Depression following traumatic brain injury: A functional connectivity perspective

Running title: Neural substrates of depression in TBI patients

Laura Moreno-López¹, Barbara J. Sahakian², Anne Manktelow¹, David K. Menon¹ and Emmanuel A. Stamatakis¹

¹Division of Anaesthesia, University of Cambridge, Box 93 Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, UK; +44 (0)1223 217892; lm618@cam.ac.uk

²Department of Psychiatry, MRC/Wellcome Trust Behavioural and Clinical Neuroscience Institute, University of Cambridge, Forvie Site, Robinson Way, Cambridge CB2 0SZ, UK; +44 (0)1223 336961. bjs-sec@medschl.cam.ac.uk

*Corresponding Author:
Laura Moreno-López
Division of Anaesthesia
Box 93 Addenbrooke's Hospital, Hills Road,
Cambridge, CB2 0QQ, UK
Tel.: +44 (0)1223 217892
Fax: +44 (0)1223 217887
lm618@cam.ac.uk
Abstract

Introduction: Despite the mounting evidence that depression is one of the most common psychiatric sequelae in survivors of traumatic brain injury (TBI), no studies so far have attempted to provide an explanation in terms of functional network integrity. This proof of concept study investigated the association between the severity of depressive symptoms and resting network integrity in a sample of patients with TBI and a group of healthy controls. Methods: We first examined the association between depression symptomatology and global functional connectivity and then attempted to characterize the extent of differences in functional network integrity. Results: The severity of depressive symptoms in patients with TBI was associated with neuroadaptations within the insula, the thalamus and the subgenual anterior cingulate cortex (ACC). Specifically, patients with TBI displayed increased connectivity between the insula and a region encompassing the rolandic operculum and the superior temporal cortex and reduced connectivity between the thalamus and the dorsolateral prefrontal cortex. Conclusions: These findings show the network level involvement of the insula, the thalamus and the subgenual ACC in the depressive symptomatology of patients with TBI and tentatively propose that TBI-induced depression may result from altered functional connectivity of a set of networks associated with emotional regulation. However, other factors including a number of adjustment issues and challenges may also lead to depression in this population.

Keywords

Traumatic brain injury, Resting state, Functional connectivity, Depression
Introduction

With more than 10 million people affected worldwide and nearly 1.6 million in Europe, traumatic brain injury (TBI) represents a significant public health issue. While our knowledge of the damage mechanisms involved in TBI has benefited somewhat from recent basic research, improvements in clinical management have not advanced considerably. Although TBI usually results in a wide variety of symptoms, the majority of the published studies have focused on the physical and cognitive sequelae of this disorder and have not taken into account that emotional sequelae such as anxiety or depression may interfere with the success of rehabilitation and patient recovery [1,2].

Major Depressive Disorder (MDD) is among the most frequent complications observed in patients following TBI. Patients experiencing depression after TBI have increased levels of anxiety, cognitive deficits and disability in comparison with those who do not develop depression [3,4]. Evidence indicates that depressed patients show abnormal regulation of negative and positive valenced information as well as preferential processing of negative information (mood-congruent processing bias). These cognitive dysfunctions may result from the alteration of two brain circuits: an amplified emotion-processing circuit and an attenuated cognitive control circuit which will normally suppress such abnormal affective responses [5-10]. In this sense, evidence from imaging studies has demonstrated that depression is associated with dysfunction in areas involved in processing emotional stimuli such as the insula, the thalamus or the subgenual anterior cingulate cortex (ACC), as well as abnormal function in brain regions involved in cognitive control such as the dorsolateral prefrontal cortex (DLPFC) [11-15]. Furthermore, the alteration of these two circuits is supported by studies of cognitive reappraisal, a form of emotion regulation in which the meaning of an emotional stimulus is reinterpreted to change its affective tone. Failure to reduce activity in limbic regions in paradigms investigating emotional reappraisal has been associated with decreased DLPFC activation [16-18]. Importantly, recovery from depression has been associated with
increased activity within the DLPFC and decreased activity within the subgenual ACC, the insula and the thalamus among other areas [19-21].

From a neuroanatomical point of view, TBI can cause both localized and diffuse damage and although the majority of TBI studies have focused on the effects of focal injuries, these have been found to be of limited value when attempting to predict clinical outcomes [22]. Here we focus on diffuse axonal injury (DAI) which may offer a broader neurobiological framework for understanding clinical outcomes following brain injury [23].

In the last few years, our understanding of TBI has benefited from the considerable interest shown in the spontaneous low frequency blood oxygen level-dependent fluctuations observed in the resting brain. The study of resting-state networks allows the exploration of activity relationships, in the absence of any specific external stimulation, between anatomically remote brain regions or, in other words, the level of functional connectivity between regions [24]. Since the functional architecture of these networks may in part reflect underlying structural connectivity integrity [25], DAI patients are a population in which the elucidation of within and between network relationships would be beneficial not only for our understanding of the disease but also brain function in general. Moreover, because the acquisition of resting functional MRI data does not require active task participation, it may be particularly useful for characterising disease processes in patients who find performing tasks in a scanner difficult.

The networks most extensively investigated in this context have been the a) default network, whose key nodes include the ventromedial prefrontal cortex (including the subgenual ACC), the posterior cingulate cortex, lateral inferior parietal and lateral and medial temporal cortices, thought to play an important role in internally directed cognitive activity (e.g. interoceptive awareness, self-focus); b) the central executive network, which includes the DLPFC and posterior parietal cortex and has been associated with the manipulation and maintenance of information in the context of
goal directed behaviours [26], and c) the salience network, a network comprising the anterior and posterior parts of the insula and the ACC, which has been associated with the integration of external and internal information in order to direct attention to salient stimuli and is thought to regulate the transitions between the default and the central executive network [27,28].

The majority of studies carried out in patients with depression outside TBI have reported abnormally increased default network activity and connectivity with the subgenual ACC, as well as in subcortical regions such as the amygdala, the thalamus, the pallidum/putamen and midbrain regions like the ventral tegmental area [29-34]. Other studies have demonstrated the alteration of specific networks associated with cognitive control. For example, reduced resting-state activity in the DLPFC [29,31] and the alteration of the salience network have been reported in patients with depression [33,35,36].

Finally, there is a small body of literature suggesting the alteration of these networks in TBI populations. These studies have reported abnormally enhanced default network connectivity [37-42] as well as the alteration of the central executive and salience networks during the execution of cognitive tasks such as rule switching and inhibitory control tasks [37,40,43-45]. Although the role of these alterations remains unclear, increased connectivity in regions associated with interoceptive awareness and self-focus along with the increased connectivity found in regions associated with the processing of emotional stimuli and the alteration of the circuits associated with their control could explain the high rates of depression found in TBI populations.

Despite the mounting evidence for altered network functional connectivity in TBI and even though MDD is one of the most common psychiatric sequelae in survivors of TBI, no studies so far have explored the relationship between severity of depressive symptoms and functional network integrity in patients with TBI. This study aimed to explore the association between an index of global functional connectivity and severity of depressive symptoms in a sample of patients with TBI.
DAI and a sample of healthy controls (HC). Additionally, we investigated between-group differences in the spatial extent of the functional network integrity in the regions we found to be associated with the severity of depressive symptoms. This proof of concept study may help to understand the mechanisms of the depressive symptoms in these patients. We hypothesised that connectivity in the default, the central executive and the salience networks, which include areas implicated in depression such as the insula, the thalamus and the subgenual ACC would be related to the severity of depressive symptoms in the TBI population.

**Materials and methods**

**Participants**

Thirteen patients with TBI and 14 HC participated in this study. Patients with TBI were referred from the Addenbrooke’s Neurosciences Critical Care Unit Follow-Up Clinic, the Addenbrooke’s Traumatic Brain Injury Clinic and the Royal London Hospital Intensive Care Unit. Inclusion criteria included the presence of brain injury and age range between 16 and 60 years old. The exclusion criteria were (1) National Adult Reading Test <70, (2) Mini Mental State Exam <23, (3) left-handedness, (4) history of psychiatric or neurologic disorders including premorbid histories of depression or substance abuse, (5) taking medication that may affect their physical or cognitive performance (including tricyclic antidepressants and benzodiazepines), contraindications for MRI scanning and pregnancy or nursing and (6) had a physical disability that could prevent them from completing the tasks either in the screening or scanning stages. Only patients with DAI, as opposed to focal lesions, were included in this study. We confirmed the absence of focal lesions from medical records completed at the time of the injury and clinical follow-ups and from additional visual inspection of the T1-weighted images acquired at the same time as fMRI data. Patients were at least 7 months post TBI (mean 21 ± 12.17 months) and were not receiving any acute hospital interventions. They sustained moderate to severe TBI –as measured by the Glasgow Coma Scale
[46], the Injury Severity Scale [47], and the Acute Physiology and Chronic Health Evaluation II [48]. Lesion information of the patients is presented in Table 1. 

HC were recruited via advertisements in Addenbrooke’s Hospital and in the local Cambridge area taking care to match them to the clinical group in the main demographic characterises. In addition to the former exclusion criteria, HC were screened to exclude a history of TBI. Participants between 16 and 60 years old were eligible.

All subjects gave written informed consent before participating in the study as approved by the Cambridgeshire 2 Research Ethics Committee in accordance with the Declaration of Helsinki of 1975, as revised in 2008.

**Depression measure**

Depressive symptomatology was evaluated with the Beck Depression Inventory (BDI) [49] which is a self-rating questionnaire used extensively in clinical settings for measuring severity of depressive symptoms [50]. The BDI is a 21-item self-report scale measuring the emotional, cognitive, somatic, and motivational symptoms of depression. Each item is scored on a scale from one to three, and total scores are calculated by summing the scores on all the items. Scores of ten or higher fall within the depressed range with scores of 10 to 16 indicating mild depression, scores of 17 to 29 moderate, and scores of 30 to 63 severe depression.

**MRI acquisition and preprocessing**

Participants were scanned on a Siemens Trio 3-Tesla MR system (Siemens AG, Munich, Germany) at the Wolfson Brain Imaging Centre of Addenbrooke’s Hospital (Cambridge, UK). The imaging session started with a localiser followed by a high resolution T1-weighted, magnetization-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) structural scan (TR=2300 ms, TE=2.98 ms, TA=9.14 min, flip angle=9°, field of view read=256 mm, voxel size=1.0x1.0x1.0
mm, slices=176). fMRI assessment involved a resting state scan with a duration of 5.5 min for which participants were instructed to not think of anything in particular and to keep their eyes closed. After the scan, participants confirmed they had not fallen asleep during the resting state scan. Our experiment also involved a set of cognitive tasks (working memory, planning, attention and decision making) which will be reported elsewhere. The functional data were acquired using an EPI (echo-planar imaging) sequence with the following parameters: TR=2000 ms, TE=30 ms, flip angle=78°, FOV read=192 mm, voxel size=3.0x3.0x3.0 mm, slices per volume=32.

Prior to preprocessing, the first five volumes of each scan were discarded to control for initial signal instability. Images were preprocessed using Statistical Parametric Mapping 8 (SPM8) (Welcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) implemented in MatLab R2007b (Mathworks, Natick, MA, USA). fMRI images need to be corrected for movement artefacts. Even the most relaxed volunteer will move slightly during a long fMRI scanning session. Here we used a two pass procedure to correct for movement artefacts. In the first instance the data were realigned to the first fMRI volume. A mean realigned image was then produced and the fMRI images were realigned again to the mean image. Resulting plots from the movement correction procedure were individually examined to ensure head motion was less than 2 mm translation and less than 2° rotation in any of the x, y and z axes. All volumes were then spatially normalized to a standard EPI template in the Montreal Neurological Institute (MNI) space and spatially smoothed with a Gaussian filter of 6 mm FWHM Gaussian kernel. Visual inspection after every step was performed to ensure quality of the preprocessing. The CONN fMRI Connectivity toolbox [51] was used to calculate the intrinsic connectivity contrast (ICC) of each participant. Before the ICC calculation we used CompCor, a strict noise reduction method, to remove data components attributable to the signal from white matter and cerebrospinal fluid [52] (utilising white matter and CSF masks from segmenting the T1 weighted images), which eliminated the need for global signal normalisation [53,54]. The subject-specific 6 realignment parameters and
their first order derivatives were also partialled out before the ICC calculation [55]. Moreover, a temporal filter of 0.009 and 0.08 Hz was applied to focus on low-frequency fluctuations [56].

**Behavioural analyses**

Independent-sample t-tests and chi-square tests were used to examine between-group differences in the main socio-demographic and clinical variables using SPSS 21.0 for Windows (SPSS Inc., Chicago IL). Significance threshold was set at p<0.05.

**Imaging analyses**

*Intrinsic connectivity contrast*

The ICC is a voxel-wise index (a single number for each voxel) that represents how well connected each voxel is to the rest of the brain [57]. Following the calculation of the resting state ICC map for each participant, associations between the severity of depressive symptoms and ICCs using a voxel-wise approach were tested within a general linear model framework. Specifically, we assessed the correlation between severity of depressive symptoms and the voxel level ICC of each group and the interaction between severity of depressive symptoms and the variable group (patients with TBI vs. HC) using a multiple regression analysis in SPM8. Both analyses were restricted to a mask encompassing a set of areas consistently associated with depression, as identified from 5,809 neuroimaging studies using the term ‘depression’ in the Neurosynth framework [58] (Supplementary Fig. 1 shows the regions included in the mask). The great number of voxels in an fMRI image creates a multiple testing problem that needs to be addressed in a principled manner. Then to correct for multiple comparisons and to determine statistical significance, we used a cluster-level threshold that was determined by 1000 Monte Carlo simulations using AlphaSim [59]. Input parameters to AlphaSim included an individual voxel threshold probability of 0.001, a cluster connection radius of 5 mm and the data smoothness after model estimation on the depression mask.
The minimum cluster size was determined to be 9 voxels to satisfy a family-wise error rate correction of pFWE<0.05.

**Seed-based analyses**

The ICC metric discussed above, is a global functional connectivity index and does not provide information on the spatial extent of functional connectivity. To calculate functional connectivity maps and network involvement, of the regions found to be associated with severity of depressive symptoms, we used the CONN fMRI functional connectivity toolbox [51]. At this stage, we also explored between-group (patients with TBI vs. HC) differences in network integrity. Spatial extent thresholds were determined by 1000 Monte Carlo simulations using AlphaSim. Input parameters to AlphaSim included an individual voxel threshold probability of 0.001, cluster connection radius of 5 mm and the estimate of the FWHM smoothness for each T map used incorporating a grey matter mask volume of 167.265 voxels (2.0x2.0x2.0 mm). The minimum cluster sizes were determined to be 96, 84 and 83 voxels for the insula, the thalamus and the subgenual ACC connectivity analyses respectively, to satisfy a family-wise error rate correction of pFWE<0.05. A schematic diagram of the procedure followed in the analysis is shown in Supplementary Fig. 2.

**Results**

**Background and clinical characteristics**

Three patients sustained moderate TBI, the rest sustained severe TBI. Both groups had statistically equivalent distributions for age (patients with TBI 33.31 ± 13.61 vs. HC 33.43 ± 10.70; p=0.98), verbal IQ (patients with TBI 111.15 ± 10.06 vs. HC 116.57 ± 6.08; p=0.11) and were matched on gender (patients with TBI 76.9% vs. HC 64.3% male; p=0.47). Patients presented with significantly higher depressive symptom scores than HC (patients with TBI 8.38 ± 6.51 vs. HC 2.36 ± 1.98; p=0.006). Demographic and clinical characteristics of the samples are presented in Table 2.
Imaging analyses

Intrinsic connectivity contrast

Within-group ICC functional connectivity maps appear similar on visual inspection, but have greater spatial extent in the HC group. In both groups, we observed within group high ICC in the dorsal and medial prefrontal cortex and posterior regions including the posterior cingulate cortex, the precuneus, the angular and the inferior parietal and occipital cortices. The group ICCs (calculated with one-sample t-tests) are shown in Fig. 1.

The within group assessment of the relationships between ICC and severity of depressive symptoms revealed a significant negative correlation in the right insula for the group of TBI (Fig. 2A). Additionally, we found significant between-group interactions in the patterns of correlation. Specifically, the correlation of ICC with severity of depressive symptoms was negative in patients with TBI and positive in HC for the right thalamus (Fig. 2B), whereas it was positive in patients with TBI and negative in HC for the right subgenual ACC (Fig. 2C).

Importantly, to confirm that our findings were not influenced by the patients’ age or time since injury, we correlated these variables with the ICC from the statistically significant peaks described above. We found no significant relationships at p<0.05.

Seed-based analyses

Within-group functional connectivity maps appear similar but more limited in the group of patients with TBI. The connectivity maps for each group were calculated by using seeds from the areas we found to be related to the severity of depressive symptoms above. When the connectivity of the insula was investigated, both groups displayed positive correlations with the insula and the dorsal ACC but HC also exhibited positive correlations with several frontal regions and negative correlation with the precuneus. When the connectivity of the thalamus was calculated, both groups
displayed similar positive correlations within the thalamus and surrounding structures. However, whereas the HC group showed additional positive correlations with several areas of the prefrontal cortex (i.e. dorsal ACC, dorsolateral prefrontal cortex), the patients with TBI showed positive correlations with the temporal and the parietal cortex. Finally, when the connectivity of the subgenual ACC was estimated, both groups showed positive correlations between this area and the rest of the default mode network, including both medial prefrontal cortex and precuneus but patients with TBI failed to show a negative correlation with the inferior parietal cortex as the HC group did. The maps for each group are shown in Fig. 3.

Between-group comparisons revealed that patients with TBI had increased functional connectivity between the insula and a region encompassing the rolandic operculum and superior temporal cortex (Fig. 4A) and reduced functional connectivity between the thalamus and the DLPFC (Fig. 4B). We did not find any significant between-group differences in the connectivity of the subgenual ACC at cluster level pFWE<0.05.

Discussion

Although TBI has been associated with altered network functional connectivity and high rates of major depressive disorder, the relationship between these two factors has not been explored. To this end, we investigated the relationship between network integrity and severity of depressive symptoms following TBI. We found a negative association between the severity of depressive symptoms and ICC in the right insular cortex of the patient group and two significant between-group interactions between severity of depressive symptoms and the ICC of the thalamus and the subgenual ACC. Specifically, the correlation with severity of depressive symptoms was negative in patients with TBI and positive in HC for the right thalamus, whereas it was positive in patients with TBI and negative in HC for the right subgenual ACC. Furthermore, when our search was extended to group level network maps of the regions associated with severity of depressive symptoms,
patients with TBI displayed increased functional connectivity between the insula and a region encompassing the rolandic operculum and the superior temporal cortex and reduced functional connectivity between the thalamus and the DLPFC.

The regions whose connectivity we found to be related with the severity of depressive symptoms have been associated with the processing of emotional stimuli and cognitive control. First, the network connectivity of the insula has been found to be reduced in patients with depression [33,60] and second, structural and functional connectivity disruptions between the cortex and the thalamus have been found in MRI studies of patients with depression [32,61,62]. Moreover, damage to the structural connections of the thalamus is a frequent finding in TBI and, thalamic dysfunction has been consistently shown to be of clinical relevance to emotion and cognition [63-65]. Additionally, altered functionality of the subgenual ACC has been reported in both patients with depression and patients with TBI. This region is part of the default network and as stated earlier, there is a growing body of literature showing atypically increased default connectivity (and in particular increased subgenual ACC connectivity) not only in patients with depression [30,32,34] but also in patients with TBI [41,42].

The increased functional connectivity found in patients with TBI between the insula, a key node of the salience network, and a region in the temporal cortex thought to be part of the default network, may in part, be contributing to the rates of depression observed in patients with TBI. We propose that a compromised interaction between salience and default networks could impact the transitions between default and task positive states. This in turn may be hindering the awareness of positive events that could beneficially influence depressive symptomatology and leaving individuals ruminating on the same negative thoughts continuously [66]. This evidence overlaps with the results of previous studies in patients with TBI showing increased resting state functional connectivity in the default network of patients with TBI [37-42], as well as with the association of this network with the integrity of the salience network [43,44]. However, the association those studies made
were not in the context of depression but instead they related altered connectivity to impaired cognitive control during the execution of paradigms involving task switching and response inhibition. Our finding also aligns agreeably with the result of a recent study in which abnormalities in the superior temporal gyrus assessed within 1 month of mild TBI were associated with the development of major depression and depressive symptomatology in the first year post-TBI [67].

The reduced functional connectivity found between the thalamus and the DLPFC could explain the difficulty of this population to control their emotions as a result of the compromised interaction between the systems involved in the processing (thalamus) and regulation (DLPFC) of emotions [18]. Whereas the thalamus is an integral part of the cingulate-pallidostriatal-thalamic-amygdala mood-regulating circuit [15] and due to its connections with the DLPFC, the OFC (orbitofrontal cortex) and the ACC, is believed to play a vital role in emotional attention and awareness [68], the DLPFC forms part of the central executive network and is a core region in emotional processing, particularly during the down-regulation of negative emotional states. Indeed, functional imaging studies indicate the involvement of these structures in tasks in which the participants have to reappraise the meaning of emotion-eliciting situations [17,69,70]. Evidence from studies of patients with MDD have consistently reported increased activation in areas associated with the processing of emotional stimuli such as the subgenual ACC, the insula or the thalamus and decreased activation in cortical regions associated with cognitive control such as the DLPFC [12,14,15,71]. Finally, compromised connectivity between the DLPFC and the thalamus has already been documented in a sample of patients with moderate to severe TBI [37].

In conclusion, the compromised network integrity we observed in the TBI group along with the difficulties that these patients encounter following their injury could be partly responsible for the high rates of depression found in patients with TBI. Nevertheless, due to the cross-sectional nature of this study, it is unclear whether the alterations we found are the result of the injury or if they represent risk markers for the development of this disorder. Note that in this study we did not
include patients with a history of psychiatric disorder thus ruling out the association of the alterations found with a pre-injury history of depression. The strengths of the study include the use of a sample of patients with TBI and HC matched in the main socio-demographic variables and the utilization of a well-validated quantitative measure of depressive symptomatology. Nevertheless, several limitations are worth noting. First, although comparable with previous studies in this population in terms of sample size [72,73], it would be important to replicate this study with a larger sample size. Second, future studies should use a more robust diagnostic process to identify patients with depression since this disorder can be only diagnosed through a clinical interview following specific diagnostic criteria (e.g. structured clinical interview for DSM-5 - SCID-5). Third, there are many circumstances surrounding TBI that may lead to experience depressive symptomatology which may not be attributed to structural or functional brain damage. In this sense, it has been postulated that in some cases, especially those in which depressive disorders develop in the late post-injury period, psychological and social factors appear to be etiologically important [74,75]. For example following their injury, many of these patients must deal with altered physical abilities, lose their jobs or develop compromised relationships with their family members, which would all contribute to depression. Nevertheless, it is worth noting here that the functional alterations we report could affect the ability of these people to cope with those circumstances and therefore they should not be underestimated. Forth, although matched with the group of patients with TBI in the main sociodemographic characteristic, the choice of the control group used in this study (a group of healthy participants with no depressive symptomatology or other non-TBI injuries), makes it difficult to attribute the differences observed specifically to depression in TBI. Fifth, it should be noted that the methods used to assess functional connectivity here cannot determine causality. In the future, techniques such as dynamic causal modelling [76] could be used. Sixth, our sample was composed for a majority of men and although the groups did not differ in gender, there is some evidence for sex differences in the neural correlates of emotion (including
perception, reactivity, regulation and experience) [77] and therefore future large scale-studies should test potential sex differences in the neural substrates of depressive symptomatology and depression in patients with TBI. Finally, as one of our reviewers pointed out, this study would have benefited from the inclusion of the years of education, race, marital status and occupational status of the samples.

In summary, with this proof of concept study we demonstrated for the first time, the altered integrity of functional networks which include the insula, the thalamus and the subgenual ACC in the depressive symptomatology of patients with TBI. These areas have been widely implicated with depression in patient samples other than TBI, allowing thus a tentative conclusion that TBI-induced depression may result from altered functional connectivity of a set of networks associated with emotional regulation. Nevertheless, as stipulated earlier, other factors including a number of adjustment issues and challenges may also lead to depression in this population.
References


