Published in final edited form as:

Bipolar Disord. 2018 September ; 20(6): 523–530. doi:10.1111/bdi.12588.

Patients with Chronic Bipolar Disorder Exhibit Widespread Increases in Extracellular Free Water

Carissa Tuozzo¹, Amanda E. Lyall^{1,2}, Ofer Pasternak^{1,3}, Anthony D.C. James^{4,5}, Timothy J. Crow⁴, and Marek Kubicki^{1,2,3}

¹Psychiatry Neuroimaging Laboratory, Department of Psychiatry, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

²Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

³Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

⁴SANE POWIC, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK.

⁵Highfield Unit, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK

Abstract

Objectives—Bipolar disorder (BP) is a debilitating psychiatric disease that is not well understood. Previous diffusion MRI (dMRI) studies of BP patients found prominent microstructural white matter (WM) abnormalities of reduced fractional anisotropy (FA). Because FA is a nonspecific measure, relating these abnormalities to a specific pathology is difficult. Here, dMRI specificity is increased by Free Water Imaging, which allows for identification of changes in extracellular space (free water (FW)) from neuronal tissue (fractional anisotropy of tissue (FA-t)). Previous studies identified increased FW in early schizophrenia (SZ) stages which was replaced by widespread decreased FA-t in chronic stages. This is the first analysis utilizing this method to compare BP patients and controls.

Methods—3T DWI data was acquired for 17 chronic BP and 28 healthy control (HC) participants at Oxford University. Tract Based Spatial Statistics was utilized to generate a WM skeleton. Free Water Imaging deconstructed the diffusion signal into extracellular FW and tissue FA-t maps. These maps were projected onto the skeleton and FA, FA-t, and FW were compared between groups.

Results—We found significantly lower FA in BP patients when compared to HC in areas that overlapped with extensive FW increases. There were no FA-t differences.

Conclusions—Our study suggests that chronic BP shows similar WM changes to early SZ, implicating that extracellular FW increases could be a transient indication of recent psychotic

Corresponding Author: Marek Kubicki, MD/PhD, Psychiatry Neuroimaging Laboratory, Department of Psychiatry, Brigham and Women's Hospital, Harvard Medical School Boston, MA, 02215, USA., Phone: (617)-525-6129 Fax: (617)-525-6150, kubicki@bwh.harvard.edu.

Financial Disclosures:

Each author has declared that there are no conflicts of interest in relation to the study presented here.

episodes. Since FW increase in SZ has been suggested to be related to neuroinflammation, we theorize that neuroinflammation might be a shared pathology between chronic BP and early SZ.

Keywords

bipolar disorder; diffusion magnetic resonance imaging; Free Water Imaging

Introduction

Bipolar disorder (BP) is a chronic, debilitating disorder that affects between 2–3% of the world's population¹. Individuals with BP suffer from various deficits in cognitive, social, and emotional functioning that can often result in difficulties in everyday abilities and interpersonal relationships. Patients and families often experience severe economic burden because treatment for BP generally includes the lifelong use of antipsychotic medication and multiple hospitalizations. In fact, recent studies estimate that BP is one of the most expensive healthcare diagnoses, with the estimated total economic burden of bipolar I and II disorders adding up to 151 billion dollars in the United States of America in 2009². There is also a heightened risk of suicide, up to 20 to 30 times greater, amongst patients who have BP than the general population³. However, despite years of research to better understand the symptoms of BP and how to prevent onset, little is known about the etiology of the disorder.

Neuroimaging has proven to be a useful tool for the identification of anatomical abnormalities in the brain that could give insight into the underlying pathophysiology behind BP. Previous structural imaging findings in patients with BP have shown reductions in gray matter density in the frontal lobe and in areas responsible for emotional processing when compared to controls⁴. Diffusion MRI (dMRI) and specifically diffusion tensor imaging (DTI) studies in patients with chronic BP have shown a decrease in fractional anisotropy (FA) in the corpus callosum^{5–7}, anterior limb of the internal capsule⁸ and anterior corona radiata^{5, 9}, concluding that the “integrity” of white matter connections within these regions is abnormal. Further studies of patients with BP have found decreased FA specifically in anatomical regions involved with cognitive, emotional, and behavioral regulation that are present from early on in the course of disease¹⁰. Lower FA in orbitofrontal and subgenual white matter in BP has also been related to dysregulation of affective systems and impulsivity symptoms¹¹. Interestingly, a comparison study of schizophrenia (SZ) and BP found that there is a significant decrease in FA in the uncinate fasciculus, anterior limb of the internal capsule, and anterior thalamic radiation in both groups when compared to controls; however, when directly comparing patient groups there was no significant difference⁸. Previous research has also found decreased FA in prefrontal white matter tracts in a sample of medication-naïve adolescents who recently experienced their first episode of mania, implicating that there are microstructural differences present even at the onset of disease¹². Despite these numerous findings, it is difficult to relate these abnormalities to a specific pathology.

Although FA is the most commonly reported DTI measure, there are inconsistencies between studies in reports of how white matter connectivity is affected in BP. While most studies report a decrease in FA, a few studies have reported instances of increased FA¹³ or

no difference when compared to controls¹⁴. In addition to these inconsistencies, FA is considered a non-specific measure in relation with pathophysiology. FA is sensitive to a variety of biological mechanisms that might include potential increases in extracellular water volume, axonal demyelination, or degeneration¹⁵. Therefore, it is hard to know which pathology, or perhaps number of pathologies, contribute to the changes evidenced in BP.

Free Water Imaging is a dMRI analysis method that provides greater biological specificity than DTI to investigate underlying white matter pathologies of disease because this method deconstructs the diffusion signal. The FW compartment is isotropic with a fixed diffusion coefficient that models water molecules diffusing freely in the extracellular space and may indicate changes caused by neuroinflammation, atrophy, or cerebral edema¹⁶. The tissue compartment is modeled as a tensor and denotes water molecules that are hindered or restricted by brain tissue¹⁶ and can be quantified by the fractional anisotropy of the tissue (FA-t). In this two-compartment model, changes in the tissue, such as greater demyelination, for example, would lead to a reduction in FA-t, while increased extracellular water would lead to an increase in FW. Both a reduction in FA-t, as well as an increase in FW, would appear as reduction in FA when using the traditional DTI diffusion method for analysis. Therefore, the Free Water method is valuable for distinguishing between structural changes that are related to underlying white matter pathology with greater biological specificity than traditional diffusion metrics, such as FA.

Previous studies have utilized the Free Water Imaging method in patients with first episode as well as recent-onset SZ, reporting that there were increased FW values in early stages of the illness, indicating extracellular changes when compared to controls^{17,18}. In contrast, reductions in FA-t were predominantly found in patients with chronic SZ when compared to healthy controls¹⁹. This finding has been proposed to reflect the presence of compromised white matter or axonal damage in chronic stages of SZ²⁰.

While Free Water Imaging has been applied to study patients with SZ and other disorders such as major depressive disorder²¹, Parkinson's disease²², and mild traumatic brain injury²⁰, this method has never before been applied to patients with BP. Current research finds that SZ and BP share many similarities such as symptoms, genetic susceptibility loci²³, elevated levels of peripheral neuroinflammatory biomarkers²⁴, and, as mentioned previously, white matter aberrations⁸. Importantly, BP and SZ patients have been found to share endophenotypic markers associated with genetic risk of white matter reductions in the left temporoparietal and frontal regions suggesting that there are shared, genetically controlled structural brain abnormalities between the disorders²⁵. There is a consistent pattern between these similarities, however, in that patients with SZ tend to have a greater severity of disease impairment than patients with BP²⁶. Based on these findings, it can be hypothesized that there will be parallel, yet less severe findings with the Free Water Imaging method in patients with chronic BP. Specifically, we hypothesize that there will be FA-t reductions in patients with chronic BP that are less severe than reductions previously reported in chronic SZ.

In this first study to utilize Free Water Imaging in BP, we aim to provide greater biological specificity of the underlying white matter changes that mediate chronic BP and thus provide a better understanding of severe mental illness.

Materials and Methods

All patients were recruited from community-based clinics and psychiatric hospitals through the Oxford Mental Health Services in Oxford, United Kingdom as part of the Cerebral Asymmetry and Functional Language in Psychosis (CAFLIP) study. This study utilized dMRI scans for 28 controls (HC) and 17 bipolar disorder I patients (BP). See Table 1 for additional demographic information. The following inclusion criteria for the patient cohort were used: 1) English spoken as their first language, 2) age between 16 and 50 years old during the study, and 3) a DSM-IV diagnosis of bipolar disorder. Patients were excluded if: 1) MINI Mental State Examination score < 28, 2) abuse of alcohol or drugs within six months as defined by the SCID and by a score of < 8 on the Alcohol Use Disorders Identification Test (AUDIT) questionnaire, 3) score of > 7 on either the 17-item Hamilton Depression Rating Scale (HAM-D) or the Young Mania Rating Scale (YMRS) at initial assessment or one month later at scanning, 4) history of other Axis I or II conditions, 5) learning disability, 6) electroconvulsive therapy in the past year, 7) neurological disorder, 8) systemic illness with potential cerebral consequences, 9) hypertension (blood pressure > 150/100mm Hg or antihypertensive drug use) and 10) diagnosis of endocrine disorder, excluding corrected hypothyroidism. Typical and atypical antipsychotics, mood stabilizers, and antidepressants were used by the patients but specific daily dose data was not available for all individuals. Positive and Negative Syndrome Scale (PANSS) scores were available for 15 of the 17 patients with BP.

The following inclusion criteria were used for the control group: 1) English used as the primary language and 2) age between 16 and 50 years old at the time of the study. Control exclusion criteria were similar to the patient group exclusion criteria with the addition of: 1) no current or previous Axis I or II psychiatric diagnosis, 2) no psychiatric history in first-degree relatives, and 3) no medication use other than oral contraceptives.

This study was approved by the Oxford Research Ethical Committee and all procedures were conducted in accordance with the Helsinki Declaration of 1975. Written informed consent was acquired from all study participants.

Diffusion weighted images were acquired on a Siemens 3T Trio scanner with spin-echo, single shot echo-planar pulse sequence. All scans were acquired at Oxford University within a two year period. The data set parameters include: 12 directions with $b = 1000 \text{ s/mm}^2$, $b = 0$, resolution of $2.5 \text{ mm} \times 2.5 \text{ mm} \times 2.5 \text{ mm}$, bandwidth = 1860 Hz/pixel, TE/TR = 89/8500 ms.

Each scan was visually checked to ensure image quality and to exclude cases with severe motion artifacts, dropped signals, or portions of the brain that were out of view. All scans were corrected for motion and eddy currents. A motion parameter was calculated so that it could be included as a covariate in the statistical analyses²⁷. One scan of a control

participant had a motion parameter more than 2 standard deviations from the mean and was omitted. Tensor masks were created for each scan using 3D Slicer software (<http://www.slicer.org>), followed by manual editing to exclude non-brain areas.

Free Water Imaging analysis was then applied to all corrected diffusion weighted images using the methods described previously in Pasternak and colleagues¹⁷. As mentioned earlier, with this method the diffusion signal is deconstructed into two components and is represented with two maps: a voxel-wise map representing the fractional volume of extracellular free water (FW) and a voxel-wise map of the fractional anisotropy of “tissue” (FA-t). A conventional DTI FA map was also estimated from the corrected diffusion weighted images.

Next, we employed the Tract Based Spatial Statistics (TBSS) processing pipeline. In this analysis, we utilized the DTI FA map to project the data onto skeleton templates provided by the Enhanced Neuroimaging Genetics by Meta-Analysis (ENIGMA) DTI Working Group at the University of California²⁸. Using this pipeline, we projected each participant’s FA, FW, and FA-t maps onto the white matter skeleton where we conducted statistical analyses²⁹.

Group differences of FA, FA-t, and FW were detected by utilizing Randomise, a program from FMRIB Software Library (FSL) that uses nonparametric permutation-based testing with 5000 permutations and a threshold-free cluster enhancement to identify clusters with significant group differences that are fully corrected for family-wise errors. Covariates for these analyses included age, sex, and the motion parameter. Areas of white matter pathology were identified through comparison with anatomical structures according to the ICBM-DTI-81 white-matter labels atlas provided by Johns Hopkins University DTI workgroup³⁰. Group differences were considered significant at $p < 0.05$.

Pearson correlations were conducted to analyze the relationship between positive and negative symptom scores from the PANSS with FA, FA-t, and FW values.

Results

Healthy controls were significantly younger than patients with BP ($t(28) = -2.81, p < 0.01$). See Table I. There were no other known significant differences between groups.

Figure I shows that we find significant reductions in FA in BP patients compared to HC when using DTI. The FA reductions were found in the genu, body, and splenium of the corpus callosum, bilateral anterior corona radiata, right superior corona radiata, right posterior corona radiata, posterior thalamic radiation, right external capsule and left cingulum ($p < 0.05$).

Figure II shows significant and even more extensive increases in FW when chronic BP patients were compared to HC ($p < 0.05$) using Free Water imaging. Areas with increased FW in BP patients almost completely overlapped with areas of decreased FA from DTI, with a greater amount of areas displaying a FW increase than FA decrease. Regions with significant increases in FW include the genu, body, and splenium of the corpus callosum, bilateral posterior limb of the internal capsule, bilateral corona radiata, bilateral external

capsule and bilateral superior longitudinal fasciculus. Average whole brain FW values were significantly higher in patients with BP than in controls, as can be seen in Figure III.

There was no significant difference of FA-t when comparing chronic BP and HC groups and there were no significant correlations between positive and negative symptoms from the PANSS with FA, FA-t or FW.

Discussion

The present study utilized, for the first time, Free Water Imaging in a sample of patients with chronic BP compared with HC. Our analyses replicated previous findings of significant reductions in DTI based FA in patients with BP. More importantly, our analyses showed that these FA reductions highly overlapped with areas of widespread, and even more extensive, FW increases. The results of the present study are similar to the increased FW found in patients with recent onset SZ¹⁷. This pattern is different than that reported in chronic SZ, where only a slight increase in FW and extensive reductions in FA-t were found²⁰. The lack of FA-t findings in the present study supports our hypothesis of less tissue changes in chronic BP compared with chronic SZ and suggests that axonal changes in chronic BP are either subtle or not present. Alternatively, our findings demonstrate that a widespread increase of extracellular free water is the predominant pathology in chronic bipolar disorder. The lack of correlation between PANSS scores and FA, FA-t, and FW signifies that there is no relationship in our sample between symptom severity and microstructural or extracellular pathology. Similarly, the previously mentioned study by Lyall and colleagues (2016) utilizing Free Water Imaging in patients with first-episode schizophrenia also found no correlation between FA, FA-t, or FW with BPRS scores at baseline or 12 week-follow-up, despite a significant widespread increase in extracellular water and localized reductions in FA-t in circumscribed regions¹⁸. This gives further support that extracellular pathology does not have a clear or direct relationship with psychopathology, and instead may reflect a biological effect such as the presence of increased neuroinflammation, cerebral edema, or atrophy. The previous FW and FA-t findings of Pasternak and colleagues^{17, 20} provide converging support that a possible neuroinflammatory cascade occurs throughout the course of SZ. Based on this interpretation, it can be hypothesized that patients with chronic BP may experience a globalized neuroinflammatory response.

Recent evidence, such as an increased risk for psychiatric disease when diagnosed with a chronic inflammatory medical condition as well as elevated pro-inflammatory markers, has implicated that neuroinflammation may play a role in many psychiatric disorders^{31, 32}. During periods of neuroinflammation, the permeability of the blood brain barrier changes and allows for a greater influx of extracellular water. Increased peripheral IL-2, IL-4 and IL-6 pro-inflammatory cytokine levels³¹ as well as upregulated TNF- α ^{33, 34} have been found during manic episodes in BP patients. Likewise, IL-6³¹ and TNF- α ³⁴ levels were reported to be increased during depressive mood states. Interestingly, a study on postmortem frontal cortex brain tissue of patients who had chronic BP discovered upregulation of microglial and astroglial markers, as well as higher protein and mRNA levels of the IL-1 receptor and IL-1 β , all of which are markers for neuroinflammation³⁵. These studies suggest the presence of an increased neuroinflammatory immune system response, especially during

manic and depressive phases of BP. The cyclic nature of the dramatic shifts between manic and depressive episodes in BP aligns well to a possible transient increase in inflammation that may mediate BP pathology and symptomatology.

In the present study, reduced FA was found in the corpus callosum, corona radiata, internal capsule, and left cingulum. The authors of Repple and colleagues (2017) suggest that reduced FA points to less white matter connectivity in the corpus callosum, which is a crucial tract for interhemispheric communication and specifically for regulating emotion, attention, and cognitive functioning, which are often impaired in patients with bipolar disorder^{5,7,36}. Further, previous studies have found the corona radiata to be important in executive functioning and information processing, both of which are also commonly compromised in bipolar disorder³⁷. Pavuluri and associates (2009) found decreased FA in the anterior corona radiata in pediatric patients with bipolar disorder, and postulate that this decreased connectivity may contribute to inattention³⁷. Similarly, Lin et al. (2011) found decreased FA involving frontal lobe white matter tracts, suggesting that mood dysregulation in bipolar disorder may result from deficient white matter tracts including the thalamic radiation and cingulum³⁸.

In sum, the present study's findings are consistent with previous studies, and thus fit into the classical presentation of bipolar disorder symptomatology. In addition, the Free Water Imaging method utilized here points to a possible biological source of observed differences, such as increased neuroinflammation, cerebral edema, or atrophy in the brain of patients with chronic bipolar disorder, rather than compromised white matter tissue. However, neither the present study nor previous studies have found a strong correlation between changes in diffusion signal and clinical presentation. This could be due to the fact that the samples in these studies were too small or heterogeneous, or that our observations reflect generalized phenomena, rather than localized connectivity disruption.

Our study identifies a clear difference in white matter changes between chronic BP and chronic SZ. Chronic SZ shows more prominent structural damage, seen by a decrease in FA-t, in place of global FW increases, as seen in chronic BP. Interestingly, Martinez-Aran and colleagues reported that patients with BP who experienced a larger number of manic episodes or had a longer duration of illness showed greater cognitive dysfunction³⁹. Taken together, this indicates that frequent cyclic episodes, thus representing possible concurrent and greater amounts of neuroinflammation and extracellular free water pathology, could lead to more severe cognitive decline. However, because it is unknown whether patients with BP were in the manic, depressive, or euthymic phase at the time of scanning in this study, it is difficult to draw definitive conclusions. Further research is needed and should record the state of patients at the time of the scan (i.e., whether patients are experiencing mania or depression) so that FA, FW, and FA-t values can be better interrogated.

Despite the numerous similarities between SZ and BP previously mentioned, there are differences that will help to distinguish the possibility of differential pathology. The effect of medication is one specific piece of evidence that will help to identify these alterations. For example, when patients with SZ are prescribed Lithium, the gold standard drug for BP, there was no change in symptoms when prescribed the drug alone in comparison to placebo⁴⁰.

Recent studies have reported inconsistent findings when testing how Lithium may affect inflammatory levels in BP, however, a number of studies have shown that Lithium may work as an anti-inflammatory agent⁴¹. Thus, future research is needed to distinguish how psychiatric medication may affect biological processes in the brain and how these interactions may differ between distinct psychiatric illnesses.

There are several limitations to this study. The first limitation is the small sample size. Future studies should aim to utilize a larger sample that records the state of the patient at the time of the scan. The sample should also be more closely matched in age and gender because this will increase the power of the study to detect more subtle differences, and help to potentially further isolate underlying pathological differences between the patient group and controls. There was also limited information available about the age of onset of illness, mood state at the time of scanning, and duration of illness. The inclusion and exclusion criteria offer some evidence from cognitive symptom scoring, though nonspecific. Future studies should investigate whether there is a relationship between diffusion changes, disease state, and symptomatology at the time of scanning in order to better understand the pathology present in chronic bipolar disorder.

Another limitation to this study is the absence of medication information that was available for both control and BP groups. Typical and atypical antipsychotics have been found to have a normalizing effect on FA⁴² as well as the mood stabilizer lithium showing significant anti-inflammatory potential in rat models⁴³. On the other hand, the previously cited study of O'Brien and coworkers³⁴ found elevated cytokine levels of IL-6, IL-8 and TNF- α when antipsychotics or mood stabilizers were taken. It is thus important to take into consideration the effects of chronic medication use, including the use of long term antibiotics and anti-inflammatory medications, and future studies should investigate the potential relationship between medication use and imaging findings.

With regards to technical aspects, the current acquisition of the CAFLIP study was designed for DTI analysis, containing a single b-value. Although Free Water analysis with a single b-value is comparable to multiple b-value analyses⁴⁴, the estimation of the Free Water analysis parameters from multiple b-values may be more accurate and provide stronger statistical power to detect subtle differences⁴⁴. Future studies should consider including a number of b-values, especially low b-values, where the effect of the fast diffusing free water is more pronounced.

The results may have been affected by image artifacts that could be more common in one group than another. For example, motion can produce a great amount of difficulty in obtaining optimal acquisition between and within each scan because patients with manic symptoms may be too restless to endure scanning⁴⁵. Additionally, tardive dyskinesia, as well as similar Parkinsonian symptoms, are possible extrapyramidal side effects for patients taking antipsychotics⁴⁶ and thus may provoke excessive head motion. Even with correction for the motion parameter in our analyses, Weinberger and Radulescu (2015) argue that head motion can still be a confounding factor that can influence DTI measurements even when corrected for⁴⁷. To minimize the effect of motion, this study had strict inclusion criteria

regarding motion artifacts, and motion was quantified and used as a covariate in the analysis in order to control for this limitation.

While there is evidence that increased FW may symbolize greater neuroinflammation in the brain, it is possible that there are alternative interpretations of what this measure signifies. FW could be related to tissue loss, which would increase the amount of extracellular space and would be expected to be more prominent in older subjects. However, in an analysis of DTI tractography in healthy subjects aged 5–83 years, Lebel and colleagues⁴⁸ showed that there is not a great amount of change in FA in the age range that this study utilizes. It is therefore unlikely that FW is related to tissue loss or aging because gray matter tissue loss in patients with BP generally tends to be more focal, as seen in the meta-analysis of voxel-based morphometry studies by Selvaraj and colleagues⁴⁹ and are not as widespread as the findings presented here. To account for the potential impact of age, we included it as a covariate in our statistical analyses. It would be expected that white matter tissue loss would be more readily represented by the FA-t component, of which we see no difference in this analysis.

This study utilized Free Water Imaging to investigate microstructural white matter abnormalities and the underlying pathophysiology of chronic BP. We found that patients with BP displayed a decrease in FA mediated by an increase in FW, indicating that extracellular free water pathology, which could be related to neuroinflammation, is evident in chronic stages of BP. This finding could lead to possible new treatment methods and therapeutic interventions. This study can be used as a building block for more research into the underlying pathophysiology of BP because it was the first time Free Water analysis was used in BP and suggests that previous findings of FA decrease in patients with chronic BP could be due to extracellular water pathology. While more research is needed for greater clarity between the relationship of diffusion abnormalities and disease progression, this study complements previous research and provides promising insight into the pathophysiology behind severe psychiatric disease.

Acknowledgements:

This work was supported in part by grants from the National Institutes of Mental Health (R01MH102377, R01MH108574, R01MH074794) and the Stuart T. Hauser Clinical Research Training Program in Biological and Social Psychiatry awarded to Dr. Lyall (5T32MHQ1659–35).

References

1. Bipolar Disorder Among Adults. National Institute of Mental Health. Available from: <http://www.nimh.nih.gov/health/statistics/prevalence/bipolar-disorder-among-adults.shtml>. Updated April 2016 Last accessed on September 17, 2016.
2. Dilsaver SC. An estimate of the minimum economic burden of bipolar I and II disorders in the United States: 2009 [Abstract]. J Affect Disord 2011; 129: 79–83. [PubMed: 20888048]
3. Pompili M, Gonda X, Serafini G, Innamorati M, Sher L, Amore M, et al. Epidemiology of suicide in bipolar disorders: a systematic review of the literature. Bipolar Disord 2013; 15: 457–490. [PubMed: 23755739]
4. Lyoo IK, Kim MJ, Stoll AL, Demopulos CM, Parow AM, Dager SR, et al. Frontal lobe gray matter density decreases in bipolar disorder I [Abstract]. Biol Psychiatry 2004; 55: 648–651. [PubMed: 15013835]

5. Barysheva M, Jahanshad N, Foland-Ross L, Altshuler LL, Thompson PM. White matter microstructural abnormalities in bipolar disorder: A whole brain diffusion tensor imaging study. *NeuroImage Clin* 2013; 2: 558–568. [PubMed: 24179807]
6. Kumar J, Iwabuchi S, Oowise S, Balain V, Palaniyappan L, Liddle PF. Shared white matter disconnectivity in schizophrenia and bipolar disorder with psychosis. *Psychol Med* 2015; 45: 759–770. [PubMed: 25089761]
7. Wang F, Kalmar JH, Edmiston E, Chepenik LG, Bhagwagar Z, Spencer L, et al. Abnormal corpus callosum integrity in bipolar disorder: A diffusion tensor imaging study. *Biol Psychiatry* 2008; 64: 730–733. [PubMed: 18620337]
8. Sussmann JE, Lymer GKS, McKindy J, Moorhead TWJ, Munoz MS, Job D, et al. White matter abnormalities in bipolar disorder and schizophrenia detected using diffusion tensor magnetic resonance imaging. *Bipolar Disord* 2009; 11: 11–18. [PubMed: 19133962]
9. Karababa IF, Bayazit H, Kilicaslan N, Celik M, Cece H, Karakas E, Selek S. Microstructural changes of anterior corona radiata in bipolar disorder. *Psychiatry Investig* 2015; 12: 367–371.
10. Barnea-Goraly N, Chang KD, Karchemskiy A, Howe ME, Reiss AL. Limbic and corpus callosum aberrations in adolescents with bipolar disorder: A tract-based spatial statistics analysis [Abstract]. *Biol Psychiatry* 2009; 66: 238–244. [PubMed: 19389661]
11. Kafantaris V, Kingsley P, Ardekani B, Saito E, Lencz T, Lim K, Szeszko P. Lower orbital frontal white matter integrity in adolescents with bipolar I disorder [Abstract]. *J Am Acad of Child Adolesc Psychiatry* 2009; 48: 79–86. [PubMed: 19050654]
12. Adler CM, Adams J, DelBello MP, Holland SK, Schmithorst V, Levine A, et al. Evidence of white matter pathology in bipolar disorder adolescents experiencing their first episode of mania: A diffusion tensor imaging study. *Am J Psychiatry* 2006; 163: 322–324. [PubMed: 16449490]
13. Yurgelun-Todd DA, Silveri MM, Gruber SA, Rohan ML, Pimentel PJ. White matter abnormalities observed in bipolar disorder: A diffusion tensor imaging study. *Bipolar Disord* 2007; 9: 504–512. [PubMed: 17680921]
14. Houenou J, Wessa M, Douaud G, Leboyer M, Chanraud S, Perrin M, et al. Increased white matter connectivity in euthymic bipolar patients: Diffusion tensor tractography between the subgenual cingulate and the amygdalo-hippocampal complex. *Mol Psychiatry* 2007; 12: 1001–1010. [PubMed: 17471288]
15. Jones DK, Knosche TR, Turner R. White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. *Neuroimage* 2013; 73: 239–254. [PubMed: 22846632]
16. Pasternak O, Sochen N, Gur Y, Intrator N, Assaf Y. Free water elimination and mapping from diffusion MRI. *Magn Reson Med* 2009; 62: 717–730. [PubMed: 19623619]
17. Pasternak O, Westin CF, Bouix S, Seidman LJ, Goldstein JM, Woo TU, et al. Excessive extracellular volume reveals a neurodegenerative pattern in schizophrenia onset. *J Neurosci* 2012; 32: 17365–17372. [PubMed: 23197727]
18. Lyall AE, Pasternak O, Robinson DG, Newell D, Trampush JW, Gallego JA, et al. Greater extracellular free-water in first-episode psychosis predicts better neurocognitive functioning. *Mol Psychiatry* 2017; 00: 1–7.
19. Pasternak O, Westin CF, Dahlben B, Bouix S, Kubicki M. The extent of diffusion MRI markers of neuroinflammation and white matter deterioration in chronic schizophrenia. *Schizophr Res* 2015; 161: 113–118. [PubMed: 25126717]
20. Pasternak O, Koerte IK, Bouix S, Fredman E, Sasaki T, Mayinger M, et al. Hockey concussion education project, part 2. Microstructural white matter alterations in acutely concussed ice hockey players: a longitudinal free-water MRI study. *J Neurosurg* 2014; 120: 873–881. [PubMed: 24490785]
21. Bergamino M, Pasternak O, Farmer M, Shenton ME, Hamilton JP. Applying a free-water correction to diffusion imaging data uncovers stress-related neural pathology in depression. *Neuroimage: Clin* 2016; 10: 336–342. [PubMed: 27006903]
22. Ofori E, Pasternak O, Planetta PJ, Burciu R, Snyder A, Febo M, et al. Increased free water in the substantia nigra of Parkinson's disease: a single-site and multi-site study. *Neurobiol Aging* 2015; 36: 1097–1104. [PubMed: 25467638]

23. Berrettini WH. Are schizophrenic and bipolar disorders related? A review of family and molecular studies. *Biol Psychiatry* 2000; 48: 531–538. [PubMed: 11018225]
24. Kim S, Hwang Y, Webster MJ, Lee D Differential activation of immune/inflammatory response-related co-expression modules in the hippocampus across the major psychiatric disorders. *Mol Psychiatry* 2015; 21: 376–385. [PubMed: 26077692]
25. McDonald C, Bullmore ET, Sham PC, Chitnis X, Wickham H, Bramon E, Murray RM. Association of genetic risk for schizophrenia and bipolar disorder with specific and generic brain structural endophenotypes. *Arch Gen Psychiatry* 2004; 61: 974–984. [PubMed: 15466670]
26. Seidman LJ, Kremen WS, Koren D, Faraone SV, Goldstein JM, Tsuang MT. A comparative profile analysis of neuropsychological functioning in patients with schizophrenia and bipolar psychoses [Abstract]. *Schizophr Res* 2002; 53: 31–44. [PubMed: 11728836]
27. Ling J, Meredith F, Caprihan A, Pena A, Teshiba T, Mayer AR. Head injury or head motion? Assessment and quantification of motion artifacts in diffusion tensor imaging studies. *Hum Brain Mapp* 2012; 33: 50–62.. [PubMed: 21391258]
28. Jahanshad N, Kochunov PV, Sprooten E, Mandl RC, Nichols TE, Almasy L, et al. Multi-site genetic analysis of diffusion images and voxelwise heritability analysis: A pilot project of the ENIGMA/DTI working group. *Neuroimage* 2013; 81: 455–469. [PubMed: 23629049]
29. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *Neuroimage* 2006; 31:
30. Mori S, Wakana S, Nague-Poetscher LM, van Zijl PCM. *MRI Atlas of Human White Matter*. Amsterdam, The Netherlands: Elsevier 2005.
31. Brietzke E, Stertz L, Simoes Fernandez B, Kauer-Sant'Anna M, Mascarenhas M, Vargas AE, et al. Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. *J Affect Disord* 2009; 116: 214–217. [PubMed: 19251324]
32. Leboyer M, Soreca I, Scott J, Frye M, Henry C, Tamouza R, Kupfer DJ. Can bipolar disorder be viewed as a multi-system inflammatory disease? [Abstract]. *J Affect Disord* 2012; 141: 1–10. [PubMed: 22497876]
33. Kim Y, Jung H, Mynt A, Kim H, Park S Imbalance between proinflammatory cytokines in bipolar disorder. *J Affect Disord* 2007; 104: 91–95. [PubMed: 17434599]
34. O'Brien SM, Scully P, Scott LV, Dinan TG. Cytokine profiles in bipolar affective disorder: Focus on acutely ill patients [Abstract]. *Journal of Affective Disorders* 2006; 90: 263–267. [PubMed: 16410025]
35. Rao JS, Harry GJ, Rapoport SI, Kim HW. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Mol Psychiatry* 2010; 15: 384–392. [PubMed: 19488045]
36. Repple J, Meinert S, Grotegerd D, Kugel H, Redlich R, Dohm K, et al. A voxel-based diffusion tensor imaging study in unipolar and bipolar depression. *Bipolar Disord* 2017; 19: 23–31. [PubMed: 28239946]
37. Pavuluri MN, Yang S, Kamineni K, Passarotti AM, Srinivasan G, Harral EM, et al. DTI study of white matter fiber tracts in pediatric bipolar disorder and attention deficit hyperactivity disorder. *Biol Psychiatry* 2009; 65: 586–593. [PubMed: 19027102]
38. Lin F, Weng S, Xie B, Wu G, Lei H. Abnormal frontal cortex white matter connections in bipolar disorder: A DTI tractography study. *J Affect Disord* 2011; 131: 299–306. [PubMed: 21236494]
39. Martinez-Aran A, Vieta E, Reinares M, Colom F, Torrent C, Sanchez-Moreno J, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. *Am J Psychiatry* 2004; 161: 262–270. [PubMed: 14754775]
40. Leucht S, McGrath J, Kissling W. Lithium for schizophrenia. *Cochrane Database Syst Rev* 3 2003; doi:10.1002/14651858.CD003834.pub3
41. Nassar A, Azab AN. Effects of lithium on inflammation. *ACS Chem Neurosci* 2014; 5: 451–458. [PubMed: 24803181]
42. Phillips ML, Travis MJ, Fagioli A, Kupfer DJ. Medication effects in neuroimaging studies of bipolar disorder. *Am J Psychiatry* 2008; 165: 313–320. [PubMed: 18245175]
43. Nahman S, Belmaker RH, Azab AN. Effects of lithium on lipopolysaccharide-induced inflammation in rat primary glia cells. *J Innate Immun* 2012; 18: 447–458.

44. Pasternak O, Shenton ME, Westin CF. Estimation of extracellular volume from regularized multi-shell diffusion MRI. *Med Image Comput Comput Assist Interv* 2012; 15: 305–312.
45. Van Hecke W, Emsell L, Sunaert S. *Diffusion tensor imaging: A practical handbook*. 1st ed. Springer-Verlag New York, 2016; p. 365 Retrieved from: doi:10.1007/978-1-4939-3118-7.
46. Gao K, Kemp DE, Ganocy SJ, Gajwani P, Xia G, Calabrese JR. Antipsychotic-induced extrapyramidal side effects in bipolar disorder and schizophrenia: A systematic review. *J Clin Psychopharmacol* 2008; 28: 203–209. [PubMed: 18344731]
47. Weinberger DR, Radulescu E. Finding the elusive psychiatric “lesion” with 21st-century neuroanatomy: A note of caution. *Am J Psychiatry* 2016; 173: 27–33. doi: 10.1176/appi.ajp.2015.15060753. [PubMed: 26315983]
48. Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. *Neuroimage* 2012; 60: 340–352. [PubMed: 22178809]
49. Selvaraj S, Arnone D, Job D, Stanfield A, Farrow TFD, Nugent AC, et al. Grey matter differences in bipolar disorder: A meta-analysis of voxel-based morphometry studies. *Bipolar Disord* 2012; 14: 135–145. [PubMed: 22420589]

Figure 1: Reduced Fractional Anisotropy in Chronic Bipolar Disorder

Fractional anisotropy reductions ($p < 0.05$) are seen by red and yellow significance clusters that were corrected for voxel-wise multiple comparisons using Randomize's threshold free cluster enhancement. The clusters are overlaid on a white matter skeleton, shown in green. Significant reductions of FA are found when comparing patients with bipolar disorder to controls primarily in the corpus callosum, bilateral anterior corona radiata, right superior corona radiata, right posterior corona radiata, posterior thalamic radiation, right external capsule and left cingulum.

Figure 2: Increased Free Water in Bipolar Disorder Compared to Control

Increased free water ($p < 0.05$) is seen by blue significance clusters that were corrected for voxel-wise multiple comparisons using Randomize's threshold free cluster enhancement. The clusters are overlaid on a white matter skeleton, shown in green. Significant increases are widespread and are found when comparing patients with bipolar disorder to controls primarily in the corpus callosum, bilateral posterior limb of the internal capsule, bilateral corona radiata, bilateral external capsule and bilateral superior longitudinal fasciculus.

Figure 3: Average Whole Brain Free Water by Group

The average Free Water (FW) level has been plotted for each participant in the study. Free Water is indicating the fractional volume of water molecules that diffuse freely in the extracellular space (ranging between 0 and 1) and may indicate changes caused by neuroinflammation, atrophy, or cerebral edema¹⁶. Chronic bipolar disorder (BP) patients had a significantly higher average FW when compared to controls ($p < 0.05$), suggesting that there may be greater extracellular change in patients with chronic BP.

Table I:

Demographic Data Table

	Controls (Mean ± SD)	Bipolar Disorder (Mean ± SD)	p-value
Subjects	28	17	-
Gender	16 Males 12 Females	8 Males 9 Females	-
Age	26.32 ± 6.00	32.41 ± 7.62	< 0.01
PANSS Subjects	-	15	-
PANSS Score (Total)	-	48.47 ± 11.75	-