THE FUNCTIONAL NEUROANATOMY OF POST-TRAUMATIC STRESS DISORDER FOLLOWING THE REDUCTION OF TRAUMATIC MEMORY RECONSOLIDATION USING PROPRANOLOL

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ABSTRACT

Reconsolidation blockade of traumatic memories has been proposed as a promising intervention for PTSD. Animal and human data suggest that memory reconsolidation may be attenuated by the beta-adrenergic receptor antagonist propranolol, which has been specifically shown to mitigate the strength of aversive memories after retrieval. The neural correlates of reconsolidation impairments have been explored in healthy participants employing fear-conditioning paradigms which have lacked ecological validity in clinical anxiety patient populations. However, the neurobiology of reconsolidation blockade as a treatment for PTSD has never been examined among individuals suffering from ingrained, traumatic memories.

This dissertation employed functional magnetic resonance imaging (f-MRI) to map the neural substrates implicated in traumatic memory reactivation relative to neutral information, (Study 1); and the neural correlates of non-trauma, threat processing (Study 2), before and after reconsolidation blockade with propranolol. Study 1 demonstrated that six weekly doses of propranolol administered prior to trauma reactivation, modulated brain activity in regions implicated in learning, memory, and attention (amygdala, hippocampus, insula, thalamus, and medial prefrontal cortex). Symptom severity was significantly diminished following reconsolidation blockade. These results suggest that

reconsolidation impairments of traumatic memories may promote PTSD remission by decreasing the emotional response to trauma specific information via inhibition of the fear-conditioning neural network, and stimulation of the prefrontal cortex to suppress stress reactivity. In the second investigation (Study 2), fearful face encoding was associated with increased limbic activity (amygdala) relative to happy or neutral stimuli, indicating hyper-vigilance to threat, although the concomitant recruitment of frontal brain regions may be an attempt to inhibit negative emotional states. Reconsolidation blockade engaged the anterior cingulate, implicated in cognitive appraisal of stimuli, without an exaggerated fear response. These results imply that the reinstatement of top-down regulation is associated with decreased fear generalization to non-trauma related stimuli, in PTSD patients, which may contribute to PTSD symptom resolution.

As a pernicious and prevalent mental health problem, PTSD requires improved treatment methods. Understanding the neural responses to trauma specific cues compared to non-traumatic emotional stimuli, before and after reconsolidation blockade provides information on biomarkers of recovery from a traumatic experience; and validates the therapeutic potential of propranolol for PTSD symptoms. Interventions aimed at altering memory reconsolidation hold promise for treating PTSD and other psychiatric conditions involving aversive recollections.

RÉSUME

Le blocage de la reconsolidation du souvenir traumatique est une intervention prometteuse pour les patients atteints du trouble de stress post-traumatique (TSPT). Les données sur les animaux et les humains suggèrent que la reconsolidation du souvenir peut être atténuée par le propranolol, un antagoniste du récepteur beta-adrénergiques, qui permet de réduire l'intensité des souvenirs aversifs après récupération.

Les corrélats neuronaux des troubles de reconsolidation ont été explorés chez des participants normaux en utilisant des paradigmes de conditionnement de la peur dont la validité écologique est toutefois manquante chez des populations de patients cliniques anxieux.

Cependant, la neurobiologie du blocage de la reconsolidation en tant que traitement pour le TSPT n'a jamais été examiné chez des individus souffrant de souvenirs traumatiques tenaces.

Cette thèse utilise l'Imagerie par Résonance Magnétique fonctionnelle (IRM-f) pour cartographier les substrats neuronaux impliqués dans la réactivation relative aux souvenirs traumatiques, (étude 1) ; et dans les corrélas neuronaux non-traumatiques du traitement de la menace, (étude 2), avant et après le blocage de la reconsolidation avec le propranolol.

L'étude 1 a montré que les 6 doses de propranolol reçues de manière hebdomadaire administrées avant la réactivation du trauma ont modulées l'activité fonctionnelle du

cerveau dans les régions impliquées dans l'apprentissage, la mémoire et l'attention (amygdale, hippocampe, insula, thalamus, cortex cingulaire antérieur, et le cortex préfrontal médial). La sévérité des symptômes a significativement diminuée après la reconsolidation du blocage. Ces résultats suggèrent que le trouble de reconsolidation des souvenirs traumatiques peut aider à la rémission des TSPT en diminuant la réponse émotionnelle aux informations spécifiques du traumatisme par l'inhibition des réseaux neuronaux du conditionnement de la peur, et en stimulant le cortex préfrontal pour supprimer la réaction au stress.

Dans la seconde étude, l'encodage de visages exprimant la peur a été associé avec une augmentation de l'activité limbique (amygdale) par rapport à des stimuli exprimant la neutralité ou la joie, indiquant une hyper-vigilance à la menace, bien que le recrutement des régions frontales du cerveau peut être une tentative d'inhiber l'état émotionnel négatif.

Le blocage de la reconsolidation engage le cortex cingulaire antérieur, impliqué dans l'appréciation cognitive des stimuli, sans réponse exagérée pour la peur.

Ces résultats impliquent que la restauration des régulations *top-down* est associé avec la diminution générale de la peur des stimuli non reliés aux traumatismes, dans les patients TSPT, ce qui peut contribuer à la résolution des symptômes TSPT.

En tant que problème pernicieux de santé mentale, le TSPT nécessite une amélioration de la méthode de traitement. Comprendre les réponses neuronales spécifiques au traumatisme comparé à des stimuli émotionnels non-traumatiques, avant et après le

blocage de la reconsolidation fournit des informations sur les biomarqueurs du rétablissement d'une expérience traumatique; et valide le potentiel thérapeutique du propranolol pour des symptômes liés aux TSPT.

Les interventions visant à altérer la reconsolidation de la mémoire sont prometteuses pour guérir les TSPT et les autres conditions psychiatriques impliquant.

To follow knowledge like a sinking star,
Beyond the utmost bound of human thought -
To strive, to seek, to find, and not to yield

- *Ulysses, Alfred Lord Tennyson*

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a. **Contribution of Authors**

As per university regulations, a thesis containing co-authored papers requires an explicit statement indicating the contribution of each individual.

Megan Mahabir, MSc. (Integrated Graduate Program in Neuroscience, McGill University), PhD Candidate and Study Co-Investigator. I wrote and designed the research protocol based in part on a project funded by the Réseau de Bio-imagerie du Québec (RBIQ) to Dr. Brunet. I prepared the ethics and Health Canada applications, and conducted all of the functional neuroimaging sessions (data acquisition) throughout the study. I was significantly involved in the statistical analysis and interpretation of the complete functional imaging datasets, and the behavioral data from the treatment sessions.

Alain Brunet, Ph.D. (Douglas Institute, McGill University), Study Principal Investigator. Dr. Brunet has extensive expertise in using the reconsolidation blocker propranolol for treating PTSD. He provided leadership to the project and supervised the implementation of the study procedures across the sites. He assisted with the interpretation of the results and manuscript preparation.

Pierre Etienne, M.D. (Douglas Institute, McGill University). Dr. Etienne is the *Qualified Investigator* for this clinical trial, in accordance with the regulations for Health Canada. He supervised the medical and pharmacological aspects of the study, and assisted with the interpretation of the data.

Daniel Saumier, Ph.D. (Douglas Institute, McGill University). Dr. Saumier assisted with study co-ordination and was responsible for participant assessments, recruitment and

follow-up throughout the study. He assisted with the preparation of the ethical and regulatory submissions, and the preparation of the manuscript, for the first study (Chapter II).

Alan Tucholka, Ph.D. (Neuroimaging Research Scientist, Radiology Department, Notre-Dame Hospital). Dr. Tucholka provided mentorship on the technical imaging protocol and analysis techniques employed in this study.

Lisa Shin, PhD (*Chair, Psychology Department, Tufts University, Medford, Massachusetts, USA).* Dr. Shin acted as a consultant on the design of the f-MRI tasks presented to the study participants and contributed to the preparation of the manuscripts.

b.**Contribution to Original Scholarship**

In accordance with the McGill Faculty of Graduate Studies and Research guidelines, "elements of the thesis that are considered original scholarship and an advancement of knowledge in the specific research domain must be clearly stated".

Over the last century, a major tenet of memory research held that once memories are consolidated into long-term memory (LTM) storage, they were permanent and could not be altered [1]. With the discovery of *reconsolidation*, research has demonstrated that memories are plastic and can be retrieved, destabilized, and altered, during this phase. This has promising implications for the treatment of chronic, recurrent traumatic memories, which undergo reconsolidation when re-experienced by individuals suffering from PTSD. However, the neural basis of reconsolidation is still a subject of investigation, as PTSD pathology involves perturbations of complex and different neuro-

anatomical pathways. Using functional magnetic resonance imaging (f-MRI), this thesis elucidated the neural substrates of traumatic memory reactivation; and non-traumatic, emotional information processing, in a PTSD patient cohort before and after 6 weekly doses of propranolol, a reconsolidation blocker. These results comprise original scholarship by contributing to the comprehension of the neural markers of PTSD remission following reconsolidation blockade, and demonstrate that noradrenergic mechanisms are implicated in the chronic phase of the illness. The two studies presented are novel undertakings that advance scientific knowledge in the *treatment* domain of PTSD.

CHAPTER I

GENERAL INTRODUCTION

1.0 STATEMENT OF PROBLEM

Post-traumatic Stress Disorder (PTSD) remains a leading international burden [2] and a formidable treatment challenge. It is characterized by involuntary and persistent memories of a traumatic event, which trigger intense emotional responses, including intrusions, avoidance of trauma-related stimuli, and increased arousal [3]. The avoidance of stimuli reminiscent of the trauma provides short-term relief, but prevents the extinction and remission of the disorder, limiting patients' social functioning, and severely affecting their quality of life.

According to a recent meta-analysis, cognitive behavioral therapy (CBT) is currently considered the best treatment for PTSD. Despite this, only one-third of patients receiving cognitive therapies show lasting, clinically meaningful improvements [4]. Furthermore, CBT is theoretically based upon extinction. Extinction does not alter the original memory, but only inhibits it, with the consequent risk of spontaneous recovery (return of symptoms over time) and symptom renewal (re-appearance of symptoms in unforeseen contexts). Current pharmaco-therapies are only modestly more helpful than placebo, [5] and need to be taken for extended periods with ensuing side effects.

Breakthroughs in PTSD treatment and in psychiatry more generally, are likely to stem from an enhanced understanding of neuroplasticity. Reconsolidation, a concept virtually unknown of 15 years ago [6], is one example of the brain's plasticity that could lead to a major paradigm shift in PTSD treatment in the near future. Reconsolidation is not based upon extinction, but rather on modifying the original memory, and conditioned responses reduced by this method do not

undergo spontaneous recovery or renewal. These considerations suggest that the treatment benefits conferred by reconsolidation blockade should be more generalized and potentially longer lasting than those conferred by CBT. Despite this, propranolol's use to block the reconsolidation of traumatic memories, has only been tested in open-label trials of a brief duration with a six-month follow-up period [7]. Therefore, more information is required about the application of reconsolidation theory to the treatment of PTSD. To date, it is not known how reconsolidation blockade affects trauma specific memories and non-traumatic emotional processing; at the neural level in chronic PTSD patients.

1.1 PRESENT STATE OF KNOWLEDGE

1.1.1 Historical Perspective

In the year 1876, Dr. Mendez DaCosta identified "Soldier's Heart", a condition which afflicted American Civil War combat veterans, who exhibited startle-responses, arrhythmia, and hyper-arousal [8]. Subsequently, in World War I and II this syndrome was referred to as "shell shock" and "war neurosis" respectively, which introduced the concept of a neurobiological correlate of these symptoms. The advent of the Vietnam War significantly influenced the current concept of PTSD, as veterans exhibited combat related nightmares, anger, depression, alcohol and/or drug dependence, disability and anxiety for several consecutive years after returning home [9]. The term "post-traumatic stress disorder" was formalized as a psychiatric condition in the Diagnostic and Statistical Manual of Mental Disorders III (DSM-III) published in 1980[10]. Subsequently, epidemiologic studies identified a high prevalence of *civilian* trauma in the general urban, population [11].

1.1.2 Post-traumatic Stress Disorder: Definition

Traumatic events, including interpersonal violence and natural disasters, can elicit stressful reactions which endure after the occurrence of these incidents. PTSD is embodied by anxiety in the aftermath of a traumatic event, and is comprised of a constellation of symptoms, currently outlined in the DSM-IV (Text Revision-TR) [3]:

The person must have experienced, witnessed, or encountered an event (*stressor*, Criteria A) involving a threat of death, or serious injury to self or others, and this exposure must have precipitated intense fear, helplessness, or horror as an emotional reaction. It is postulated that the stress reaction, rather than the stressor, negatively alters homeostatic function.

The *re-experiencing* criterion (Criterion B) includes intrusive recollections of the trauma, nightmares and flashbacks to internal or external cues that can be persistent for decades or a lifetime, invoking intense psychological distress (e.g. panic, despair) and physiological reactivity. Persistent *avoidance* (Criterion C) consists of symptoms reflecting behavioral, cognitive, or emotional strategies by PTSD patients to reduce the likelihood that they will be exposed to trauma-related stimuli. Avoidance symptoms are associated with contextual phobias of places, people, and experiences that remind the person of their trauma; or can manifest as emotional numbing characterized by loss of memory for a specific aspect of the trauma, anhedonia, reclusive behavior (detachment), and a sense of a foreshortened future. *Hyper-arousal* (Criterion D) includes symptoms such as insomnia, irritability, an exaggerated startle response and an inability to concentrate after the trauma. PTSD patients also exhibit hyper-vigilance in which they survey the environment for potential threats or danger that could elicit a traumatic episode.

At least one re-experiencing symptom, three avoidance/numbing symptoms, and two hyper-arousal symptoms must be present for at least one month (Criteria E) and these must cause clinically significant distress or functional impairment (Criteria F) for an initial diagnosis of PTSD to be assigned to an individual.

If these symptoms persist for less than three months, they are considered *acute*, whereas *chronic* symptoms will last greater than three consecutive months. If there is a six month period between the event and the development of symptoms, the condition is classified as *delayed onset* PTSD.

1.2 Epidemiology

To date, PTSD is the fourth most common psychiatric diagnosis for which the etiology is known, as cases must correspond to specific precipitating events. It is currently estimated that 90% of Americans will be exposed to a severe traumatic event during their lifetime [12]. It has been estimated that 76.1% of the Canadian population has had exposure to at least one traumatic event during their lifetime [13]. Approximately 10–25% of individuals who experience a traumatic circumstance will develop a diagnosis of PTSD after one month [14].

In the general population of the United States (US), 60% of men experience a traumatic event during their lifetime compared to 50% of women [15]. With the exception of rape and sexual assault, there is a greater likelihood that males will experience every other type of trauma [15] and at higher frequencies than females. The probability of PTSD as a result of rape is equivalent in both males and females, followed by combat and physical assault [12, 15]. Despite this, women are twice as likely as men to develop PTSD in response to trauma, overall [16].

A steep dose-response curve between trauma frequency and PTSD symptom severity has been identified, such that the more traumatic events a person experiences, the greater the intensity of PTSD symptoms [17]. In addition, the re-experiencing and avoidance symptoms of the disorder are highly co-morbid with major depressive disorder and substance abuse [18-20], which can further contribute to chronic disability, as these conditions negatively impact the emotional, physical, occupational, and social functioning of PTSD individuals and are associated with significant financial costs to society [8].

1.3 Risk Factors

Previous exposure to trauma [15], specifically interpersonal violence exposure is more likely to precipitate PTSD than a traumatic event without a human perpetrator, such as a natural disaster. Additional pre-trauma risk factors include a young age, since the peak exposure period for all traumatic types occurs between 16 and 20 years of age [12, 21]. Inter-individual characteristics, including education level, socioeconomic status, a history of childhood maltreatment, the type and severity of the trauma, individual and family psychiatric history, and race are also antecedents for PTSD development. In low income populations, there is approximately a 20 to 30% lifetime prevalence of chronic PTSD, following exposure to a severely stressful event [15].

Genetic [22], social and environmental factors [23] play a role not only in the development of PTSD, but in the severity, and likelihood of recovery. Understanding of the neurobiological underpinnings of PTSD may explain individual differences in vulnerability to the disorder, and facilitate the development of more effective interventions.

2.0 PTSD PATHOPHYSIOLOGY

2.1 The Contribution of the Noradrenergic System

There is substantial evidence that the neurotransmitter norepinephrine (NE) has a pivotal role in generating fear and anxiety [24], and is implicated in plasticity mechanisms, mediating learning and memory [24-26]. Stress-induction in animals, activates the amygdala and hypothalamic pituitary axis (HPA-axis), eliciting the release of epinephrine and corticosterone via the adrenal glands [27]. The basolateral amygdala receives noradrenergic input from two sources: the vagus nerve which contains beta-adrenergic receptors that project to the nucleus of solitary tract (NST), stimulating the flow of norepinephrine; and via corticosterone-mediated action of the glucocorticoid receptors on the NST [24]. Pharmacological challenge studies in rodent models have shown that epinephrine administered locally, induces stress and this effect is reduced by β-adrenergic (i.e. propranolol) or glucocorticoid (i.e. RU486) receptor antagonists applied to the amygdala.

Human based studies with PTSD patients indicate augmented concentrations of NE, at baseline (rest) and during stress-responsivity, in the cerebrospinal fluid metabolites (CSF) and in the periphery [28]. These results may reflect increased CNS *pre-synaptic* adrenergic tone via the locus coeruleus (at night) contributing to PTSD symptomatology**.** Increased heart rate at the time of a traumatic event has been predictive of PTSD. These patients also exhibit elevated heart rates relative to controls when exposed to β-adrenergic stimulation, indicating hyper-responsive *post-synaptic* β-adrenoreceptors. The medial prefrontal cortex (mPFC) regulates peripheral biological responses to stress, including alterations in heart rate, blood pressure, and cortisol response [29], and has emerged as a neural region, adversely impacted by chronic stress.

3.0 NEUROBIOLOGY OF POST-TRAUMATIC STRESS DISORDER

3.1. Translational Theory of PTSD pathophysiology

Learning, classical Pavlovian and instrumental conditioning are fundamental to behavioral adaptation. With respect to the development of PTSD, it has been postulated that the trauma (unconditioned stimulus, UCS), elicits a strong release of stress hormones (unconditioned response, UCR), over-consolidating the memory of the event which leads to intrusions. Subsequently, traumatic event cues (conditioned stimulus, CS) trigger the recollection of the event, accompanied by a surge of stress hormone release (conditioned response, CR), which strengthens the trauma memory via a positive feedback mechanism [30].

For the brain to accomplish new learning, neural synapses must be held into stable neural representations, a process that requires protein synthesis and synaptic plasticity. *Memory consolidation* is the time-dependent process by which memories are transferred from short-term (**STM**) to long-term memory (**LTM**). Animal and human data indicate that stress hormones potentiate memory consolidation. Their effects are mediated by noradrenergic activity in the amygdala and are opposed by β-adrenergic blockers such as ***propranolol*** [31]. Interfering with memory consolidation (i.e. immediately after learning) yields a degraded memory trace.

Conceptually, memory formation involves the synaptic coupling between neurons, which become strengthened by their activity, in a process known as long-term potentiation (LTP) [32]. A type of synaptic plasticity, LTP reinforces neuronal connections throughout networks that correlate with learned information or an experience. It is postulated that PTSD memories become over-consolidated by a cellular mechanism which is perturbed by a psychologically stressful antecedent [33]. Key brain regions which mediate learning and memory are

preferentially affected by stress, and include the hippocampus, amygdala, hypothalamus, medial prefrontal (mPFC), and anterior cingulate cortex [34]. The limbic system structures, particularly the amygdalae and hippocampi, have been studied extensively due to their role in fear conditioning and the pathophysiology of PTSD [35, 36]. Within the lateral amygdala (amygdaloidal-thalamic pathway) LTP is presumed to underlie the pathological memory associations between conditioned and unconditioned stimuli (trauma) in patients with PTSD [32, 37]. Neurotransmitters have been shown to alter the efficacy of LTP in the amygdala. Of significance, norepinephrine (noradrenalin) is provided to the basolateral amygdala by the locus coeruleus, a cluster of neurons in the brainstem [38]. Concurrent activation of β-adrenergic and glucocorticoid mechanisms modulate memory via the amygdala during stress exposure [39-41]. Moreover, the hippocampi have a seminal function in the formation, storage, and consolidation of memories, as well as contextual fear conditioning [36, 42], which may also contribute to the symptoms of PTSD.

Previous research has illustrated that NE contributes to aversive memory tasks, such as inhibitory avoidance (IA) in animals [40, 43] and that via β-adrenergic receptors (βARs), NE modulates the consolidation of long-term memory (LTM) of IA. Similarly, animal models demonstrated that administration of epinephrine (EPI) or norepinephrine (NE) post-training enhances the storage of emotional (fearful) memories [40, 44]. At the time of the traumatic event, excess epinephrine release, cements strong emotional memories and fear conditioning that subsequently manifest as PTSD symptoms [45]. Clinical research studies suggest that the persistence and severity of PTSD symptoms are connected to increased noradrenergic activity, many years after the traumatic experience [35]. Within the noradrenergic system, the *postsynaptic* β-adrenoreceptor may mediate PTSD symptoms by over-consolidating emotional memories [46].

Thus, modifications in noradrenergic signaling affect the acute stages of memory; from the initial encoding, to the maintenance and the exacerbation of symptoms associated with long-term traumatic memories. Taken together, animal and human studies reveal that norepinephrine enhances memory consolidation processes whereby new learning is deeply engraved into long-term memories. It is possible that the deeper encoding and consolidation of the traumatic memories may impede extinction and recovery from PTSD.

3.2. Neuroadaptive Memory Mechanisms

Reconsolidation theory posits that the recall of a previously consolidated memory returns it to a labile state from which it must re-stabilize in order to persist [6]. Thus, consolidation is recapitulated each time a memory is recalled. After activation of the long-term memory, the structure has to be consolidated by the synthesis of new proteins [39].

Classic theories of memory maintain that following the occurrence of an event, there exists a labile period during which the consolidation of the memory trace can be interfered with or even occluded [1]. Early behavioural work demonstrated that electroconvulsive shock; disrupted fearful memories when actively recalled by obsessive compulsive disorder patients, thereby improving their symptoms [47]. In addition, pharmacological agents such as propranolol, a β-adrenergic blocker, have been shown to reduce subsequent physiological arousal when administered on a short-term basis during this labile period. In a small double-blind, randomized, placebo-controlled trial, 40 mg of propranolol was administered twice daily for 10 days, to acute trauma victims with six hours after a traumatic event. Propranolol administered during this critical period reduced physiological responses (a hallmark of PTSD) during script-driven imagery of the event three months later [48].

Other evidence indicates that a memory trace may be modifiable, even after consolidation of the memory trace has occurred. For example, Nader, Schafe and Le Doux (2000) [39] trained animals in a conditioning task, and the memory trace was allowed to consolidate. The conditioned stimulus was later presented unreinforced by the unconditioned stimulus, after which the animal was given either, a saline solution or anisomycin, the latter of which is known to interfere with the protein synthesis process responsible for memory consolidation. Subsequent exposure to the conditioned stimulus resulted in a diminished conditioned response for those receiving the drug but not the saline solution. These results show that a previously consolidated memory, once re-activated, returns to a labile state; in order to return the memory to long-term storage and consolidate again (hence the term reconsolidation). More importantly, they also show that the administration of the protein synthesis inhibitor may disrupt the reconsolidation process of the fear memory, and thereby interfere with it. Interestingly, injection of *propranolol* during the reconsolidation phase in animals has been found to have similar effects as anisomycin [49, 50].

It is postulated that consolidation and reconsolidation are mediated by different brain regions, cellular and molecular circuits, yet both have a critical phase during which they can be disrupted by stress hormones [1, 51]. Both require protein synthesis, the extracellular signal-regulated MAP kinase pathway and the transcription factor, cyclic adenosine monophosphate response binding element (CREB) protein. In contrast, other studies have noted that reconsolidation is resistant to blockade [52], or that the effects are temporary [53] and thus there are boundaries under which reconsolidation occurs, such that it is not a direct recapitulation of consolidation processes. Factors effecting reconsolidation include the age of the memory, the type and length of the memory reactivation procedure.

4.0 PHARMACOLOGY OF PROPRANOLOL HYDROCHLORIDE

Propranolol hydrochloride (*5- [2- [4- (1, 2-benzisothiazol-3-yl)-1-piperazinyl] ethyl] -6-chloro-1, 3-dihydro-2H-indol-2-one mono-hydrochloride monohydrate)* is a competitive, synthetic β-adrenergic antagonist, which reduces sympathetic activity. It is a well-known drug typically prescribed to individuals suffering from hypertension, tachycardia, cardiac arrhythmia, tremors, thyroid disease, or migraine. Propranolol is lipophilic, readily crosses the blood-brain barrier, and exhibits non-selective binding among β -adrenoreceptor subtypes [54].

Propranolol is absorbed via the gastrointestinal tract and metabolized by the liver. According to Dey et al. (1986)[55], a short-acting propranolol dose of 80 mg (at a dose of 1 mg/kg, and assuming a mean weight of 80 kg) should produce a peak blood level of approximately 100ng/ml, at two hours post-dose. The biological half-life is approximately four hours [56]. It is important to note that mean concentrations of approximately 200 ng/ml have been documented with a dose of 160 mg of fast acting propranolol, which is greater than the levels that we expect to obtain with the doses used in the current study. In addition, the fast acting doses will not exceed the maximum therapeutic daily dose of 320 mg that is indicated for the treatment of hypertension and angina [57]. Toxic levels are associated with plasma concentrations above 2000 ng/ml.

5.0 PTSD TREATMENT STUDIES WITH PROPRANOLOL TO BLOCK CONSOLIDATION OR RECONSOLIDATION

Given the role of noradrenergic activity in facilitating amygdala-dependent fear memories, researchers examined the administration of adrenergic receptor antagonists that could potentially

mitigate the severity of PTSD symptoms. Lipid-soluble anti-adrenergic drugs that are CNS active when administered peripherally are available to reduce CNS adrenergic activity by several mechanisms. These include the administration of adrenergic receptor modulators, prazosin (alpha 1 receptor antagonist) [58], clonidine (alpha 2 receptor agonist) to decrease norepinephrine outflow [59] and propranolol (β adrenergic receptor antagonist) [7, 48, 60-63]. These inexpensive, generic anti-adrenergic drugs have been frequently and safely prescribed, to treat hypertension since the 1970's [10].

Based on the current animal research, propranolol has emerged as the most promising candidate drug to impair the consolidation strength of the traumatic memory trace via blockade of the post-synaptic β-adrenergic receptors in the basolateral amygdala. Consequently, early human studies examined propranolol as a prophylactic for PTSD, following a traumatic event. In one study, 41 participants who had experienced a traumatic event, within four hours of emergency department arrival, received 40 mg of propranolol or placebo three times a day, for 10 days, with a 9-12 day taper period. Patients presented with a pulse rate greater than 80 beats per minute (BPM), which was considered a biomarker of a hyper-adrenergic state. One-month post-trauma, the treated participants showed a trend for decreased scores on the Clinician-Administered PTSD Scale relative to the placebo group, demonstrating that propranolol reduced the conditioned fear response. At three months follow-up, physiological responses to script-driven imagery, indicated that propranolol was effective, in preventing PTSD [48].

Similarly, in a preliminary study (controlled, non-blind/non-randomized) 19 patients with a heart rate greater than 90 beats per minute, were enrolled from two emergency departments in France

approximately 2–20 hours after a motor vehicle accident (MVA) or physical assault. Of these, 11 participants agreed to receive 40 mg of propranolol three times a day for seven days, followed by an 8–12 day taper period. Two months after the traumatic events, PTSD symptoms were significantly lower in the propranolol treated patients, relative to the eight untreated participants [60]. The two groups did not differ on demographics, exposure characteristics, physical injury severity, or peri-traumatic emotional responses, indicating that these factors were not significantly implicated in the treatment response.

6.0 FUNCTIONAL NEUROIMAGING STUDIES OF POST-TRAUMATIC STRESS DISORDER

Aberrant activity in a triad of brain regions has been the hallmark of post-traumatic stress disorder: the *amygdala* which is involved in the encoding of emotional memories, the *hippocampus* which notably mediates the contextual encoding and retrieval of the event, and the *medial prefrontal cortex* (including the *anterior cingulate cortex* [ACC], the sub-callosal gyrus, and the medial frontal gyrus) which regulate affect. The ACC exerts top-down control of the limbic areas, that mediate threat response, and is implicated in extinguishing exaggerated fear responses, that are cardinal characteristics of chronic PTSD [64].

Neurobiological models posit that augmented amygdala reactivity to fearful, trauma-related, stimuli represents impaired inhibition of the limbic structures by the medial PFC [65]. Functional imaging studies have indicated decreases in mPFC activity and simultaneous hyper-activation of the amygdala in PTSD patients [66-69] Furthermore, decrements in mPFC activation, are inversely related to PTSD symptomatology, and associated with greater symptom severity [68, 70].

6.1 Affective Facial Tasks as probes of limbic circuit activity in PTSD patients

Unmedicated PTSD patients with acute symptoms, exhibit increased right amygdala activity, similar to chronic cases, when exposed to masked fearful faces [71]. Combat veterans with PTSD, vs. healthy veterans, significantly activate the amygdala, without engaging the prefrontal (PFC) circuits during the same task [72]. In a longitudinal study, Dickie et al. (2008) [73] examined the relationship between PTSD symptom severity and neural activity, during the memory encoding of emotional faces using f-MRI. Amygdala activation and PTSD symptom severity were associated only with successful encoding of fearful, rather than neutral faces. Reduced activation of the ventromedial PFC (vmPFC) in highly symptomatic PTSD individuals, was also predictive of decrements in memory encoding [74], and correlated with the subsequent forgetting of facial stimuli [73]. The vmPFC in concert with the hippocampus confers inhibition of amygdala function during fear extinction [75]. Alterations in the activity of the hippocampal and sub-genual ACC (sgACC) during emotional face encoding in PTSD subjects, has been reflective of changes in symptom severity [76]. Moreover, the hippocampus has a seminal function in the formation, storage, and consolidation of memories, as well as contextual fear conditioning [36, 42], also contributing to the symptoms of PTSD.

It has been postulated that the limbic hyperactivity in PTSD is reflective of "bottom-up" reactivity stimulated by the semblance of threat based emotional signals, that are indicative of amygdala [77] and insula [78] responsivity to the non-conscious perception of fear. Both cognitive (conscious) as well as associative, (unconscious) mechanisms contribute to the behavior [79, 80]. The limbic system activation pattern in intimate personal violence victims with PTSD is consistent with exaggerated and functionally disconnected processing of threat-

related affective stimuli (i.e. emotional faces). Fonzo et al. (2010) [81] demonstrated that the anterior insula and amygdala were significantly activated in female PTSD participants following interpersonal violence vs. non-trauma exposed controls during the presentation of fearful or angry faces relative to happy faces. It has been previously postulated that hyper-vigilance is related to aberrant activation of "top-down" emotional and cognitive-appraisal networks that mediate attentional mechanisms and arousal [82, 83]. Insula activity has been linked to interoception as well as the anticipation and avoidance of fear. The ACC in conjunction with the insula has been implicated in reflecting internal body states such as feelings of disgust and, aversion which are commonly experienced by PTSD patients.

With regards to noradrenergic mechanisms, studies which incorporate threat based stimuli, such as negative emotional faces, are thought to evoke self-preservation responses associated with hyperactivity of the sympathetic nervous system. The ensuing amygdala hyper-activation may orient PTSD patients to novel or salient stimuli in the environment that may be menacing, thereby perpetuating the disorder.

6.2 The neural correlates of PTSD symptom provocation

The neural substrates of traumatic memory recall have been examined using script-driven imagery paradigms in conjunction with neuroimaging methods. Motor vehicle collision patients who experienced *acute* distress, but were *resilient* to PTSD, exhibited right perirhinal cortex regional cerebral blood flow (rCBF) decreases during traumatic script replay (relative to a personalized neutral script), which correlated with symptom improvement three months after trauma [84]. These patients also showed lower resting state amygdala and parahippocampal blood flow relative to non-trauma exposed healthy controls. The perirhinal cortex is implicated

in memory processes, including the mediation of "meaningfulness" and lesions to this region in conditioned rodents abolishes fear-potentiated startle [84]. It is plausible that decrements in limbic circuit blood flow at rest, and in the perirhinal cortex in the acute aftermath of a traumatic event, confer an inhibitory mechanism curtailing the development of PTSD.

PET imaging has also been conducted during script-driven imagery of a traumatic event in *chronic* PTSD patients, healthy controls without combat experience, and combat veterans without PTSD [85]. While the healthy controls demonstrated increased amygdala activity and decreased vmPFC function, the PTSD patients deactivated the rostro-dorsal ACC (rACC). The authors interpreted the compromised rACC activity in the PTSD group as limiting the normal regulation of emotion to traumatic cues. Both the combat groups with and without PTSD did not show amygdala activity during traumatic memory reactivation, which may have indicated a compensatory response or resiliency to the stressors. In a separate study, Lanius et al. (2001) [86] conducted (f-MRI) while PTSD patients listened to a description of their traumatic event. PTSD participants underwent a medication wash-out period of two-weeks prior to scanning and had notably decreased activity in the mPFC, rACC and thalamus during traumatic memory re-activation. The ACC region in particular has reciprocal connections with the amygdala, and modulates responses to affective stimuli [87] whereas altered thalamic function may reflect disruptions of sensory processing (dissociation) in PTSD subjects during traumatic memory recall. Similarly, emotional gating through deactivation of the limbic system may enhance cognitive performance for neutral material. These findings further highlight the complex interaction between the prefrontal cortex and the limbic system in modulating the fear response, during traumatic memory reactivation.

Symptom provocation has been employed with PET imaging, to study individuals with a history of sexual abuse who developed PTSD, compared to those who were abused, but did not experience PTSD symptoms [88]. Both groups exhibited increased orbital frontal cortex (OFC) and anterior temporal lobe activation during traumatic memory reactivation, but these findings were more significant in the PTSD group. The PTSD group also exhibited rCBF decreases in the anterior frontal regions bilaterally, as well as a decrease in the left inferior frontal gyrus, the latter which is associated with decreased linguistic processing while recollecting a traumatic event. In comparison, the non-PTSD group showed greater rCBF in the insular cortex and ACC during traumatic imagery. However, amygdala activation was not observed in either group. The main study findings corroborate previous reports that patients with PTSD exhibit hypo-frontal activation during traumatic recall, but the authors stipulate that this may mediate the effortless, intrusive memories, rather than indicating dis-inhibition of the amygdala.

Decrements in activation of the thalamus, ACC and mPFC have been observed during the induction of different mood states (sad or anxious) in PTSD subjects, using script-driven imagery. Additionally, these patients reported symptoms of hyper-arousal, and flashbacks relative to trauma exposed participants *without* PTSD. These early results indicated that dysregulation in these brain regions could mediate aversive memories and the processing of negative emotional reactions which are experienced by PTSD participants. Other PFC regions implicated in the pathogenesis of PTSD include the sub-callosal anterior cingulate gyrus, and the left inferior frontal cortex [89]. Previous studies have shown that individuals with PTSD have smaller ACC and mPFC volumes [90, 91].

In summary, the PFC regions inhibit aberrant cognitive and emotional responses that are mediated, in part, by the amygdala [20]. PTSD patients exhibit hypo-activation or in some cases, a failure to activate, PFC brain regions when presented with trauma cues [49, 85, 86]. Converging evidence from imaging studies on the pathophysiology of PTSD; demonstrate that afflicted individuals have impaired PFC functioning, which leads to amygdala hyperactivity and exaggerated emotional responsiveness.

7.0 NEURAL CORRELATES OF POST-TRAUMATIC STRESS DISORDER RECOVERY

7.1 The neural correlates of anti-depressant effects on chronic PTSD symptoms

The plausible neural mechanisms of PTSD recovery have been examined following serotonin selective reuptake inhibitor (SSRI) treatments, in conjunction with neuroimaging techniques. An 8 week, preliminary randomized controlled trial of citalopram, an anti-depressant in conjunction with single photon emission tomography (SPECT) imaging, showed a negative correlation between increased activity in the mPFC and PTSD symptom reports, whereas after the treatment, there was significantly decreased activity in the left medial temporal lobe [92]. Additionally, SPECT imaging conducted before and after 12 weeks of SSRI administration reported that the treatment significantly decreased activity in the ACC, left hippocampus, and right thalamus across a group of anxiety patients, including PTSD, obsessive compulsive disorder (OCD) and social anxiety disorder (SAD) [93].

In a separate positron emission tomography (PET) imaging study, six months of fluoxetine treatment, *increased* regional cerebral blood flow in the orbitofrontal, prefrontal and inferior frontal cortices during the presentation of war-related sounds to a torture victim diagnosed with

PTSD, and this was correlated with a 48% decrease in symptoms post-treatment [94]. Similarly, in a second pilot PET study, PTSD participants who were treated with paroxetine for 12 weeks exhibited increased rCBF in the orbito-frontal cortex, a region implicated in extinction responses during script-driven imagery [95]. However, both placebo and paroxetine increased blood flow in the ACC, which the authors thought was indicative of a general treatment response, possibly to regulate fear. Although the study was conducted in a PTSD sample, it was a small randomized double blind placebo controlled trial, comprised of six placebo and seven paroxetine completers, which limited the generalizability of the findings. In addition, participants listened to their script while being scanned at baseline and 12 weeks after, when the treatment phase was completed. However, habituation effects on neural activation, during the script-driven imagery task at post-treatment, were not addressed in the study.

7.2 Neural activation following reconsolidation impairments using fear extinction

Interestingly, with the use of f-MRI, Agren et al (2012) [96] demonstrated that *extinction* is correlated with altered amygdala activity. *Healthy* women underwent fear conditioning via a cued shock pairing paradigm, on the first day. On the following day, participants were randomized to receive *extinction* within 10 minutes after fearful memory reactivation (within the reconsolidation window) or 6 hours after reactivation (outside of the reconsolidation phase), by re-presenting the cue without the shock. On the third day, participants underwent f-MRI scanning during fear renewal, in which they were outfitted with shock electrodes, although no shocks were administered. Fear memory was abolished in the group that received extinction after 10 minutes, as indicated by *decreased* amygdala activation, indicative of disrupted reconsolidation. On the fifth day, participants were tested for the "return of fear", whereby they

were exposed to non-cued shocks. *Increased* BOLD signal activity was reported in the amygdala bilaterally, only in the six-hour group following the return of fear. Amygdala activity also correlated with their fear recall during extinction, but this was not observed in the 10 minute group. This amygdala response suggested that the fear memory trace remained superior in the group who had received extinction 6 hours after reactivation, and this neural response predicted the reinstatement of fear. Other neural regions significantly activated (bilaterally) in the 6 hour group included the hippocampus, insula and the ACC which indicated that the amygdala is part of a neural network which modulates human reconsolidation and fear memory plasticity. However, vmPFC activity did not negatively correlate with fear responses, following successful extinction in the 10 minute group. Therefore, the authors surmised that the fear memory was *erased*, rather than suppressed by PFC activation in a sample of healthy participants.

7.3 Naturalistic PTSD recovery studies

A recent longitudinal fMRI study from our group [76], employed a fearful face encoding paradigm, which demonstrated that altered hippocampal activity is involved in recovery from PTSD. Alterations in the activity of the hippocampus and sub-genual ACC (sgACC) during emotional face encoding in PTSD subjects, has reflected changes in symptom severity [76]. Other longitudinal imaging studies have noted that augmented cortical thickness in the dorsolateral pre-frontal cortex (DLPFC) and sub-genual anterior cingulate cortex (ACC) are associated with greater PTSD symptom improvement [97, 98].

In conclusion, while the reinstatement of PFC function is implicated in PTSD symptom improvement; it has also been shown with neuroimaging, that directly altering amygdala function may be important to extinguish fear. Furthermore, aversive memories can be modified

by both pharmacological and behavioral interventions applied during the reconsolidation phase. The neuroimaging paradigms which have been employed to examine the brain regions implicated in recovery, have provided support for the hypothesis that fear conditioning is a valid model of PTSD, and that reconsolidation blockade may be effective in reducing amygdala activity.

8.0 PHARMACOLOGICAL NEUROIMAGING STUDIES OF EMOTION AND ADRENERGIC AGENTS

Studies have investigated the contribution of both increased and decreased noradrenergic signaling to fear-processing and emotional memory. Using f-MRI, Onur et al. [99] observed that a single 4mg dose of reboxetine, a pre-synaptic NE reuptake inhibitor, increased right basolateral amygdala activation in response to fearful face stimuli, while decreasing activation to the neutral faces, in *healthy adults*. Other limbic brain regions activated during the fearful faces condition, include the right hippocampus, right temporal lobe (Heschl's gyrus), inferior frontal gyrus (bilaterally), and the fusiform "face" gyrus (bilaterally). It is possible that these results support the notion that augmented NE signaling (post-stress), can selectively increase the signal to noise ratio for fear, by mobilizing a "fear-module" comprised of a subset of amygdala neurons [99]. Although the amygdala mediates aversive responses through NE stimulation, its cyto-architecture is also involved in functions such as vigilance, novelty detection, salience of perceptual stimuli, and the integration of emotional responses [100].

To date, there are two f-MRI studies that have examined the neural effects of propranolol. In the first study, Hurlemann et al. (2010) [38] conducted a randomized, double-blind, placebo controlled study in *healthy participants*, to test the hypothesis that β-adrenoceptor blockade with

propranolol would reduce basolateral amygdala activation. Participants received a 40 mg oral dose of propranolol, and after a two hour interval, they viewed movie clips of happy, sad and neutral faces while undergoing f-MRI scanning. Indeed, propranolol attenuated human amygdala responses to the facial stimuli, regardless of emotional expression type. Despite this, implications regarding the impact of propranolol on memory reconsolidation cannot be concluded from this study, as other brain regions implicated in learning and memory were not examined. Additionally, the study is limited in its' generalizability to anxiety populations, as the investigators administered a low dose of propranolol to healthy participants, whereas several doses may be required for the treatment of chronic PTSD symptoms [101, 102].

In a subsequent imaging study, healthy participants encoded a series of neutral and negative pictures. One day after (i.e. 24 hours), participants were randomized to receive either 40 mg of propranolol or placebo. Approximately 70 minutes post-drug, they were scanned during a retrieval (reactivation) exercise in which they were asked to recall the pictures they had previously learned, without external cuing. On the third day, participants were scanned during a recognition test, in which the pictures were presented in a randomized order. Imaging results yielded increased activation in the amygdala and hippocampus during the recognition phase, indicating compensatory up-regulation of limbic activity, following reconsolidation blockade [103].

To date, functional neuroimaging studies have probed the short-term neural effects of propranolol in *healthy participants*, and thus the treatment implications of reconsolidation blockade, were not extrapolated to clinical populations who experience chronic disturbances in emotional memory.

9.0 STUDY RATIONALE

PTSD involves the development of conditioned emotional responses through Pavlovian fear conditioning at the time of trauma exposure. Peri-traumatic distress [104] and arousal leads to the release of endogenous stress hormones, which enhance memory consolidation, subsequently leading to an excessively powerful, persistent, and aversive fear memory. Trauma memories are too easily activated by reminders, resulting in anxiety and dysfunction [45, 105, 106].

Researchers have been trying to block memory consolidation shortly following traumatic exposure as a means of preventing PTSD. It has been shown that the drug propranolol administered to acute trauma victims reduced consolidation of the traumatic memory, as manifest by lower physiologic responding during script-driven mental imagery of the event three months later [48], and by lower PTSD symptoms two months later [107] when the medication was no longer exerting its effects. However, preventing PTSD by blocking *consolidation* of the traumatic memory has conspicuous limitations. First, there is only a brief window of opportunity; a few hours after the traumatic event, after which the memory trace has already consolidated [108]. Only a small fraction of trauma-exposed individuals present for help within this window. Second, for this prophylactic approach to work, all trauma-exposed individuals would need to be treated. Targeting *reconsolidation* may circumvent these limitations. With reconsolidation, each time the memory is reactivated it may be possible to weaken it by blocking its reconsolidation even after it has been consolidated. Considering the pivotal role of negative emotional experiences in the development and persistence of mental disorders, interfering with the consolidation/reconsolidation of such experiences would open the door to a novel treatment approach in psychiatry, which could also benefit, for example, alcohol and drug abuse, phobias,

complicated grief, and obsessive-compulsive disorder. Unfortunately, the amount of translational research remains extremely limited.

The advantage of the reconsolidation approach is that it is more appealing than SSRIs, as a treatment for PTSD. Contrary to SSRIs, which need to be taken for months to years, propranolol dosing may be required six times according to our treatment protocol. This provides a strong rationale for developing alternative pharmaco-therapies for individuals suffering from PTSD.

Propranolol is safe, has few temporary and reversible side effects in contrast to SSRIs whose side effects (e.g., weight gain, abnormal ejaculation, suicidality) decrease compliance. Furthermore, research suggests that in the worst case scenario minor peripheral details of an episodic memory may be lost [109], but the most salient effect is that the emotional and not the declarative, component memory is toned down [110]. The reason the properties of propranolol as a reconsolidation blocker were not discovered earlier, in spite of its widespread use, lies perhaps in the recent research showing that in order for a memory to undergo reconsolidation, it needs to be actively retrieved for a sufficient amount of time [111-113]. This is especially true for older memories. Failure to do so will result in the memory not undergoing reconsolidation.

Previous research indicated that after six weeks of propranolol administration and traumatic memory reactivation, PTSD symptom severity was alleviated by 45% on average and 70% of the participants no longer met the diagnostic criteria for chronic PTSD [7]. These considerations suggest that the treatment benefits conferred by reconsolidation blockade should be more generalized and *longer lasting* than those conferred by CBT. While considered an effective treatment outcome, the neuro-anatomical targets mediating propranolol's therapeutic action, merit further investigation in individuals with chronic PTSD symptomatology. Acknowledging

that the development of such experimental treatments is lengthy and costly, neuroimaging markers of drug response may inform researchers of how interventions alter their intended brain targets and key variables, which may be predictive of treatment outcome. By quantifying brain responses, neuroimaging biomarkers can facilitate the delivery of novel treatments to patients with PTSD, that are more timely than those based solely on clinical data.

10.0 SPECIFIC AIMS AND HYPOTHESES

We proposed to evaluate the activity of the brain regions mediating PTSD symptomatology and recovery, before and after reconsolidation blockade treatment, using a randomized, double-blind, placebo-controlled trial.

Objectives

a. The primary objective was to explore longitudinally, the neural correlates associated with PTSD symptom improvement obtained via reconsolidation blockade using propranolol, relative to placebo.

b. A secondary objective was to demonstrate the efficacy of reconsolidation blockade with propranolol for treating PTSD among the study completers.

Hypotheses

In order to achieve this, we tested the following hypotheses:

a. An involvement of hippocampus, amygdala, medial prefrontal cortex, and the sub-genual ACC will be observed in the mediation of reconsolidation blockade. Specifically, there would be increased blood flow to the mPFC and decreased blood flow

to the amygdala following six weekly trauma reactivation sessions, under the influence of propranolol. Although brain activation in the fear circuit may decline, brain regions involved in memory (i.e., hippocampus), but not in fear, may be preferentially activated in response to trauma cues, post-treatment. This would provide evidence of neurobiological changes associated with reconsolidation blockade.

b. Combining trauma memory reactivation and *propranolol* administration will induce a significant reduction in PTSD symptoms from pre-treatment to post-treatment, relative to a placebo treated group

Such results would not only validate the therapeutic potential of reconsolidation blockade for PTSD, but they would also suggest applicability to many other psychiatric conditions presumably involving an aversive emotional memory.

11.0 METHODOLOGY

11.1 Trial Design

This was a 10-week, randomized, double-blind, placebo-controlled trial consisting of twenty participants diagnosed with chronic PTSD. Participants were randomized to one of two treatment arms (placebo group + treatment as usual, or propranolol group + treatment as usual) and remained in that arm for the duration of the study. An f-MRI scan was conducted one-week prior to starting the study treatment, and repeated one-week post-treatment. Participants were administered two f-MRI tasks which have been previously utilized to probe the neural markers of chronic PTSD symptoms. One neuroimaging task employed the script-driven imagery

paradigm, and entailed participants listening to a 30 second excerpt of their traumatic event relative to a neutral story, before and after six weekly doses of propranolol or placebo (Chapter II). The second task, presented emotional, non-traumatic facial stimuli to participants before and after the six week treatment interval (Chapter III).

In between the scan sessions, participants in each drug group were required to undergo trauma reactivation in which they read an account of their traumatic event, under the influence of their assigned medication. The same treatment procedures were repeated once a week for a total of six weeks. Results from these studies may provide a mechanistic explanation for the potential treatment effects of propranolol on trauma specific information, relative to non-trauma information in PTSD patients.

We expected to retain twenty study completers with valid data out of a possible thirty consented participants. Patients from the PTSD Clinic at the Douglas Institute served as study participants and were treatment seeking individuals, recruited from local newspaper and radio advertisements, as well as from medical center referrals.

11.2 Randomization and Blinding

Study medication was allocated according to a randomization list issued by personnel who are not otherwise conducting the study, using a random number generator. Participants were randomized to the propranolol or the placebo condition (50% probability) according to a pre-established randomization schedule using a double-blind procedure. The randomization schedule was balanced by blocks of six treatment assignment numbers (TANs), with one TAN assigned to

each condition in order to ensure an equivalent ratio of subjects across the treatment conditions[114].

Twenty participants were randomized to the trial. At the end of the study, it was determined that within the *propranolol* group, two participants discontinued after the pre-treatment (baseline) scan, and the corresponding treatment data was not acquired. There was significant attrition in the *placebo* group, and an analysis of the data could not be undertaken. Thus, complete pre- and post-treatment datasets were available for seven propranolol participants. The socio-demographic information, clinical scores and physiological measures for each of the study *non-completers* is presented in the Appendix section.

As a result of attrition in the placebo group, and the loss of randomization, the study was altered to a within-subjects design, consisting of the propranolol group completers, only. Our hypotheses were reformulated and tested as follows:

a) Reconsolidation blockade treatment using propranolol would decrease fear conditioning circuit activity, predominantly in the amygdala and hippocampus, while stimulating prefrontal/anterior cingulate cortex during *trauma reactivation* in patients diagnosed with long-standing PTSD.

b) If reconsolidation blockade effectively mitigates PTSD symptoms, this would also be reflected in decreased activation of the amygdala, with concomitant increases in activation of the hippocampus and ACC during a *'non-traumatic', affective task.*

The following Chapters (II and III) present the experimental results obtained from the propranolol group completers (N=7), only.

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CHAPTER II

Cortico-Limbic System Responses to the Reconsolidation Impairment of Traumatic Memories

Chapter II – Preface

Prior research by our group, produced data supporting the conclusions that trauma memory reactivation under the influence of the β-blocker propranolol subsequently leads to (a) a large decrease in physiologic responding during trauma mental imagery [1] and (b) a substantial and sustained decrease in posttraumatic stress symptoms [2]. It is hypothesized that β-adrenergic receptor antagonism via propranolol inhibits the reconsolidation of the traumatic memory when it is reactivated, reducing its' strength and the emotional arousal to the event. As such, the aim of the following study was to test the hypothesis that the successful treatment of PTSD will correlate with reduced limbic system activation, in the amygdala and hippocampus, which modulate emotional responses, during traumatic memory reactivation. Specifically, f-MRI was conducted to reveal the functional neuro-anatomical substrates of traumatic memory retrieval, after the manipulation of reconsolidation blockade with propranolol.

Manuscript 1

The functional neuroanatomy of traumatic memory reactivation following reconsolidation blockade treatment

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ABSTRACT

Background: Post-traumatic stress disorder (PTSD) involves conditioned emotional responding and memory dysfunction, whereby extinction is impaired and traumatic memories are over-consolidated. Patients afflicted with PTSD experience significant, recurrent traumatic recollections, and it remains challenging to disentangle the neural mechanisms underlying symptom remission. We aimed to identify the neural correlates of traumatic memory before and after reconsolidation blockade, a proposed treatment for PTSD. **Methods:** A f-MRI symptom provocation task was conducted within subjects, before and following six weekly doses of propranolol, in conjunction with traumatic memory reactivation, in PTSD patients *(N=7)*. Statistical parametric mapping (SPM) was employed to compare neural activation before and after the medication for the group. **Results:** Before treatment, trauma relative to neutral script-driven mental imagery elicited significant activation in the amygdala and hippocampus. However, post-treatment, PTSD symptom severity was significantly ameliorated, and trauma imagery activated the right middle prefrontal cortex. **Limitations:** As a pilot study, the small sample size, limits the generalizability of these results. **Conclusion**: Reconsolidation blockade treatment was associated with functional changes in the amygdala and hippocampus, and future, larger studies will need to assess the significance of these results in psychiatric conditions involving aversive memories.

Keywords:

Posttraumatic Stress Disorder (PTSD), reconsolidation, neuroimaging, emotional memory, propranolol.

1.0 INTRODUCTION

Perturbations in memory function, particularly involuntary and intrusive recollections of a traumatic event are cardinal characteristics of post-traumatic stress disorder (PTSD). It is postulated that peri-traumatic distress [3] and arousal potentiate endogenous stress hormone release, consolidating an excessively powerful and aversive emotional memory. Prior PTSD symptom provocation studies have revealed limbic system, thalamic and prefrontal cortex disturbances during the mental re-experiencing of traumatic events [4, 5]. Altered noradrenergic, glucocorticoid [6] and glutamatergic (N-Methyl-D-aspartate) [7] neurotransmission have also been implicated in memory consolidation, retrieval and extinction in PTSD patients. Despite this, current pharmacotherapies for PTSD, are modestly more effective than placebo [8], and require prescription for extended periods, with significant side-effects.

Reconsolidation, a protein synthesis dependent process, stabilizes memories after retrieval and yet, presents an interval in which memory representations can be susceptible to alteration [9]. Pre-clinical studies demonstrated that consolidated aversive memories are sensitive to beta (β)-adrenergic receptor blockade after their reactivation. Specifically, propranolol administered during the memory reconsolidation phase reduced inhibitory avoidance [10], contextual fear conditioning [11, 12], and auditory fear conditioning in rodents [13]. A meta-analytic review of healthy human studies reported that memory retrieval and reconsolidation under propranolol reduced subsequent recall of negatively valenced emotional words and decreased the expression of cue-elicited fear responses [14]. Traumatic memory reactivation under propranolol's influence lead to a sustained decrease in PTSD symptom severity [15], and psychophysiological responding during script-driven imagery [16][Brunet et al., In Press], indicating a possible therapeutic benefit of β-adrenergic receptor blockade.

Cross-sectional, neuroimaging studies have employed script-driven imagery to characterize the neural mediators of trauma mental re-experiencing, in unmedicated PTSD patients [4, 17]. Converging evidence indicates that amygdala hyperactivity coupled with hypo-activation in the rostral anterior cingulate (ACC), medial prefrontal cortex (mPFC) and inferior frontal gyrus, could sub-serve the intrusive, emotional thoughts and traumatic memories. The neural correlates of traumatic memory reconsolidation blockade in PTSD patients are not currently known. To date, f-MRI studies have revealed hippocampal and amygdala mediation of emotional material retrieval in *healthy participants*, after β-adrenergic receptor antagonism, relative to placebo [18, 19]. However, these studies did not include individuals with ingrained traumatic memories. In this pilot study, we examined the functional neuroanatomy of traumatic and neutral script-driven imagery before and after 6 sessions of trauma reactivation, under the influence of propranolol. We hypothesized that reconsolidation blockade treatment would a) decrease fear conditioning circuit activity, notably in the amygdala and hippocampus, b) upregulate prefrontal/anterior cingulate cortex during trauma reactivation and c) mitigate symptom severity in patients diagnosed with long-standing PTSD.

2.0 METHODS

2.1 Participants

Nine traumatized individuals (age range: 18-65 years) were recruited through local advertisements, to a 10-week study. Consented participants were medically screened to ensure that they could receive propranolol and undergo neuroimaging procedures. Urinalyses for toxicology and pregnancy screening were negative for each participant. All participants were free of a history of head injury, and other significant neurological and medical disorders, which

would preclude propranolol administration or scanning. Participants taking selective serotonin or norepinephrine reuptake inhibitors *(n = 2)*, agreed to delay their morning dose on treatment days to minimize propranolol drug interactions [20]. Individuals *(n = 3)* receiving stable doses of anxiolytics and antidepressants, for more than 1 month prior to screening, remained on these medications. Two participants discontinued the study (moved away, *n = 1* and opted to pursue other treatment, *n = 1*). The final cohort consisted of 5 females and 2 males (*n =7, M* age = 33.1 years, *SD* = 7.0). At visit 1, patients completed the medical and clinical assessments. The neuroimaging sessions were conducted on visits 2 and 9; while visits 3-8 comprised the treatment sessions. At visit 10, the participants' symptoms were re-evaluated by a trained clinician. Procedures received approval from Health Canada; the Institutional Review Boards at the Centre Hospitalier de l'Université de Montréal, and the McGill University, Faculty of Medicine.

2.2 Clinical Assessment

The semi-structured Clinician-administered PTSD scale (CAPS) version 2 [21], evaluated DSM-IV-TR [22] PTSD symptom severity before and after treatment (range: 0-136). Trauma etiology included sexual assault *(n = 3)*, a motor vehicle accident, a physical assault, a traumatic bereavement, and verbal threats to one's life and family. At treatment onset, participants met the criteria for chronic PTSD with an estimated symptom duration of 7.6 years (*SD* = 6.5) and all had a CAPS score above 50. This cohort presented with the following DSM-IV-TR co-morbid Axis I psychiatric conditions: major depressive disorder *(n = 4)*, agoraphobia *(n = 1),* panic disorder, obsessive compulsive disorder and generalized anxiety disorder *(n = 2)*, as assessed by the structured Mini International Neuropsychiatric Interview [23]. The Impact of Events Scale-

Revised (IES-R) [24], was completed by participants at enrollment, at each treatment session, and at the final re-evaluation.

The treatment procedure has been described elsewhere [15]. Briefly, 75 minutes after ingesting 1 mg/Kg of propranolol under medical supervision, participants read aloud once to the investigator the 1-page traumatic event description they had produced to create the script-driven imagery vignettes. This procedure, henceforth referred to as the treatment, required 5-10 minutes depending on the participant, and was repeated once a week for 6 consecutive weeks. A paired *t*-test (pre – post-treatment) revealed a significant 49% CAPS score improvement ($M = 80.4$, $SD = 17.6$ vs. $M = 41.0$, $SD = 27.2$; $p < .003$), and a 61% IES-R score reduction ($M = 62.8$, $SD = 12.9$ vs. $M = 24.4$, $SD = 23.4$; $p < .001$) suggesting that the treatment was beneficial to participants.

2.3 Script-driven Imagery Task

Script-driven traumatic imagery is a well-validated, standardized symptom provocation paradigm that has been described previously [5, 25]. Participants provided a written, detailed description of a personal traumatic event. Based on this, a male interviewer composed two, 30-second trauma script recordings, portraying the most poignant aspects of the traumatic experience in the second person, present tense, to be played back to the participant in the scanner through headphones. The same interviewer recorded two 30-second, standardized, neutral scripts for the same purpose. In the scanner, the task began with a 30-second block of silence, with a fixation cross to focus upon (baseline period). Next, participants were instructed to close their eyes while carefully listening to the 30-second script (listening period). Immediately afterwards, participants imagined the described event for 30 seconds (imagery period) until a tone ended the imagery period. Participants relaxed for 90 seconds (recovery period) in the scanner after which

the baseline block was presented followed by the next script. Scripts were presented once in a standard order with a neutral script always played before a traumatic excerpt, to minimize potential emotional transfer between conditions [4, 5].

2.4 Imaging Procedure

F-MRI was performed using a whole-body 3.0 Tesla (Philips Achieva X) MRI system with an 8-channel head coil. Imaging sessions were comprised of: i) a 1-minute functional scan to adjust the headphone volume; ii) one functional Gradient Echo Planar Imaging session with the following parameters: 255 volumes, TR/TE = 3000/30ms, voxel size = 3 x 3 x 3mm^3, slice gap = 0 mm, FOV = 240 x 240 mm, 46 axial slices, flip angle = 90°, EPI factor = 41, duration = 12:57; iv) a Gradient Echo 3D with inversion recovery T1-weighted image: TR/TE/TI = 8.1 / 3.7 / 1011.4 ms, FOV = 240 x 240 mm, voxel size = 0.86 x 0.86 x 1 mm^3, flip angle = 8, sense factor = 2, 160 slices in sagittal orientation, duration = 5:35. Instructions and auditory scripts were presented using E-Prime Professional 2.0 (Psychology Software Tools, PA, USA), which ensured proper timing of the stimulation blocks.

2.5 Imaging Analyses

Imaging datasets were analyzed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK). Functional images were motion corrected, realigned on the T1 weighted images, segmented and spatially normalized into an MRI stereotactic space (Montreal Neurological Institute [MNI] co-ordinates), and spatially smoothed with a 8 mm Gaussian kernel. Voxel effects were estimated using the general linear model with the first and second hemodynamic response function derivatives; and motion parameters, as regressors. For each individual, a first-level of analysis contrasted the neural activation during the trauma script listening versus neutral

script listening blocks; and the trauma imagery versus neutral imagery conditions. The resulting t-contrast maps for each participant were submitted to a second-level paired t-test random effects analysis (pre versus post-treatment). For this pilot study, the paired-test maps were generated at an uncorrected threshold of $p < .05$ with a spatial extent of 5 voxels, as exploratory analyses. Subsequently, neural activation in a priori regions of interest were examined with an *a*-level of $p < .05$ (small volume corrected) in the amygdala (BA 34), hippocampus (BA 32), superior PFC (BA 10), middle PFC (BA 9), and thalamus, according to the automated anatomic labelling (AAL) atlas [26]. However, activations did not survive the conservative False Discovery Rate (FDR) multiple comparison correction at $p < .05$.

3.0 RESULTS

The paired t-test analysis, (*pre* > *post-treatment*) for the trauma vs. neutral script *listening* contrast, yielded greater activation in the right hippocampus, cingulate bilaterally, right insula, and right thalamus, ($p < .01$ uncorrected). Trauma vs. neutral *imagery* comparisons revealed significantly left lateralized neural activity in the amygdala, and thalamus, and the hippocampus bilaterally (see Table 1).

In the *Post* > *Pre-treatment* contrast, patients exhibited a significant blood oxygenation level dependent (BOLD) signal increase in the left middle and superior frontal gyrus during the trauma relative to the neutral script listening, ($p < .01$ uncorrected) (Figure 2). Similarly, the trauma vs. neutral imagery comparisons revealed greater neural activity in the medial frontal gyri bilaterally; and the right superior frontal gyrus.

Table 1: Brain regions with increased activation pre- and post-treatment during script-driven imagery task (Paired *t*-test analysis, $k > 5$ voxels; R , right; L, left).

Contrast	MNI Co-ordinates (x,y,z)	Region	*p*-value uncorr.	*p*-value (SVC) FDR
Pre > Post-treatment				
Trauma Listening > Neutral Listening	-12 -4 43	L Mid-Cingulum	.01	ns
	12 -8 37	R Mid-Cingulum	.01	ns
	-38 -4 -18	L Insula	.01	ns
	31 -34 -8	R Hippocampus (+ R Parahippocampus)	.01	ns
Trauma Imagery > Neutral Imagery	-18 -2 -17	L Amygdala (BA 34)	.01	ns
	-31 -19 -11	L Hippocampus	.002	ns
	24 -31-8	R Hippocampus	.002	ns
	0 -19 7	L Thalamus	.01	ns
	-9 -58 25	L Precuneus	.001	ns
Post > Pre-treatment				
Trauma Listening > Neutral Listening	-27 50 28	L Mid Frontal	.01	ns
	-33 41 37	L Mid Frontal	.01	ns
	-15 32 58	L Superior Frontal	.01	ns
	30 -1 10	R Putamen	.01	ns
Trauma Imagery > Neutral Imagery	45 32 31	R Mid Frontal	.01	ns
	27 -4 64	R Superior Frontal	.01	ns

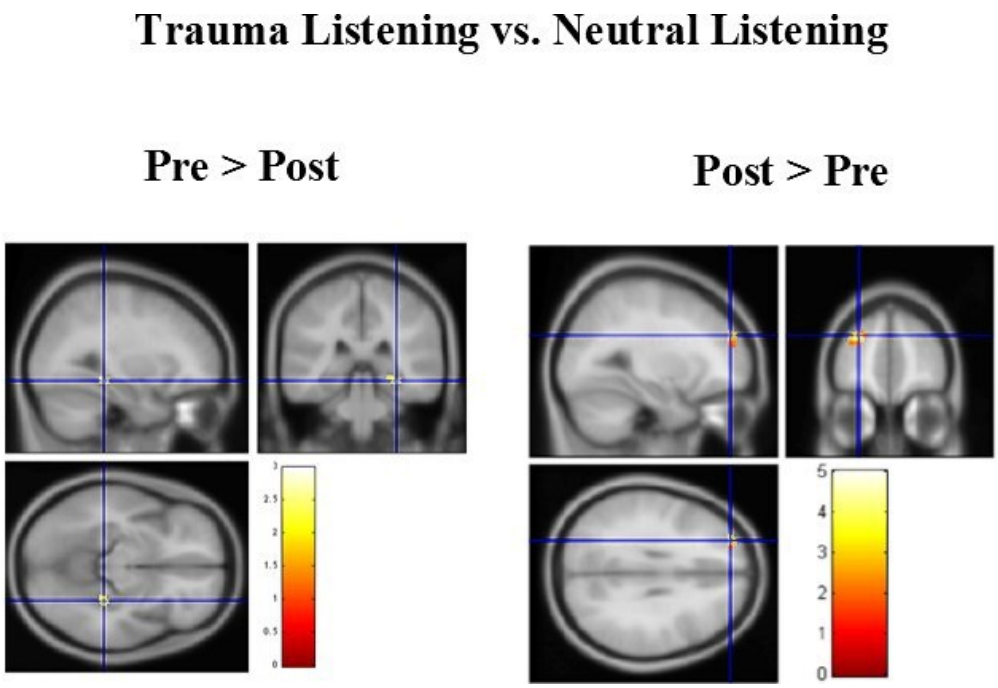

Figure 1: *Example of the brain activation in PTSD patients (N = 7) before and after reconsolidation blockade treatment during the listening phase of the script driven imagery task.*

Left panel: Pre > Post-treatment

Significantly increased BOLD signal in the right hippocampal gyrus (x = 31, y = -34, z = -8), during the trauma relative to the neutral listening condition ($p < .01$ uncorrected), before treatment.

Right Panel: Post > Pre-treatment

After treatment, patients exhibited greater activation in the left middle frontal gyrus (x = -27, y = 50, z = 28) while listening to the trauma versus neutral scripts ($p < .01$ uncorrected). Activations are superimposed on the T1 MNI template.

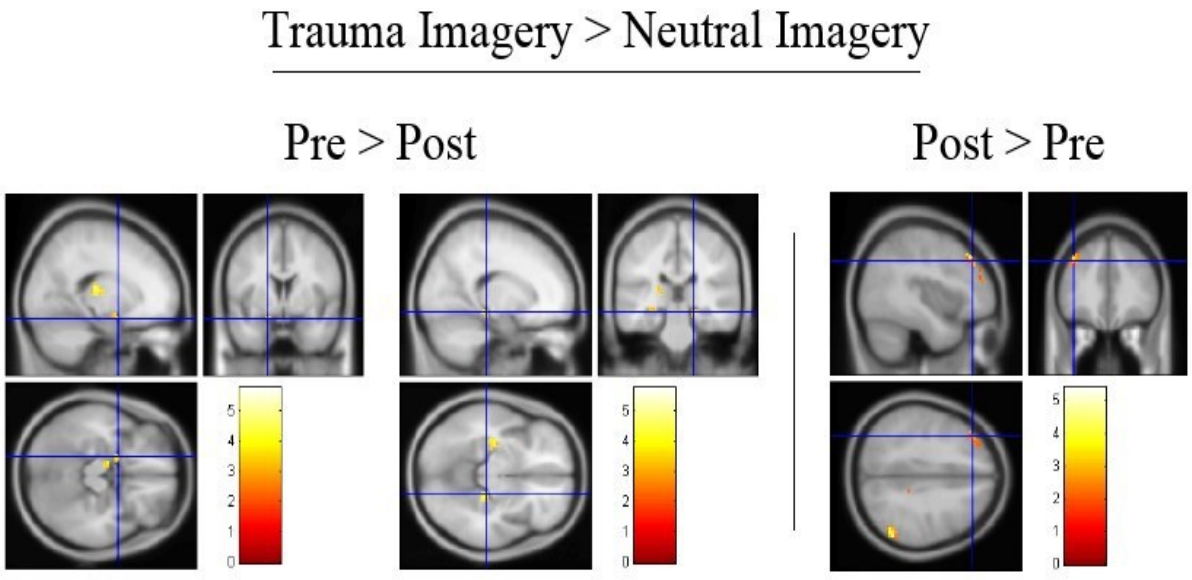

Figure 2: *Paired t-test analysis comparing brain activation in PTSD patients (N = 7) before and after reconsolidation blockade during the imagery phase of the script driven imagery task.*

Pre > Post-treatment

Left Panel: Significantly increased BOLD signal in the left amygdala (x = -18, y = 2, z = -17) ($p < .01$ uncorrected), and right hippocampus (x = 33, y = -28, z = -11; $p = .002$) (middle panel) of PTSD participants, during the traumatic relative to the neutral imagery condition, before treatment.

Post > Pre-treatment

Right panel: a greater activation in the right middle frontal gyrus (x = 45, y = 32, z = 31) was observed during trauma imagery vs. neutral imagery in PTSD participants after treatment ($p < .01$ uncorrected).

4.0 DISCUSSION

As expected, our pre-treatment results demonstrated that imagining traumatic events elicited activation in the amygdala and hippocampus in chronic PTSD patients, consistent with the memory modulation hypothesis [19]. Both increased and decreased amygdala responses have been reported in PTSD participants during script-driven traumatic imagery [27] while hippocampal activity has positively correlated with symptom severity [5]. The regions activated during trauma listening before treatment, are consistent with the resting state PTSD circuit [28], and may represent β-adrenergic mediated neuropathology. The current PTSD cohort increased thalamic, hippocampal and insular activity during trauma listening and imagery pre-treatment, whereas, these effects were abrogated following β-adrenergic blockade. Heightened emotional arousal during trauma reactivation is associated with disrupted sensory transmission via the thalamus to the prefrontal cortex, hippocampus and amygdala. Thalamic nuclei receive projections from the nucleus of the solitary tract, which stimulates the noradrenergic system. Despite this, prior script-driven imagery studies have reported both enhanced thalamic activity in PTSD cases with dissociation [29, 30]; and hypoactive signals in medication-free PTSD patients during dissociation relative to healthy controls [4]. The relationship of these cortico-thalamic circuit activity differences during PTSD *treatment*, merit further investigation.

Decreased prefrontal cortex (PFC) inhibition and concomitant amygdala hyperactivity have been implicated in governing the intrusive, emotional memories in PTSD. Our results demonstrate significant medial and superior PFC engagement, in the trauma listening and imagery conditions relative to their neutral counterparts, post-treatment. Increased mPFC activation has been observed in PTSD individuals who responded to cognitive behavioural therapy [31]. In this study, upregulated PFC activity may reflect reinstated inhibitory control accounting for the absent amygdala and hippocampal activity during the trauma imagery, post-

treatment. A prior study demonstrated that trauma-exposed individuals, without PTSD, exhibit attenuated amygdala reactivity to traumatic cues [17], which in turn suggests that amygdala inhibition after treatment, could be a potential neural marker of PTSD recovery [32]. Pharmacotherapies have been aimed at abating amygdala responsivity to mitigate anxiety in human participants [33] and previous pharmacological inhibition of the hippocampus has diminished the renewal of fear in rodents who underwent fear-conditioning [34]. Post-treatment, PTSD participants' symptom severity significantly decreased, which may reflect reduced emotional memory strength, corresponding to the tapered limbic activity.

4.1 Limitations

This pilot study is limited by the small sample size, although the within-subject design yields increased statistical power, relative to between group comparisons with the same number of observations. Future studies should incorporate control groups to distinguish between the effects of trauma reactivation with propranolol, trauma reactivation without propranolol and propranolol without reactivation.

4.2 Conclusion

Brief recall of one's trauma under the influence of propranolol on six occasions, was associated with lessened limbic activity during script-driven imagery of the event. This intervention procedure is consistent with reconsolidation theory, although other causal explanations for symptom improvement, such as accelerated extinction, remain plausible [35]. However, extinction-based therapies typically require 15.6 hours of therapy [36], rather than 60 minutes (total trauma reactivation time), to achieve similar results. These preliminary results signify that β-adrenergic receptor antagonism during guided trauma recall could facilitate PTSD

remission possibly by increasing medial PFC function and/ or diminishing amygdala function, through disrupted reconsolidation of fear conditioning.

Conflict of Interest

The authors declare that they have no conflict of interest.

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CHAPTER III

The Role of the Noradrenergic System in Post-trauma Emotional Processing

Chapter III: Preface

Emotional self-regulation is a complex process that involves endogenous neurobiological factors, and is moderated by the external environment. The neurotransmitter, norepinephrine has been implicated in stress-mediated emotional responses, through its action on the amygdala which contains a high density of beta-adrenergic receptors. Other neural regions affected by the endogenous stress response include the hippocampus, hypothalamus, medial prefrontal cortex, and cingulate cortex. The amygdala has a pivotal function in regulating fear-conditioning, and a memory's strength associated with emotional arousal; while its' output is modulated by projections from the medial prefrontal regions.

In the first experiment (see chapter II), the script-driven imagery f-MRI task reactivated traumatic memories and we investigated amygdala activity in participants with PTSD before and after treatment with the reconsolidation blocker, propranolol. Despite its' strong ecological validity, it has been noted that re-experiencing paradigms do not ascertain whether the neural responses are markers of the specific disorder. Participants with chronic PTSD experience emotional dysregulation, in the absence of trauma specific stimuli, which may also reflect limbic pathophysiology and alterations in the noradrenergic system. Consequently, presentation of emotive facial stimuli is considered a standardized method to compare and assess amygdala aberrations. While the presentation of *masked* facial stimuli is aimed at amygdala activation in PTSD participants, the *overt* presentation of faces could reveal other neural regions which mediate emotional expression.

This study employed f-MRI to explore the neural underpinnings of reconsolidation blockade treatment on the perception of non-traumatic, emotional stimuli in participants with chronic PTSD.

Manuscript 2

Emotional Face Processing in Post-Traumatic Stress Disorder after Reconsolidation Blockade using Propranolol: A Pilot f-MRI Study

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ABSTRACT

Individuals with post-traumatic stress disorder (PTSD) exhibit exaggerated emotional reactions to threatening stimuli, which may represent dysregulated fear-conditioning, associated with long-term adaptations in the sympathetic nervous system. Within a repeated measures design, functional magnetic resonance imaging (f-MRI) was employed to investigate the neural correlates of threat reactivity in seven PTSD participants, during the overt presentation of emotional facial expressions. Scan sessions were separated by a six-week intervention period, in which participants performed traumatic memory reactivation, under the influence of the reconsolidation blocker, propranolol. Prior to treatment, chronic PTSD participants exhibited significantly greater activations in the thalamus, amygdala, hippocampus, and insula in response to fearful, relative to neutral faces. Post-treatment, PTSD symptoms significantly improved (d = 1.75); and neural activation increased in the medial prefrontal and rostral anterior cingulate cortices during the fearful stimuli conditions. These results suggest that aberrant emotional responding is modulated by noradrenergic plasticity within the amygdala-prefrontal cortex circuit; a neural substrate for the pharmacological treatment of PTSD.

Keywords:
Posttraumatic Stress Disorder (PTSD), reconsolidation, neuroimaging, memory, propranolol.

1.0 INTRODUCTION

Recurrent traumatic memories, avoidance, and hyper-vigilance to trauma related stimuli, are cardinal characteristics of post-traumatic stress disorder (PTSD). Additionally, heightened threat perception post-trauma may continue to impact catecholamine release in the locus coeruleus, and central nucleus of the amygdala which comprise the fear conditioning neuro-circuitry [1]. In particular, trauma-induced neural sensitization to norepinephrine has been associated with the hyper-vigilant symptoms which perpetuate the disorder by eliciting attentional and emotional biases to environmental threat [2], rendering PTSD a treatment challenge.

After retrieval, long-term emotional memories undergo reconsolidation, a protein-synthesis dependent process, in which they can be re-stabilized or occluded [3]. Several animal and healthy human studies have proposed that blocking the reconsolidation of a traumatic memory might present a promising translational treatment for PTSD [4]. In a small randomized controlled trial, we showed that post-retrieval propranolol compared to placebo, reduced psychophysiological responding during script-driven imagery of the traumatic event one week later [5]; and that six sessions of open-label, pre-retrieval propranolol induced a clinically significant and durable decrease in PTSD symptoms [6] and physiologic responding (Brunet et al., In Press). Subsequently, using functional magnetic resonance imaging (f-MRI), we demonstrated that participants treated with this protocol successfully inhibited amygdala activity during traumatic script-driven imagery and reported decreased PTSD symptoms (Mahabir et al, submitted). However, script-

driven imagery paradigms are highly idiosyncratic and do not assess fear generalization to non-traumatic stimuli, in PTSD patients.

Several cross-sectional studies have employed fearful face stimuli as non-specific-threat signals to probe the extent of limbic system dysfunction in PTSD [7]. Amygdala hyper-responsivity was exhibited in PTSD participants during masked fearful face presentations, relative to happy [7, 8] or neutral facial stimuli [9]. Similarly, viewing overtly presented fearful faces was associated with greater right amygdala activity, and concomitant decrements in dorsal medial and ventromedial prefrontal cortex activations compared to happy face counterpart images [10]. Left amygdala activation and PTSD symptom severity have been associated only with the successful encoding of fearful, rather than neutral faces [11]. These studies support the notion that PTSD is characterized by a generalized dysfunction within the neural circuitry mediating threat detection [12]; and it is likely that a sensitized amygdala has a lower activation threshold, in response to both traumatic and non-traumatic emotional stimuli in this population.

The pathophysiology of PTSD implicates hyper-arousal of the sympathetic nervous system, particularly elevated noradrenergic activity [13] which primarily inhibits the prefrontal cortex, curtailing emotional control and extinction capacities, while stimulating the amygdala to generate phobic behaviors [14]. In healthy participants, a single dose of reboxetine, a norepinephrine reuptake inhibitor, pharmacologically induced right amygdala, right hippocampal and bilateral inferior frontal gyri activation, in response to fearful but not neutral stimuli, when administered 2-hours before scanning [15]. In a

separate f-MRI study, an oral dose of the beta-adrenergic receptor antagonist propranolol, attenuated amygdala activity to neutral, positive, and aversive facial stimuli in healthy controls [16]. Although, emotional dysregulation and symptom severity interact to maintain PTSD, the neural basis of this relationship and the effect of reconsolidation blockade treatment on non-specific threat processing have not been explored in a traumatized cohort.

Functional imaging studies have applied fearful face paradigms to examine the neural correlates of recovery; and predict treatment responses in PTSD patients. In a longitudinal study, a subsequent memory f-MRI task for emotional faces [17], demonstrated that hippocampal activity was associated with recovery potential, whereas sub-genual anterior cingulate cortex (sgACC) activation negatively correlated with PTSD symptom improvement after 9 months. However, as a naturalistic recovery paradigm, the former study had not controlled the PTSD intervention type, between scan assessments. In contrast, increased bilateral amygdala and ventral anterior cingulate activation in response to masked fearful faces predicted poor treatment response, specifically to cognitive behavioral therapy for PTSD [18].

In the current pilot study, participants were exposed to affective facial expressions to explore the neural correlates of PTSD remission after reconsolidation blockade treatment using, propranolol. Congruent with the observation that PTSD involves aberrant, emotional conditioning and expression, we hypothesized that if reconsolidation blockade effectively alleviates trauma symptoms, this would be reflected in decreased activation of

the amygdala, with concomitant increases in hippocampal and ACC activation during a 'non-traumatic', affective task.

2.0 METHOD

2.1 Participants

Nine traumatized individuals (age range: 18-65 years) were recruited through newspaper advertisements in the Montreal metropolitan area. Of these, one individual relocated to another city during the study, and a second participant withdrew to pursue other treatment. The final sample included 5 females and 2 males (n = 7, M = 33.1 years of age, SD = 7.0). Consented participants were medically screened to ensure their eligibility to receive propranolol and undergo f-MRI procedures. Individuals did not present with a clinical history of head injury, neurological, or major medical conditions contraindicated for propranolol administration. Urinalyses confirmed that none of the participants were pregnant or had a substance dependence disorder. Three individuals were on stable doses of Selective Serotonin Reuptake Inhibitors (SSRIs) or Serotonin–Norepinephrine Reuptake Inhibitors (SNRIs) for at least one month before study enrolment. They accepted to post-pone their morning dose on the study days to minimize interactions with propranolol [19]. Two participants were taking stable doses of anxiolytics (> 1 month), and remained on these medications during the study. The trial duration was 10 weeks as follows: At visit 1, medical and clinical PTSD assessments were completed; the f-MRI sessions occurred at visits 2 and 9, while visits 3-8 were dedicated to the treatment. At visit 10, participants' PTSD symptoms were reassessed through a semi-structured clinical

interview. Procedures were approved by the Institutional Review Boards at the McGill University, Faculty of Medicine, the *Centre Hospitalier de l'Université de Montréal*, and Health Canada.

2.2 Clinical Assessment

A trained interviewer assessed DSM-IV-TR [20] current PTSD symptom severity using the Clinician Administered PTSD Scale (CAPS, range: 0-136) [21], before and after treatment. All 7 participants were diagnosed with chronic PTSD (M = 7.6 years duration, SD = 7.6), and yielded CAPS scores above 50 at enrollment. PTSD developed following sexual assaults *(n =3)*, a motor vehicle accident, a physical assault, a traumatic bereavement, and threats to harm one's life and family. Participants provided written, 1-page traumatic scripts, describing their visceral and emotional reactions to their specific events [22]. The Impact of Event Scale (IES)- Revised [23] evaluated the PTSD symptoms in the 7 days preceding study enrolment, and at each subsequent treatment visit. The Mini International Neuropsychiatric Interview [24] determined the presence of current DSM-IV-TR Axis I co-morbid psychiatric conditions as follows: major depressive disorder *(n = 4)*; panic disorder *(n = 2*); agoraphobia *(n = 1);* obsessive compulsive disorder *(n = 2)* and generalized anxiety disorder *(n = 2)*.

The treatment procedures have been described in detail previously [6]. Once a week, for 6 consecutive weeks, participants received an oral dose of 1 mg/kg of propranolol under medical supervision. Seventy-five minutes after having ingested propranolol, participants read aloud once, their traumatic event description to the investigator. Paired *t*-tests revealed significant symptom improvements as indicated by a 49% CAPS score

change pre- (M = 80.4, SD = 17.6) vs. post-treatment (M = 41.0, SD = 27.2) ($t[6]$ = 4.9, p < .003), and a 61% IES-R score decrease pre- (M = 62.8, SD = 12.9) vs. post-treatment (M = 24.4, SD = 23.4) ($t[6]$ = 3.6, p < .011).

2.3 Imaging Procedure

F-MRI was performed using a whole-body 3.0 Tesla (Philips Achieva X) MRI system with an 8-channel head coil, comprising: i) one functional Gradient Echo Planar Imaging (EPI) session with the following parameters: 235 volumes, repetition time (TR) 3000 milliseconds (ms), echo time (TE) 30 ms, voxel size = 3 x 3 x 3 mm^3, slice gap = 0 mm, Field of View (FOV) = 240 x 240 mm, 46 slices in axial orientation, flip angle = 90°, EPI factor = 41, duration = 11 minutes, 57 seconds. Following the f-MRI scans, a high resolution anatomical scan was performed for each participant using a Gradient Echo 3D inversion recovery T1-weighted image with the following parameters: TR = 8.1 ms, TE = 3.7 ms, inversion time (TI) = 1011.4 ms, FOV = 240 x 240 mm, voxel size = 0.86 x 0.86 x1 mm, flip angle = 8°, sense factor = 2, 160 slices in sagittal orientation, duration = 5 minutes, 35 seconds.

2.4 Overt Faces f-MRI Task

A description of the Overt Faces f-MRI task is available elsewhere [10]. Briefly, stimuli were comprised of 6 fearful, 6 happy and 6 neutral facial expressions. Each facial expression was posed by 3 men and 3 women, in black and white photograph format, for a total of 18 pictures. The task was programmed as a block-design paradigm using E-Prime Version 2.0 Professional (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Faces were presented to the participants in the scanner via a video projector with a mirror

located above the head-coil for 200 ms each, with a 300 ms inter-stimulus interval with a fixation cross, in a pseudorandom order to avoid successive repetition of a single identity. Within a block, each face was presented 9 times for a total of 54 faces with the same expression. Facial expression blocks were 27 seconds each, separated by a 15 second block of fixation. Each fearful, happy, and neutral facial expression block was presented five times across the run, in a randomized order for a total of 15 blocks. A 27 second block of low-level fixation was also presented at the beginning and end of the run.

2.5 Imaging Analysis

Neuroimaging data were analyzed with SPM8 (Wellcome Department of Imaging Neuroscience, London, UK). Standard f-MRI pre-processing was performed including motion correction; realignment of the average functional image on the T1 weighted image, segmentation and normalization of the anatomy on the Montreal Neurological Institute (MNI) referential image, normalization of the realigned functional data, and smoothing with an 8 mm FWHM Gaussian kernel. A General Linear Model was performed on the normalised and smoothed datasets incorporating the motion regressors; and the first and second temporal derivatives of the hemodynamic response function. For each participant, a random effects model was used to compute specific, separate t-contrast maps between the (fearful – neutral) and (fearful – happy) facial expression blocks. Contrast maps were submitted to a second level group analysis in which paired *t*-tests (pre- and post-treatment) assessed the treatment's influence on the neural processing of different facial expressions. Exploratory whole brain analyses were conducted at $p < .05$ (small volume correction) with a minimum cluster extent of 5 voxels. Each region of

interest (ROI) was determined by the Automated Anatomical Labelling (AAL) masks [25].

3.0 RESULTS

The brain regions activated during the fearful face comparisons before (*pre > post-treatment*), and after treatment (*post > pre-treatment*) are shown in Table 1. Before treatment, *fearful faces relative to neutral faces*, elicited activation in the right amygdala, left para-hippocampal gyrus, left insula and thalamus bilaterally ($p < .05$ uncorrected). Contrasting the *fearful with happy faces,* yielded greater activation, sub-cortically in the right pallidum; mid-frontal gyrus bilaterally; and the inferior frontal gyrus ($p < .05$ uncorrected). After treatment, PTSD participants exhibited increased activation in the right parahippocampal gyrus ($p < .05$ corrected), mid-frontal gyri bilaterally and the right supra-marginal gyrus for the *fearful vs. neutral face* comparison ($p < .05$ uncorrected). Contrasting *fearful vs. happy* faces (*post > pre-treatment*) yielded greater activation in the right ACC, right mid-cingulate bilaterally, the left putamen and left precuneus. Neural activity changes in the a priori regions of interest were not significantly correlated with decreases in the CAPS and IES-R symptom severity measures.

Table 1:

Paired *t*-test analyses indicating brain regions exhibiting greater activation before and after treatment, k > 5 voxels (n = 7).

Contrast	MNI Co-ordinates (x, y, z)	Brain Region	*p*-value uncorrected	*p*-value corrected (FDR)
Pre > Post-treatment				
Fear vs. Neutral	-18 -16 7	L Thalamus	.05	na
	6 -16 4	R Thalamus	.05	na
	18 -28 -11	R Para-hippocampal	.05	ns
	21 -4 -14	R Amygdala	.05	ns
	33 -1 13	R Insula	.05	ns
Fear vs. Happy	18 -1 -5	R Pallidum	.05	ns
	51 29 31	R Mid-Frontal Gyrus	.05	ns
	-33 29 10	L Inferior Frontal	.05	ns
Post > Pre-treatment				
Fear vs. Neutral	27 -40 -5	R Parahippocampal	.005	.05
	24 56 28	R Mid Frontal Gyrus	.005	ns
	-42 14 43	L Mid Frontal Gyrus	.005	ns
	66 -28 28	R Supra-marginal	.005	ns
Fear vs. Happy	3 29 16	R Anterior Cingulate	.001	.05
	9 -16 43	R Mid Cingulate	.001	.05
	-6 -16 46	L Mid Cingulate	.001	.05
	-21 5 1	L Putamen	.001	na
	-6 -82 46	L Precuneus (BA 7)	.001	na

FDR = false discovery rate (at $p = .05$), ns = non-significant, na = not applicable/not an a priori region of interest

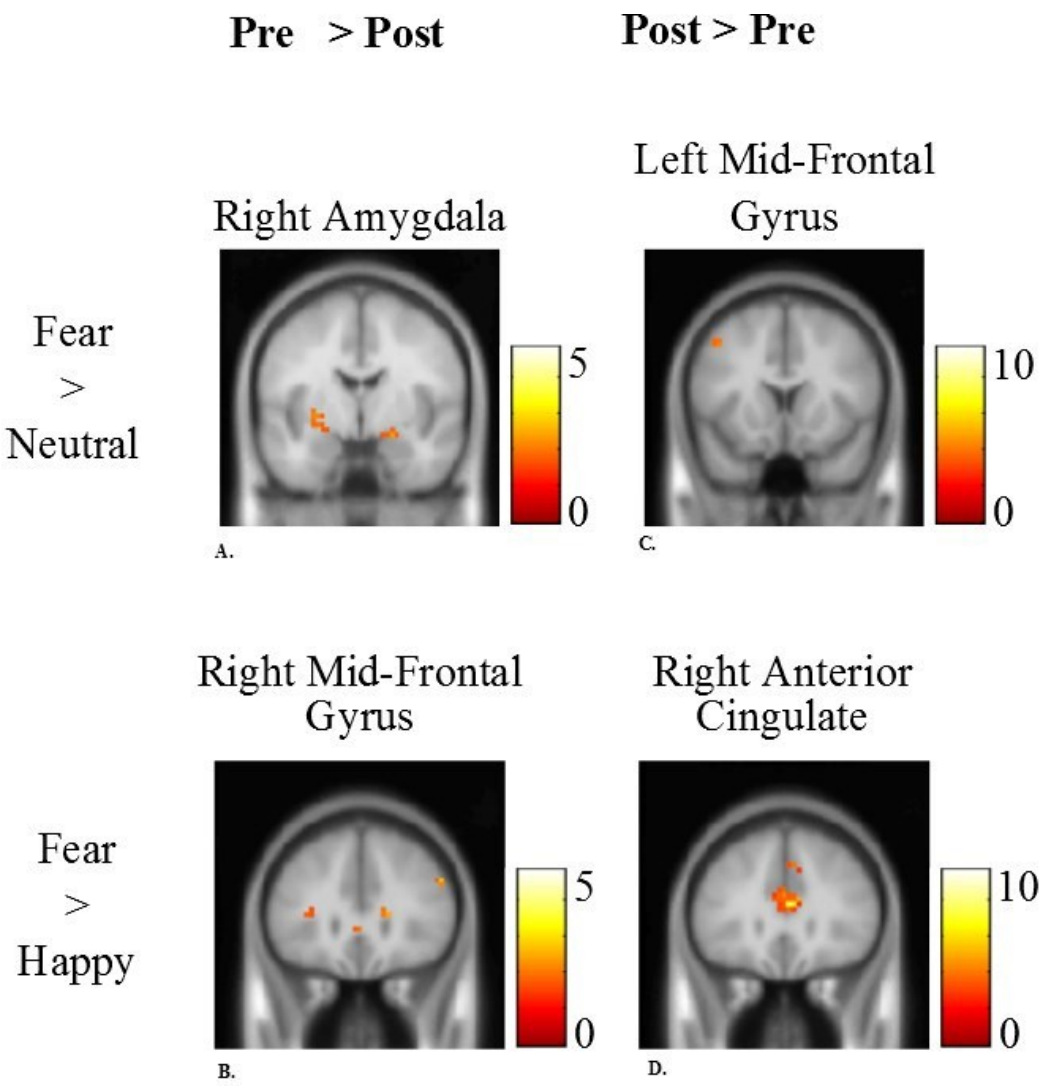
Pre > Post
Post > Pre
Right Amygdala
Left Mid-Frontal Gyrus
Fear > Neutral
5
0
10
0
A.
C.
Right Mid-Frontal Gyrus
Right Anterior Cingulate
Fear > Happy
5
0
10
0
B.
D.

Figure 1: Neural responses to emotional face stimuli before and after treatment

Pre-Treatment > Post-treatment

A. Greater activation is noted in the participants' (n = 7) right amygdala (x = 21, y = -4, z = -14, p < .05, uncorrected) during the fearful vs. neutral faces comparison, before treatment (upper line, left side). **B.** For the fearful vs. happy faces contrast (lower line, left side), increased activation occurred in the mid-frontal gyri bilaterally (right side shown, x = 51, y = 29, z = 31, p < .05 uncorrected).

Post-Treatment > Pre-Treatment

C. Participants (n = 7) displayed greater activation in the mid-frontal gyri bilaterally (left side shown, x = -42, y = 14, z = 43, p < .005 uncorrected) in response to the fearful vs. neutral faces following treatment (upper line, right side). **D.** During the fearful vs. happy face comparison (lower line, right side), participants engaged the right anterior cingulate (x = 3, y = 29, z = 16) to a greater degree, after treatment (p < .05 corrected).

Blood oxygenation level dependent (BOLD) signal activation is superimposed on the T1 MNI template. Corresponding T-values appear at the left of each image.

4.0 DISCUSSION

Participants with chronic PTSD reported significantly decreased symptom severity, subsequent to treatment with the reconsolidation blocker propranolol, as reported in our previous studies [6]. The neuroanatomical regions activated during the fearful face conditions, notably the thalamus, amygdala, insula, hippocampus and pallidum, contain high densities of beta-receptors [26], and comprise part of the threat-detection and affective processing circuitry. Before treatment, activation of these regions may indicate that chronic fear reactivity in PTSD patients is mediated by dis-inhibition of "bottom-up" locus coeruleus – noradrenergic signaling [15].

Diminished activation has been noted in the medial prefrontal cortex (mPFC) and rostral anterior cingulate (rACC) in response to fearful vs. happy faces in PTSD [10]. The inhibitory level conferred by the mPFC governs the strength of the physiological endogenous stress response [27]. The treatment-related increase in mPFC responses to fearful faces may reflect a degree of reinstatement of beta-adrenergic receptor mediated inhibition in the limbic system. Evidence from animal and human studies, have documented that propranolol blocks the amygdala beta-adrenoreceptors during emotive processing [28] and that direct mPFC projections to the amygdala mediate the suppression of fear responding. Shin et al. [10] reported a negative correlation between PTSD symptom severity and rACC activation during fearful relative to happy face processing. In the current trial, increased ACC engagement during the fear condition after treatment might reflect the modulation of both arousal [29] and vigilance.

As an extension of pathological fear conditioning, participants with PTSD experience difficulty discriminating between fearful and other emotional cues. Exposure based therapies (with trauma cues), have shown that improved emotional regulation predicted greater PTSD symptom reduction [30]. In this pilot study, we observed that six 5 to 10-minute trauma reactivations under the influence of propranolol subsequently reduced amygdala reactivity to non-traumatic, threat-relevant stimuli in PTSD participants, and decreased their symptom severity. Although these results are tentative, they may support the idea that the ability to discriminate emotions may be linked to PTSD remission.

We acknowledge that the primary limitations of this study are the small sample size and the absence of a control group. However, longitudinal f-MRI studies with PTSD patients are uncommon and the within-subject design of this study yields increased statistical power, relative to between group comparisons. Additionally, the Overt Faces f-MRI task has demonstrated test-retest reliability such that amygdala activation is elicited in response to fearful faces, when the paradigm is administered longitudinally [31]. Consequently, absent amygdala activity during the post-scan session may be attributable to the reconsolidation blockade intervention, rather than task habituation. Future studies may consider including a propranolol treated PTSD group without trauma memory reactivation, to elucidate the neural responses to threat-processing, independent of reconsolidation mechanisms following β-adrenergic antagonism.

In conclusion, cortico-limbic neuroadaptations in the aftermath of trauma may disrupt daily activities, reflecting abnormal processing of threatening information [32]. This study has shown that reconsolidation blockade with propranolol may facilitate the

regulation of abnormal threat processing, by eliciting neural changes that have been associated with PTSD remission. These results provide mounting evidence that reconsolidation blockade represents a promising treatment for traumatic stress and therefore, may aid in identifying interventions which reverse abnormalities in the noradrenergic pathway and limbic system which mediate PTSD pathology.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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CHAPTER IV

GENERAL DISCUSSION

1.0 OVERVIEW

Evolutionarily, it is paramount to one's functioning and survival to remember important life events. While "significance facilitates remembrance" [1], the over-consolidation of an emotional memory can become maladaptive, especially in the case of trauma victims who are incapacitated by fearful memories and anxiety. It is postulated that the reconsolidation of fearful memories can be influenced by neurobiological manipulations *before* or *during* the reactivation period [2], by directly modulating protein synthesis [3], or altering neurotransmitter release (e.g. norepinephrine) in the amygdala. Subsequently, reconsolidation blockade may alter the expression of fearful memories at the behavioral level.

The functional imaging investigations reported in this dissertation have mapped the neural underpinnings of PTSD symptoms prior and subsequent to beta-adrenergic receptor blockade during: a) trauma specific memory reactivation (Study 1); and b) the presentation of non-specific threat signals (Study 2). These studies yielded the following main results in a chronic PTSD cohort: 1) in the symptomatic state (pre-treatment), areas such as the amygdala, hippocampus, and thalamus were significantly responsive, implicating emotional, memory and sensory networks; 2) attenuated activation in the limbic system were observed while the PFC regions were notably more active and 3) the treatment's effect on the amygdala was associated with significantly decreased PTSD symptom severity.

This final chapter will discuss the physiological link between these studies; the relationship of these findings to reconsolidation theory; the limitations and implications of this work, as well as directions for future PTSD research.

2.0 NEUROBIOLOGICAL LINK BETWEEN THE TRAUMA SPECIFIC AND NON-TRAUMA PROCESSING EXPERIMENTS

Several studies have illustrated a neuro-circuitry model of PTSD in which patients exhibit *hypo-functional* prefrontal activity, and *hyper-functionality* in the emotional circuit, particularly the amygdala, relative to healthy or trauma-exposed control groups [4]. It is hypothesized that individuals with compromised mPFC function are not able to regulate their stress response [5]. These factors, contribute to the mental re-experiencing of the traumatic event [6]. Additionally, increased salience to daily emotional events interacts with noradrenergic circuitry adaptations observed in PTSD [7], providing feedback which can amplify the individual's stress reactions. A *stressor* is defined as an external stimulus which challenges the homeostasis of an organism, including a potential disturbance in the environment, to which the individual must adapt. When fear is activated disproportionately, in a chronic manner, outside of actual threat, it is maladaptive and related to the development of anxiety disorders [8].

In these experiments we have shown for the first time that provision of a treatment approach that may be consistent with reconsolidation blockade, correlates with altered activity within the fear conditioning neuro-circuitry in PTSD patients. In Study 1, f-MRI data was presented from the script-driven imagery task, indicating greater medial and superior frontal cortex activation in PTSD patients during the trauma condition, after treatment. Similarly, in Study 2, we observed a decreased amygdala response to *non-trauma fearful (aversive) emotional* cues, while the anterior cingulate cortex became engaged.

Frontal cortical areas modulate emotional responsiveness by inhibiting amygdala function, and it is hypothesized that dysfunction in these regions may underlie pathological emotional responses

in patients with PTSD, and possibly other anxiety disorders [9]. The mPFC has reciprocal projections to the limbic system, which are involved in suppressing the amygdala's responsiveness to fearful cues [10]. Additionally, according to Yin et al (2011) [11], the thalamus has been implicated in the *functional resting state* of PTSD. Both the medial prefrontal cortex and the thalamus have been activated in response to *externally and internally* generated emotions; and in response to a spectrum of affective states including happiness, sadness, and disgust [12] indicating that their activation is independent of the source of the emotion.

Before treatment, the amygdala, hippocampus, and thalamus were significantly activated during both trauma imagery and fearful facial stimuli processing, relative to neutral information. These results re-affirm that amygdala dysfunction is a marker of sympathetic arousal, and that the aforementioned regions of the hippocampus, thalamus and prefrontal cortex, comprise a PTSD circuit. Furthermore, noradrenergic system dysfunction is involved in mediating trauma specific responses (Study 1) and emotional deregulation (Study 2), characteristic of PTSD, while the inhibition conferred by the prefrontal regions (mPFC, ACC, superior and inferior frontal gyri) may be implicated in alleviating both traumatic and other fearful responses.

In addition, to the cortico-limbic activity during the Overt Faces Task, we observed sub-cortical activation in the pallidum pre-treatment and the putamen, post-treatment. The amygdala has extensive projections to the striatum, nucleus accumbens and hippocampus [13]. Putamen activity has been previously reported while *processing* human faces [14], whereas the amygdala has governed processing of the emotional component. However, it is plausible that the striatal

activation may be sub-served by dopamine (DA) as well as norepinephrine which are both derived from the tyrosine metabolic pathway [15]. Pre-clinical experiments have shown stress-mediated release of DA, although not specifically in response to fear. As such, aversive stimuli activate the DA system, although the pattern of activation differs from NE stimulation. For example, DA has been shown to stimulate the PFC. In contrast, NE can inhibit the frontal cortices as hypothesized, in the pathophysiology of PTSD. During the *fearful vs. happy* comparison, we expected to see decreased PFC activity *pre-treatment*, but instead we observed increased activation in the medial and inferior PFC. This result has been found previously in unmedicated PTSD patients [16], and may reflect methodological differences in face stimuli imaging paradigms, although PFC activation during the symptomatic state may be a function of severity differences in the samples.

3.0 THE FUNCTIONAL NEUROANATOMY OF TRAUMATIC MEMORIES AND RECONSOLIDATION THEORY

The prevailing model has provided substantial evidence that PTSD patients exhibit *hypo-functional* prefrontal activity and *hyper-functionality* in the emotional circuit [17]. This altered neural activity may also reflect the overall affective dysfunction that they present in clinical sessions [18]. However, to date, no single neurotransmitter or neurochemical mechanism can account for all of the PTSD symptoms. Since neurotransmitters interact in a complex manner, a dysfunction of any particular pathway (e.g., norepinephrine, dopamine, etc.) is likely to affect other systems. Thus, the various PTSD symptoms could represent several deregulated neurochemical systems. In particular, animal models have noted that β-adrenergic receptors mediate consolidation through interaction with gamma amino butyric acid (GABA)-ergic, cholinergic and opioid transmitter systems in the amygdala [19]. Despite this, previous clinical

studies have shown that anxiolytics, particularly $GABA_A$ receptor agonists (benzodiazepines), are ineffective for assuaging the core PTSD symptoms [20], and are associated with behavioral dis-inhibition, an inability to process the trauma during psychotherapy; and high abuse liability.

While we acknowledge the contribution of other transmitter systems in the pathophysiology of PTSD, *propranolol* administered peripherally in humans, traverses the blood–brain barrier; achieving a ratio of brain/plasma concentrations of 20:1 [21]. The observation that adrenergic antagonists that do not cross the blood-brain barrier, do not affect memory processes, has provided strong evidence that the CNS β-adrenergic receptors modulate reconsolidation processes (in addition to blood pressure and heart rate). It has been proposed that after β-adrenergic blockade, there is a dissociation of the fear memory from its declarative component where by the memory of the event is intact, but the emotional component is reduced [22]. This has been attributed to the functional differences in the amygdala and hippocampus in memory processes. Kindt et al. (2009) have suggested that beta-adrenergic blockade of reconsolidation *selectively* "deconsolidates" the amygdala fear memory via protein synthesis inhibition, while the declarative memory remains intact in the hippocampus [23]. After treatment, we observed hippocampal activity in the Overt Faces and Script Driven Imagery Tasks. Therefore, for the trauma specific task, it is plausible that the fearful memory was impaired via direct blockade of the amygdala beta-adrenergic receptors, which subsequently down regulated activity (blood flow) in the hippocampus. Hippocampal synaptic plasticity is dependent on β-receptor mediated modulation [24].

In addition, the hippocampus and mPFC regulate *stress anticipation*, but not necessarily pharmacological challenge. Regarding the Overt Faces Task, activity in these regions, in concert

with the amygdala could be mediating aberrant encoding of non-traumatic, fearful facial stimuli in the symptomatic state. In contrast, *post-treatment*, the hippocampal and mPFC activation may reflect the modulation of stress reactivity to potential threat or fear, as the amygdala hyper-activation is absent.

4.0 METHODOLOGICAL CONSIDERATIONS

4.1 Functional Imaging Paradigm

Imaging cognitive and emotional states can be challenging in clinical populations, and factors such as the order of tasks, duration of the scan and the patients' symptomatic behaviour can introduce confounds in these types of experiments. Both f-MRI tasks employed in this dissertation have been validated in chronic PTSD patients, previously. However, the tasks were presented in a standard order such that the Overt Faces task was completed first, followed by the Script-Driven Imagery task within a 45 minute scanning period. This was implemented to increase the likelihood that both tasks would be completed in the same session; while best capturing the neural responses during non-traumatic emotional processing and symptom provocation respectively. Based on prior experience with trauma reactivation in a lab setting, it was anticipated that if the Script-Driven Imagery Task was presented first in the scanner, it might elicit emotional responses that could prompt PTSD participants to discontinue the session, or produce carry-over effects that would influence the neural responses during the Overt Faces task. As such, task counterbalancing was not considered ideal for the two experiments presented to the participants. In addition, PTSD participants exhibit high levels of anxiety and the scanning environment can intensify their general arousal, and influence the brain activation pattern that is captured during the task. In particular, healthy participants' anxiety at the time of

a scan has correlated positively with neural activation to neutral faces [25], and may be a factor that has influenced the brain activation during our task.

Upon analysis of the data, there were neural regions activated that were not part of our a priori regions of interest. For example, pre-treatment, during the trauma vs. neutral imagery comparison, we observed activation in the left middle and right superior temporal gyri (5 voxels only), right pre-central and post-central gyri, and in the right para-central lobule. The temporal lobe activity may be associated with general memory processes during trauma reactivation, while the central and para-central lobule activation may correlate with the movement of the extremities (e.g. finger and toe movements) in the scanner. In general, longer scan sessions have been associated with movement artifacts, fatigue and habituation effects in participants, regardless of the paradigm.

The Overt Faces task is designed as a passive viewing experiment which has been associated with reliable amygdala activation, whereas button pressing in response to faces inhibits the limbic circuitry [26]. According to Johnstone et al (2005) [25], the Overt faces task has good test-retest reliability, when administered approximately 8 weeks apart, as in our study. Therefore, it is plausible that the decreased amygdala activity is not attributable to habituation during the post-scan, but rather to our reconsolidation blockade intervention, with propranolol, although our single group study design cannot ascertain that. A randomized trial would have been required to show that the effect on the amygdala was due to the intervention. Although the propranolol was administered under blinded conditions, the randomization did not work out for the intended control group.

The script-driven imagery task has been utilized in a PET imaging study, with PTSD patients before and after receiving placebo or SSRI treatment for 12 weeks [17]. However, the authors did not address habituation to the trauma scripts, as a potential study confound, although it is plausible when the narratives were presented in a pre-post design. In addition, it is possible that even very short trauma reactivation sessions can elicit extinction in some individuals. However, the treatment and brief trauma reactivation sessions were spanned a week apart which might have limited the likelihood of extinction, as the main explanation for our results. Given that PTSD participants experience extinction memory deficits as well, it has been challenging to determine a consensus on practice effects with the script-driven imagery task in a PTSD population.

4.2 Co-morbidity and concomitant medications

There has been a paucity of randomized controlled trials (RCTs) aimed at the treatment of PTSD. Consequently, commonly available anti-depressant, anxiolytic and sedative-hypnotic medications for other disorders have been prescribed "off label" for the relief of traumatic symptoms. Of these, the SSRI and SNRI anti-depressants have been considered as the first-line treatment for PTSD. Therefore, some of our PTSD participants had been prescribed these anti-depressants for at least 30 days prior to enrolment; and remained on stable doses during our trial. However, these medications could have influenced the effectiveness of propranolol and the pattern of activation in our neuroimaging results. We controlled for this potential drug interaction by requiring participants to skip their morning dose of SSRI/SNRI's on both the neuroimaging and study treatment days.

In particular, SNRIs are mostly indicated for Major Depressive Disorder (MDD), which is associated with decreased levels of serotonin and norepinephrine in the synaptic cleft, which then induces aberrant CNS signaling. By inhibiting the re-uptake of these transmitters, there is an increase in their extracellular concentrations, which facilitates neural functions such as attention and arousal [9]. In contrast, propranolol inhibits the effect of norepinephrine, and therefore is not a treatment for MDD.

Evidence suggests that PTSD *with* comorbid MDD differs both behaviorally and neurobiologically from PTSD *without* MDD. Chronic PTSD patients with and without comorbid MDD have been examined using the script-driven imagery f-MRI task, which yielded different neural activation patterns in these groups [27]. Participants were required to undergo a medication washout period for 2 weeks, prior to the study. When symptom severity was controlled for, PTSD participants activated the insula to a greater degree during the trauma imagery condition, whereas the co-morbid group showed enhanced activity in the anterior and posterior cingulate gyri. The authors concluded that decreased activity in the left anterior-insula is considered a neural marker of comorbid MDD and PTSD, in these participants. However, the study lacked a depression only (without PTSD) comparison group, and none of the participants had been taking fluoxetine, prior to the washout period. Thus, examining the influence of SSRIs on neural activation patterns during trauma re-experiencing in *co-morbid PTSD-MDD* patients was limited in the study.

Other neuroimaging studies have examined the neurobiology of PTSD symptoms in medication-free patients (treatment naïve), some of whom have concurrent disorders, in addition to patients

who were taking several medications but underwent a wash-out period prior to study participation. However, it has been argued that only examining unmedicated patients does not accurately represent the PTSD population, and therefore is an approach that can lack ecological validity. For example, only scanning medication free patients can introduce sampling biases and potential confounds by including individuals who a) were easily persuaded to voluntarily discontinue their treatment; b) may be less symptomatic or have less severe co-morbid conditions from the outset, and c) may develop withdrawal symptoms after ending antidepressant treatment, which can alter cerebral blood flow [28]. To control for these factors, the studies conducted in this dissertation included PTSD patients who were taking stable doses of psychotropic medications.

Studies have noted that 77% and 80% of domestic and military PTSD patients, respectively, were prescribed psychotropic medications [29]. Consequently, it is important to determine if the brain mechanisms of unmedicated PTSD research participants are congruent with those of traumatized individuals who are seen in daily clinical practice. Currently, there's a lack of empirical evidence showing that commonly prescribed psychoactive medications, confound task performance or rCBF in PTSD patients, when medication status is applied as a co-variate in data analyses. However, it has been shown that psychotropic medications alter task responses and brain activation in *healthy participants* [28], whose neurophysiology differs from PTSD patients.

The current dissertation results suggest that at baseline, the neural correlates of unmedicated and medicated PTSD patients are similar during the symptomatic state. This may suggest that the other psychotropic medications are marginally effective in treating PTSD, but may be managing

the co-morbid conditions. Therefore, medicated PTSD patients should be included in studies, as long as their current treatments are not impacting the trauma symptoms of interest [28]. Consequently, our current study design may be more representative of the PTSD patients seen in clinical practice. Sampling biases can emerge as methodological constraints when recruiting clinical populations. Future studies may consider examining the neural characteristics of non-treatment seeking PTSD individuals.

4.3 Sample Size

First, there are small sample sizes in the dissertation studies reported, here. While a pilot study with a small sample size can yield results that are informative and promising, such results cannot yet modify clinical practice until a more definitive study with larger samples are conducted possibly with additional groups. Second, the studies published so far in the area of PTSD cannot conclusively demonstrate that the symptomatic improvement observed is *caused* by reconsolidation blockade, and not by another mechanism. The work presented is congruent with a reconsolidation account but does not rule out other possible explanations. It is legitimate to a certain extent to first ascertain *whether* a novel treatment works, and then in follow-up studies to ascertain *how* it works.

Additionally, comprehensive, but necessary eligibility criteria can influence the number of study candidates, especially when recruiting a clinical population with a specific disorder of interest. In some cases, an individual is qualified to take the study medication, but does not fulfill the scanning criteria or vice-versa. For these dissertation studies, PTSD participants must have been eligible to take both placebo and propranolol. According to our prior pilot data from an open-

label study, the combination of trauma reactivation with propranolol, significantly improved PTSD symptoms relative to trauma reactivation under placebo [30]. However, these studies did not include a neuroimaging arm, and therefore, had less inclusion criteria.

In addition, the randomization charter with a 50% chance of receiving either medication did not work, for the current dissertation studies. In general, improper randomization may result in a bias toward either study group. In this case, the placebo group sample size was significantly reduced (due to attrition) which increased the likelihood of individual variability confounding the results among the few placebo completers; while limiting the interpretation of the neural responses to propranolol. Attempting to obtain a sufficiently large control group for a small number of participants can be challenging for research involving individuals with debilitating medical conditions.

As the placebo group for this study was no longer feasible, the influence of reconsolidation blockade treatment on brain activation during fearful face presentation, and trauma reactivation is considered correlational, rather than causal. It is also possible that this correlation is driven by elements that have nothing to do with reconsolidation blockade treatment per se but rather to other extraneous variables such as the passage of time, practice effects, and clinical attention (eg. monitoring) [30]. Despite this, our remaining propranolol group comprised a within-subjects design (pre vs. post) which has more sensitivity to treatment effects (power) than a between-subjects design employing the same number of observations.

In the current project, the treatment effect size reported (*d =1.75)* is consistent with our previous studies, in which six reconsolidation blockade sessions with propranolol, yielded effect sizes

ranging from *(d =1.32 - 2.19)* relative to an *untreated* PTSD group *(d = 0.24)* [30]. To date, rather than calculating placebo effect sizes, randomized controlled studies have reported using a standard $p < .05$, that propranolol has significantly reduced PTSD symptoms and psychophysiological responding during script driven imagery [31], particularly in compliant participants [32].

Additionally, individual variability in neural function is inherent in all random samples. Individual factors such as age and comorbid mental illness may influence reconsolidation processes. Pre-clinical literature on traumatic memory reconsolidation indicates that more *recent* memories are susceptible to reconsolidation impairments, whereas older traumatic memories are not as prone to blockade using pharmacological agents [33, 34]. One study demonstrated that the trauma memories of *younger* post-Vietnam veteran cohorts are less chronic, and therefore are more likely to be modified during reconsolidation blockade [35].

Within the current study, the participants' age range was 21.0 – 45.0 years old, in the propranolol group. There was only one participant who was older than 60 years of age, who had been randomized to the placebo group. As expected, this participant's symptoms did not improve after six weekly doses of placebo, in conjunction with trauma reactivation. This individual's imaging data was not acquired, as a technical malfunction occurred during his f-MRI session (see Appendix I), thereby limiting an assessment of age effects on reconsolidation. Furthermore, a prominent symptom of Alzheimer's disease, an age-related neurodegenerative disorder, includes deficient consolidation of long-term memories, which manifests as retrograde amnesia in advanced stages of the disease [36]. Neurological, including age-related neurodegenerative

disorders were *exclusionary* for the study, to reduce the likelihood of these factors impacting memory processes in our sample.

Finally, Van der Kolk et al (1996)[18] have posited that endogenous opioids may be associated with numbness in a subset of PTSD patients, by decreasing (not eliminating) their emotional intensity, as a coping mechanism. Although, it is plausible that this biological process could have altered task responsiveness in this study, emotional numbing and autobiographical memory alterations in PTSD patients have been associated with *dissociation,* which was an exclusionary criterion for this pilot study.

5.0 TIME DEPENDENT FACTORS IN RECONSOLIDATION BLOCKADE

An element which limits causal inferences from our data has to do with the fact that, contrary to animals receiving a post-retrieval injection (or infusion) of a reconsolidation blocker, propranolol *per os* takes 75-90 minutes to peak in the blood. Therefore, practical circumstances dictate that in order for propranolol to exert its effect, it must be given *prior* to memory reactivation, as in these dissertation studies. However, it is possible that the medication also influences the retrieval of the memory. For instance, retrieving a traumatic memory under a more relaxed bodily state might be helpful. This, of course, does not rule out the fact that propranolol could also block the reconsolidation of the memory, as the preclinical studies strongly suggest. In order to test this, propranolol could be injected after memory reactivation (retrieval). However, the risks and intrusiveness of propranolol injections as a route of administration outweigh the benefits in human participants.

One other possibility to limit the number of alternate explanation for our results would be to use a β-blocker that does not traverse the blood-brain barrier (and therefore cannot block the protein

synthesis presumably involved in blocking reconsolidation). However, this has already been investigated within the framework of consolidation in healthy participants [37]: nadolol did not block consolidation, and therefore it is unlikely that it would block reconsolidation either.

6.0 FUTURE DIRECTIONS

Potential factors for treatment targets

Individuals who have been trauma exposed do not always develop PTSD, and it remains unclear as to whether there are structural and functional brain anomalies that predispose one to PTSD or whether alterations in the brain are a consequence of the disorder. Currently, imaging studies suggest that there are vulnerability factors such as reduced hippocampal volume [38] but this pathology could also occur after the traumatic event, and continue to deteriorate in chronic cases. As such, these functional imaging dissertation results are predicated on the idea that the brain regions were modified after the stressor. However, there may have been pre-existing cortico-limbic sensitivity or noradrenergic system imbalance prior to the trauma that cannot be determined from this work. Therefore, future studies could address whether these neural abnormalities are familial risk factors or acquired after the trauma [16].

Recent scientific advancements have shown that single nucleotide polymorphisms (SNPs) in genes that code catecholaminergic proteins [e.g., neuropeptide Y (NPY), catechol-O-methyl-transferase (COMT), dopamine receptor D2 (DRD2), serotonergic proteins (e.g. serotonin 2A receptor (5-HTR2A) and other *plasticity-related proteins* e.g., gamma-amino butyric acid receptor alpha 2 (GABARA2), and brain-derived neurotrophic factor (BDNF) are linked to increased susceptibility for development of PTSD [39]. Presence of the deletion variant ADRA2B, seems to intensify the magnitude of emotional memories, which renders individuals

with this gene variant more susceptible to develop intense intrusive memories compared to individuals without the gene variant. Therefore, it is possible that a subset of people, based on their genetic profile, process traumatic events with greater cognitive efficiency, increasing the probability of developing the intrusive memory components of PTSD [40]. It may be informative to observe the effects of polymorphisms on stress-induced brain activation. For example, prior studies have indicated that PTSD symptoms profiles (e.g. dissociation) are associated with different neural activation patterns, which could potentially aid in tailoring appropriate treatments [16]. The treatment of PTSD is extremely challenging, requiring many years of therapy with variable results. Therefore, genetic screening for vulnerability and resilience may offer an important option for the prevention of PTSD and the amelioration of symptoms. In the future, the application of these genetic markers in conjunction with symptomatic, remitted and PTSD resilient individuals could be implemented with structural or functional imaging techniques to better understand the neural correlates of various treatment effects.

In addition, according to Besnard et al. 2012 [41], all disorders that have a strong learning component in their etiology could potentially be helped by harnessing the mechanisms involved in memory consolidation and reconsolidation, notably the anxiety and addiction disorders. However, this needs to be demonstrated convincingly in one disorder before it can be tested in other psychopathologies. In the future, the inclusion of other psychiatric patients as control groups could determine the generalizability of the post-treatment neuroimaging results in a PTSD cohort, reported in this dissertation.

7.0 CONCLUSION

Prior imaging studies were aimed at identifying the neural substrates mediating the active symptoms of PTSD in treatment naïve subjects, or functional differences in trauma exposed individuals without PTSD. In addition, early studies have tested propranolol's prophylactic ability, following a traumatic event. As such, longitudinal studies examining the functional neuro-anatomical changes following recovery or pharmacotherapy are limited in a PTSD sample. The projects presented in this dissertation extend these previous studies and examine the behavioural and *neural effects* of propranolol (reconsolidation blockade treatment) in a chronic PTSD population.

These preliminary neuroimaging results lend credence to the idea that altered noradrenergic signaling contributes to limbic BOLD signal decreases in PTSD participants, which may represent fear-conditioning reconsolidation impairments subsequent to propranolol administration. Additionally, the increased neurophysiological activity in the ACC/mPFC, in conjunction with the symptom improvement reported by our participants, suggests the potential therapeutic application of reconsolidation blockade and provides positive prognostic significance for chronic PTSD resolution. Future longitudinal studies which incorporate a randomized design, will further aid in establishing the role of reconsolidated blockade in these results.

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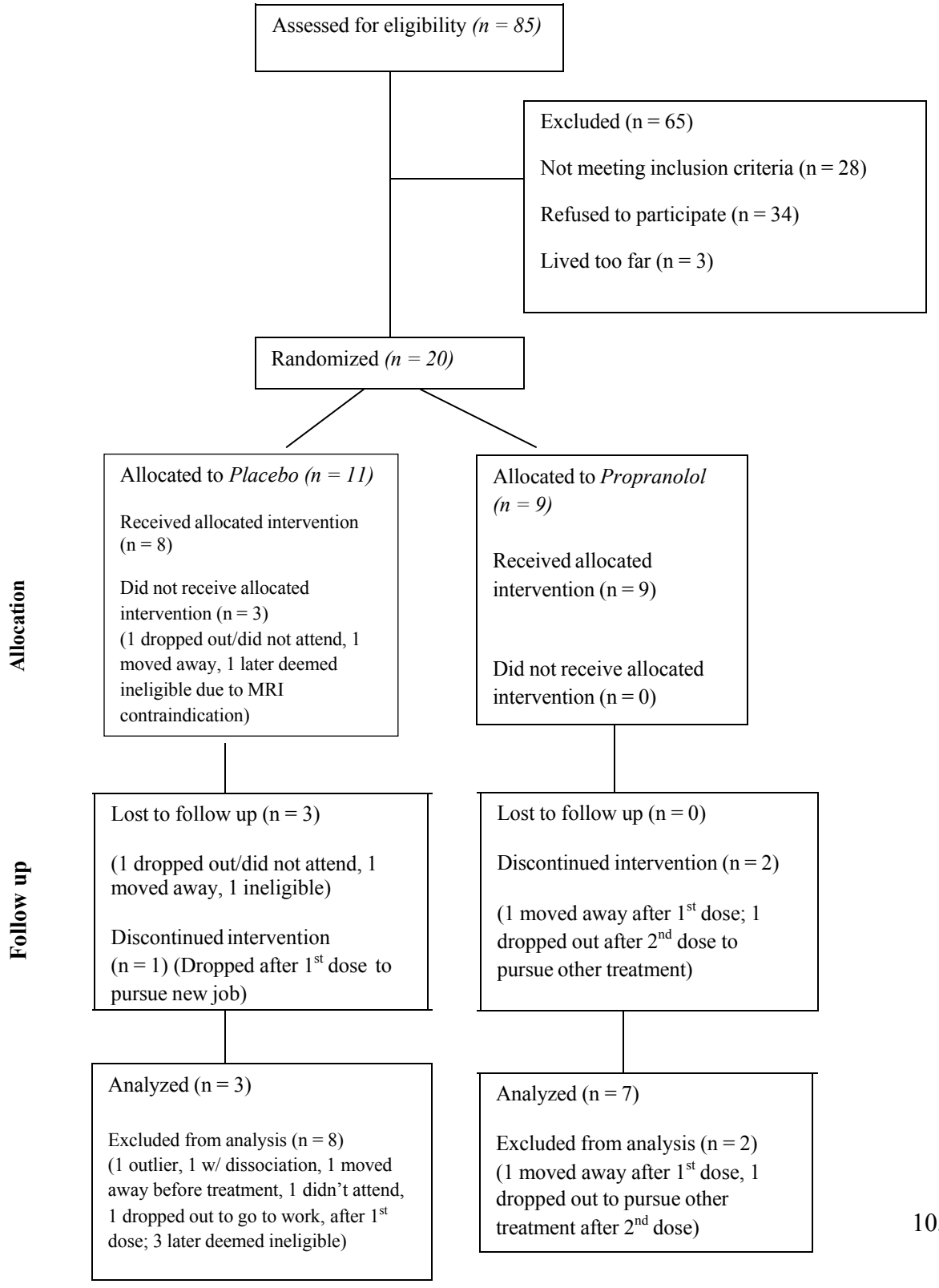
APPENDIX

APPENDIX I: RECRUITMENT STATISTICS

Figure A1.0: CONSORT diagram illustrating the flow of participants through each stage of this randomized trial.



A1.0 Recruitment and Attrition

This study was initially designed as a randomized double-blind placebo controlled trial. The blind was removed at the end of the study, according to prescribed procedures for clinical trials. Twenty participants were randomized to the study, as shown in the Consolidated Standards of Reporting Trials (CONSORT) diagram (Figure A1). Of the 11 participants randomized to the placebo condition, only 3 participants completed both the neuroimaging and six treatment (reactivation) sessions of the trial. As a result of the high attrition rate in the placebo group, statistical analysis of their data was not possible.

Additionally, 9 participants were randomized to the propranolol arm of the study. Of these, 7 propranolol participants completed the f-MRI experiments, and the six treatment sessions. Two propranolol participants discontinued the treatment session after the baseline scan, and were excluded from the statistical analyses.

The socio-demographic data for the placebo group (completers and non-completers) and propranolol (non-completers) is presented in Table A1. The corresponding baseline PTSD clinical symptom scores for these participants, who were excluded from the analysis, are shown in Table A2.

Table A1.0: Socio-demographic summary for the participants *excluded* from the analysis *(N=13)*

Age (years)	Sex	Years of PTSD	Years of Education	Socioeconomic status	Marital Status
62.00	M	50.00	11.00	$15,000 and less	single
27.00	F	1.00	-	-	single
46.00	F	14.00	17.00	$70,001-90,000	married
34.00	F	4.00	16.00	$30,000-50,000	common law
32.00	F	2.00	-	$15,000 and less	married
54.00	F	4.00	15.00	$15,000 and less	single
45.00	F	30.00	13.00	$15,001-30,000	single
35.00	F	6.00	16.00	$15,000 and less	common law
32.00	M	17.00	16.00	$15,000 and less	single
21.00	F	ineligible	16.00	$15,000 and less	single
40.00	F	2.00	16.00	$30,000-50,000	single
40.00	M	10.00	11.00	$30,000 -50,000	common-law
28.00	F	5.00	16.00	$30,000 -50,000	single

Table A1.1: Socio-demographic summary for the *propranolol group completers (N=7)*

Age (years)	Sex	Years of PTSD	Years of Education	Socioeconomic status	Marital Status
21.00	F	1.50	11.00	$15,000 and less	common-law
45.00	F	3.00	-	$15,000 and less	single
34.00	F	15.00	-	$30,000 - 50,000	common-law
33.00	F	18.00	9.00	$15,000 and less	married
35.00	M	1.00	13.00	$90,000 and more	married
31.00	M	1.00	16.00	$30,000 - 50,000	single
33.00	F	14.00	-	$15,000 and less	common-law

Table A1.2: Socio-demographic summary for participants *excluded from the final analysis (N=13)* and the *Propranolol completers (N=7)*

Demographic Measure	**Excluded Participants *(N =13)***	**Propranolol Group Completers *(N =7)***
Characteristics		
Age (mean yrs.) (SD)	38.1 (11.4)	33.3 (6.4)
Sex Ratio (% female)	76.9 %	71.4%
PTSD Duration (mean yrs.) (SD)	12.8 (14.6) *(n =12)*	7.6 (6.5)
Education (mean yrs.) (SD)	14.8 (2.1) *(n =11)*	12.2 (2.9) *(n =4)*
Individual socio-economic status		
$15,000 and less	*n = 6*	*n = 4*
$15,000 – 30,000	*n = 1*	-
$30,001 – 50,000	*n = 4*	*n = 2*
$50,001 -70,000	-	-
$ 70,001 -90,000	*n = 1*	-
$ 90, 000 and more	-	*n = 1*
Unknown	*n = 1*	-
Marital Status		
Single	*n = 8*	*n = 2*
Married	*n = 2*	*n = 2*
Common Law	*n = 3*	*n = 3*

APPENDIX II: Clinical Assessment Tools

A2.0 Description of Measures

Self-report measures

(1) The Peritraumatic Distress Inventory (PDI)[1] is a validated 13-item instrument that quantifies the intensity of peri-traumatic emotional distress, reflecting the DSM-IV A.2 (traumatized response) PTSD criterion. As a brief scale, the PDI can be completed within 5 minutes. The PDI was administered at baseline on the screening day (Week 0). The PDI was used to ensure that the two traumatic episodes of the two groups are comparable.

(2) The Peritraumatic Dissociative Experiences Questionnaire (PDQ) [2] is a validated 10-item instrument that quantifies the intensity of dissociative reactions at the time of the traumatic event. The PDQ takes approximately 5 minutes to administer, and was completed by the participants at screening (Week 0).

(3) The Impact of Event Scale-Revised (IES-R)[3]. The IES-R is a validated 22-item questionnaire that addresses the three PTSD symptom clusters for the selected traumatic event: re-experiencing, avoidance, and hyper-arousal; all items are Likert-type scales. The standard time period covered by the IES-R is the week prior to administration. The IES-R was administered each time the CAPS is administered (Week 0 & Week 10). Additionally, the IES-R was administered prior to each traumatic memory reactivation session to cover the week elapsed since the last such session. Approximately 10 minutes are required to complete the IES-R.

Structured interviews

Each participant was administered the following structured diagnostic interview instruments by a trained clinician:

(1) Clinician-Administered PTSD Scale (CAPS)[4]. The CAPS is widely regarded as the state-of-the-art structured clinical interview instrument for PTSD. It yields a categorical (present/absent) score according to DSM-IV PTSD criteria as well as severity scores for each of the 17 DSM-IV PTSD symptoms, for each of the three PTSD symptom clusters (re-experiencing,

avoidance, and hyper-arousal) and for total PTSD. The CAPS can be administered in 45 minutes. The CAPS was administered at baseline (Week 0) and the final evaluation (Week 10).

(2) Mini International Neuropsychiatric Interview (MINI)[5] will evaluate the life-time prevalence of most Axis I mental disorders of the DSM-IV. The MINI will be administered at the screening visit (Week 0). The MINI is a short structured clinical interview which enables researchers to make diagnoses of psychiatric disorders according to DSM-IV. The administration time of the interview is approximately 30 minutes.

Table A2.0: Summary of clinical scores for non-completers/excluded participants *(N =13)*

PDI T0	PDEQ T0	CAPS T0	CAPS T8	IES-R T0	IES-R T8	Trauma Etiology	Scan T1	Scan T8	Treatment Notes
30	24	61	69	51	31	Physical assault	TM*	TM	Completed treatment but scan incomplete
29	23	55	-	56	-	Combat or exposure to a war-zone	Yes	-	Dropped out at T2 (lost to follow-up) No treatment administered
52	50	78	6	81	1	Sudden, violent death of someone close	TM	Yes	Completed, but excluded as an outlier Technical malfunction during T1 scan
43	41	67	-	83	-	Combat or exposure to a war-zone	-	-	Moved away from Montreal No fMRI scan or treatment administered
52	36	105	83	68	59	Captivity	Yes	Yes	Completed but excluded due to dissociation, headache with nose-bleed and agitation
40	39	100	-	69	-	Physical assault	-	-	Ineligible, due to MRI contraindication (metal fragments in eye) at T1
27	23	65	56	46	19	Other unwanted or uncomfortable sexual experience	Yes	Yes	Completed
46	40	71	12	35	4	Physical assault	Yes	Yes	Completed, but may have tried other treatment (psychotherapy) while in the study
35	20	74	-	76	-	Sexual Assault	Yes	-	Dropped out to pursue employment after 1st dose (T2)
24	18	39	-	55	-	Accident	Yes	-	Ineligible; traumatic event doesn't meet PTSD criteria
39	30	84	-	56	-	Other unwanted or uncomfortable sexual experience	Yes	-	Ineligible; multiple traumatic events
40	40	106	71	64	-	Combat or exposure to war-zone	Yes	-	Moved away after 1st dose (T2)
31	35	85	57	73	65	Sexual assault	Yes	-	Pursued other treatment after T3

*TM = technical malfunction

A2.1 Treatments Effects on PTSD Symptom Severity

Table A2.1: *Between Group Analysis of PTSD symptom severity differences after six weekly treatment sessions*

Measure	Group	Mean	Standard Deviation (SD)	Percentile		
				25	50	75
IES-R Difference Score	Placebo	-26.75	4.60	-31.00	-27.50	-20.75
	Propranolol	-37.71	25.47	-69.00	-44.00	-13.00
CAPS Difference Score	Placebo	-20.50	28.98	-59.00	-9.00	8.00
	Propranolol	-39.43	19.70	-61.00	-40.00	-16.00

An *exploratory analysis* was performed to determine the change in PTSD symptom severity before and after treatment for *study completers* in both the placebo *(N = 3)* and propranolol *(N = 7)* conditions. Difference scores (Week 0 – Week 8) for the IES-R and CAPS measures were computed for each participant in both treatment groups (Table A2.2). Using the Shapiro-Wilks statistical test of normality, it was determined that the mean difference scores on the IES-R and the CAPS were not normally distributed in each treatment group (IES-R difference normality: placebo $p < .0001$; propranolol $p < .0001$; CAPS difference normality: placebo $p < .0001$; propranolol $p < .0001$). The Mann-Whitney U test was then applied, and significant differences emerged between groups, in symptom severity change after treatment on the CAPS ($p < .0001$). There was a trend toward a significant group difference on the IES-R ($p < .07$). These preliminary findings suggest that among the study completers, PTSD symptoms as measured by

the CAPS, significantly decreased in the propranolol group compared to the placebo group. The IES-R results suggest that there was a trend toward a significant group difference, with the propranolol group reporting a greater decrease in weekly symptom change. However, this IES-R result is considered a tentative outcome and may be a function of the small placebo sample size.

APPENDIX III PHYSIOLOGICAL MEASUREMENTS

A3.0 Heart rate and Blood Pressure Data

Heart rate, systolic and diastolic blood pressures were measured as a reliability check, following the administration of 1mg/Kg of placebo or propranolol. The mean significant decrease in these vital signs upon receiving propranolol indicates that the medication was physiologically acting as per its' indication and that the dosing regimen was in all likelihood adequate.

The mean heart rate (HR), systolic and diastolic blood pressure measures were computed for each participant at four time points (0, 30, 60 and 75 minutes after drug ingestion) collapsed across the six week intervention period. At baseline, the mean blood pressure (BP) (systolic/diastolic) for the propranolol group was 120.3/76.3 mmHg. A repeated measures analysis of variance (ANOVA; 4 time points) revealed a significant overall effect of time for the systolic ($F\ (3,\ 4) = 7.7$, $p < .03$) and diastolic ($F\ (3,\ 4) = 8.56$, $p < .03$) BP. Both BP measures decreased over time, post-propranolol (mean BP post-treatment = 110.4/71.3 mmHg). There was also a significant overall effect of time on HR which significantly decreased by 18% 75 minutes after propranolol administration (mean HR pre-treatment = 76.1 bpm and post-treatment = 62.5 bpm, $F\ (3,\ 4) = 12.9$, $p < .02$. The expected decrease is typically between 15-30% [6]. The physiological results for the propranolol completers *(N =7)* are illustrated in Figures A3.1 to A3.3.

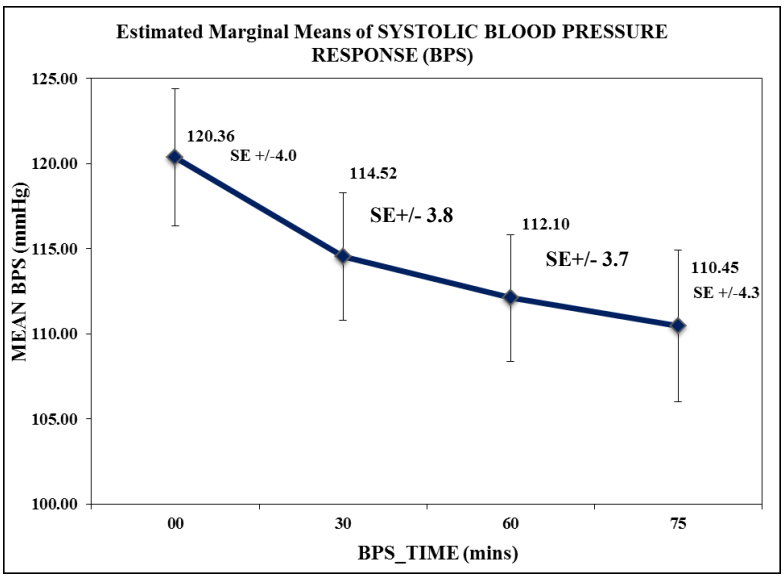


Figure A3.1

Mean decrease in systolic *(Figure A3.1)* and diastolic *(Figure A3.2)* blood pressure at 00, 30, 60 and 75 minutes after propranolol administration, collapsed across 6 weeks *(N = 7)*.

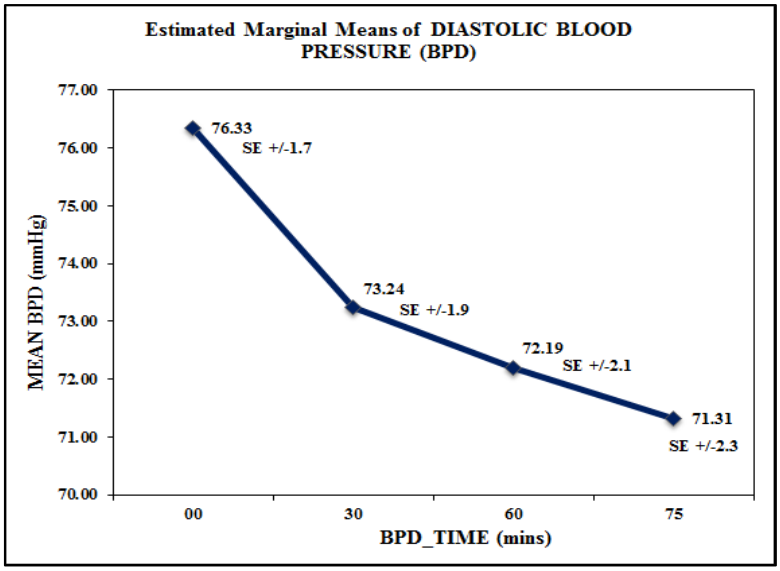


Figure A3.2

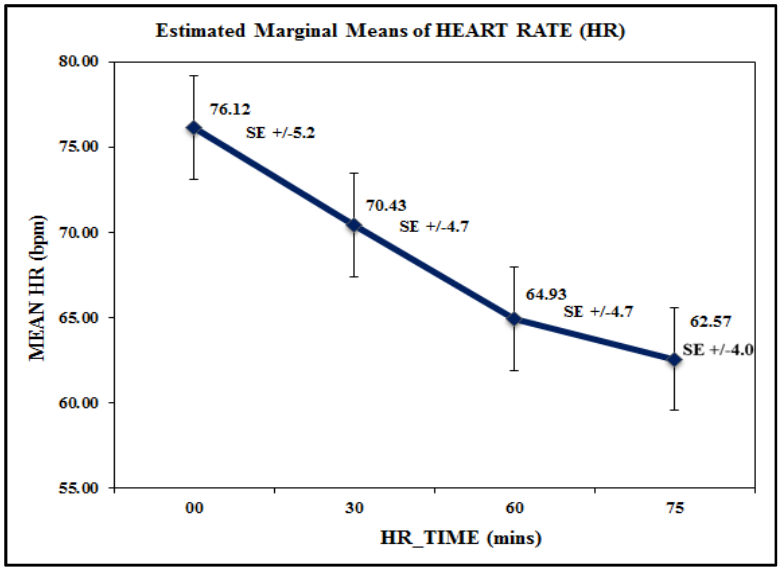


Figure A3.3

Figure A3.3: Mean decline in heart rate at 00, 30, 60 and 75 minutes after propranolol administration, collapsed across 6 weekly treatment sessions *(N = 7).*

The physiological datasets *excluded* from the analyses are presented in Table A3.1 for the placebo and propranolol groups.

Table A3.1: Physiological Data Summary for *participants excluded from analyses (N=13)*

BPS00 MEAN	***BPS30 MEAN***	***BPS60 MEAN***	***BPS75 MEAN***	***BPD00 MEAN***	***BPD30 MEAN***	***BPD60 MEAN***	***BPD75 MEAN***	***HR00 MEAN***	***HR30 MEAN***	***HR60 MEAN***	***HR75 MEAN***
112.83(3.7)	109.83(1.7)	113.50(1.0)	111.33(2.3)	74.67(2.7)	73.33(1.4)	73.17(0.9)	72.17 (1.8)	60.83(2.7)	60.50(2.5)	60.67(2.4)	60.83(1.4)
[a] -	-	-	-	-	-	-	-	-	-	-	-
[b]108.83(2.5)	104.83(2.9)	109.83(3.1)	106.00(1.1)	74.00(1.8)	71.50(2.1)	74.00(1.5)	73.83(1.7)	70.83(1.5)	66.33(1.5)	74.17(1.8)	67.50(0.8)
[a] -	-	-	-	-	-	-	-	-	-	-	-
[c] -	-	-	-	-	-	-	-	-	-	-	-
[d]103.33(1.5)	109.50(2.0)	102.5(1.1)	103.50(1.2)	70.33(1.7)	72.17(2.2)	68.67(1.9)	68.83(1.9)	81.33(2.3)	77.00(1.8)	73.33(3.2)	75.50(3.0)
118.67(3.1)	122.67(3.0)	123.83(2.8)	121.33(2.8)	76.17(1.6)	78.67(1.3)	79.33(2.4)	77.17(1.9)	69.33(1.7)	68.67(2.2)	70.17(2.0)	68.00(1.2)
96.00(2.1)	91.83(1.7)	92.67(1.1)	94.17(2.1)	64.50(1.5)	62.17(1.2)	61.00(1.4)	63.00(1.8)	71.17(2.8)	69.50(1.1)	72.67(3.2)	72.00(3.2)
[e]101.00	104.00	100.00	99.00	64.00	68.00	65.00	59.00	74.00	71.00	78.00	74.00
[c] -	-	-	-	-	-	-	-	-	-	-	-
[c] -	-	-	-	-	-	-	-	-	-	-	-
[e]129.00	121.00	111.00	110.00	82.00	74.00	80.00	66.00	55.00	61.00	52.00	51.00
[f]120.00(4.0)	110.50(10.0)	107.50(1.0)	101.50(6.5)	79.50(1.5)	71.00(6.0)	69.50(2.5)	67.00(6.0)	86.50(10.5)	78.50(6.5)	69.00(7.0)	72.00(4.0)

BPS = systolic blood pressure, BPD = diastolic blood pressure, HR = heart rate

Each *row* corresponds to a participant. BPS, BPD and HR were recorded at 4 time points (00 min, 30min, 60 min and 75 min) post-drug every week for 6 weeks. Data are presented as the *mean (with standard error of the mean)* for each time point collapsed across six weeks, unless otherwise indicated:

[a] dropped out without treatment administered

[b] removed as an outlier

[c] randomized but later deemed ineligible.

[d] completed treatment, but later excluded due to notification of dissociative episodes, and a headache with significant nose-bleeding, requiring a separate diagnostic scan by participant's physician

[e] data shown corresponds to the first treatment session (not the mean of six sessions). Participant discontinued after first treatment visit. *Mean and standard error calculations are not applicable for single session scores.*

[f] data shown corresponds to the *mean* of two treatment sessions (not the mean of six sessions). Participant discontinued after the second treatment visit.

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APPENDIX IV: Supplementary Group Analysis Reports

A4.0: ADDITIONAL CROSS-SECTIONAL GROUP RESULTS

As part of the exploratory analyses, whole brain analyses were also conducted at $p < .005$ (uncorrected) for the propranolol group completers. The main effects (PRE-TREATMENT ONLY and POST-TREATMENT ONLY) for each task are presented here.

SCRIPT-DRIVEN IMAGERY TASK

Contrast 1: Trauma Listening > Neutral Listening
Pre-treatment only

Type: T, df: 6, Threshold - p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebellum, Cerebellum Posterior Lobe, Declive, Cerebelum_6_R (aal)	5	12 -79 -20	4.701
Right Cerebrum, Occipital Lobe, Cuneus, Gray Matter, Brodmann area 17, Calcarine_R (aal)	8	9 -94 -2	4.322
Left Cerebrum, Occipital Lobe, Cuneus, White Matter, Occipital_Sup_L (aal)	49	-12 -91 4	5.499
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, White Matter, Temporal_Mid_R (aal)	10	57 -55 -2	7.279
Left Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Putamen, Putamen_L (aal)	22	-21 8 4	5.759
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, White Matter, Frontal_Inf_Oper_R (aal)	8	51 20 -2	6.471
Right Cerebrum, Sub-lobar, Extra-Nuclear, White Matter, Caudate_R (aal)	33	18 14 10	5.816
Left Cerebrum, Sub-lobar, Insula, Insula_L (aal)	41	-33 20 7	9.605
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Gray Matter, Brodmann area 21, Temporal_Mid_L (aal)	13	-63 -49 4	4.826
Right Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Putamen, Putamen_R (aal)	13	27 -7 7	7.125
Right Cerebrum, Sub-lobar, Extra-Nuclear, White Matter, Corpus Callosum	8	3 20 4	5.699
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, White Matter, Frontal_Inf_Tri_R (aal)	5	54 23 4	4.450
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, White Matter, Insula_R (aal)	8	33 32 4	6.297
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, White Matter, Frontal_Sup_L (aal)	50	-12 56 13	6.755
Right Cerebrum, Frontal Lobe, Sub-Gyral, White Matter	9	39 -1 19	6.992
Right Cerebrum, Frontal Lobe, Sub-Gyral, White Matter	88	36 -22 37	11.312

Left Cerebrum, Parietal Lobe, Inferior Parietal Lobule, White Matter, SupraMarginal_L (aal)	31	-51 -34 34	11.457
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, White Matter, Angular_R (aal)	5	39 -58 28	5.743
Left Cerebrum, Parietal Lobe, Sub-Gyral, White Matter	5	-30 -3 34	4.732
Right Cerebrum, Parietal Lobe, Sub-Gyral, White Matter	70	21 -40 52	8.791
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Precentral_R (aal)	16	39 -7 49	4.571
Right Cerebrum, Parietal Lobe, Superior Parietal Lobule, Gray Matter, Brodmann area 7, Parietal_Sup_R (aal)	30	27 -64 52	5.726
Right Cerebrum, Parietal Lobe, Precuneus, Gray Matter, Brodmann area 7, Precuneus_R (aal)	11	3 -70 52	5.313

Contrast 1: Trauma Listening vs. Neutral Listening
Post-treatment Only

Type: T, df: 6, Threshold - p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, White Matter, undefined, Frontal_Sup_R (aal)	15	24 59 10	7.281
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, White Matter, undefined, Frontal_Mid_L (aal)	8	-27 50 16	4.437
Right Cerebrum, Limbic Lobe, Anterior Cingulate, Gray Matter, Brodmann area 24, Cingulum_Ant_R (aal)	13	3 29 22	4.530
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Mid_L (aal)	26	-27 50 31	12.001
Right Cerebrum, Parietal Lobe, Inferior Parietal Lobule, Gray Matter, Brodmann area 40, SupraMarginal_R (aal)	11	63 -40 31	5.511
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Gray Matter, Brodmann area 9, Frontal_Mid_R (aal)	9	33 41 40	6.090
Left Cerebrum, Parietal Lobe, Precuneus, White Matter, undefined, Precuneus_L (aal)	14	-6 -76 46	5.495

Contrast 2: Trauma Imagery > Neutral Imagery
Pre-treatment Only

Type: T, df: 6, Threshold - p < 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Sub-lobar, Extra-Nuclear, White Matter, undefined, Putamen_L (aal)	8	-21 11 -8	7.646
Left Cerebrum, Sub-lobar, Extra-Nuclear, White Matter, undefined, Thalamus_L (aal)	10	-15 -10 7	6.157
Left Cerebrum, Parietal Lobe, Precuneus, White Matter, undefined, Occipital_Mid_L (aal)	8	-27 -67 34	6.256
Right Cerebrum, Frontal Lobe, Precentral Gyrus, White Matter, undefined, Precentral_R (aal)	11	15 -28 76	6.800

Contrast 2: Trauma Imagery vs Neutral Imagery

Post-treatment Only

Type: T, df: 6, Threshold- *p* value = 0.005 (uncorrected), intensity = none, cluster size = 5
Number of clusters found: 0

OVERT FACES TASK

Contrast 1: Fear > Neutral
Pre-treatment Only

Type: T, df: 6, Threshold - *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Sub-lobar, Thalamus, Gray Matter, Ventral Posterior Lateral Nucleus, Thalamus_L (aal)	20	-18 -19 4	5.940
Right Cerebrum, Sub-lobar, Thalamus, Gray Matter, Medial Dorsal Nucleus, Thalamus_R (aal)	6	6 -16 4	6.894
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, White Matter, undefined, Frontal_Sup_Medial_L (aal)	6	-12 44 19	4.909

Region of Interest (ROI) Analysis

Contrast 1: Fear > Neutral

Pre-treatment, we did not observe significant activation in the amygdala, during the fear condition (PRE only, $p < .005$). Subsequently, region of interest analyses were conducted in the amygdala (ROI threshold level, $p < .05$ uncorrected).

Pre-treatment Only

Region	*p < .05 (uncorr)*	*(FWE) (.05)*
Left Amygdala	**activations**	-
Right Amygdala	**activations**	-

Contrast 1: Fear > Neutral
Post-treatment Only

Type: T, df: 6, Threshold - *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Sup_L (aal)	5	-21 50 1	5.290
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, White Matter, undefined, Frontal_Mid_L (aal)	7	-33 47 13	5.407
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, White Matter, undefined, Frontal_Sup_R (aal)	9	21 47 16	5.566
Right Cerebrum, Parietal Lobe, Postcentral Gyrus, Gray Matter, Brodmann area 43, Postcentral_R (aal)	5	63 -7 19	4.155
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Sup_R (aal)	10	15 56 25	9.394
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, undefined, undefined, Frontal_Sup_Medial_R (aal)	8	6 50 31	8.126
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Gray Matter, Brodmann area 9, Frontal_Sup_Medial_L (aal)	6	-3 32 37	4.995

Contrast 2: Fear > Happy
Pre-treatment Only

Type: T, df: 6, Threshold- p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Occipital Lobe, Fusiform Gyrus, White Matter, undefined, Fusiform_L (aal)	36	-36 -73 -17	5.188
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, White Matter, undefined, Temporal_Inf_L (aal)	5	-51 -55 -14	4.798
Right Cerebrum, Temporal Lobe, Sub-Gyral, White Matter, undefined, Occipital_Inf_R (aal)	5	39 -70 -5	4.915
Right Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Lateral Globus Pallidus, Pallidum_R (aal)	32	18 -4 -2	5.843
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, White Matter, undefined, Insula_R (aal)	12	33 23 7	4.914
Left Cerebrum, Frontal Lobe, Sub-Gyral, White Matter, undefined, Frontal_Inf_Oper_L (aal)	16	-39 8 19	8.855
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Gray Matter, Brodmann area 46, Frontal_Mid_R (aal)	12	48 32 22	6.385
Left Cerebrum, Frontal Lobe, Sub-Gyral, White Matter, undefined, Frontal_Mid_L (aal)	10	-33 32 22	5.178

ROI ANALYSIS

Contrast 2: Fear > Happy

Pre-treatment, we did not observe significant activation in the amygdala, during the fear condition (PRE only, $p < .005$). Subsequently, region of interest analyses were conducted in the amygdala (ROI threshold level, $p < .05$ uncorrected).

Pre-treatment Only

Region	*p < .05 (uncorr)*	*(FWE) (.05)*
Left Amygdala	-	-
Right Amygdala	-	-

Contrast 2: Fear > Happy
Post-treatment Only

Type: T, df: 6, Threshold - *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebellum, Cerebellum Anterior Lobe, Culmen, undefined, undefined, Cerebelum_4_5_L (aal)	22	-9 -46 -20	5.299
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Sup_R (aal)	6	21 53 4	4.684
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Sup_L (aal)	11	-12 56 25	4.673
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Gray Matter, Brodmann area 46, Frontal_Mid_R (aal)	9	48 32 22	5.480
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Gray Matter, Brodmann area 9, Frontal_Sup_Medial_R (aal)	13	12 47 34	6.150

A4.1: ADDITIONAL PRE vs POST-TREATMENT GROUP RESULTS

Whole brain exploratory analyses were also conducted at $p < .005$ for the PRE-POST and POST-PRE comparisons for each task in the propranolol group completers.

SCRIPT-DRIVEN IMAGERY TASK

Contrast 1: Trauma Listening > Neutral Listening
PRE > POST

Type: T, df: 6, Threshold - p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Occipital Lobe, Lingual Gyrus, White Matter, undefined, Occipital_Mid_L (aal)	101	-18 -85 -5	5.429
Right Cerebellum, Cerebellum Anterior Lobe, Culmen, undefined, undefined, Lingual_R (aal)	22	9 -67 -11	4.208
Right Cerebrum, Occipital Lobe, Lingual Gyrus, White Matter, undefined, Lingual_R (aal)	29	24 -76 -2	5.020
Left Cerebrum, Occipital Lobe, Middle Occipital Gyrus, White Matter, undefined, Occipital_Mid_L (aal)	10	-27 -76 1	4.439
Right Cerebrum, Sub-lobar, Lateral Ventricle, Cerebro-Spinal Fluid, undefined, Calcarine_R (aal)	61	30 -67 10	6.460
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, White Matter, undefined, Rolandic_Oper_R (aal)	5	54 2 1	3.963
Right Cerebrum, Frontal Lobe, Sub-Gyral, White Matter, undefined, Precentral_R (aal)	10	36 -10 43	4.514
Left Cerebrum, Frontal Lobe, Sub-Gyral, White Matter, undefined, Paracentral_Lobule_L (aal)	24	-12 -22 61	5.550
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, White Matter, undefined, Paracentral_Lobule_R (aal)	11	6 -25 73	4.444

ROI ANALYSIS

Significant activations were not observed in our a priori regions of interest using whole brain analyses (pre > post, $p < .005$). Subsequently, region of interest analyses were conducted in the amygdala, hippocampus, insula, cingulate and prefrontal cortex regions (ROI threshold level, $p < .05$ uncorrected, no minimum cluster size). Small activations refer to those clusters that disappear at $k = 5$.

Contrast 1: Trauma listening vs. Neutral listening
PRE > POST

Region	***p < .05 (uncorr)***	***p < .01(uncorr)***	***(FWE) (.05)***
Amygdala left			
Amygdala right			
Cingulum ant left	small activations		
Cingulum ant right			
Cingulum mid left	**activations**	**activations**	
Cingulum mid right	**activations**	**activations**	
Cingulum post left	**activations**		
Cingulum post right	**activations**		
Frontal mid left	small activations		
Frontal mid right	**activations**		
Frontal sup medial left	small activations		
Frontal sup medial right	small activations		
Frontal sup left	small activations		
Frontal sup right	**activations**	small activations	
Hippocampus left	small activations		
Hippocampus right	**activations**	small activations	
Insula left	**activations**	**activations**	
Insula right	**activations**	small activations	
ParaHippocampal left			
ParaHippocampal right	**activations**		
Thalamus left	**activations**	small activations	
Thalamus right	small activations		

Contrast 1: Trauma listening vs Neutral listening
POST > PRE

Type: T, df: 6, Threshold- p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Number of clusters found: 0

Contrast 2: Trauma imagery > Neutral imagery
PRE > POST

Type: T, df: 6, Threshold - *p* value = 0.005(uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Limbic Lobe, Cingulate Gyrus, Gray Matter, Brodmann area 31, Precuneus L (aal)	7	-9 -58 25	5.613

ROI ANALYSIS

PRE > POST

Region	*p < .05 (uncorr)*	*p < .01(uncorr)*	*(FWE) (.05)*
Amygdala left	**activations**	**activations**	
Amygdala right	small activations		
Cingulum ant left	small activations		
Cingulum ant right	small activations		
Cingulum mid left	**activations**		
Cingulum mid right	**activations**	small activations	
Cingulum post left	small activations		
Cingulum post right	small activations		
Frontal mid left	**activations**		
Frontal mid right	small activations		
Frontal sup medial left			
Frontal sup medial right			
Frontal sup left	small activations		
Frontal sup right	small activations		
Hippocampus left	**activations**	**activations**	
Hippocampus right	**activations**		
Insula left	**activations**		
Insula right	**activations**	small activations	
ParaHippocampal left	**activations**		
ParaHippocampal right	**activations**		
Thalamus left	**activations**	**activations**	
Thalamus right	**activations**		

Contrast 2: Trauma imagery vs. Neutral imagery
POST > PRE

Type: T, df: 6, Threshold- p value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebrum, Parietal Lobe, Supramarginal Gyrus, Gray Matter, Brodmann area 40, Angular_R (aal)	5	57 -52 31	-5.388

OVERT FACES TASK

Contrast 1: Fear > Happy
PRE > POST

Type: T, df: 6, Threshold - *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Lateral Globus Pallidus, Pallidum_R (aal)	7	18 -1 -5	6.221

POST > PRE

Type: T, df: 6 Threshold- p value = 0.005 (uncorrected); intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebrum, Occipital Lobe, Middle Occipital Gyrus, White Matter, undefined, Occipital_Inf_R (aal)	15	45 -70 -14	-7.864
Right Cerebellum, Cerebellum Anterior Lobe, Cerebellar Lingual, undefined, undefined, Vermis_4_5 (aal)	15	3 -46 -11	-5.062
Right Cerebrum, Occipital Lobe, Lingual Gyrus, undefined, undefined, Calcarine_L (aal)	19	0 -85 -8	-6.856
Right Cerebrum, Limbic Lobe, Parahippocampa Gyrus, White Matter, undefined, ParaHippocampal_R (aal)	5	27 -40 -5	-8.971
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Gray Matter, Brodmann area 22, Temporal_Mid_R (aal)	5	60 -43 1	-4.960
Right Cerebrum, Sub-lobar, Lateral Ventricle, Cerebro-Spinal Fluid, undefined, Calcarine_R (aal)	7	27 -64 4	-5.157
Left Cerebrum, Limbic Lobe, Posterior Cingulate, Gray Matter, Brodmann area 30, Calcarine_L (aal)	11	-15 -67 7	-5.933
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Gray Matter, Brodmann area 39, Temporal_Mid_L (aal)	5	-45 -55 10	-4.780
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, White Matter, undefined, Temporal_Mid_R (aal)	6	63 -49 10	-4.352
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Gray Matter, Brodmann area 10, Frontal_Mid_R (aal)	5	24 56 28	-4.335
Right Cerebrum, Occipital Lobe, Cuneus, Gray Matter, Brodmann area 19, Occipital_Sup_R (aal)	9	21 -85 28	-4.133
Right Cerebrum, Parietal Lobe, Inferior Parietal Lobule, Gray Matter, Brodmann area 40, SupraMarginal_R (aal)	16	66 -28 28	-4.944
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, White Matter, undefined, Frontal_Mid_L (aal)	11	-42 14 43	-4.239
Inter-Hemispheric, undefined, undefined, undefined, undefined, Supp_Motor_Area_L (aal)	13	0 8 61	-4.763

Contrast 2: Fear > Neutral
PRE > POST

Type: T, df: 6, Threshold - *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Right Cerebrum, Sub-lobar, Thalamus, Gray Matter, Medial Dorsal Nucleus, Thalamus R (aal)	6	6 -16 4	5.276

POST > PRE

Type: T, df: 6, Threshold- *p* value = 0.005 (uncorrected), intensity = 3.7074, cluster size = 5

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Putamen, Putamen_L (aal)	93	-21 5 1	-9.523
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, White Matter, undefined, Temporal_Mid_R (aal)	6	54 -34 -5	-5.316
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Sup_R (aal)	162	21 53 1	-11.052
Left Cerebrum, Temporal Lobe, Transverse Temporal Gyrus, White Matter, undefined, Temporal_Sup_L (aal	20	-51 -16 10	-4.540
Right Cerebrum, Sub-lobar, Lentiform Nucleus, Gray Matter, Putamen, Pallidum_R (aal)	14	15 5 1	-5.860
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Gray Matter, Brodmann area 41, Temporal_Sup_R (aal)	58	39 -31 13	-7.307
Left Cerebrum, Sub-lobar, Insula, White Matter, undefined, Insula_L (aal)	5	-33 -13 10	-4.825
Right Cerebrum, Frontal Lobe, Precentral Gyrus, White Matter, undefined, Frontal_Inf_Oper_R (aal)	9	54 8 7	-5.067
Left Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, White Matter, undefined, Frontal_Inf_Oper_L (aal)	12	-51 14 13	-6.477
Left Cerebrum, Frontal Lobe, Precentral Gyrus, White Matter, undefined, Precentral_L (aal)	5	-48 5 13	-4.842
Left Cerebrum, Parietal Lobe, Inferior Parietal Lobule, White Matter, undefined, Temporal_Sup_L (aal)	52	-54 -37 22	-8.720
Left Cerebrum, Parietal Lobe, Sub-Gyral, White Matter, undefined, Occipital_Sup_L (aal)	6	-21 -64 25	-5.359
Right Cerebrum, Frontal Lobe, Precentral Gyrus, White Matter, undefined, Postcentral_R (aal)	6	51 -10 28	-4.468

Contrast 2: Fear > Neutral (Continued)
POST > PRE

Cluster / Overlapping regions/ Peak MNI coordinate region	Number of voxels	Peak MNI coordinates	Peak intensity
Left Cerebrum, Parietal Lobe, Precuneus, Gray Matter, Brodmann area 7, Precuneus_L (aal)	135	-6 -82 46	-8.151
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, White Matter, undefined, Frontal_Mid_L (aal)	12	-21 47 31	-8.040
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Gray Matter, Brodmann area 6, Cingulum_Mid_R (aal)	54	6 26 40	-6.585
Right Cerebrum, Parietal Lobe, Inferior Parietal Lobule, Gray Matter, Brodmann area 40, Parietal_Inf_R (aal)	28	57 -55 40	-5.395
Left Cerebrum, Parietal Lobe, Postcentral Gyrus, Gray Matter, Brodmann area 3, Postcentral_L (aal)	20	-45 -22 40	-9.452
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Gray Matter, Brodmann area 9, Frontal_Mid_L (aal)	6	-30 32 40	-4.801
Right Cerebrum, Parietal Lobe, Precuneus, Gray Matter, Brodmann area 7, Precuneus_R (aal)	8	12 -52 40	-4.377
Right Cerebrum, Limbic Lobe, Cingulate Gyrus, White Matter, undefined, Cingulum_Mid_R (aal)	216	9 -16 43	-12.535
Left Cerebrum, Parietal Lobe, Postcentral Gyrus, Gray Matter, Brodmann area 3, Postcentral_L (aal)	6	-42 -22 58	-4.173
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Gray Matter, Brodmann area 6, Frontal_Mid_L (aal)	8	-30 17 61	-4.999
undefined, undefined, undefined, undefined, undefined, Supp_Motor_Area_L (aal)	6	-3 23 64	-6.898
Left Cerebrum, Parietal Lobe, Postcentral Gyrus, White Matter, undefined, Parietal_Sup_L (aal)	9	-15 -52 70	-4.131

APPENDIX V: Individual Participant Imaging Data Reports

The imaging results for each participant were submitted to t-contrasts (first level of analysis) at PRE-ONLY and POST-ONLY for each task.

SCRIPT-DRIVEN IMAGERY TASK

PARTICIPANT 1:

Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p< .001, nb vox min = 5

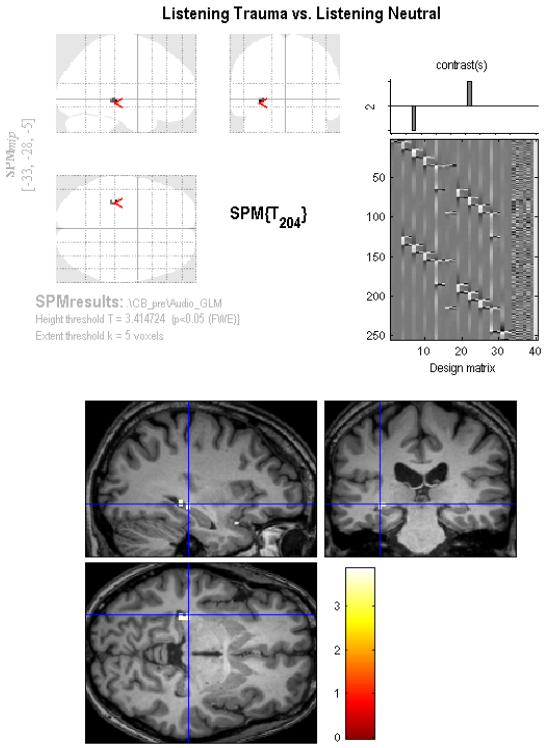
Clusters		**Peaks inside the cluster**							
							MNI coordinates (mm)		
Structures overlapped	*nb vox*	*p (FWE-corr)*	*p (FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Lobe, brodmann area 37, Fusiform_L (aal)	364	0.069	0.246	4.80	4.67	< .001	-42	-64	-20
		0.470	0.43	4.21	4.11	< .001	-48	-52	-23
		0.631	0.43	4.07	3.99	< .001	0	-73	-17
Right Cerebrum, Temporal Lobe, Inferior Temporal Gyrus, brodmann area 20, Temporal_Inf_R (aal)	169	0.190	0.362	4.52	4.41	< .001	51	-52	-20
		0.514	0.43	4.17	4.08	< .001	21	-52	-23
		0.633	0.43	4.07	3.98	< .001	54	-43	-17
Right Cerebrum, Occipital Lobe, Fusiform Gyrus, Cerebelum_6_R (aal)	45	0.360	0.43	4.31	4.21	< .001	36	-73	-20
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, brodmann area 10, Frontal_Sup_Orb_L (aal)	41	0.827	0.48	3.89	3.82	< .001	-30	62	-2
		0.838	0.48	3.88	3.81	< .001	-18	71	4
Left Cerebrum, Sub-lobar, Caudate, Caudate Tail, Hippocampus_L (aal)	41	0.867	0.494	3.85	3.78	< .001	-33	-28	-5
		0.988	0.716	3.58	3.52	< .001	-39	-37	-5
Left Cerebrum, Parietal Lobe, Inferior Parietal Lobule, Angular_L (aal)	29	0.894	0.515	3.81	3.74	< .001	-39	-55	40
		0.984	0.716	3.6	3.54	< .001	-42	-61	46
Right Cerebrum, Sub-lobar, Caudate, Caudate Tail, Hippocampus_R (aal)	16	0.988	0.716	3.58	3.52	< .001	33	-28	-8
		0.999	0.885	3.28	3.23	< .001	33	-34	1
Left Cerebrum, Sub-lobar, Caudate, Caudate Body, Caudate_L (aal)	15	0.993	0.719	3.53	3.48	< .001	-3	11	7
		0.999	0.885	3.24	3.2	< .001	12	17	7
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_Orb_R (aal)	14	0.999	0.745	3.41	3.36	< .001	30	59	-2
Left Cerebellum, Cerebellum Anterior Lobe 11	6	0.999	0.752	3.37	3.32	< .001	-9	-55	-26
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Temporal_Sup_R (aal)	9	0.999	0.796	3.34	3.29	< .001	51	-19	-2

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 1:

ROI ANALYSIS: ***Trauma Listening vs. Neutral Listening (PRE-ONLY)***

Clusters		**Peaks inside the cluster**					**MNI Coordinates (mm)**		
ROI	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Hippocampus (aal)	9	0.013	0.437	3.85	3.78	< .001	-33	-28	-5



Left Hippocampus

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<.001, nb vox min = 5

Clusters		Peaks inside cluster					MNI Coordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Parietal Lobe, Inferior Parietal Lobe, Gray Matter, Brodmann area 40, Supramarginal_L	26	0.290	0.608	4.29	4.19	< .001	-63	-40	34
Right Cerebrum, Parietal Lobe, Supramarginal Gyrus, Gray Matter, Brodmann area 40, Parietal_Inf_R (aal)	6	0.294	0.608	4.28	4.19	< .001	60	-46	37
Inter-hemispheric, Cingulum_Mid_L(aal)	6	0.342	0.608	4.23	4.13	< .001	0	-4	46
Left Cerebrum, , Limbic Lobe, Cingulate Gyrus, Cingulate_Mid_L (aal)	13	0.548	0.729	4.04	3.95	< .001	-9	-34	43
		0.574	0.729	4.01	3.93	< .001	0	-34	49

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 1:

Contrast 2: Trauma Imagery vs Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Lobe, Brodmann area 37, Fusiform_L (aal)	165	0.954	0.975	3.70	3.64	< .001	-39	-64	-20
Right Cerebellum, Cerebellum Anterior Lobe, Culmen, Cerebellum_6_R(aal)	75	0.998	0.975	3.46	3.40	< .001	30	-49	-20
		0.999	0.975	3.18	3.14	< .001	54	-49	-17
		0.999	0.975	3.07	3.03	< .001	21	-52	-23
Right Cerebrum, Limbic Lobe, Parahippocampal gyrus, Parahippocampal_R (aal)	9	0.999	0.975	3.35	3.31	< .001	15	-10	22
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Precentral_R (aal)	8	0.999	0.975	2.94	2.90	< .002	45	-1	40
Right Cerebrum, Transverse Temporal Lobe, Heschl Gyrus_R (aal)	7	0.999	0.975	2.93	2.90	< .002	33	-31	-11
Right Cerebrum, Sub-lobar, Extra-Nuclear Gyrus, Hippocampal_R(aal)	7	0.999	0.975	2.87	2.84	< .002	21	-16	-11

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Para-central Lobe, Brodmann area 5, Precuneus_R (aal)	9	0.993	0.761	3.41	3.36	< .001	9	-46	58
		0.996	0.761	3.37	3.32	< .001	15	-40	55
Right Cerebrum, Sub-lobar, Claustrum, Insula_R (aal)	5	0.998	0.761	3.32	3.28	< .001	36	-16	-5

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 2:

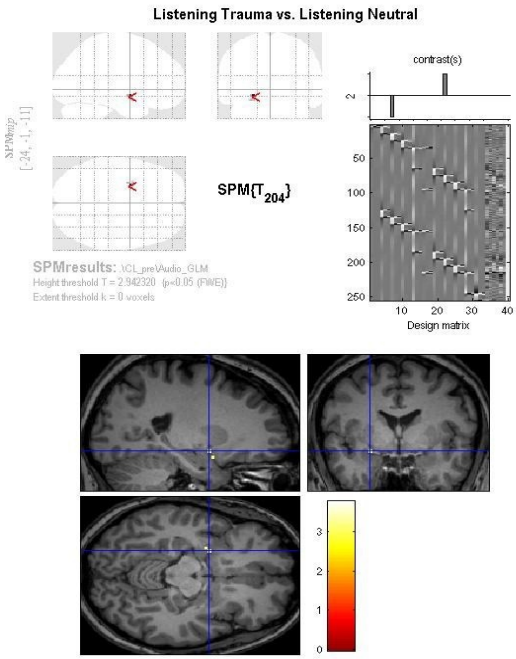
Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p< .001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Sub-lobar, Claustrum, Insula_R (aal)	128	0.998	0.761	3.32	3.28	< .001	36	-16	-5
*Right Cerebrum, Limbic Lobe, Posterior Cingulate, Precuneus_R(aal)	92	0.003 0.004	0.304 0.304	5.51 5.45	5.32 5.26	< .001 < .001	6 -6	-52 -58	7 4
*Left Cerebrum , Occipital Lobe, Cuneus, Brodmann area 23, Calcarine_L(aal)	17	0.018	0.611	5.1	4.94	< .001	-3	-76	-8
*Right Cerebrum, Temporal Lobe, Sub-gyral, Occipital_Sup_R(aal)	6	0.018	0.611	5.09	4.94	< .001	27	-67	22
*Left Cerebrum , Occipital Lobe, Cuneus, Brodmann area 18, Cuneus_L(aal)	5	0.034	0.760	4.94	4.79	< .001	-9	-82	13

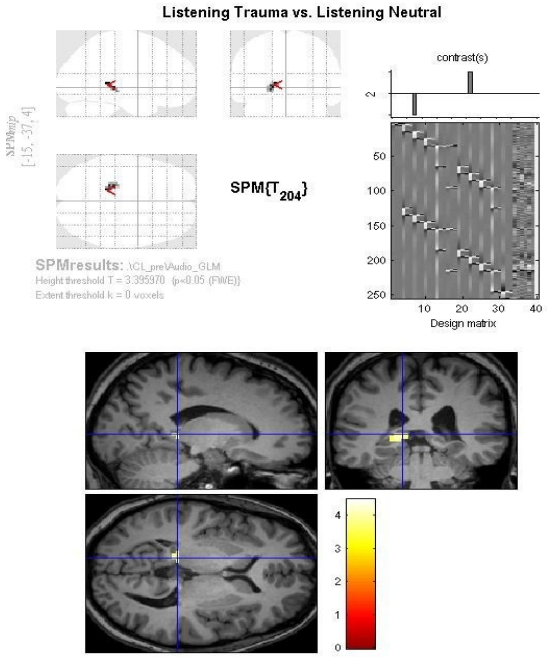
Indicates regions which survived at p < .05 (FWE)

ROI ANALYSIS: ***Trauma Listening vs. Neutral Listening (PRE- ONLY)***

Cluster		**Peaks within Clusters**					**MNI Coordinates (mm)**		
ROI	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Amygdala (aal)	2	0.004	0.255	3.77	3.70	< .001	-24	-1	-11
	3	0.034	0.668	3.09	3.05	< .001	-27	2	-17
Left Hippocampus (aal)	30	0.001	0.055	4.48	4.37	< .001	-15	-37	4
		0.001	0.055	4.46	4.35	<.001	-18	-31	-2



Left Amygdala



Left Hippocampus

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
N/A	-	-	-	-	-	-	-	-	-

No supra-threshold voxels survived at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Cingulate, Precentral_R(aal)	19	0.913	0.975	3.72	3.65	< .001	51	-10	40

Right Cerebrum, Frontal Lobe, Precentral Gyrus, Cingulate, Postcentral_R(aal)	7	0.973	0.975	3.58	3.52	< .001	60	-1	37
Right Cerebellum, Cerebellum Declive, Cerebellum_6_R(aal)	5	0.999	0.975	3.15	3.11	<.001	12	-64	-23
Left Cerebrum , Occipital Lobe, Cuneus, Calcarine_L(aal)	7	0.994	0.975	3.45	3.4	< .001	-3	-94	1
Interhemispheric, Supp_Motor_Area_L(aal)	5	0.996	0.975	3.42	3.37	< .001	0	-13	58
		0.999	0.975	3.2	3.16	<.001	-12	-61	-29

No supra-threshold voxels survived at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (POST-ONLY) p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vo*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L(aal)	11	0.808	0.987	3.81	3.74	< .001	-27	41	22
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Supp_Motor_Area_R (aal)	55	0.876	0.987	3.73	3.66	< .001	9	-5	73
		0.994	0.987	3.44	3.36	< .001	-3	-52	70
Right Cerebrum, Limbic Lobe, Parahippocampal Gyrus, Fusiform_R(aal)	11	0.996	0.987	3.37	3.32	< .001	33	-49	-8

No suprathreshold voxels survived at p < .05 (FWE)

PARTICIPANT 3:

Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY) p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Parietal Lobe, Postcentral Gyrus, Rolandic_Oper_R (aal)	37	0.245	0.274	4.28	4.18	< .001	60	-16	13
Right Cerebrum, Parietal Lobe, Inferior Parietal Gyrus, Parietal_Inf_R (aal)	51	0.267	0.274	4.25	4.15	< .001	57	-37	34
		0.444	0.295	4.06	3.97	< .001	54	-37	43
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Brodmann area 44, Frontal_Inf_Oper_R (aal)	121	0.487	0.295	4.02	3.93	< .001	54	14	7
		0.75	0.403	3.78	3.71	< .001	42	17	4
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, Frontal_Inf_Oper_R (aal)	37	0.788	0.403	3.74	3.68	< .001	48	11	19

No suprathreshold clusters at p < .05 (FWE)

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p< .001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Parietal Lobe, Postcentral Gyrus, Rolandic_Oper_R(aal)	32	0.274	0.212	4.24	4.14	< .001	60	-16	13
Right Cerebrum, Parietal Lobe, Inferior Parietal Lobe, SuperMarginal_R(aal)	47	0.337	0.212	4.16	4.07	< .001	57	-37	34
		0.460	0.212	4.04	3.96	< .001	54	-37	43
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Brodmann area 44, Frontal_Inf_Oper_R (aal)	111	0.504	0.212	4.00	3.92	< .001	51	17	7
		0.635	0.254	3.89	3.81	< .001	48	14	22
Right Cerebrum, Sub-lobar, Lentiform Nucleus, Putamen, Putamen_R (aal)	7	0.863	0.430	3.66	3.60	< .001	24	11	7

No suprathreshold clusters at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (PRE-ONLY)
p< .001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Sub-lobar, Insula, Brodmann 13, Insula_R(aal)	32	0.657	0.321	3.87	3.79	< .001	39	17	4
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_R (aal)	17	0.817	0.321	3.71	3.65	< .001	39	38	22

No suprathreshold clusters at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (POST-ONLY)
p< .001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Sub-lobar, Insula, Brodmann 13, Insula_R(aal)	28	0.442	0.294	4.06	3.97	< .001	39	17	4
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_R (aal)	16	0.845	0.470	3.68	3.62	< .001	39	35	22

No suprathreshold clusters at p < .05 (FWE)

PARTICIPANT 4:

Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Parietal Lobe, Postcentral Gyrus, Precuneus_L (aal)	5	0.999	0.924	2.79	2.76	<.003	-15	-37	67

No suprathreshold clusters at p < .05 (FWE)

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Frontal_Sup_Medial_L (aal)	98	0.087	0.291	4.69	4.57	< .001	-9	32	34
		0.897	0.612	3.74	3.68	< .001	3	29	46
		0.964	0.626	3.61	3.55	< .001	9	29	31
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Frontal_Medial_L (aal)	47	0.405	0.612	4.21	4.12	< .001	-33	56	10
		0.872	0.612	3.78	3.71	< .001	-21	47	7
		0.993	0.789	3.47	3.42	< .001	-24	62	13
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Cingulum_Ant_R(aal)	59	0.695	0.612	3.96	3.88	<.001	12	47	7
		0.730	0.612	3.93	3.85	<.001	0	59	1

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Parietal Lobe, Postcentral Gyrus, Postcentral _R(aal)	50	0.999	0.998	2.87	2.84	<.002	12	-37	73
Left Cerebrum, Temporal Lobe, Sub-gyral, Lingual_L(aal)	13	0.999	0.998	2.54	2.52	<.006	-18	56	-2
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 41, Temporal_Sup_L (aal)	27	0.999	0.998	2.28	2.27	<.012	-39	-16	10

Right Cerebrum, Limbic Lobe, Parahippocampal Gyrus, Brodmann area 30, Lingual_R(aal)	16	0.999	0.998	2.25	2.24	<.013	18	53	-2
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Insula_R (aal)	35	0.999	0.998	2.2	2.19	<.014	48	-4	1
Left Cerebrum, Sub-lobar Insula, Rolandic_Oper_L (aal)	27	0.999 0.999	0.998 0.998	2.11 2.09	2.10 2.08	<.018 <.019	-45 -42	-1 11	1 -2
Right Cerebrum, Sub-lobar Extra-Nuclear, Putamen_R(aal)	10	0.999	0.998	2.04	2.03	<.021	24	20	-8
Inter-hemispheric, Lingual_R(aal)	6	0.999	0.998	2.01	2.00	<.023	6	-37	1
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_R(aal)	20	0.999	0.998	1.99	1.98	<.024	24	17	55
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Frontal_Sup_Med_L(aal)	5	0.999	0.998	1.86	1.86	<.032	-9	-49	-5

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Inter-hemispheric, Superior Frontal Gyrus, Frontal_Sup_Medial_R(aal)	7	0.999	0.994	1.92	1.91	<.028	6	62	31

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 5:

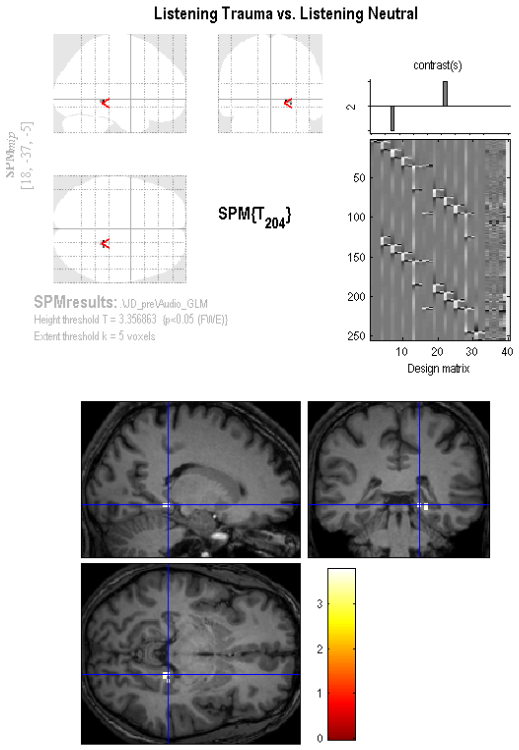
Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Mid_L (aal)	8	0.004	0.492	5.38	5.19	<.001	-51	-58	4
*Left Cerebrum, Sub-lobar, Left Extra_Nuclear, Corpus Callosum, Precuneus_L (aal)	7	0.025	0.626	4.95	4.80	< .001	-12	-46	7
*Right Cerebrum, Limbic Lobe, Cingulate Gyrus, Brodmann area 31, Cingulum_Mid_R (aal)	5	0.026	0.626	4.93	4.79	< .001	6	-34	43

**Indicates areas that survived at p < .05 (FWE)*

ROI ANALYSIS: ***Trauma Listening vs. Neutral Listening (PRE-ONLY)***

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
ROI	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Para-hippocampal Gyrus (aal)	5	0.014	0.421	3.77	3.70	< .001	18	-37	-5



Right Parahippocampal Gyrus

PARTICIPANT 5:

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Sub-lobar, Lenticular Nucleus, Lateral Globus Pallidus	12	0.658	0.659	3.90	3.83	< .001	-18	-4	7

Left Cerebrum, Sub-lobar, Thalamus, Pulvinar, Thalamus_L (aal)	23	0.669	0.659	3.89	3.82	< .001	-15	-25	-5
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Brodmann area 9, Frontal_Mid_L (aal)	8	0.770	0.659	3.80	3.73	< .001	-36	29	49
Right Cerebrum, Sub-lobar, Left Extra_Nuclear, Optic Tract	7	0.960	0.808	3.53	3.47	< .001	24	-19	-5

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Parietal Lobe, Precuneus, Brodmann area 7, Precuneus_R(aal)	24	0.886	0.850	3.66	3.60	< .001	6	-70	52
		0.999	0.945	3.23	3.19	<.001	12	-76	49
Right Cerebrum, Frontal Lobe, Superior Frontal Lobe, Frontal_Mid_Orb_R (aal)	7	0.936	0.850	3.58	3.52	< .001	12	62	-5

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Mid_R (aal)	11	0.998	0.939	3.29	3.24	<.001	60	-28	-8
		0.999	0.939	2.86	2.83	< .002	60	-31	1
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 38, Temporal_Sup_L (aal)	7	0.999	0.939	3.20	3.15	<.001	-48	11	-17
		0.936	0.850	3.58	3.52	< .001	12	62	-5

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 6:

Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Parietal Lobe, Postcentral Gyrus, Brodmann area 40, SupraMarginal_L(aal)	67	0.143	0.265	4.34	4.24	<. 001	-66	-22	19
		0.733	0.818	3.67	3.61	< .001	-60	-28	4
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Sup_R(aal)	44	0.463	0.714	3.92	3.85	< .001	60	-55	10
		0.905	0.818	3.47	3.41	< .001	63	-46	10
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Mid_L(aal)	21	0.571	0.728	3.82	3.75	<.001	-45	-10	-5
Left Cerebrum, Frontal Lobe, Precentral Gyrus, Frontal_Inf_Oper_L(aal)	13	0.782	0.818	3.62	3.56	<.001	-48	11	10
Left Cerebrum, Sub-lobar, Insula, Brodmann area 13, Rolandic_Oper_L(aal)	6	0.917	0.818	3.45	3.39	<.001	-42	-1	13
Right Cerebrum, Sub-lobar, Insula, Brodmann area 13, Insula_R(aal)	9	0.966	0.839	3.33	3.28	<.001	42	-4	-5

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Superior Frontal Lobe, Brodmann area 8, Frontal_Mid_Orb_R(aal)	12	0.991	0.867	3.13	3.09	<.001	12	35	58

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Brodmann area 21, TemporaL_Mid_L(aal)	14	0.999	0.985	2.78	2.75	<.003	-60	-1	-26
		0.999	0.985	2.75	2.72	<.003	-54	8	-20

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Trauma Imagery vs. Neutral Imagery (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
N/A	-	-	-	-	-	-	-	-	-

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 7:

Contrast 1: Trauma Listening vs. Neutral Listening (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Rectus_R (aal)	16	0.878	0.329	3.73	3.66	< .001	12	23	-11
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Brodmann area 21, Temporal_Mid_L (aal)	5	0.999	0.915	3.22	3.18	< .001	-66	-49	-5

No suprathreshold voxels survived at p < .05 (FWE)

Contrast 1: Trauma Listening vs. Neutral Listening (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
N/A	-	-	-	-	-	-	-	-	-

No suprathreshold voxels survived at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_R (aal)	8	0.999	0.996	2.83	2.80	0.003	15	50	25

No suprathreshold voxels survived at p < .05 (FWE)

Contrast 2: Trauma Imagery vs Neutral Imagery (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
N/A	-	-	-	-	-	-	-	-	-

No suprathreshold voxels survived at p < .05 (FWE)

OVERT FACES TASK

PARTICIPANT 1:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Sub-lobar, Caudate, Caudate Body, Caudate_L (aal)	11	0.999	0.976	2.84	2.81	< .002	-12	20	10
Right Cerebrum, Parietal Lobe, Precuneus, Precuneus_R (aal)	7	0.999	0.976	2.57	2.55	< .005	12	-52	37

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
N/A	-	-	-	-	-	-	-	-	-

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Limbic Lobe, Parahippocampal Gyrus, Hippocampus_L (aal)	7	0.999	0.974	2.87	2.84	< .002	-30	-22	-17
Right Cerebrum, Limbic Lobe, Parahippocampal Gyrus, Brodmann 36, Parahippocampal_R (aal)	6	0.999	0.974	2.73	2.70	< .003	24	-28	-20

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (POST-ONLY)
p< .001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Gyrus, Middle Temporal Lobe, Brodmann area 21, Temporal_Mid_L (aal)	12	0.999 0.999	0.979 0.979	2.79 2.64	2.76 2.62	0.003 0.004	-63 -60	-55 -61	1 10
Left Cerebrum, Parietal Gyrus, Inferior Parietal Lobe, Brodmann area 40, Parietal_Inf L(aal)	5	0.999	0.979	2.5	2.48	0.007	-57	-40	43

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 2:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_L(aal)	8	0.999	0.991	3.15	3.11	<.001	-21	56	10
Right Cerebrum, Frontal Lobe, Sub-gyral, Corpus Callosum	5	0.999	0.991	2.81	2.78	<.003	12	32	1
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Angular_L (aal)	9	0.999	0.991	2.77	2.74	<.003	-42	-67	28
Left Cerebrum, Sublobar, Insula, Rolandic_Oper_L (aal)	8	0.999 0.999	0.991 0.991	2.75 2.73	2.72 2.71	<.003 <.003	-39 -30	-22 -22	13 13

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Lobe, Inferior Temporal_Inf_L (aal)	20	0.745 0.999	0.956 0.956	3.91 3.27	3.83 3.23	< .001 < .001	-57 -60	-34 -22	22 22

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Frontal_Sup_Medial_R (aal)	13	0.165	0.122	4.58	4.46	< .001	12	62	13
Interhemispheric, Cingulum_Ant_L (aal)	5	0.998	0.588	3.48	3.43	< .001	0	35	-2

No suprathreshold voxels at p < .05 (FWE)

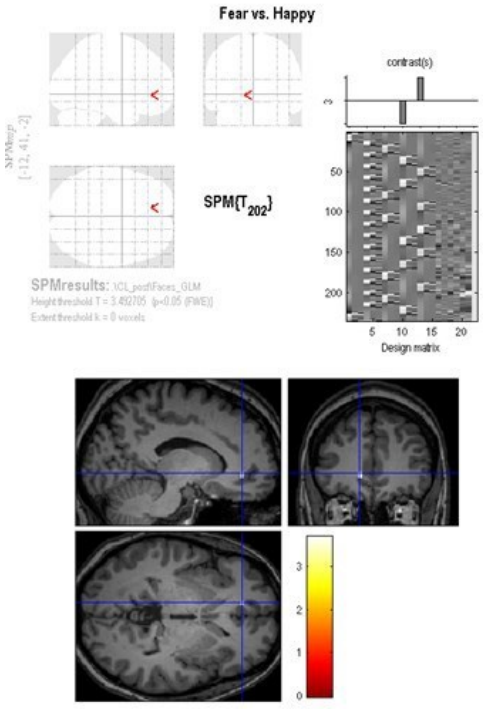
Contrast 2: Fear vs Happy (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Sub-gyral, Cingulum_Anterior_R (aal)	32	0.840 0.993	0.547 0.598	3.81 3.46	3.74 3.41	< .001 < .001	12 24	38 41	1 -11
Left Cerebrum, Cerebellum Posterior Lobe, Cerebellar Tonsil, Cerebellum _9_L(aal)	15	0.881	0.547	3.76	3.69	< .001	-6	-49	-8
Right Cerebrum, Frontal Lobe, Sub-gyral, Caudate_R (aal)	9	0.983	0.56	3.54	3.48	< .001	15	23	-5

No suprathreshold voxels at p < .05 (FWE)

ROI ANALYSIS: ***Fear vs Happy (POST-ONLY)***

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
ROI	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Sub-gyral, Cingulum_Anterior_L (aal)	8	0.016	0.498	3.81	3.74	< .001	-12	41	-2



Left Anterior Cingulate

PARTICIPANT 3:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Brodmann area 22, Temporal_Mid_R (aal)	76	0.974 0.999	0.586 0.928	3.72 3.12	3.65 3.08	< .001 <.001	60 57	-34 -52	4 7
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Mid_L (aal)	12	0.999	0.928	3.19	3.15	<.001	-57	-43	7
Left Cerebrum, Sub-lobar, Extra-nuclear, Caudate_L (aal)	5	0.999	0.978	2.75	2.73	<.003	-18	8	22

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster				MNI Co-ordinates (mm)			
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L (aal)	14	0.864	0.647	3.84	3.77	< .001	-30	56	25
Right Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Brodmann area 22, Temporal_Sup_R (aal)	11	0.903	0.647	3.79	3.72	< .001	60	-34	1
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_R(aal)	14	0.929	0.647	3.75	3.68	< .001	15	59	28
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L(aal)	7	0.978	0.647	3.62	3.56	< .001	-42	44	25
Left Cerebrum, Frontal Lobe, Sub-gyral, Frontal_Inf_Tri_L (aal)	22	0.981	0.647	3.61	3.55	< .001	-36	17	28

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_R (aal)	41	0.149	0.066	4.65	4.52	< .001	45	26	31
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Mid_R (aal)	29	0.771	0.304	4.01	3.93	< .001	57	-52	7

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Mid_R (aal)	110	0.592	0.321	4.09	4.01	<.001	60	-55	10
		0.982	0.775	3.60	3.54	<.001	66	-37	-2
		0.999	0.874	3.04	3.00	<.001	69	-34	10
Right Cerebrum, Occipital Lobe, Fusiform Gyrus, Fusiform_R (aal)	120	0.671	0.321	4.03	3.94	<.001	45	-67	-17
		0.999	0.874	3.31	3.26	<.001	45	-52	-23
		0.999	0.874	3.21	3.17	<.001	36	-88	-5
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Brodmann area 22, Temporal_Mid_L (aal)	14	0.999	0.874	3.30	3.26	<.001	-60	-52	-2

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 4:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Limbic Lobe, Sub-Anterior Cingulate, Brodmann area 32, Cingulum_Anterior_L (aal)	29	0.527	0.725	4.06	3.97	< .001	-12	35	22
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, Insula_R (aal)	22	0.594	0.725	4.00	3.92	< .001	42	26	-2
Right Cerebrum, Cerebellum Posterior Lobe, Cerebellar Tonsil, Cerebellum _9_R(aal)	43	0.597	0.725	4.00	3.91	< .001	9	-58	-41
		0.967	0.898	3.55	3.49	< .001	-6	-55	-41
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Mid_L (aal)	14	0.831	0.898	3.78	3.71	< .001	-51	-16	-5
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Inf_L (aal)	9	0.914	0.898	3.67	3.61	< .001	-42	2	-32
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Mid_L (aal)	10	0.975	0.898	3.53	3.47	< .001	-51	2	-17
		0.995	0.898	3.39	3.34	< .001	-57	-4	-17
Right Cerebrum, Cerebellum Anterior Lobe, Culmen, Cerebellum _Crus1_R(aal)	7	0.987	0.898	3.46	3.41	< .001	42	-46	-29
		0.999	0.898	3.21	3.17	< .001	33	-49	-26
Left Cerebrum, Cerebellum Posterior Lobe, Declive, Cerebellum _Crus1_L(aal)	5	0.996	0.898	3.38	3.33	< .001	-3	-73	-23
Inter-hemispheric, Precuneus_L(aal)	6	0.996	0.898	3.37	3.32	< .001	0	-67	-38
Right Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, Frontal_Mid_R (aal)	7	0.999	0.898	3.28	3.23	< .001	42	44	4

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Temporal Lobe, Middle Temporal Gyrus, Temporal_Inf_L (aal)	7	0.062	0.734	4.82	4.69	< .001	-54	-22	-5
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_R (aal)	12	0.105	0.803	4.68	4.56	< .001	30	44	13

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Brodmann area 10, Frontal_Mid_L(aal)	16	0.639	0.561	3.96	3.88	< .001	-33	53	4
Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 22, Temporal_Mid_R (aal)	6	0.988	0.561	3.45	3.40	< .001	42	23	-2
Right Cerebrum, Occipital Lobe, Lingual Gyrus, Brodmann area 18, Calcarine_L (aal)	9	0.99	0.561	3.44	3.39	< .001	3	-88	-8

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Limbic Lobe, Anterior Cingulate, Brodmann 10, Frontal_Sup_Med_R (aal)	21	0.470	0.522	4.20	4.10	< .001	9	50	1
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_L (aal)	7	0.558	0.522	4.12	4.03	< .001	-12	20	55
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Temporal_Mid_L (aal)	19	0.661	0.522	4.03	3.95	< .001	-66	-40	7
Right Cerebrum, Limbic Lobe, Cingulate Gyrus, Cingulum_Mid_R (aal)	7	0.974	0.889	3.63	3.57	< .001	12	17	43

Left Cerebrum, Limbic Lobe, Anterior Cingulate, Brodmann area 32, Cingulum_Mid_L (aal)	8	0.976	0.889	3.63	3.57	< .001	-6	35	25
Left Cerebrum, Limbic Lobe, Parahippocampal Gyrus, Fusiform _L (aal)	28	0.981	0.889	3.61	3.55	< .001	-27	-16	-32
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Brodmann area 38, Temporal_Pole_Mid_L (aal)	16	0.993	0.889	3.53	3.47	< .001	-33	8	-35
		0.999	0.935	3.25	3.20	< .001	-30	-1	-26
Left Brainstem, Pons, Cerebellum_4_5_L	6	0.998	0.889	3.45	3.40	< .001	-15	-28	-26
Left Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Frontal_Sup_Med_L (aal)	8	0.999	0.889	3.34	3.29	< .001	-9	59	10
		0.999	0.924	3.27	3.22	< .001	-9	53	4

No suprathreshold voxels at p < .05 (FWE)

PARTICIPANT 5:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p((FWE)-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Brodmann area 6, Supp_Motor_Area_L (aal)	18	0.999	0.999	3.24	3.20	<.001	-12	8	70
Right Cerebrum, Cerebellum Posterior Lobe, Uvula, Cerebellum_8_R(aal)	7	0.999	0.999	2.79	2.76	<.003	18	-73	-44

No suprathreshold voxels at p < .05 (FWE)

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Parietal Lobe, SupraMarginal Gyrus, SupraMarginal_L (aal)	108	0.111	0.15	4.54	4.43	< .001	-54	-43	31
		0.997	0.893	3.3	3.25	< .001	-51	-58	43
		0.997	0.893	3.28	3.24	< .001	-45	-34	37
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Brodmann area 10, Frontal_Mid_L (aal)	32	0.661	0.549	3.89	3.82	< .001	-33	47	28
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_R (aal)	13	0.881	0.702	3.67	3.60	< .001	15	50	31
Left Cerebrum, Cerebellum Posterior Lobe, Cerebellar Tonsil, Cerebellum_8_L(aal)	16	0.898	0.702	3.64	3.58	< .001	-36	-52	-47

Left Cerebrum, Occipital Lobe, Fusiform Gyrus, Brodmann area 19, Fusiform _L (aal)	10	0.959	0.756	3.52	3.47	< .001	-27	-70	-14
Right Cerebrum, Frontal Lobe, Precentral Gyrus, Brodmann area 6, Precentral_R (aal)	6	0.962	0.756	3.51	3.46	< .001	51	-4	37
Left Cerebrum, Sub-lobar, Insula, Insula_L (aal)	12	0.982	0.786	3.44	3.38	< .001	-36	14	7

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Limbic Lobe, Cingulate Gyrus	12	0.993	0.846	3.46	3.41	< .001	15	-13	31
Left Cerebrum, Limbic Lobe, Cingulate Gyrus	5	0.993	0.846	3.46	3.41	< .001	-15	-10	31

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Brodmann area 10, Frontal__Medial (aal)	19	0.001	0.007	5.65	5.44	< .001	-18	68	4
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Brodmann area 10, Frontal__Sup_Med_R (aal)	5	0.300	0.639	4.23	4.14	< .001	3	56	4
Left Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Temporal_Sup_L (aal)	5	0.408	0.705	4.12	4.03	< .001	-60	-46	16

**Indicates region that survived at p < .05 (FWE)*

PARTICIPANT 6:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Brodmann area 10, Frontal_Sup_Medial_R (aal)	24	< .001	0.002	6.38	6.08	< .001	9	68	13
		0.002	0.05	5.55	5.35	< .001	-3	65	16

**Indicates region that survived p < .05 (FWE)*

Contrast 1: Fear vs Neutral (POST-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Temporal_Mid_R (aal)	13	0.799	0.454	3.69	3.62	<.001	69	-46	4

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (PRE-ONLY)
p<0.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Frontal_Sup_Med_L (aal)	26	< .001	0.026	5.85	5.61	< .001	-6	65	16
		0.002	0.065	5.49	5.30	< .001	9	68	13
		0.004	0.084	5.34	5.16	< .001	18	65	10

**Indicates region that survived at p < .05 (FWE)*

Contrast 2: Fear vs Happy (POST-ONLY)
p<0.0001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Right Cerebrum, Temporal Lobe, Superior Temporal Gyrus, Temporal_Mid_R (aal)	33	0.020	0.222	4.94	4.79	< .0001	69	-40	4
Right Cerebrum, Temporal Lobe, Superior Frontal Gyrus, Temporal_Mid_R (aal)	31	0.094	0.398	4.53	4.42	< .0001	54	-58	19
		0.138	0.399	4.42	4.31	< .0001	51	-67	19
		0.376	0.888	4.08	4.00	< .0001	45	-67	25
Right Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Frontal_Sup_R (aal)	7	0.105	0.398	4.50	4.39	< .0001	18	53	25
Right Cerebrum, Parietal Lobe, Precuneus, Brodmann area 7, Precuneus_R (aal)	5	0.511	0.888	3.95	3.87	< .0001	3	-73	46

**Indicates region that survived at p < .05 (FWE)*

PARTICIPANT 7:

Contrast 1: Fear vs Neutral (PRE-ONLY)
p<.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Parietal Lobe, Inferior Parietal Lobe, Parietal_Sup_L(aal)	13	0.999	0.997	2.07	2.06	<.020	-30	-46	58
Left Cerebrum, Frontal Lobe, Superior Frontal Gyrus, Brodmann area 10, Frontal_Mid_L (aal)	30	0.999	0.997	2.50	2.48	<.006	-36	-55	-23
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_Orb_R (aal)	9	0.999 0.999	0.997 0.997	2.32 2.25	2.30 2.24	<.011 <.013	33 -27	47 -46	-14 16
Interhemispheric, Sup_Motor_Area_L(aal)	24	0.999	0.997	2.21	2.20	<.014	0	8	58

No suprathreshold voxels at p < .05 (FWE)

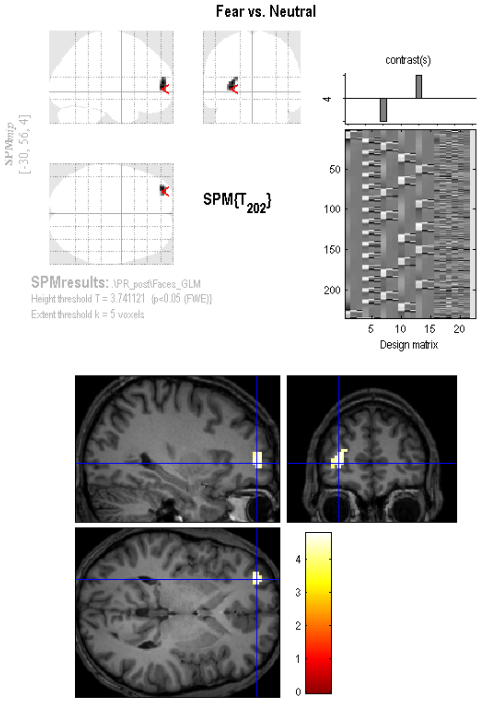
Contrast 1: Fear vs Neutral (POST-ONLY)
p<.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
*Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L (aal)	387	0.049 0.063 0.244	0.125 0.125 0.398	4.76 4.69 4.30	4.63 4.57 4.2	< .001 < .001 < .001	-30 -27 -15	56 59 56	4 13 7
Left Cerebrum, Frontal Lobe, Paracentral Lobule, Brodmann area 4	79	0.912 0.962 0.975	0.828 0.828 0.828	3.61 3.50 3.46	3.55 3.45 3.40	< .001 < .001 < .001	0 15 -6	-34 -40 -34	73 79 82
Right Cerebrum, Limbic Lobe, Cingulate Gyrus, Supp_Motor_Area_R (aal)	25	0.980	0.828	3.44	3.38	< .001	12	-1	49
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Brodmann area 10, Frontal_Mid_L (aal)	5	0.988	0.828	3.39	3.34	< .001	-36	38	16
Right Cerebrum, Frontal Lobe, Medial Frontal Gyrus, Brodmann area 6, Paracentral_Lobule_R (aal)	12	0.997	0.911	3.28	3.24	< .001	3	-22	76

**Indicates region that survived at p < .05 (FWE)*

ROI ANALYSIS: ***Fear vs Neutral (POST-ONLY)***

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
ROI	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L (aal)	44	0.001	0.08	4.76	4.63	< .001	-30	56	4



Left Middle Frontal Gyrus

Contrast 2: Fear vs Happy (PRE-ONLY)
p<.001, nb vox min = 5

Cluster		Peaks within Cluster					MNI Co-ordinates (mm)		
Structures Overlapped	*nb vox*	*p(FWE-corr)*	*p(FDR-corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Right Cerebrum, Occipital Lobe, Lingual Gyrus, Lingual _R (aal)	89	0.904	0.995	3.72	3.65	< .001	3	-82	-2
		0.999	0.995	2.91	2.87	< .002	-6	-79	10
Right Cerebrum, Temporal Lobe, Sub-gyral, Fusiform_R (aal)	5	0.999	0.995	2.95	2.91	< .002	39	-43	-14

No suprathreshold voxels at p < .05 (FWE)

Contrast 2: Fear vs Happy (POST-ONLY)
p<.001, nb vox min = 5

Cluster		**Peaks within Cluster**					**MNI Co-ordinates (mm)**		
Structures Overlapped	*nb vox*	*p(FWE) -corr)*	*p(FDR -corr)*	T	Z	*p(unc)*	*x*	*y*	*z*
Left Cerebrum, Frontal Lobe, Inferior Frontal Gyrus, Frontal_Inf_Tri_L(aal)	36	0.998	0.727	3.25	3.21	<.001	-42	44	1
		0.998	0.855	2.76	2.74	<.003	-33	53	7
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L (aal)	15	0.999	0.727	3.15	3.11	<.001	-27	56	16
Left Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_L(aal)	14	0.999	0.727	3.00	2.97	<.002	-39	44	16
Right Cerebrum, Frontal Lobe, Middle Frontal Gyrus, Frontal_Mid_R (aal)	12	0.999	0.727	2.97	2.94	<.002	39	41	16

No suprathreshold voxels at p < .05 (FWE)