Magnetic Resonance Spectroscopy to Monitor Neurochemical Changes in PTSD Study Protocol

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Introduction:

This research project is being conducted in collaboration with The Australian Defence Force, The Princess Alexandra Hospital, Herston Imaging Research Facility, Hunter Medical Research Institute and University of Newcastle. This project is supported by an Australian Defence Force and United States Defence Force grant.

Background:

Post-traumatic Stress Disorder (PTSD) is a mental health disorder that can develop after a person is exposed to a traumatic event, such as sexual assault, warfare, accidents, or other threats on a person's life (Shin, Rauch, & Pitman, 2006). Symptoms may include disturbing thoughts, feelings, or dreams related to the events, mental or physical distress to trauma-related cues, attempts to avoid trauma-related cues, alterations in how a person thinks and feels, and increased arousal (Brailey, K., Vasterling, Proctor, Constans, & Friedman, 2007). These symptoms last for more than a month after the event (Shin et al., 2006; Brailey et al, 2007). Exposure to trauma also increases ones risk for major depression, panic disorders, generalised anxiety and substance abuse (Shin et al., 2006).

In order for a diagnosis of PTSD to be made a person must have experienced a traumatic event typified by:

- Actual or threatened death or serious injury, or a threat to the physical integrity of self or others;
- A response of intense fear, helplessness or horror;

- Have three main symptoms consisting of persistent re-experiencing of the traumatic event; avoidance of stimuli related to the trauma; and hyperarousal for at least one month (American Psychiatric Association 1994).

Anyone can develop PTSD following a traumatic event, but people are at greater risk if the event involved deliberate harm such as physical or sexual assault or they have had repeated traumatic experiences such as childhood sexual abuse or living in a war zone. Apart from the event itself, risk factors for developing PTSD include a past history of trauma or previous mental health problems, as well as ongoing stressful life events after the trauma and an absence of social supports.

Around 12% of Australians will experience PTSD in their lifetime. Serious accidents are one of the leading causes of PTSD in Australia. Post-traumatic stress disorder is also the most common anxiety disorder in the general and military populations with an estimated twelve month prevalence of 5.2% vs. 8.3% respectively.

PTSD has been described as the "signature injury" of the Iraq and Afghanistan wars, affecting up to 300,000 US soldiers. Despite the increased public awareness of PTSD, the condition remains challenging to diagnose.

Purpose and Rational

Currently, the diagnosis of PTSD is based solely on a psychological assessment leaving the condition open to interpretation (Ozer, Best, Lipsey& Weiss 2008). As a step towards better understanding and treating PTSD, an objective diagnostic tool is required**.** One-dimensional Magnetic Resonance Spectroscopy (MRS) has been used in a previous research to study neurochemical changes in PTSD. The most consistent finding has been a statistically significant reduction in N-Acetylaspartate (NAA) levels in the hippocampus. Additionally NAA is reduced in the anterior cingulate cortex. Up until recently no previous studies had used MRS to measure GABA levels in the brain of PTSD suffers.

The primary aim of the study is to develop a diagnostic test for PTSD. If this study confirms that there is altered biochemical markers in the brains of those who are suffering PTSD compared to healthy controls, this information would be useful in the early identification and treatment of PTSD.

Hypothesis:

We hypothesise that (1H) and two-dimensional COSY MR Spectroscopy can be used to document and monitor PTSD in a civilian population. To test this hypothesis the following objectives will be pursued.

1. Use 1 Dimensional and 2 Dimensional MR spectroscopy to characterise neurochemical changes in patients with PTSD
2. Compare any spectral changes found in the PTSD population to healthy controls.
3. Determine if a 'biomarker' can be found for PTSD using statistical classification algorithms.

Methodology

The Australian Defence Force will be responsible for the recruitment of enlisted soldiers as detailed in the Australian Defence Human Research Ethics Committee approved protocol (ADHREC/OUT/2014/R18910579). Upon recruitment, the participants will be referred to the Clinical Research Nurse at the Clinical Research Facility (CRF) located at the Princess Alexandra Hospital.

The research team located within the CRF are responsible for the recruitment of civilian participants. The civilian recruits (male and female) aged between 18-60 with confirmed PTSD and healthy controls will be recruited from social media, local PTSD support groups and through a patient referral processes from private clinicians working at Toowong Private Hospital and the Princess Alexandra Hospital. A control cohort of 'healthy' volunteers will be recruited through the same processes. PTSD participants will be matched to controls by gender and age.

Inclusion/Exclusion Criteria

The study will have the following Inclusion criteria for experimental groups:

- Male or Female aged between 18-60
- Diagnosis of PTSD made according to the DSM-V

The study will have to following Inclusion criteria the healthy control group:

- Male or Female aged between 18-60
- No previous diagnosis of PTSD
- No history of psychiatric illness
- No chronic medical conditions

The study will have to following exclusion criteria:

- Current substance use disorder.
- Lifetime history of substance dependence.
- Current diagnosis of Major Depressive Disorder, Schizophrenia, Psychosis or Bipolar disorder.
- History of childhood trauma
- Previous history of head injury or loss of consciousness (significance of this will depend on when the injuries occurred – will be assessed with questionnaire).
- Use of benzodiazepines; anticonvulsants; mood stabilisers within the last 4 weeks of MR scan.
- Contraindication to MRI scanning including shrapnel or any mental implants.
- Current pregnancy.
- History of meningitis.
- History of a chronic serious medical condition.
- History of chronic inflammatory disease.
- Structural brain injury or abnormality.
- Neurological disorders.
- High dependency on medical care.

Participant Withdrawal

Participants are able to withdraw from the project at any time for any reason. Participants will be informed that all data collected up to the point of withdrawal will be used in the data analysis.

Advertising

Social media will be used as a tool for advertising and recruitment. Study specific flyers have been created and attached (See Appendix – Advertising Material). The flyers detail the requirement of those diagnosed PTSD and healthy controls for the purpose of investigating the physical effects of PTSD on the brain. The flyer also specifies the age requirement of 18-60 years and provides contact details for further information. Social media will be utilised in the following ways;

- Paid advertisement on Facebook that targets by groups such as gender, age and Facebook algorithms such as age, location, interest groups and hash tags (ie PTSD, Military, Medical Imaging, MRI) to recruit participants.
- Advertising for recruitment through the TRI existing Facebook page
- Advertising for recruitment through the TRI existing Twitter account.
- Advertising through existing local PTSD Facebook groups and their websites (ie Mates4Mates, and Picking up the Peaces).

In the case of those potential participants who are attending an outpatient clinic at Toowong Private Hospital, the Princess Alexandra Hospital, private clinics or a local PTSD group, an invitation letter will be given or sent to the potential participant informing them of the MRS PTSD study. Individuals who wish to volunteer for the study are then free to contact the study team for further information.

Potential participants who call the allocated number to enquire about the study, will be referred to the Principal Investigator for medical based enquires or to the study's clinical psychologist for psychological assessment based enquires. The study nurse is also available for general enquires. Individuals who wish to volunteer for the study will be provided with information and consent forms. After verbal consent is obtained potential participants will undergo a pre-screening questionnaire via the phone conducted by the clinical research nurse to ensure eligibility according to the set inclusion and exclusion criteria.

Treatment of Participants

Questionnaires and Psychological Assessment Process

There are 3 participant groups involved in this study:

1. Military personnel with PTSD as recruited and referred by the Australian Army
2. Non-military (civilian) with PTSD
3. Civilian with no PTSD

Upon successful completion of the pre-screening questionnaire via telephone, a mental health assessment will be performed by the Clinical Psychologist to ensure a diagnosis of PTSD (for Group 1) and to rule out any co-morbid psychological illness (eg anxiety disorder, depression, substance abuse).

The Clinical Psychologist will then organise either a telephone or face-to-face interview, depending on the participant's location, to administer the following psychological diagnostic tests:

- SCID 5 – A structured interview process performed by a Clinical Psychologist. The modules used are dependent upon the answers provided by the participant.
- CAPS-5 – A 30 item structured interview that is used to diagnose PTSD.

Once the participant is enrolled into the study, they will be required to complete an online questionnaire. The questionnaire was developed using the online Lyme Survey tool, which is hosted on the University of Newcastle Server. All data that is provided by the participant will be de-identified and communication between the University server and the participant's computer is encrypted.

The online screening questionnaire includes the following modules:

- Demographics

- Previous medical history
- PTSD Checklist – Civilian version (PCL-C)
- Illicit drug screen and Alcohol Use Disorders Identification Test (AUDIT)
- Graded chronic pain scale
- Generalised anxiety assessment (GAD-7)
- Primary Health Questionnaire (PHQ- 9 and PHQ-15)
- Ohio State TBI identification method

Prior to the scan, the participant is required to complete the Brain Resource companies 'WebNeuro' package. The participant will be provided the option of completing it at home or have the opportunity to use a private room with access to a computer within the CRF. Webneuro provides an assessment of general and emotional cognition. Webneuro will consist of the following tasks:

- Motor tapping
- Choice reaction time
- Verbal memory recall
- Digit span
- Verbal interface
- Switching of attention
- Maze; Go/NoGo;
- Continuous Performance Test
- Emotional Identification and Emotional Recognition.

MRI and MRS Process

The participant will be booked in to the closest imaging site convenient to the participant for an MRI scan. While magnetic resonance imaging identifies the anatomical location of any abnormalities, magnetic resonance spectroscopy compares the chemical composition of brain tissue.

The participant will lie on a moveable bed with their head cradled on a headrest and arms at their sides. An antenna device called a "coil" will be placed over the participant's head and the table will slowly move into the magnetic field. The two scans will be conducted, which take approximately 45 minutes each.

For further information on the MRI and MRS process, please refer to the Appendix _ MRI and MRS Scan Information.

Assessment of Safety

Prior to imaging, the participant will complete an MRI Safety Questionnaire. This questionnaire will identify any conditions that could interfere with the MRI. If the participant identifies any of the conditions outlined in the MRI safety questionnaire, this can present significant health and safety hazards in the MRI environment and the participant will not proceed with the study. For further information on the MRI Safety Questionnaire, please refer to the Appendix – MRI Safety Questionnaire.

Upon completion of the MRI, an experienced Radiologist will perform a safety check. The purpose of this check is to report on any incidental findings. If there is an incidental finding, the Radiologist will report to the Principal Investigator, who will then contact the participant and their General Practitioner for follow up.

Ethics

Consent

Every participant is required to provide written informed consent prior to the commencement of the study. Participants will be provided with study information and a contact number to call if they have any enquires. All participants are required to sign the study specific consent and are able to withdraw consent at any time.

Duty of Care

Due to the potential for mental health problems in participants presenting with traumatic experiences, a standard operation policy has been implemented to ensure researchers meet their "duty of care" obligations to the study participants.

Once the participant has completed the questionnaire the staff member collecting it should immediately review the answers pertaining to depression and anxiety. This should be done prior to allowing the participant to leave the facility. Where the questionnaire is not administered in person (e.g. to be returned by mail, completed over the telephone, completed electronically), this SOP regarding meeting duty of care requirements will still apply.

The Researcher involved should determine the level of anxiety and/or depression ("None" "Mild" "Moderate" or "High") according to the participant's responses to the questionnaire. If the participant falls under mild, moderate or high levels of depression and/or anxiety, the 'Duty of Care Procedure' must be followed.

Ethics Approval

This study will be performed under the regulatory approval of the Metro South HREC (EC00167) and the Australian Defence Human Research Ethics Committees.

Governance

Additional site specific approval has been granted for the HMRI Imaging Centre, Herston Imaging Research Faculty.

Monitoring/Audits

The Principal Investigators alongside the Translational Research Institute will permit project-related monitoring, audits and regulatory inspections, providing direct access to source data and documents. This may include, but not limited to, review by the HREC committees involved and institutional governance review bodies.

Quality Control

This study will be conducted in accordance with the National Statement of Enthical Conduct in Human Research, The Australian Code for Ther Responsible Conduct of Research, relevant policies and procedures and under the guidelines of ICH GCP as annotated by the Australian Therapeutic Goods Administration.
All data that is entered by a member of the research team will be checked by another member of the research team to safeguard the integrity of the data.

Key Milestones and Estimated Timelines:

- Submission to the Metro South Human Research Ethics Committee July 2016 for protocol amendment and approval of advertising materials.
- Begin advertising for recruitment in August 2016.
- Commence psychological assessment for Queensland based participants in July of 2016.
- Commence MRS scanning at Herston Imaging Research Facility in August 2016.
- Commence MRS scanning at the Princess Alexandra Hospital in September 2016.
- Complete data analysis by November 2018.
- In the event that subject recruitment is slower than anticipated, the study could potentially extend to the first half of 2019.

Public Disclosure Plan and Publishing

After obtaining approval from the Australian Defence Force, the results of the study will be presented at medical and scientific meetings and will be published in peer-reviewed medical or scientific journals.

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Appendices

1. **MRI and MRS Methods**
2. **Patient Information and Consent Form**
3. **Advertising Material**
4. **Nurse Pre-Screening Questionnaire**
5. **GP Follow-up Letter**
6. **SOP: Duty of Care**

MRI and MRS Methods:

Scanner:

Data will be obtained using the Siemens 3T Prisma (Siemens AG, Erlangen, Germany) using a sixty-four (64) channel head and neck coil.

Imagine:

For anatomical localisation and voxel placement a three dimensional high resolution MPRAGE (Magnetisation-Prepared Rapid Acquisition with Echo Gradient) scan will be obtained (TR/TE=2530/1.7 ms, 12 degree flip angle, FOV= 256x256mm, voxel size 1x1x1mm, NEX 4, acquisition time 6 minutes).

1D Spectroscopy:

Single voxel PRESS sequence will be obtained using the following parameters: TR 1.5s; TE 30ms; voxel size 2×2×2cm=8cm^3; 96 averages. One-dimensional spectra will be obtained in the following regions: amygdala; hippocampus; anterior cingulate cortex (ACC); and insular cortex. Each voxel will take approximately 5 minutes to obtain.

2D Spectroscopy:

Will be obtained in the ACC using the following acquisition parameters: RF carrier frequency at 2.0 ppm; TR 1.5s; water suppression; spectral width of 2000Hz; increment size of 0.8 ms in 96 t1 increments giving an indirect spectral width of 1250Hz; 12 averages per increment; and 1024 data points. Scan time will be approximately 30 minutes.

Diffusion Tensor Imaging (DTI):

35-directions scan; TR: 5520ms; TE: 89.5ms; FOV: 210×180; slice thickness 1.25mm; Multiband = 2; IPAT = 2; b-value = 0, 1000 and 3000 s/mm^2.

Resting state fMRI: TR/TE = 2390/24ms, FOV = 260mm×260mm, flip angle = 90°, slice thickness = 3.0mm without gap, interleaved scanning, 47 slices covering the whole brain, 250 volumes acquired in 10 minutes.

Post-Acquisition Analysis:

One-dimensional spectroscopy will be processed using LC-Model. LC-Model is an automated package that uses a time domain-fitting loop. The best fit is found by varying a basis set if concentration-calibrated model spectra of individual metabolites. LC – Model provides identification and estimation of the absolute concentrations of metabolites. The average metabolite ratios will be compared between each group and tested using a t-test or Mann-Whitney if the data are not normally distributed.

Statistical classification algorithms:

Raw one dimensional spectroscopy data will be input into a comprehensive set of statistical classification algorithms. Before data is analysed it is post-processed using the following steps: 1st spectral alignment; water removal; apodization; phase correction; baseline removal; and 2nd spectral alignment. Feature extraction is performed using a wavelet-based transform, undertaken on the entire spectrum. Candidate biomarkers are compared using a two-sided, equal variance, student t-test and are considered statistically if $p < 0.01$. Extracted features can then be correlated to clinical measures that have been collected throughout the project such as the McGill pain questionnaire and the State-Trait Anxiety Inventory. The clinical measures will be compared to features using a spearman correlation co-efficient. Biomarkers that have been identified can be tested to determine their sensitivity and specificity.

Two-Dimensional COSY:

Raw 2D spectroscopy is transferred from the scanner to Matlab. Within Matlab the signal is combined from multiple elements, rows concatenated into a 2D matrix and reformatted. The resulting 2D file is now processed and analysed using Felix, a specialised 2D nuclear MR processing software. In Felix each prominent diagonal and cross peak is selected and integrated to determine the peak chemical shift; intensity and volume. These values are standardised (using creatine diagonal cross peak at 3.02 ppm) and can then be compared statistically for each group.