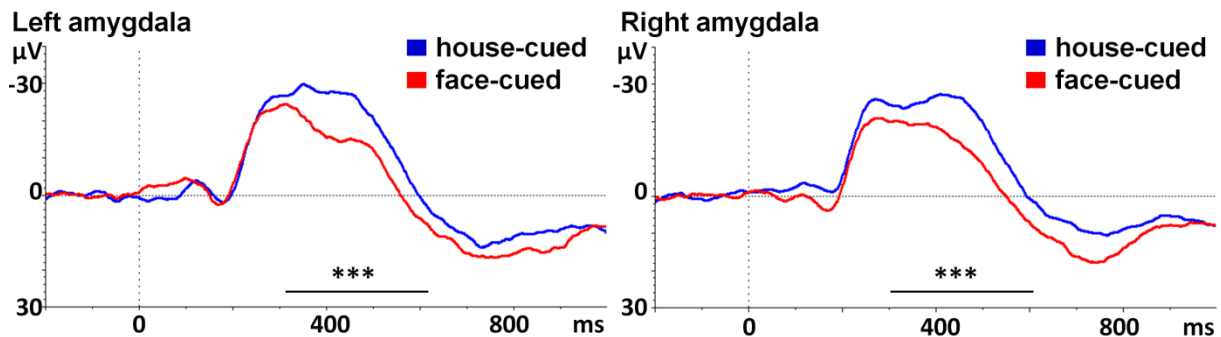


Figure 4. Effect of attention at the group-level (n=10).

Stimulus-locked grand average iERP waveforms recorded in the amygdala in response to house-cued trials (blue lines) and face-cued trials (red lines). Note the prolonged differential responses starting at 300 ms. At the group-level, the amplitude of the negative component was significantly larger for house-cued relative to face-cued trials from 300-600 ms post-stimulus in both left and right amygdala. *** $p < 0.001$.

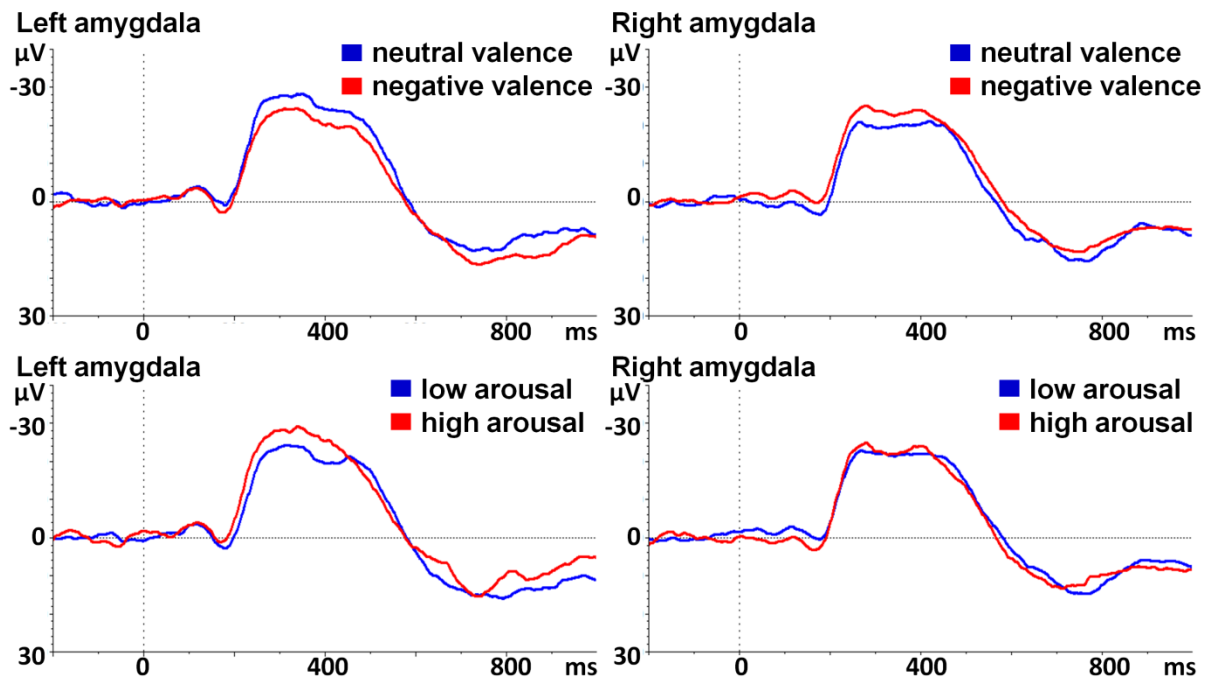


Figure 5. Effects of arousal and valence at the group-level (n=10). Stimulus-locked grand average iERP waveforms recorded in the amygdala: upper panels show amygdala iERP separately for neutral faces (blue line) and negative faces (sad and fear, red line); lower panels for low arousal faces (neutral and sad faces, blue line) and high arousal faces (fear faces, red line). At the group-level, amygdala iERPs were not systematically modulated by either emotional arousal or emotional valence of the face stimuli, for both attended and unattended conditions.

2.2 Individual analysis of single trial potentials

Statistical analysis of the single trial potentials in the amygdala of the individual patients are summarized in Table 2. An effect of attention (face-cued vs. house-cued trials) was significant in nine out of ten patients (Table 2, Figure 3). The negative component between 300 and 600 ms post stimulus onset was significantly larger for making same/different judgment for houses compared to faces in seven patients (patients 1, 2, 3, 4, 5, 9 and 10). Furthermore, the positive component between 600 and 1000 ms was significantly larger for face-cued relative to house-cued trials in five patients (patients 2, 3, 5, 8 and 9). In addition, amygdala ERPs were significantly modulated by emotional valence and arousal in six out of ten patients (patients 1, 2, 3, 5, 7 and 10), however, occurring at different latencies from 110 to 920 ms following stimulus onset depending on the patient considered (Table 2, Figure 6-8). Valence (neutral vs. negative) had a significant effect in 4 different time windows varying between 150 and 920 ms after stimulus onset in patients 1, 3 and 7 (Table 2, Figure 6). Amygdala iERPs were systematically modulated by arousal in five out of ten patients (patients 1, 2, 3, 5 and 10) in time windows varying between 110 and 900 ms post stimulus onset (Figure 6-8).

Table 2. Single trial potential analysis: attention, valence and arousal differences ($p < 0.05$ for > 10 ms)

Patient	Side	Attention	Valence	Arousal
1	Left	348-424, 971-977 ms	721-771, 314-375 ms	615-658, 779-836, 928-936 ms
	Right	236-252, 631-691, 740-876 ms	588-604, 703-723, 393-734, 748-785 ms	365-387, 518-559, 588-605, 625-637 ms
2	Left	744-1000 ms	n.s.	188-215, 229-246, 555-563 ms
	Right	316-402 ms	n.s.	n.s.
3	Left	453-674, 795-836 ms	154-248, 877-912 ms	n.s.
	Right	469-828 ms	n.s.	113-139, 148-158 ms
4	Left	383-559 ms	n.s.	n.s.
	Right	943-957 ms	n.s.	n.s.
5	Left	426-449, 748-766 ms	n.s.	789-838
	Right	402-482, 645-734 ms	n.s.	715-738, 748-895 ms
6	Left	828-846 ms	n.s.	n.s.
7	Left	n.s.	582-596 ms	521-594 ms
8	Right	797-807 ms	n.s.	n.s.
9	Right	252-279, 713-791, 916-1000 ms	453-680, n.s.	n.s.
10	Right	162-184, 398-518 ms	n.s.	380-490 ms

n.s. not significant

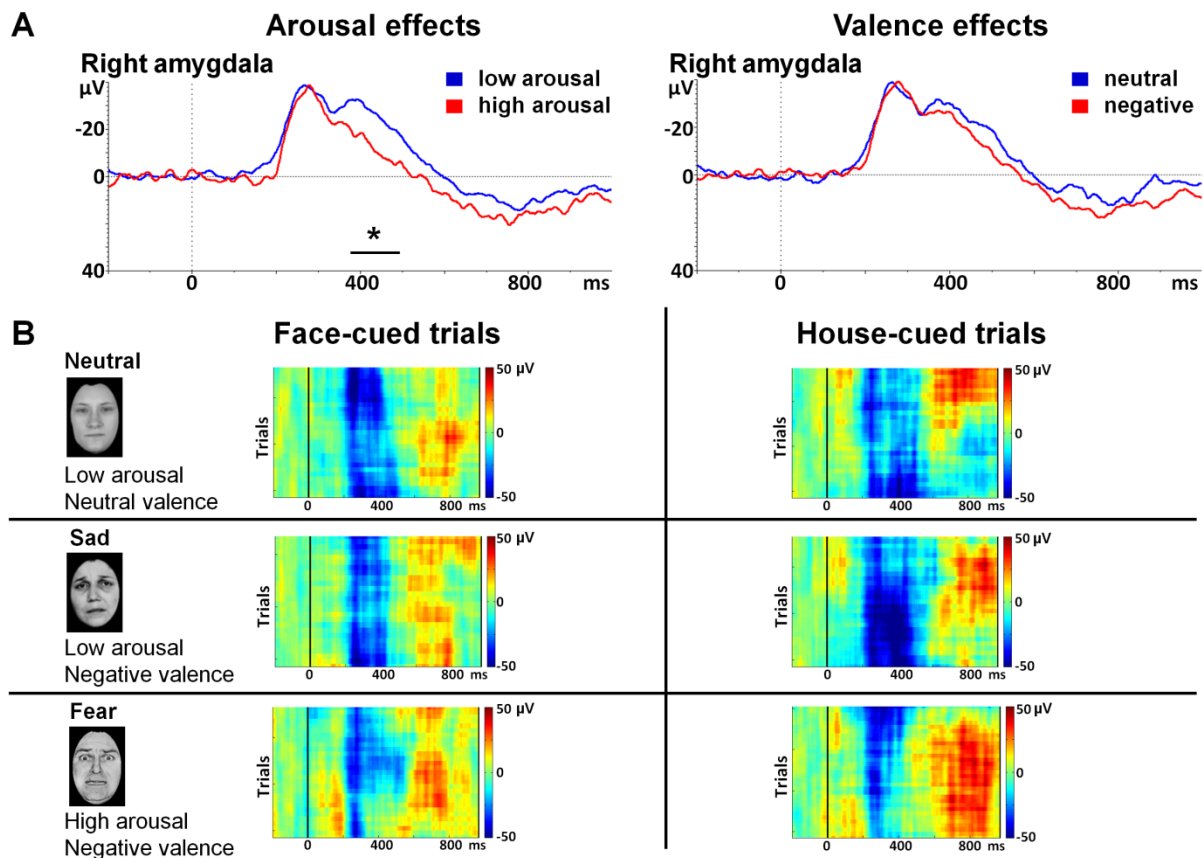


Figure 8. Effects of arousal and valence on single trial level in patient 10.

(A) Averaged intracranial ERP from the right amygdala of patient 10: the left panel shows the average response to low arousing (blue) and high arousing faces (red); the right panel shows the average response to neutral (blue) and negatively valenced faces (red). (B) ERP image showing a two-dimensional representation of amplitude x time data for all consecutive trials of patient 5 for each condition. The amplitude of single-trial responses is plotted from $-50 \mu\text{V}$ (blue) to $+50 \mu\text{V}$ (red). Differential responses to high arousing faces compared to low arousing faces arose during the time window spanning from 380-490 ms in the right amygdala and remained generally stable across the successive trials. Single trial analysis showed no significant effect of valence.

DISCUSSION

This study explored the effects of attention and emotion on the processing of visual face stimuli in ten patients implanted with intracranial electrodes that directly measured the neuronal activity in the amygdala. The results indicate that there is a systematic gating by object-based attention on the amygdala responses. Furthermore, our data suggest that neuronal processing in the amygdala can be modulated by both emotional valence and arousal, but these effects occur at variable latencies following stimulus onset in the different patients. Taken together, our findings therefore suggest that both attention and emotional factors can independently influence the sensory processing in the amygdala, but at distinct, sometimes overlapping, latencies following stimulus onset. This dissociation in time-course cannot be resolved with fMRI due to the slow temporal resolution of this technique and may explain previous discrepancies reported in the literature based on fMRI studies using the same task [Vuilleumier et al., 2001; Vuilleumier et al., 2004; Bishop et al., 2004; Fales et al., 2008]. The results of our study are in line with the notion that emotion processing may take place in parallel and independently from the strong and systematic gating effect exerted by voluntary attention and mediated by a large fronto-parietal network [Pourtois et al., 2013].

Previous intracranial recordings from the left amygdala in one patient during the face- or house-matching task showed early (140 to 290 ms) modulation of the amygdala iERPs by fearful faces (arousal) that preceded the effects of attention at a much later latency (750 to 950 ms) [Pourtois et al., 2010b]. The results of this study are very comparable with recordings in the left amygdala of patient 2 that showed a significant early effect of arousal in three time windows ranging from 188 to 563 ms, followed by a much later effect of attention ranging from 744-1000 ms. However, in other patients effects of emotion occurred at different latencies, suggesting that influence of emotion on visual processing in the amygdala varies among individuals, with some patients showing a significant early effect of emotional valence or arousal, while in other patients, early stimulus processing in amygdala seems rather insensitive to the (negative) emotional expression conveyed by the faces. These findings are in agreement with a previous study [Krolak-Salmon et al., 2004] which observed larger negative amplitudes of amygdala iERP specifically for fearful faces, but auxiliary analyzes of the single trial responses of the individual patients revealed that this fear-specific effect was actually significant only in half of the patients and in variable time windows ranging from 200 to 1000 ms post-stimulus onset. In addition, the emotion effects in these patients were only observed during a task where patients had to recognize the emotional expression of the faces, but not when they had to discriminate their gender. In our paradigm, recognition of the emotional expression was not explicitly relevant for the matching task and stimuli were

presented very briefly (300 ms), which might potentially explain the lack of systematic modulation of the amygdala iERPs by emotional expressions during this task.

The intracranial recordings in the amygdala carried out in this study showed a systematic difference between face-cued and house-cued trials. Single-unit activity recordings in the amygdala of monkeys reported that some neurons distinguish between faces and non-face objects, while other neurons encode identity or expression of faces [Nakamura et al., 1992; Gothard et al., 2007]. Findings of category-specific neurons in monkey amygdala have been generalized to humans in single-unit recordings in patients with epilepsy [Fried et al., 1997; Kreiman et al., 2000; Quiroga et al., 2005; Mormann et al., 2011]. These studies have demonstrated that single neurons in the human amygdala can discriminate objects from faces, with some neurons firing selectively to images of faces while others show category-specific response to objects [Fried et al., 1997; Kreiman et al., 2000]. There are even amygdala neurons with a visual categorical selectivity for animals and this selectivity appeared to be independent of emotional valence and arousal [Mormann et al., 2011]. Taken together, single-unit recordings suggest that during visual processing, amygdala neurons encode information about stimulus identity and category [Nakamura et al., 1992; Fried et al., 1997; Kreiman et al., 2000; Quiroga et al., 2005; Gothard et al., 2007; Mormann et al., 2011], which is necessary to coordinate the activation of cortical networks (interconnected with this amygdala) that are required to prioritize the processing of behaviorally relevant stimuli [Sander et al., 2003]. There are multiple parallel routes for visual information processing that may lead to substantial temporal dispersion of evoked responses. Notably, the amygdala may not be unique in the processing of affective visual stimuli as there are other largely parallel networks that enable comparable functions, such as the network operated by the connections between the pulvinar and the cortex [Pessoa and Adolphs, 2010].

It should be pointed out that the new neurophysiological results obtained here with intracranial recordings performed in patients with chronic refractory epilepsy are necessarily limited and cannot be generalized without caution. However, ERPs recorded in the amygdala during the face- or house-matching task showed a very reproducible wave morphology across patients. Moreover, the inclusion and systematic comparison of 10 different patients in this study are rather exceptional, as most of the previous studies available in the literature were actually conducted with a much smaller sample size [Murray et al., 2014]. In all ten patients, a first negative component was found arising between 300 to 600 ms post stimulus onset, followed by a positive component between 600 to 1000 ms. The observed amygdala ERP wave morphology is highly similar and consistent to previous studies that recorded amygdala ERPs in response to human face stimuli [Krolak-Salmon et al., 2004; Pourtois et al., 2010b; Meletti et al., 2012; Murray

et al., 2014]. Moreover, no clear lateral effects (left vs. right amygdala) were observed in our study; a result which is again in line with previous studies of amygdala ERPs [Krolak-Salmon et al., 2004; Meletti et al., 2012]. In general, lateralized activations by emotional stimuli are most supportive for the asymmetry framework in which the left hemisphere is dominant for approach and positive emotions and both hemispheres are involved in withdrawal and negative emotions [Davidson et al., 2000; Pizzagalli et al., 2003; Wager et al., 2003]. Lanteaume and coworkers have shown that electrical stimulation of the right amygdala induced negative emotional states (fear and sadness), while stimulation of the left amygdala could induce either positive (happiness) or negative (fear, sadness) emotions [Lanteaume et al., 2007]. Although several neuroimaging studies have reported lateralization of amygdala responses as a function of valence, meta-analysis by Sergerie and colleagues found no evidence for functional asymmetry of the amygdala during emotional processing. The magnitude of activation in the left and right amygdala was similar in response to both positive and negative valenced stimuli, with a preference for faces depicting emotional expressions [Sergerie et al., 2008]. This is in agreement with our iERP results that showed no evidence of lateralized amygdala specialization.

A second limitation of the present study is that some patients with temporal lobe epilepsy can have deficits in the processing and recognition of facial expressions [Meletti et al., 2009], which might eventually explain why effects of emotion (either valence or arousal) in the amygdala were not systematic across patients, unlike attention. Nevertheless, in our study, patients rated the stimuli as being more negative when fearful and sad faces were presented, compared to neutral faces, and they also judged fearful faces more arousing for than either neutral or sad faces. Hence, these results agree with the assumption of preserved visual emotional processing abilities for the epileptic patients included in our study.

In sum, this study provides direct evidence for asymmetric effects of attention and emotion in the human amygdala during visual stimulus processing. While object-based attention exerts a systematic effect on amygdala ERPs visible at the group-level, only a subset of the patients shows amplitude modulations of amygdala ERPs by emotion at variable latencies following stimulus onset. Therefore, these results suggest that attention and emotion can differentially influence sensory processing in the amygdala, likely via different pathways, with stable gating by attention but versatile modulation by emotion.

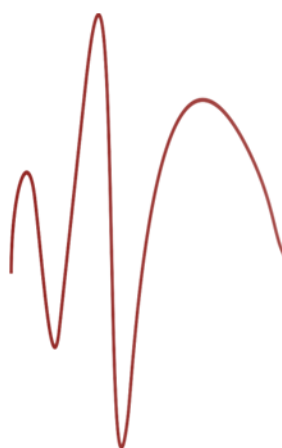
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GENERAL DISCUSSION & FUTURE PERSPECTIVES

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

This PhD project investigated three central research questions (see “Research aims” page 7). The first section will discuss each of the research questions in light of the new insights revealed by our studies and mark out future research challenges and opportunities. In the second section, advantages and limitations of ERP recordings in patients with epilepsy will be discussed in turn and a general conclusion will be drawn.

1 Current insights and future avenues

1.1 The P3 is a potential biomarker for the response to VNS in epilepsy

The first chapter provides new neurophysiological evidence which supports the hypothesis that VNS-induced activation of the locus coeruleus noradrenergic signaling is associated with the therapeutic response to VNS in patients with epilepsy. Our ERP results showed that VNS induces a significant increase of the oddball P3 amplitude at the parietal midline electrode, in VNS responders only. In addition, logistic regression analysis revealed that modulation of the P3 amplitude can be used as a non-invasive indicator for VNS responders and non-responders with relatively good sensitivity and specificity. Amplitude modulations of the P3 should be further investigated as a non-invasive biomarker to predict the treatment response to VNS in patients with refractory epilepsy. A biomarker for the efficacy of VNS could help neurologists to identify the best patient candidates for VNS and fine-tune the patients’ individual stimulation parameters in a more objective way [Vonck and Boon, 2015].

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention and that is quantifiable, precise and reproducible [Atkinson et al., 2001; Leiser et al., 2011]. The relative increase in P3 amplitude is an objective, quantifiable measure with a reasonably good precision: when a cutoff value of > 20 % amplitude increase is used, the sensitivity and specificity are 70 % and 90 % respectively. Nonetheless, the P3 is definitely not a perfect biomarker because 30 % of responders and 10 % of non-responders are misclassified with this cut-off. Previous work from our laboratory in a rat model for epilepsy has found that using a VNS-induced increase of hippocampal NE levels of at least 70 % as a classifier for the anticonvulsive effect of VNS had a sensitivity and specificity of 100 % [Raedt et al., 2013]. Several factors could underlie the lower sensitivity of the P3 in our patient study compared to the preclinical results. Patients included in our study had different types of epilepsy and heterogeneous clinical characteristics in comparison to the uniform and controlled rat model for epilepsy. In addition, the P3 component of the ERP is only an indirect measure

if LC-NE activity and other confounding factors (e.g., anti-epileptic drugs, brain lesions, type of epilepsy) could influence the amplitude of the P3. We have tried to control for this factors by calculating relative amplitude differences and comparing the P3 amplitude between the ON and OFF condition within each patient. Future research including more patients is needed to confirm the reproducibility of these P3 results. At this moment there are no biomarkers available for VNS treatment efficacy in patients with epilepsy. In this context, a further exploration of indexes of the LC-NE system as providing such biomarker appears valuable as it could potentially help to solve this problem.

Novel techniques have been designed to deliver non-invasive VNS, including transcutaneous VNS (tVNS) that stimulates the auricular branch of the vagus nerve through the skin of cymba conchae of the ear [Ellrich, 2011; Frangos et al., 2015]. A proof of concept trial showed that tVNS is a safe and well-tolerated neurostimulation therapy for patients with epilepsy [Stefan et al., 2012]. Combination of non-invasive tVNS with a (neurophysiological) biomarker that predicts the therapeutic efficacy of VNS could help identifying responders prior to surgery.

Besides the P3 component of the ERP, other non-invasive markers of the noradrenergic system could also be potential biomarkers for VNS. Recent studies have suggested that the pupil diameter is an indirect index for a LC-NE activity in the human brain [Gilzenrat et al., 2010; Murphy et al., 2011; Nassar et al., 2012; Jepma and Nieuwenhuis, 2011]. Simultaneous intracranial recordings of the locus coeruleus discharge frequency and pupil diameter in monkeys showed that these two are remarkably closely correlated [Rajkowski et al., 1993; Aston-Jones and Cohen, 2005]. In addition, there is strong pharmacological evidence that pupil dilation is controlled through alpha-2 adrenoreceptors, by the release of noradrenaline from the LC [Phillips et al., 2000]. In healthy participants, administration of the alpha-2 adrenoceptor agonist clonidine (which inhibits LC activity and decreases NE release) significantly decreased the pupil diameter, whereas the alpha-2 adrenoceptor antagonist yohimbine (which increases LC activity and NE release) caused a significant pupil dilation compared to placebo. Therefore, pupil dilation should be a reliable measure of LC-NE activity, especially under moderate levels of illumination [Phillips, 2000; Sara, 2009]. Another frequently used non-invasive marker is the sympathetic skin conductance response (SCR), which is a momentary change of the electrical potential of the skin [Vetrugno et al., 2003; Nieuwenhuis et al., 2011]. Analogous to the pupil dilation response, pharmacological studies have shown that clonidine decreased the SCR, while yohimbine substantially increased the amplitude of the SCR [Yamamoto et al., 1994]. Hence, the SCR is an additional non-invasive approach to investigate the function of the LC-NE system [Nieuwenhuis et al., 2011]. The combination of various multimodal types of non-invasive biomarkers could substantially

increase the specificity and sensitivity of the prediction for therapeutic response to VNS in patients with refractory epilepsy.

In our first study, we recruited patients who received VNS therapy for at least 18 months. Presumably, this chronic stimulation had led to long-term changes in specific neuronal networks and neurotransmitter systems [Boon et al., 2001]. Therefore, it remains unclear whether these VNS-induced effects on P3 amplitude will also be observed in epilepsy patients prior to initiation of VNS-therapy. To resolve this issue, we are currently performing a longitudinal prospective study, in which there is a baseline measurement before the chronic VNS is initiated, and subsequently, a second measurement after one year of standard cycling VNS. This study is still ongoing, until now 15 patients have been tested a first time before initiation of VNS and 5 of these patients have been tested a second time after 1 year of VNS. In these patients different non-invasive markers of noradrenergic activity (i.e., P3 component, sympathetic skin response and pupil diameter) are compared between three conditions that are performed in random order: (i) no stimulation, (ii) invasive VNS and (iii) non-invasive tVNS. This longitudinal study is required to assess whether we can predict, based on the baseline measurement (acute effects), which patients will eventually become responders to the VNS therapy (chronic effects) and whether non-invasive markers of LC-NE activity can be used as a prospective measure to predict the therapeutic efficacy of VNS. If non-invasive tVNS affects the biomarkers of LC-NE activity similarly as invasive VNS, the use of these biomarkers in combination with tVNS could help to detect responders prior to surgery and hence avoid unnecessary implantations of a VNS device in non-responders and consequently improve the clinical efficacy of VNS.

It should be noted that the mechanism of action of VNS not only involves activation of the LC-NE system, but more likely results from a complex cascade in which different networks and neuromodulatory systems are involved as well [Henry, 2002]. The vagus nerve has widespread projections to nuclei in the brainstem, the thalamus, hippocampus, amygdala, and to all cortical regions, where they modulate the activity of target cells and networks [Boon et al., 2001; Ben-Menachem, 2002; Vonck et al., 2009]. It has been demonstrated that VNS modulates the GABAergic, glutamatergic, dopaminergic, serotonergic and cholinergic systems [Henry, 2002; Vonck et al., 2009; Manta et al., 2013]. Given that these different neuromodulatory systems have strong reciprocal connections and influence each other, it is very hard to delineate the role of one single system [Sara, 2009]. Consequently, the noradrenergic system does not act alone, but interacts with other modulatory neurotransmitter pathways that may also contribute to the therapeutic effect of VNS. In addition, VNS is also being explored as a neuroimmunomodulatory treatment and has proven to change the activity of the

hypothalamic-pituitary-adrenal axis [Hosoi et al., 2000; de Herdt et al., 2009], with possible implications for disorders characterized by an abnormal stress reactivity, such as major depression [O'Keane et al., 2005; Pizzagalli, 2014]. Further exploration of the exact mechanism of action should improve the identification of functional biomarkers for VNS.

1.2 Refractory epilepsy is associated with dysfunction of emotion regulation

Patients with refractory epilepsy have a high prevalence of comorbid mood disorders. The second and third chapter gathered in this thesis aimed to evaluate whether negative affect in epilepsy was associated with dysfunction of emotion regulation. In the second chapter, ERPs were used in order to investigate whether a possible dysfunction arose during early (attention) and/or late (regulation) stages of emotional stimulus processing. Two ERP components were analyzed: the early vertex positive potential (VPP) which is normally enhanced when faces are presented as visual stimuli (compared to non-facial objects), and the late positive potential (LPP) that is typically larger for emotional stimuli. The face-sensitive VPP results revealed that early object-based attention processes were not compromised and that the early structural encoding of faces was normal and preserved in patients with refractory epilepsy, regardless of the amount of negative affect experienced by the patients and the actual emotional expression conveyed by the faces. By contrast, the amplitude of the LPP component, which is sensitive to emotion regulation, was significantly enhanced for unattended faces with negative emotional expressions, but only in patients with comorbid negative affect. The modulation of the LPP component by unattended emotional stimuli during the late stages of stimulus processing suggests that emotion regulation is disturbed in patients with epilepsy and comorbid negative affect.

In this study, deficits of emotion regulation were only present in the group of patients with negative affect, which is in line with previous fMRI studies of the face- or house-matching task performed in patients with negative affect disorders. For example, earlier fMRI results showed that patients with major depressive disorder had an enhanced amygdala response to unattended fearful faces compared to control subjects [Fales, 2008]. In addition, during the processing of task-irrelevant fearful faces, the control subjects recruited the dorsolateral PFC, while patients with depression did not. Furthermore, comparison of participants with high and low state anxiety levels revealed that the "high-anxious" group showed increased amygdala response to unattended fearful versus neutral faces [Bishop et al., 2004a] and reduced recruitment of lateral PFC for unattended fearful faces [Bishop et al., 2004b]. These results support the proposal that negative affect is associated with both enhanced bottom-up input from the

amygdala, as well as reduced top-down control from the lateral PFC, resulting in dysregulation of the emotion regulation network [Bishop et al., 2004b; Ochsner and Gross, 2005; Fales et al., 2008].

The aim of the third chapter was to investigate whether the ERP response to negatively valenced stimuli in patients with refractory epilepsy was sensitive to reappraisal, an antecedent-based cognitive emotion regulation strategy that can decrease negative affect. The main findings were that: (i) negative emotional stimuli induced a stronger LPP response than neutral stimuli, (ii) this emotional LPP response was not modulated by reappraisal, (iii) deficient reappraisal of the LPP did not correlate with comorbid negative affect, and (iv) there was no relation between the efficacy of reappraisal and location of the epileptogenic zone or brain lesions in medial temporal and frontal lobe. Taken together, our results provide novel evidence that the normal modulation of the LPP component as a function of cognitive emotion regulation is absent in patients with refractory epilepsy. This suggests abnormal cognitive emotion regulation brain processes in these patients, which could in turn underlie a higher vulnerability to negative affect.

In the third study, abnormal reappraisal was found across the entire sample of patients with refractory epilepsy and no differences were found between group of patients with and without negative affect, a finding which contrasted with the results reported in the second study. This discrepancy could tentatively be explained by the use of different paradigms in these two studies, which required two different forms of emotion regulation. The matching task used in the second study was characterized by brief stimulus durations (only 300 ms) and the emotional content of the faces was not explicitly relevant for the matching task. Hence, emotions were processed in a more automatic manner, in a sense of being implicit and not directly task-relevant. By comparison, the reappraisal task used in the third study had a much longer stimulus presentation (3 s) and required cognitive the implementation of an effortful strategy meant to downplay the impact of negative emotions, a regulation process which relies primarily on regions involved in cognitive control, such as the prefrontal cortex [Ochsner and Gross, 2005; McRae et al., 2010]. Therefore, we hypothesize that defective reappraisal in patients with refractory epilepsy potentially reflects an increased vulnerability to develop negative affect disorders. Not all patients with refractory epilepsy had a current negative affect state (as reflected by the self-reported BDI and STAI scores), but our results suggest that they could have a higher risk of "getting stuck" in the negative affect state because of impaired cognitive emotion regulation [Holtzheimer and Mayberg, 2011].

It is important to consider the localization of epileptogenic zone and brain lesions because it is possible that some of these lesions could have influenced our ERP results to a greater extent than other ones, especially lesions located in regions that are presumably important for emotion regulation processes, including frontal and medial temporal areas. Therefore, we performed additional data analyses and grouped the patients based on location of the HEZ and the presence of lesions in either frontal or medial temporal lobe structures, but these analyses failed to show any significant effect on the LPP amplitude, in both the second and the third study. Recent neuroimaging evidence have highlighted that recurrent seizures in refractory epilepsy can cause widespread reorganization of overlapping neuronal networks (for reviews, see [Spencer, 2002; Richardson, 2010]). Hence, the deficient emotion regulation in patients with refractory epilepsy could not be easily linked to damage in one specific lobe but is more likely the result from dysfunction in a distributed network underlying emotion regulation in which both cortical and subcortical structures interact with each other [Ochsner and Gross, 2005; Price and Drevets, 2010].

Quantitative volumetric MRI imaging studies in patients with epilepsy and depression found significant reduction in the volumes of the hippocampus, amygdala and prefrontal brain structures [Richardson et al., 2007; Kondziella et al., 2007; Kanner and Schachter, 2010]. Comparable structural MRI abnormalities have been reported in patients with major depressive disorder alone [Arnone et al., 2012]. Examination of the cortical thickness in a group of patients with seizures originating from the left medial temporal lobe and with pathologically confirmed hippocampal sclerosis revealed that these patients showed up to 30 % decrease in cortical thickness, with noticeable thinning of frontal poles, frontal operculum, orbitofrontal, lateral temporal, and occipital regions. Interestingly, reductions in cortical thickness were evident in both cerebral hemispheres, despite unilateral seizure onset [Lin et al., 2007; Bell et al., 2011]. Furthermore, PET studies revealed that patients with unilateral mesial temporal lobe epilepsy show hypometabolism in bilateral frontal lobes, which correlates with frontal lobe deficits during neurophysiological testing [Arnold et al., 1996; Nelissen et al., 2006]. Ictal SPECT showed that during a seizure originating in the medial temporal lobe, there is hyperperfusion in the ipsilateral temporal lobe, but hypoperfusion in the bilateral frontal lobes [Van Paesschen et al., 2003]. The hypoactivity observed in the frontal regions in patients with temporal lobe epilepsy may be related to surround inhibition, which is a dynamic defense mechanism against seizure propagation [Nelissen et al., 2006]. These extratemporal abnormalities form a possible explanation for the observed broad cognitive dysfunction, with impaired executive function and working memory in mesial temporal lobe epilepsy that extends beyond memory function [Bell et al., 2011; Stretton and

Thompson, 2012]. Resection of the epileptogenic zone often results in a gain in frontal lobe functions, with cognitive improvement and normalization of the neuroimaging abnormalities [Stretton and Thompson, 2012]. These findings highlight the importance of looking at broader network functioning, rather than discrete regions, when examining psychophysiological measures of (cognitive) emotional regulation in epilepsy. These dynamic changes in the functional brain network could be investigated using advanced functional connectivity analyses [Van Mierlo et al., 2014].

An important limitation of the studies described in chapter 2 and 3 is that there was no psychiatric evaluation of the patients with epilepsy and therefore it remains unclear how many patients would meet the DSM-5 diagnostic criteria for mood or anxiety disorders. In our studies, presence of negative affect was only assessed with self-report inventories of the severity of depression (BDI) and anxiety (STAI). Negative affect is a general factor of subjective distress, and reflects a broad range of negative mood states, including anxiety and depression. Negative affect is considered a broad and pervasive predisposition to experience negative emotions that influences cognition, self-perception and world view [Watson, 1988]. In the highly influential tripartite model of Clark and Watson, negative affect is seen as a common underlying factor contributing to both anxiety and depression and this may explain the high rates of comorbidity and similarity between these disorders [Clark and Watson, 1991]. Future studies about emotion regulation in epilepsy should include a standard psychiatric interview of every patient. In addition, results should be compared with different control groups of patients without epilepsy but with other types of negative affect disorders, such as major depressive disorder, dysthymic disorder, bipolar disorder, general anxiety disorder, in order to evaluate whether this is a general effect found across these negative affect disorders, or instead, whether it is specific for negative affect in epilepsy.

We have used the LPP to study emotion regulation in patients with refractory epilepsy. Previous ERP studies of emotion regulation have proved that the LPP is a sensitive, valid and effective neurophysiological marker for investigating emotion regulation processes in both healthy individuals and patients [Hajcak et al., 2010; Gross, 2013; Moran et al., 2013]. However, LPP measures at centro-parietal scalp sites are only indirect measures of the underlying network for emotion regulation and therefore the contributions of the involved structures, including the amygdala and prefrontal cortex, remain to be elucidated. Source localization of high-density scalp EEG could help to further localize the active neuronal sources of the observed effects [Michel, 2004]. In addition, combination of EEG with functional neuroimaging techniques such as fMRI [Sabatinelli et al., 2007; Liu et al., 2013] in the same patients might help to gain additional insight into the disturbed activation patterns of the brain regions giving rise to the emotion regulation

dysfunction observed in our ERP studies and further support the hypothesis that negative affect in epilepsy is caused by dysfunction in the neurocircuitry underlying emotion regulation.

1.3 Visual processing in the amygdala is modulated by attention and emotion

In the fourth chapter we explored the effects of attention and emotion on the processing of visual face stimuli in ten patients with intracranial electrodes that directly measured the neuronal activity in the amygdala. The results indicated that there was a systematic gating by object-based attention on the amygdala responses. Further, our findings indicated that both attention and emotional valence or arousal factors could independently influence the sensory processing in the amygdala, but at distinct, non-overlapping latencies following stimulus onset. As such, these results are in line with the notion that emotion processing may take place in parallel and independent from gating by voluntary attention control mediated by fronto-parietal attention networks [Pourtois et al., 2013].

With respect to intracranial EEG studies, there has been recently a growing interest in the analysis of frequency-specific oscillatory power changes that take place during cognitive tasks or the processing of emotional stimuli. These changes are termed event-related desynchronization (ERD) or event-related synchronization (ERS), defined as a localized decrease or increase in oscillatory power in specific frequency bands [Pfurtscheller et al., 1999]. Two types of EEG oscillations should be considered: evoked and induced oscillations. An evoked response appears at the same latency and phase in each single trial and hence can be detected in the averaged ERP. An induced response appears with a jitter in latency from one trial to another, centered around a given latency and therefore this induced activity tends to partly or totally cancel out by the averaging process classically used in ERP studies. Specific detection methods, such as time-frequency analysis with wavelet transformation, are therefore needed to detect them [Tallon-Baudry and Bertrand, 1999; Pfurtscheller et al., 1999]. The functional role of coherent oscillations remains speculative. One possibility is the temporal binding of information, particularly related to the coordination of neural networks [Singer, 1999; Varela et al., 2001]. The properties of neural networks indicate that low frequency oscillations (theta band) are likely associated with connectivity of larger scale neuronal networks, while higher frequency oscillations (gamma band) are constrained in smaller networks, and may be modulated by activity in the slower oscillating larger networks [Broyd et al., 2009]. Theta waves have also been described as "carrier waves," in which gamma oscillations representing perceptual details are embedded. In particular, it has been proposed that theta band activity plays an important role in integrating and

synchronizing neural responses during emotional processing across sub-cortical (amygdala) and cortical structures, such as frontal and visual cortices [Lewis, 2005; Jensen and Colgin, 2007; Meletti et al., 2012].

In this line, time–frequency analyses of amygdala EEG revealed increases in the theta frequency band power during the processing of emotional faces. The increase in power of theta oscillations was sensitive to both the emotional valence of the stimulus and to the part of the face presented to subjects with a stronger increase in theta band power in response to isolated fearful eyes [Meletti et al., 2012; Murray et al., 2014]. In addition, analysis of induced oscillations in the amygdala in response to pleasant, unpleasant and neutral IAPS pictures revealed that oscillations in the gamma power band could discriminate between stimuli in different emotional categories, with significant increase in gamma band power only to aversive pictures [Oya et al., 2002]. The gamma band changes were of the induced type and not phased locked to stimulus onset, which indicates that gamma-band changes probably resulted from a temporally dispersed evaluation of the emotional meaning of the stimulus. Furthermore, time-frequency analysis of the human amygdala observed stronger gamma band activity for faces compared to non-face stimuli, especially when processing of faces was task-relevant [Musch et al., 2014]. The results showed strong attentional modulation over the frequency-specific changes in the gamma band and differences between fearful and neutral faces were rarely observed. The attention effects on the gamma band oscillations in the amygdala peaked between 350 and 450 ms after stimulus onset, which is consistent with the timing of the attentional gating effect observed in our iERP study. It would be very interesting to combine iERPs with time-frequency analysis of induced oscillations during the same paradigm, in order to compare the effects of attentional and emotional factors on both induced and evoked activity of the amygdala.

In future studies using the face- or house-matching task, we plan to use an eye tracking device in order to have an objective measure of which stimuli were attended at the single trial level. Eye tracking technology allows direct and continuous measurement of overt visual attention which may provide an important supplement to the behavioral and ERP measures [Armstrong and Olatunji, 2012]. In addition, future research on the processing of emotional stimuli should combine (intracranial) ERP measurements with physiological markers of emotional response, such as electrodermal activity, facial muscle activity, cardiovascular responses, pupil dilation and changes in the neuroendocrine system [Collet et al., 1997; Bradley et al., 2001; Andreassi, 2013]. These objective peripheral physiological measures can provide complementary information about the emotional response of the participant. The physiological markers each have a specific response pattern that is sensitive to differentiate between specific aspects of emotional stimuli

[Collet et al., 1997]. It has been demonstrated that heart rate is a sensitive index for the valence of emotional stimuli. Heart rate accelerated during viewing of positive valenced IAPS pictures, whereas heart rate decelerated during viewing of negative valenced IAPS pictures [Bradley et al., 2001; Ohira et al., 2006]. By contrast, electrodermal activity (sympathetic skin conductance response) has been found to reflect emotional arousal regardless of the valence of the emotional stimuli [Bradley et al., 2001; Ohira et al., 2006]. On the other hand, a considerable amount of data has indicated that emotional experiences are accompanied by changes in the neuroendocrine system [De Kloet, 2004]. The most extensively studied endocrine system in the context of emotion and stress is the hypothalamic-pituitary-adrenocortical axis which is sensitive to emotional valence. Previous research has shown that levels of cortisol and adrenocorticotropic hormone increased after viewing affectively negative emotional stimuli but were unchanged after viewing neutral and positive stimuli [Codispoti et al., 2003].

2 Advantages and limitations of ERP recordings in patients with epilepsy

The main advantage of the ERP technique is its high temporal resolution in the order of milliseconds. In addition, ERPs provide a direct measure of electrical neuronal activity in response to specific stimuli or events. This makes it a powerful technique for investigating the electrophysiological time course of neuronal activity during specific cognitive tasks. ERPs allow continuous analysis of the ongoing neuronal processes from stimulus onset until response selection and decision making. Behavioral responses reflect the output of a large number of individual cognitive processes. Therefore, variations in reaction time and accuracy are difficult to attribute to changes to (or combination of) specific cognitive processes. In contrast, ERPs provide a continuous measure of the different processing stages, making it possible to determine which stage(s) of processing are likely to be affected by a specific experimental manipulation. ERPs can give information about the underlying neuronal process of behavioral responses and can frequently detect specific effects in the absence of clear differences at the behavioral level [Luck, 2014].

Our P3 results corroborate the notion that ERPs may provide a valuable tool as potential biomarkers in medical applications, by measuring aspects of brain function that are impaired in specific psychiatric and neurologic disorders, such as schizophrenia, mood disorders, attention deficit hyperactivity disorder, Alzheimer's disease and aphasia [Jackson and Snyder, 2008; Duncan et al., 2009; Leiser et al., 2011; Luck, 2014]. First, ERPs may serve as brain function biomarkers that are more closely related to the underlying neurobiology of the disease than the traditional clinical symptoms and measures. Second, preclinical animal models are available for various ERP components

providing opportunities for translational research [Leiser et al., 2011; Luck et al., 2011]. Third, ERP markers could be used in clinical studies for evaluation of new treatments, to determine whether a given medication or neurostimulation therapy has an effect on the specific cognitive process, neural circuit, or neurotransmitter system of interest. Subsequently, ERP biomarkers could also be used in clinical practice to monitor therapy follow-up. Finally, ERP biomarkers could help to identify patients who are the best candidates for early interventions or for specific treatments and fine-tune the individual treatments [Leiser et al., 2011; Luck et al., 2011; Luck, 2014].

However, several limitations of the ERP technique should be pointed out. ERP amplitude measurements can be influenced by individual differences in nuisance variables [Luck et al., 2011], such as skull thickness [Frodl et al., 2001] and cortical folding patterns [Ahlfors et al., 2010]. Scalp ERP recordings are mainly sensitive to activity from radial sources, in particular currents in the apical dendrites of the pyramidal cells in the cortical gyri near the surface of the head [Kropotov, 2009; Luck, 2014]. Scalp recorded EEG gives no information about activity in neurons that are not spatially aligned, because in this case dipoles will not summate but rather cancel each other out, resulting in “closed” electrical fields [Handy, 2005].

It should be noted that the high temporal resolution of the ERPs is accompanied by a relatively low spatial resolution and it is difficult to determine the neuroanatomical origins of a given ERP effect purely on the basis of the observed scalp distribution [Luck, 2014]. This is because of the inverse problem: any given voltage distribution at the scalp can be explained by an infinite number of intracranial source configurations [Rugg and Coles, 1996; Kropotov, 2009]. Therefore, intracranial recordings in patients with depth electrodes for diagnostic purposes offer a unique opportunity to merge temporal and spatial precision into one single recording and measure local field potentials or even single unit activity directly from neurons located deep inside the brain. When interpreting human intracranial ERP data it should be taken into consideration that participants of intracranial EEG studies generally belong to a clinical population suffering from refractory epilepsy and thus do not represent healthy participants [Murray et al., 2014]. Accordingly, generalization about neuronal functioning from recordings in patients with chronic refractory epilepsy to the healthy brain remains a potential limitation.

Our scalp ERP results in patients with refractory epilepsy should be interpreted with caution as well because epilepsy is a complex, heterogeneous and variable neurological condition and various potential confounding factors need to be taken into account [Kubota et al., 1998; Caravaglios et al., 2001]. Epilepsy affects the whole age range from neonates to elderly people, and has variable causes and manifestations, with many

distinct seizure types and several identifiable electroclinical syndromes [Duncan et al., 2006; Berg et al., 2010]. The type and location of the structural epileptogenic lesions can be very heterogeneous, including brain tumors, hippocampal sclerosis, focal cortical dysplasia, cysts and cavernomas. Even if lesions are limited to hippocampal sclerosis in the mesial temporal lobe, this can lead to frontal lobe dysfunction and broad cognitive deficits [Bell et al., 2011; Stretton and Thompson, 2012]. Moreover, recurrent seizures in refractory epilepsy can cause widespread reorganization of overlapping neuronal networks [Spencer, 2002; Richardson, 2010]. In addition, there are many comorbidities that may confound the results obtained, including learning disabilities, fixed neurological deficits, progressive conditions, psychological and psychiatric problems, and concomitant medical conditions [Duncan et al., 2006]. Finally, patients with refractory epilepsy take a wide range of different AEDs with common adverse effects on mood, cognition and behavior [Aldenkamp et al., 2003; Reijs et al., 2004].

In summary, the ERP technique provides a valuable neurophysiological tool for studying specific neuronal processes or neurotransmitter systems in patients with refractory epilepsy. In addition, ERPs could provide potential biomarkers that will help to predict response to treatment. However, our ERP results should be interpreted with caution, bearing in mind that epilepsy is a very heterogeneous disorder with many comorbidities and various confounding factors. Future research should combine ERPs with complementary methods and techniques, such as time-frequency analysis of induced oscillations, functional neuroimaging and measurements of physiological markers of emotional response, in order to bring complementing evidence and eventually strengthen the new insights gained from the ERP findings gathered in this thesis.

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SUMMARY

The event-related potential (ERP) technique is a sensitive neurophysiological method for investigating human brain functioning in both healthy individuals and patients. ERPs are electrical brain potentials, recorded from scalp or intracranial electrodes, that are time-locked to specific sensory, motor or cognitive events. This doctoral project used the ERP technique to study specific neuronal processes in patients with refractory epilepsy.

Epilepsy is a brain disease characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition. Worldwide, there are about 65 million patients with epilepsy, of whom about a third remain refractory to treatment with antiepileptic drugs. For those patients resective surgery is the first treatment of choice. When epilepsy surgery is not an option or patients in whom resection fails to control seizures alternative treatment options are available including vagus nerve stimulation (VNS).

VNS is a neurostimulation therapy known to be safe and effective for both refractory epilepsy and depression. The mechanism of action of VNS is currently not fully understood and it is unclear which factors determine the patient's response to the treatment. Recent preclinical experiments have highlighted the pivotal role of the locus coeruleus noradrenergic system in the antiepileptic effect of VNS. The **first study** aimed to evaluate the effect of VNS on noradrenergic signaling in the human brain through a non-invasive marker of this neurotransmitter system, i.e., the oddball P3 component of the ERP. We investigated whether VNS differentially modulated the amplitude of the P3 component in VNS responders versus VNS non-responders. Our results revealed that VNS induced a significant increase of the P3 amplitude at the parietal midline electrode, in VNS responders only. In addition, logistic regression analysis showed that the increase of P3 amplitude can be used as a non-invasive indicator for VNS responders. These results support the hypothesis that activation of the locus coeruleus noradrenergic system is associated with the antiepileptic effect of VNS. Modulation of the P3 amplitude should be further investigated as a non-invasive biomarker for the therapeutic efficacy of VNS in epilepsy.

Patients with refractory epilepsy have a high prevalence of comorbid mood disorders. The second and third study aimed to evaluate whether refractory epilepsy is associated with dysfunction of emotion regulation. In the **second study**, ERPs were recorded in order to investigate whether a possible dysfunction arises during early (attention) and/or late (regulation) stages of emotional stimulus processing. This ERP study revealed that early object-based attention processes are not compromised by epilepsy, regardless of negative affect. By contrast, the amplitude of the late positive potential (LPP), a

component that is sensitive to emotion regulation, was significantly enhanced for negative emotional expressions when visual face stimuli were unattended, but only in patients with comorbid negative affect. The modulation of the LPP component by unattended emotional stimuli during the late stages of stimulus processing suggests that emotion regulation is disturbed in patients with refractory epilepsy and comorbid negative affect.

The aim of the **third study** was to investigate whether the ERP response to negatively valenced stimuli in patients with refractory epilepsy is sensitive to reappraisal, a cognitive emotion regulation strategy that can decrease negative affect. This study showed that modulation of LPP amplitude, as electrophysiological correlate of cognitive emotion regulation, is absent in patients with epilepsy. This suggests abnormal functioning of the brain during cognitive emotion regulation in patients with epilepsy, which could explain the high susceptibility of these patients to negative affect and comorbid mood disorders.

How emotion and attention influence visual processing in the amygdala remains a matter of controversy in the literature. In the **fourth study**, we hypothesized that attention and emotional factors operate independently and in parallel on the sensory processing in the amygdala in order to increase the saliency and selection of behaviorally relevant information. To test this hypothesis ERPs were recorded directly in the amygdala of ten patients with refractory epilepsy implanted with intracranial depth electrodes. This study provided direct evidence for asymmetric effects of attention and emotion in the human amygdala during visual face stimulus processing. While object-based attention exerts a systematic effect on amygdala ERPs at group-level, only a subset of the patients shows amplitude modulations of amygdala ERPs by emotion at variable delays after stimulus presentation. These results suggest that attention and emotion can differentially influence sensory processing in the amygdala, likely via different pathways, with stable gating by attention but versatile modulation by emotion.

In conclusion, the ERP technique provides a valuable neurophysiological tool for studying specific neuronal processes or neurotransmitter systems in patients with refractory epilepsy. In addition, specific ERPs could provide potential biomarkers that will help to predict response to treatment. However, our ERP results should be interpreted with caution, because epilepsy is a very heterogeneous disorder with many comorbidities and several confounding factors should be taken into account. Future research should combine ERPs with complementary methods, such as time-frequency analysis of induced oscillations, functional neuroimaging and measurements of physiological markers of emotional response, in order to bring complementing evidence and eventually strengthen the new insights gained from the ERP findings gathered in this thesis.

SAMENVATTING

Event-gerelateerde potentialen (ERPs) worden gebruikt in neurofysiologische onderzoek naar de werking van de menselijke hersenen in zowel gezonde individuen als patiënten. ERPs zijn elektrische hersenpotentialen die gemeten worden met elektrodes op de scalp of intracraniëel en die tijdsgebonden voorkomen na specifieke sensorische, motorische of cognitieve gebeurtenissen. In dit doctoraatsproject werd gebruik gemaakt van ERPs om specifieke neuronale processen te bestuderen in patiënten met refractaire epilepsie.

Epilepsie is een chronische hersenaandoening die gekenmerkt wordt door het herhaaldelijk optreden van spontane epileptische aanvallen. Er lijden wereldwijd 65 miljoen patiënten aan epilepsie, waarvan ongeveer een derde niet afdoende reageert op behandeling met anti-epileptica. Voor de refractaire patiënten waarbij een chirurgische ingreep geen optie is of waarbij de chirurgie geen aanvalscontrole bracht, zijn er alternatieve behandelingsopties beschikbaar, waaronder nervus vagus stimulatie (NVS).

NVS is een veilige en effectieve neurostimulatie behandeling voor zowel refractaire epilepsie als depressie. Het werkingsmechanisme van NVS is momenteel nog niet volledig gekend en het is onduidelijk welke factoren bepalen of een patiënt op de behandeling zal reageren. Preklinisch onderzoek wijst erop dat het noradrenerg systeem van de locus coeruleus een kritische rol speelt in het anti-epileptisch effect van NVS. In de **eerste studie** werd getracht het effect te achterhalen van NVS op de noradrenerge signalisatie in de menselijke hersenen. Dit werd gedaan door analyse van een niet-invasieve marker van dit neurotransmitter systeem, namelijk de P3 component van de ERP. Onze resultaten toonden aan dat NVS enkel in NVS-responders een significante toename induceerde van de P3 amplitude. Logistische regressie analyse toonde bovendien aan dat de toename van de P3 amplitude een niet-invasieve marker is voor de NVS respons. Deze resultaten ondersteunen de hypothese dat activatie van het noradrenerg systeem geassocieerd is met het anti-epileptisch effect van NVS. Ze suggereren ook dat modulatie van de P3 amplitude een mogelijke niet-invasieve biomarker is voor de therapeutische respons op NVS in epilepsie, een bevinding die noopt tot verder onderzoek.

Een hoog aantal patiënten met refractaire epilepsie hebben stemmingsstoornissen als comorbiditeit. Het doel van de tweede en derde studie was om te onderzoeken of refractaire epilepsie gepaard gaat met een verstoring van de emotie regulatie. In de **tweede studie** werden ERPs gebruikt om na te gaan of er een verstoring optreedt tijdens de vroege (aandacht) en/of late (regulatie) stadia van de verwerking van emotionele stimuli. Deze ERP studie toonde aan dat vroege aandachtsprocessen niet verstoord zijn door epilepsie, onafhankelijk van negatief affect. Daarentegen was de amplitude van de late positieve potentiaal (LPP), een component die gevoelig is voor

emotie regulatie, significant toegenomen door taak-irrelevante negatieve emotionele expressies. Dit was echter enkel het geval in patiënten met comorbiditeit van negatief affect. De modulatie van de LPP component door taak-irrelevante emotionele stimuli tijdens de late stadia van stimulus verwerking suggereert dat emotie regulatie verstoord is in patiënten met refractaire epilepsie en comorbiditeit van negatief affect.

Het doel van de **derde studie** was om te onderzoeken of de ERP respons op stimuli met een negatieve valentie gevoelig is voor herwaardering, een cognitieve emotie regulatie strategie die negatief affect kan doen afnemen. Deze studie toonde aan dat modulering van de LPP amplitude door herwaardering, als elektrofysiologisch correlaat van cognitieve emotie regulatie, afwezig is in patiënten met refractaire epilepsie. Deze bevindingen wijzen op een verstoord functioneren van de hersenen tijdens cognitieve emotie regulatie in patiënten met refractaire epilepsie. Dit zou kunnen verklaren waarom deze patiënten een verhoogde predispositie vertonen voor negatief affect.

Hoe emotie en aandacht een invloed uitoefenen op de visuele verwerking in de amygdala blijft een onderwerp van discussie in de literatuur. Onze hypothese was dat aandachts en emotionele factoren onafhankelijk van elkaar een effect uitoefenen op de sensorische verwerking in de amygdala om zo gedragsrelevante informatie te selecteren. Om deze hypothese te testen werd een **vierde studie** opgezet, waarin ERPs werden gemeten in de amygdala van tien patiënten met refractaire epilepsie bij wie intracraniële diepte elektrodes geïmplanteerd waren. Hoewel er op groepsniveau een systematisch effect was van objectgebaseerde aandacht op de ERPs in de amygdala, werd er slechts bij een deel van de patiënten effecten van emotie geobserveerd en dit op variabele tijdstippen na presentatie van de stimulus. Deze studie leverde zo direct bewijs voor asymmetrische effecten van aandacht en emotie op de sensorische verwerking in de humane amygdala, met stabiele invloed door aandacht maar een variabele modulatie door emotie.

Deze studies tonen gezamenlijk aan dat de ERP-techniek een waardevolle methode is voor het bestuderen van specifieke neuronale processen of neurotransmitter systemen in patiënten met refractaire epilepsie. Bovendien zijn ERPs potentiële biomerkers die kunnen helpen bij het voorspellen van de respons op de behandeling. Desalniettemin moeten onze ERP resultaten voorzichtig geïnterpreteerd worden, daar epilepsie een uitermate heterogene aandoening is met veel comorbiditeit en er verschillende mogelijke versturende factoren bestaan. In verder onderzoek moeten ERP metingen gecombineerd worden met complementaire methodes, zoals tijd-frequentie analyse van geïnduceerde oscillaties, functionele beeldvorming en metingen van fysiologische merkers van emoties. Op deze manier kan aanvullend bewijs geleverd worden voor de nieuwe inzichten bekomen in dit ERP onderzoek.

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WORK EXPERIENCE

2011 - 2015: **Doctoral researcher** | FWO Aspirant scholarship
Faculty of Medicine and Health Sciences, Ghent University
Topic: '*Event-related potentials in patients with refractory epilepsy*'
Promotors: Prof. Dr. Robrecht Raedt and Prof. Dr. Gilles Pourtois

UNIVERSITY EDUCATION

2009 - 2011: **Master in Biomedical Sciences** | Major in Neurosciences
Ghent University
Thesis topic: '*Functional characterization of a new specific Cx43 hemichannel inhibitor*'. Promotor: Prof. Dr. Luc Leybaert
Graduated: Summa Cum Laude

2006 - 2009: **Bachelor in Biomedical Sciences**
Ghent University
Graduated: Summa Cum Laude

SCIENTIFIC AWARDS & HONORS

Oct 4th 2014: **Best poster** according to patient associations for the poster: '*Preserved attention allocation but impaired emotion regulation in epilepsy with comorbid negative affect: electrophysiological time course*'
5th Belgian Brain Congress, Ghent, Belgium

Feb 14th 2014: **Clinical junior award data blitz and poster presentation prize.**
Topic: '*The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy*'
SWO midwinter meeting of the Dutch League against epilepsy, Amsterdam, The Netherlands

Mar 22nd 2013: **Poster presentation prize** for the poster: '*Attentional emotion control in dysphoric and non-dysphoric patients with epilepsy*'
15th clinical symposium Kempenhaeghe, Heeze, The Netherlands

- Oct 20th 2011: **Prize for Best Master Thesis in Biomedical Sciences**
Thesis topic: '*Functional characterization of a new specific Cx43 hemichannel inhibitor*'. Promotor: Prof. Dr. Luc Leybaert
- Oct 20th 2011: **Prize for Best Student in Biomedical Sciences**
Ghent University, Ghent, Belgium

PUBLICATIONS IN INTERNATIONAL PEER-REVIEWED JOURNALS (A1)

- De Taeye L**, Pourtois G, Meurs A, Boon P, Vonck K, Carrette E, Raedt R (2015) *Event-related potentials reveal preserved attention allocation but impaired emotion regulation in patients with epilepsy and comorbid negative affect*. PLOS ONE. 10(1): e0116817.
- Grimonprez A, **De Taeye L**, Delbeke J, Larsen LE, Raedt R, Boon P and Vonck K (in press) *Laryngeal motor-evoked potentials mark vagus nerve activation: a preclinical study*. International Journal of Neural Systems.
- De Taeye L**, Vonck K, van Bochove M, Boon P, Van Roost D, Mollet L, Meurs A, De Herdt V, Carrette E, Dauwe I, Gadeyne S, van Mierlo P, Verguts T, Raedt R (2014) *The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy*. Neurotherapeutics. 11(3): 612-622.
- De Letter M, Aerts A, Van Borsel J, Vanhoutte S, **De Taeye L**, Raedt R, van Mierlo P, Boon P, Van Roost D, Santens P (2014) *Electrophysiological registration of phonological perception in the subthalamic nucleus of patients with Parkinson's Disease*. Brain and Language. 2138C: 19-26.
- De Taeye L**, Pourtois G, Meurs A, Van Roost D, Achten R, Boon P, Vonck K, Carrette E, Raedt R (Under review) *Systematic gating of attention but versatile influence of emotion in the human amygdala revealed by intracranial event-related potential recordings*. Human Brain mapping.
- De Taeye L**, Pourtois G, Meurs A, Boon P, Vonck K, Carrette E, Raedt R (Under review) *Abnormal reappraisal during emotion regulation in refractory epilepsy: an ERP study*. Epilepsy & Behavior.

ABSTRACTS IN INTERNATIONAL JOURNALS (C3)

- De Taeye L**, Pourtois G, Meurs A, Carrette E, Vonck K, Boon P, Raedt R (2014). *Preserved attention allocation but impaired emotion regulation in epilepsy with comorbid negative affect: electrophysiological time course*. Conference Abstract: Belgian Brain Council. Frontiers in Human Neuroscience.
- De Letter M, Aerts A, Vanhoutte S, Van Borsel J, Raedt R, **De Taeye L**, van Mierlo P, Boon P, Van Roost D, Santens P (2014). *Direct electrophysiological registration of phonological and semantic perception in the human subthalamic nucleus*. Conference Abstract: Belgian Brain Council. Frontiers in Human Neuroscience.
- De Letter M, Aerts A, Vanhoutte S, Van Borsel J, Raedt R, **De Taeye L**, van Mierlo P, Boon P, Van Roost D, Santens P (2014). *Direct electrophysiological registration of phonological and semantic perception in the human*

subthalamic nucleus. Conference: 18th International Congress of Parkinson's Disease and Movement Disorders Location: Stockholm, SWEDEN. Abstracts. Movement disorders. 29(1):S278-S278.

De Letter M, Aerts A, Vanhoutte S, Van Borsel J, Raedt R, **De Taeye L**, van Mierlo P, Boon P, Van Roost D, Santens P (2014). *Corticosubcortical interactions for semantic and phonological perception in Parkinson's disease*. VVL-congres, 35e, Abstracts. Vlaamse Vereniging voor Logopedisten (VVL): 18-18.

Staljanssens, W, Strobbe G, De Taeye L, Van Roost D, Vonck K, Raedt R, Van Hoen R, Vandenberghe S, van Mierlo P (2014). *Source Reconstruction of the P300 Event-related Potential as a Biomarker for the Efficacy of Vagus Nerve Stimulation in Patients with Epilepsy*. 19th International Conference on Biomagnetism Abstracts.

De Taeye L, Vonck K, van Bochove M, Boon P, Van Roost D, Mollet L, Meurs A, De Herdt V, Carrette E, Dauwe I, Gadeyne S, van Mierlo P, Verguts T, Raedt R (2013). *Enhancement of the P300 positively correlates with the therapeutic effect of vagus nerve stimulation in patients with refractory epilepsy*. Proceedings of the 68th Annual meeting of the American Epilepsy Society.

van Bochove M, **De Taeye L**, Vonck K, Raedt R, Meurs A, Boon P, Dauwe I, Notebaert W, Verguts T (2013). *Increased cognitive control during norepinephrine release through acute vagus nerve stimulation*. Journal of cognitive neuroscience. 25 (suppl.): 246-246.

Pourtois G, Koban L, **De Taeye L**, Van Roost D, Boon P, Fias W, Raedt R (2013). *Rapid affective marking of self-generated actions in the anterior insula: evidence from human IEEG*. Journal of cognitive neuroscience. 25 (suppl.): 207-207.

De Taeye L, Pourtois G, Meurs A, Carrette E, Vonck K, Boon P, Raedt R (2012). *Attentional emotion control in dysphoric and non-dysphoric patients with epilepsy*. Conference Abstract: Belgian Brain Council. Frontiers in Human Neuroscience.

CONFERENCES AND COURSES

Jul 7 - 11th 2015: 9th World Congress International Brain Research Organization (IBRO)
Sulamerica Convention Center, Rio De Janeiro, Brazil

Apr 20 - 23th 2015: Doctoral schools course: 'Writing for non-specialists and the press'
Ghent University, Ghent, Belgium

Oct 4th 2014: 5th Belgian Brain Congress
International Convention Centre, Ghent, Belgium

May 5th 2014: Knowledge for Growth 2014
International Convention Centre, Ghent, Belgium

Oct 29 - 30th 2013: BrainVision Analyzer Workshop
PC College, Berlin, Germany

- Aug 9 - 12th 2013: Birmingham Boot Camp 2.0
University of Birmingham, Edgbaston, Birmingham, UK
- Jun 13nd 2013: Symposium on Cognitive and Affective Control
Het Pand, Ghent, Belgium
- Feb - May 2013: Doctoral schools course: 'Advanced Academic English Writing Skills:
Life Sciences and Medicine'
De Brug, Ghent, Belgium
- Dec 12th 2012: 2nd Ghent Institute for Neuroscience Symposium
Het Pand, Ghent, Belgium
- Oct 27th 2012: 4th Belgian Brain Congress
Palais des Congrès, Liège, Belgium
- May 9 - 12th 2012: 1st Conference of the European Society for Cognitive and Affective
Neuroscience (ESCAN)
Université de Provence, Marseille, France
- Jan - Sep 2012: Statistical analysis with SPSS beginner and advanced courses
Ghent University Hospital, Ghent, Belgium
- Jan - Jun 2012: Epilepsy course 2012 of the Flemish League Against Epilepsy
Brussels University Hospital, Brussels, Belgium
- Nov 11th 2011: Kick-off Institute for Neuroscience Symposium
Het Pand, Ghent, Belgium

ORAL PRESENTATIONS

- Feb 13th 2015: '*Intracranial event-related potentials from the amygdala*'
LCEN3 PhD project Workshop, Het Pand, Ghent, Belgium
- Feb 14th 2014: '*The P3 event-related potential is a biomarker for the efficacy of vagus
nerve stimulation in patients with epilepsy*'
SWO midwinter meeting of the Dutch League Against Epilepsy,
Academic Medical Center, Amsterdam, The Netherlands
- Nov 21st 2013: '*Emotion control in patients with comorbidity of epilepsy and
depression*'
Staff meeting of the Neurology Department, UZ Ghent, Belgium
- Jun 27th 2013: '*Epilepsy and psychiatric comorbidity*'
Scientific education meeting. Psychiatric Center Ghent Sleidinge
(PCGS), Ghent, Belgium
- Feb 1st 2013: '*Attentional emotion control in dysphoric and non-dysphoric patients
with epilepsy*'
SWO midwinter meeting of the Dutch League Against Epilepsy,
Academic Medical Center, Amsterdam, The Netherlands
- Oct 18th 2012: '*Effect of vagus nerve stimulation on noradrenergic signaling in the
human brain*'
Neuroscience Forum, Institute of Neuroscience, Faculty of Psychology
and Educational Sciences, Ghent, Belgium

POSTER PRESENTATIONS

- Jul 9th 2015: *'Systematic gating of attention but versatile influence of emotion in the human amygdala revealed by intracranial event-related potential recordings'*
9th World Congress International Brain Research Organization (IBRO)
Sulamerica Convention Center, Rio De Janeiro, Brazil
- Oct 4th 2014: *'Preserved attention allocation but impaired emotion regulation in epilepsy with comorbid negative affect: electrophysiological time course.'* Certificate: Best posters according to patient associations
5th Belgian Brain Congress
International Convention Centre, Ghent, Belgium
- May 5th 2014: *'The P3 is a biomarker for efficacy of vagus nerve stimulation in patients with epilepsy'*
Knowledge for growth
International Convention Centre, Ghent, Belgium
- Mar 28th 2014: *'The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy'*
16th clinical symposium 'Update@kempenhaeghe.nl'
Heeze, The Netherlands
- Feb 14th 2014: *'The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy'*
Awarded with poster presentation prize
SWO midwinter meeting of the Dutch League against epilepsy,
Academic Medical Center, Amsterdam, The Netherlands
- Jun 13nd 2013: *'Prolonged attentional bias towards negative emotional stimuli in dysphoric patients with epilepsy'*
Symposium on Cognitive and Affective Control
Het Pand, Ghent, Belgium
- Mar 22nd 2013: *'Attentional emotion control in dysphoric and non-dysphoric patients with epilepsy'*
Awarded with poster presentation prize
15th clinical symposium 'Update@kempenhaeghe.nl'
Heeze, The Netherlands
- Oct 27th 2012: *'Attentional emotion control in dysphoric and non-dysphoric patients with epilepsy'*
4th Belgian Brain Congress
Palais des Congrès, Liège, Belgium

TEACHING, MASTER STUDENTS & SCIENCE COMMUNICATION

- 2015: **Guidance of the research internship** of Honours project student Fleur Mak: *'The mechanism of action of neurostimulation'*.
- 2014 - 2015: **Guidance of the research internship** of Master student Sema Kaya: *'Onderzoek naar een niet-invasieve biomarker voor het therapeutisch effect van nervus vagus stimulatie bij patiënten met refractaire epilepsie'*.
- Oct 20 - 24th 2014: **Science Week** *'Wetenschap in de Kijker'* when students of secondary education can participate in scientific research. Subject *'modulation of the brain'* with practical sessions in which students could measure their own EEG and test different types of noninvasive neurostimulation devices.
- Mar 13th 2014: **Lecture** about event-related potentials in the in the context of the course *'Neurobiology'* to students of the 1st Master Biomedical sciences of the Ghent University.
- 2013 - 2014: **Guidance of the research internship** of Master student Sarah Van Belle: *'Onderzoek naar een niet-invasieve biomarker voor het therapeutisch effect van nervus vagus stimulatie bij patiënten met refractaire epilepsie'*.
- Mar - Dec 2013: **Member of the organizing committee** of the PhD day of the Institute of Neuroscience *'Connecting the regions of interest'*.
- Nov 25th 2012: **i-Brain Festival** 2012: hands-on EEG workshop to record EEG of participants of the festival.
- 2012 - 2015: **Lecture on epilepsy research** at Ghent University and **scientific guided tour** to the Center for Neurophysiological Monitoring (CNM) and the Reference center for Refractory Epilepsy (RCRE) in the Ghent University Hospital to Master students Biomedical Sciences and Biotechnology.
- 2012 - 2015: **Hands-on epilepsy workshop** to Master students Biomedical sciences in context of the course *'Neurobiology'* about recording their own EEG and ERP signals, neuromodulation with non-invasive brain stimulation devices and analyzing ERP data with EEGLAB and ERPLAB.
- 2011 - 2012: **Guidance of the master thesis** of Stefanie Gadeyne: *'De rol van de noradrenerge transmissie bij nervus vagus stimulatie, een behandeling voor epilepsie'*.