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The role of childhood adverse events and immunological parameters in influencing white matter microstructure in bipolar and unipolar disorders

Il ruolo delle esperienze infantili avverse e dei parametri immunologici nell'influenzare la microstruttura della sostanza bianca nel disturbo bipolare e unipolare

Relatore:
Chiar.mo Prof. **Carlo Marchesi**

Controrelatore:
Chiar.mo Prof.ssa **Veronica Aggio**

Laureanda:
Marta Sciascia

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A Veronica,
la luce che ha illuminato questo cammino.

A mia mamma, per tutto quello che fa ogni giorno.

A Giorgio, la mia persona:
a te dedico la mia intera vita.

All'impegno e ai sacrifici di una vita intera, per la mia libertà.

Abstract

Il disturbo depressivo è una delle malattie mentali più comuni oggi e sembra essere in costante crescita. È stato stimato che il 3,8% della popolazione è affetta da depressione, circa 280 milioni di persone nel mondo. Soprattutto quando ricorrente e con intensità moderata o grave, la depressione può diventare una grave condizione di salute e nel peggiore dei casi può portare al suicidio. Il presente lavoro si propone di comprendere l'influenza che eventi avversi infantili, quali abuso e maltrattamento, possano avere sull'integrità della sostanza bianca, predicendo la probabilità di sviluppare un disordine depressivo maggiore o un disturbo bipolare. Si è cercato dunque di individuare possibili marker neuroinfiammatori che potessero predire l'esistenza di un disturbo dell'umore.

Depressive disorder is one of the most common mental illnesses today and seems to be constantly growing. It has been estimated that 3,8% of the population is affected by depression, about 280 million people in the world. Especially when recurrent and with moderate or severe intensity, depression can become a serious health condition and in the worst case can lead to suicide. The present work aims to understand the influence that childhood adverse events, such as abuse and maltreatment, may have on white matter integrity, predicting the likelihood of developing major depressive disorder or bipolar disorder. An attempt was therefore made to identify possible neuroinflammatory markers that could predict the existence of a mood disorder.

Introduzione

La nuova pandemia virale mondiale ci ha fatto affrontare la dimensione reale di molti disturbi psichiatrici: il periodo di quarantena e lockdown ha portato a conseguenze quali disturbi dello stress, disturbi del sonno, ansia ed episodi depressivi. In Cina, i risultati hanno evidenziato una prevalenza della depressione durante la quarantena fino al 37% e dell'ansia fino al 35% (Ahmed et al., 2020); in Italia, la prevalenza dei sintomi depressivi è aumentata dal 5.4%, prima della pandemia, al 32.4% della popolazione, che ha mostrato livelli di depressione da moderati a estremamente elevati (Gualano, Lo Moro et al., 2020).

Il presente lavoro si concentra proprio sul disturbo depressivo maggiore e sul disturbo bipolare. Quest'ultimo, in particolare, è un disturbo dell'umore che si manifesta con un primo episodio depressivo maggiore e, dopo circa 6-7 anni, con un episodio maniacale o ipomaniacale di variabile entità. Non esistono ancora biomarcatori specifici che identifichino il disturbo ma la ricerca in tale ambito è ancora un campo aperto: i primi studi a individuare marker biologici in grado di spiegare l'origine del BD si sono concentrate inizialmente su singoli geni, per poi passare all'ipotesi che alla base del disturbo vi sia un polimorfismo, quindi l'implicazione di più geni insieme, ma nessuno di essi è risultato indispensabile.

Oggi la ricerca studia possibili marcatori grazie alle neuroimmagini: fino ad oggi possibili marcatori potrebbero essere i neurofilamenti leggeri (Nfl) che rappresentano la proteina del filamento intermedio del citoscheletro, espressa nei neuroni. Un altro marker che potrebbe avere un ruolo è la CRP, risultata ad alta concentrazione proprio nei pazienti con disturbo bipolare.
È stata scoperta anche una correlazione tra BD e abuso o maltrattamento infantile, con conseguente alterazione dell'asse ipotalamo-ipofisi-surrene, ed un effetto moderato sui telomeri (la parte finale degli alleli), che sembrano ridursi nel tempo e in modo marcato nei soggetti abusati.
È infatti un dato ormai confermato da numerose ricerche che lo stress infantile è legato ad una alterazione dei marker infiammatori. La letteratura internazionale identifica in particolare quattro tipi di maltrattamento infantile associati a vari esiti avversi in età adulta: abuso sessuale, abuso fisico, abuso psicologico e neglect (Afifi et al., 2011; Dube et al., 2003; Felitti et al., 1998; Green et al., 2010; Widom, Czaja, & Dutton, 2014) - essi verranno trattati all'interno del capitolo 2.

Quando si parla di eventi precoci spiacevoli (stress infantile), infatti, si fa riferimento al maltrattamento infantile che racchiude tutti quei comportamenti attivi o omissivi da parte dei genitori o di altri significativi nei confronti del bambino e che procurano un danno reale o potenziale per la salute, la sopravvivenza, lo sviluppo o la dignità del bambino (Gilbert et al., 2009). L'esposizione a eventi precoci avversi sembra essere associata a un aumento della vulnerabilità a disturbi psichiatrici e medici come depressione maggiore, disturbo da stress post-traumatico, diabete, malattie cardiovascolari e altre.

Vi è un'ampissima documentazione che testimonia gli effetti del maltrattamento e stress infantile sull'età adulta: Putnam e colleghi, nel 2013, per esempio, analizzando il National Comorbidity Survey Replication Sample di 9.282 individui, hanno scoperto che punteggi più alti nell'ACE (Adverse Childhood Experiences) hanno portato a una complessa psicopatologia adulta, definito da tassi più elevati di comorbidità e un numero maggiore di sintomi; il Mexican National Comorbidity Survey di 5.826 individui ha evidenziato un aumento dell'umore, dell'ansia, dell'abuso di sostanze e dei disturbi esternalizzanti (disturbo da deficit di attenzione e iperattività, disturbo oppositivo provocatorio e disturbi della condotta) dopo l'esposizione a disfunzioni e abusi familiari (Benjet et al., 2010).

Dal punto di vista biologico, è stato osservato che la marcatura epigenetica del gene dell'arginina vasopressina (AVP) da parte dello stress precoce nei topi è alla base di un'espressione sostenuta e di un aumento dell'attività dell'asse ipotalamo-ipofisi-surrene, innescando alterazioni endocrine e comportamentali che sono caratteristiche frequenti nella depressione.

È proprio sulla depressione di pazienti bipolari e unipolari che ci si concentrerà nel presente lavoro. In particolare, la ricerca si propone di capire se lo stress infantile, attraverso l'alterazione di marker infiammatori, possa andare ad influenzare la microstruttura della sostanza bianca nei pazienti affetti da depressione bipolare e unipolare. Verrà quindi effettuato un confronto tra i livelli neuroinfiammatori dei due gruppi clinici.

Andando ad indagare i meccanismi fisiologici alla base dei disturbi dell'umore, numerosi studi hanno osservato la presenza di una disregolazione del sistema immunitario, che risulta quindi essere un meccanismo patologico sottostante il disturbo bipolare. Queste alterazioni a livello infiammatorio, come la produzione di citochine pro-infiammatorie, risultano essere

legate ad altri biomarcatori del disturbo bipolare, come la sopracitata iperattività dell'asse ipotalamo-ipofisi-surrene, l'alterazione del metabolismo, la degradazione del triptofano e l'alterazione nell'integrità della sostanza bianca. Proprio quest'ultimo aspetto verrà preso in considerazione nella nostra indagine.

Nel progetto di ricerca sviluppato, sono stati reclutati 200 pazienti con diagnosi di depressione, in cura presso l'Ospedale San Raffaele di Milano. Di questi, 100 presentavano una depressione di tipo bipolare e 100 unipolare. Il primo gruppo era costituito da soggetti di età compresa tra i 21 e 69 anni, di cui 65 uomini e 35 donne. Il secondo gruppo era invece costituito da 63 uomini e 37 donne, di età compresa tra i 26 e 65 anni. Ogni soggetto è stato sottoposto a Risonanza Magnetica e prelievo venoso: i campioni di plasma, prelevati dai pazienti, sono stati analizzati con Bio-Plex Pro Human Cytokine 27-plex. Le analisi di imaging del tensore di diffusione (DTI) sono state invece effettuate mediante Tract-Based Spatial Statistics (TBSS) e Combat.
Poiché sono state considerate immagini di Risonanza pre e post 2016 (anno in cui è stata acquisita una nuova macchina di risonanza), queste non risultavano armonizzate, quindi confrontabili tra loro. È stato dunque utilizzato il software Combat che rileva le differenze di gruppo tra pazienti con vecchia e nuova risonanza e le armonizza rendendole così confrontabili e analizzabili. È stato possibile così indagare se al variare dei valori di CTQ infantile varia l'integrità della bianca, in termini di parametri L1 (diffusività assiale-AD), L2L3 (diffusività radiale-RD), MD (mean diffusivity).

I primi risultati hanno rilevato un'associazione tra traumi infantili e alterazioni dell'integrità della sostanza bianca nel BD; sono state inoltre identificate alterazioni di marker infiammatori associati a traumi infantili. Tra questi, uno dei marker (CCL3) era associato ad una riduzione diffusa di FA: in particolare, i livelli di CCL3 sono risultati essere associati negativamente ai valori di FA in diversi tratti WM che comprendono radiazioni talamiche, corona radiata e fascicolo longitudinale superiore ($p < 0,05$).

Dunque nei pazienti bipolari è stata osservata un'associazione tra il maltrattamento infantile e l'integrità della struttura della sostanza bianca. Aspetto invece non rilevato nei pazienti con depressione unipolare.

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Chapter 1 – Mood disorders

Mood can be defined as a pervasive and sustained emotion that influences the behavior of the individual and the perception he has of the surrounding world. In the lifetime of a person, fluctuations in mood are normal. This adaptive function is lost when these oscillations appear to be persistent, resulting in an impairment of behavior rather than in a state of psychological distress suggesting the existence of an underlying affective disorder.

Mood disorders represent, in fact, a set of pathologies characterized by alteration of mood that can interfere with the normal social and relational functioning of the person, as well as cause psychological discomfort.

1.1.Bipolar disorder

1.1.1 Clinical features

The DSM-V, in classifying mood disorders, divides bipolar and depressive disorders into two different chapters.

Bipolar disorder is characterized by major depression associated with manic or hypomanic episodes.

The manic episode involves a defined period of unusually high, expansive and irritable mood, together with an abnormal increase in energy or targeted activity, for at least a week. During the period of mood alteration and increased energy or activity, three (or more) of the following symptoms were persistent and present at a significant level (four if the mood is only irritable):

1) hypertrophic self-esteem or grandiosity

2) decreased need for sleep

3) greater talkativeness than usual, or push continues to talk

4) escape of ideas or subjective experience that thoughts happen quickly

5) distractibility

6) increase in targeted activity (social, work, school or sexual), or psychomotor agitation

7) excessive involvement in recreational activities that have a high potential for harmful consequences.

The mood alteration is severe enough to cause a marked impairment of work functioning or habitual social activities or interpersonal relationships, or to require hospitalization to prevent harm to oneself or others, or psychotic manifestations are present.

The hypomanic episode instead provides for a defined period of unusually high, expansive or irritable mood and an abnormal increase in energy and activity, which lasts continuously for 4 days. Such a state is clearly different from the habitual non-depressed mood.

During the period of mood alteration and increased energy and activity, three (or more) of the following symptoms were persistent and present at a significant level (4 if the mood is only irritable): analogous to those of mania

1) hypertrophic self-esteem or grandiosity

2) decreased need for sleep

3) greater talkativeness than usual, or push continues to talk

4) escape of ideas or subjective experience that thoughts happen quickly

5) distractibility

6) increase in targeted activity (social, work, school or sexual), or psychomotor agitation

7) excessive involvement in recreational activities that have a high potential for harmful consequences.

The episode is associated with a clear change in the way of acting, which is not characteristic of the person when he is asymptomatic.

Therefore, the alteration of mood and the change in the way of acting is observable by others.

The episode is not severe enough to cause marked impairment in the workplace or social environment, or to require hospitalization, and there are no psychotic manifestations.

The onset of bipolar disorder is around late adolescence/early youth. It has been estimated that in bipolar II disorder there is a female prevalence, while in type I disorder there are no relevant differences between males and females. The disorder is associated with about 10 (or more) episodes of illness over a lifetime and the duration of remission appears to be shortened after the second episode.

In addition, the risk of relapse appears to be greater as the number of previous and postpartum episodes increases.

We speak of a mixed episode meaning the co-presence of depressive and manic symptoms. In particular, the two episodes must develop for at least 1 week and the mood alteration must be severe enough to cause marked impairment of work functioning or habitual social activities or interpersonal relationships, or to require hospitalization to prevent harm to oneself or others, or psychotic manifestations are present. Finally, the symptoms are not due to the direct physiological effects of a substance (e.g., drug, medication or other treatment), or a general medical condition (e.g., hyperthyroidism).

Mixed-like episodes induced by antidepressant somatic treatment (e.g., medications, electroconvulsive therapy, light therapy) should not be considered for a diagnosis of Bipolar I Disorder.

1.1.2 BD type I and BD type II

In the Bipolar disorder category we found:

• Bipolar disorder type I

• Bipolar disorder type II

• Cyclothymia

• Bipolar disorder induced by substances or drugs

• Bipolar disorder due to medical condition

• Unspecified bipolar disorder

The main feature of bipolar disorder type I is a clinical course characterized by the presence of one or more manic episodes or mixed episodes that last at least a week. Often individuals also presented with one or more major depressive episodes. Episodes of substance-induced mood disorder (due to the direct effects of a medication, other somatic treatments for depression, a drug of abuse or exposure to a toxin), or mood disorder due to a general medical condition, do not constitute a useful criterion for a diagnosis of bipolar I disorder. In addition, the episodes are not better explained by a schizoaffective disorder and are not superimposed on schizophrenia, schizophreniform disorder, delusional disorder or psychotic disorder not otherwise specified.

The episodes of excitement are always of a manic type therefore of such intensity as to determine an important alteration of the functioning that frequently involves the need for treatment in a hospital regime.

The essential characteristic of bipolar II disorder is, instead, a clinical course characterized by one or more major depressive episodes, accompanied by at least one hypomanic episode. Hypomanic episodes should not be confused with the days of euthymia[1] that follow remission from a major depressive episode. The presence of a manic or mixed episode precludes the

[1] State of balance of mood

diagnosis of bipolar ii disorder. The expansive phases are in hypomania, that is, of mild or moderate intensity, however, such as not to determine the complete loss of social and work functioning.

One core feature of BD is a neurocognitive impairment that involves several domains, such as attention, verbal memory, and executive functions (Sole, Jimenez et al., 2017), with a substantial impact on the psychosocial ability and the quality of life in general. Even if this impairment is more prominent during acute episodes (both depressive and manic ones), it is still present during euthymia (Bortolato, Miskowiak et al., 2015).

Cyclothymia, on the other hand, occurs when, for at least 2 years, there is the manifestation of hypomanic symptoms that do not meet the criteria of the hypomanic episode and numerous periods of depressive symptoms that do not meet the criteria for the major depressive episode. There is therefore the alternation of hypomanic and depressive episodes below the threshold (we speak of minor depression).

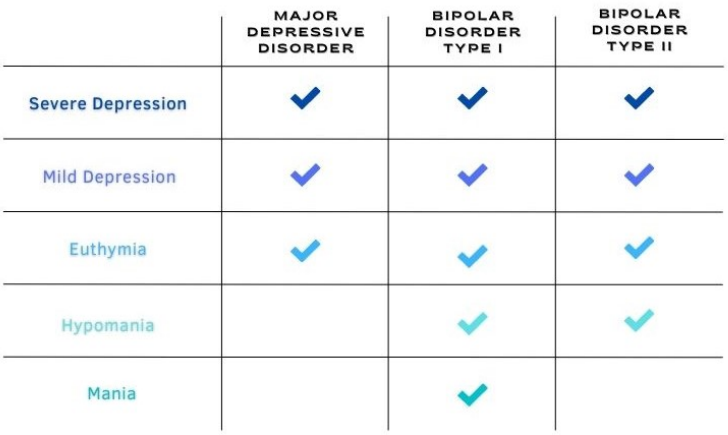


Figure 1 *- Polarity of symptoms for bipolar disorders subtypes (freely taken from O'Connell & Coombes, 2021).*

1.1.3 Differential diagnosis

It can often be difficult to distinguish bipolar disorder from other types of psychiatric disorder: the mood may vary due to several factors, such as hormonal pathologies, stress, personality disorders, biological disorders of the brain or alcohol and drugs. People with bipolar disorder may have difficulty describing their moods and giving an accurate history of their illness. These difficulties concern both introspective abilities and the tendency not to report mood changes as symptomatic, as they are pleasant. Approaching the bipolar patient can be particularly difficult, concerning specific phases of the disorder: in depressive phases, the patient may tend to mask his suffering, through the denial of symptoms and depressive experiences. In the phases of euphoria, the patient may not report as symptomatic some mental states and behaviors since he does not identify them as such; in these cases, he must be helped to recognize all the variations in his mood and report them. In the manic phase, disinhibition and distractibility can make interviewing on specific topics difficult. Professionals are therefore required to have excellent training to recognize the most subtle forms of the disorder, because certain symptoms are characteristic of several different pathologies.

For example, bipolar disorder is often confused with attention deficit hyperactivity disorder (ADHD), as people with ADHD have constant problems with attention and impulsivity: even bipolar people can become impulsive, especially during the manic crisis, the mixed or depressive episode.

Bipolar disorder can also be confused with borderline personality disorder, as it too is characterized by significant changes in mood tone. However, this last specificity, in borderline disorder manifests itself in a limited period and usually constitutes the reaction to a rejection by acquaintances or interpersonal stimuli.

1.2 Unipolar disorder

Among the depressive disorders are placed: major depression, persistent depressive disorder (dysthymia), premenstrual dysphoric disorder, depressive disorder due to medical cause, substance-induced depressive disorder, disruptive mood dysregulation disorder and unspecified depressive disorder.

In details, major depressive disorder (MDD) is characterized by:

1. five (or more) of the following symptoms (of which at least one is depressed mood or loss of interest or pleasure)
2. simultaneously present during a two-week period:

• Depressed mood for most of the day, almost every day

• Marked decrease in interest or pleasure for all, or almost, activities

• Significant loss or weight gain or appetite

• Insomnia or hypersomnia

• Agitation or psychomotor slowdown

• Fatigue or lack of energy

• Excessive or inappropriate feelings of self-devaluation or guilt

• Reduced ability to think or concentrate, associated with indecision

• Recurrent thoughts of death, suicidal ideation or suicide attempt.

3. They represent a change from the normal functioning of the person.

The onset of major depression usually falls in the third decade of life with an average duration of about 20 weeks and a probability of relapse, according to the American Psychiatric Association, of about 50-80%. Depression can also have characteristics and symptoms not present in the common experience, such as lack of feelings, suicide and delusions. Sadness is then associated with a wide range of symptoms such as: reduction of activity, physical exhaustion, reduction of interests and social relationships, physical malaise, insomnia etc. It,

therefore, impairs the normal functioning of the individual and has a longer duration than normal mood fluctuations. It has been estimated that among suicides, 45-70% are depressed people. Among individuals suffering from depression, 15% commit suicide.

Instead, we speak of minor depression when criterion n. 1 is not respected and recurrent short depression when criterion n. 2 is not met.

Cassano and collaborators (2004) state that there is a significant number of so-called unipolar patients who present slight hypomanic symptoms. Based on this unitary conceptualization of mood disorders, it is hypothesized that almost all recurrent depressions are to some extent bipolar and that the number of manic/hypomanic symptoms is concerning the number of depressive symptoms experienced over a lifetime: manic/hypomanic symptoms are associated with indicators of greater severity of depression.

There are several significant pieces of evidence for this:

- Symptoms such as paranoid ideation, hallucinations and suicide attempts are more frequent in bipolar disorder than in unipolar.
- In patients with recurrent unipolar depression, the greater the number of manic/hypomanic episodes, the higher the likelihood that suicidal ideation will be reported. This aspect confirms the idea that the presence of manic symptoms, although mild, can change a depressive state into a mixed state and increase the likelihood of psychotic symptoms.
- The number and severity of depressive aspects experienced by bipolar and unipolar patients are linked to the extent of manic and hypomanic symptoms experienced throughout life, so manic symptomatology even below the threshold is related to more severe depression.
- Reporting paranoid symptoms, such as "feeling like others are the cause of all your problems" or "feeling surrounded by hostility", and auditory hallucinations, is linked

to manic/hypomanic symptoms in both patients with recurrent unipolar depression and patients with bipolar disorder, although generally such symptoms are found more frequently in the type I bipolar patient population.

Overall, these data suggest that in patients with recurrent depression the manic/hypomaniacal aspects are not benign, rather they aggravate the psychopathological picture because they are related to an early onset of the disease, a greater probability of developing psychotic symptoms and to an increased risk of suicide current and over a lifetime.

Although there is a recent debate on the existence or not of unipolar depressions, it is currently useful to distinguish BD from recurrent major depressive episodes. The most common situation is the person who has had repeated depressive episodes and who develops, later, short periods or a few days in which he feels "thrilled"; a person who simply feels good after feeling depressed, but does not have any of the other symptoms listed in the group of those classified as hypomanic is probably a patient suffering from unipolar depression. In case of uncertainty between unipolar or bipolar depression, a mood stabilizer should be prescribed before introducing an antidepressant, since the antidepressant, in the case of BD, could facilitate the onset of a hypomanic episode. Significant mood swings can be induced by substance abuse, and in this case it is not possible to make a diagnosis of BD. Manic or depressive symptoms can also be simulated by taking certain drugs (cocaine, amphetamine, heroin, LSD, alcohol). Substance-induced mood disorders are short-lived and fade more quickly than substance-induced mood disorders; they are usually treated with detoxification and an addiction program. Substances can contribute to the onset of the first bipolar episode; changes in mood tone make you predisposed to drug or substance abuse, and these, in turn, can worsen mood changes.

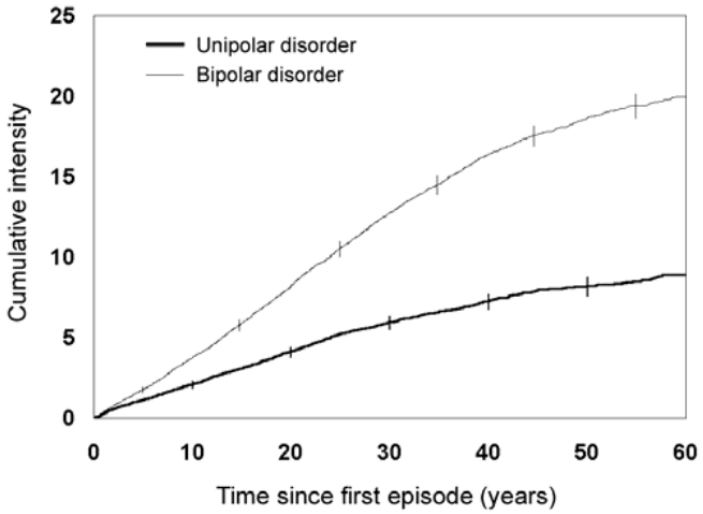


Figure 2 *- The cumulative intensity curves of the transition from states of remission to new episodes. In BP there is a doubled recurrence risk (Angst et al. 2003).*

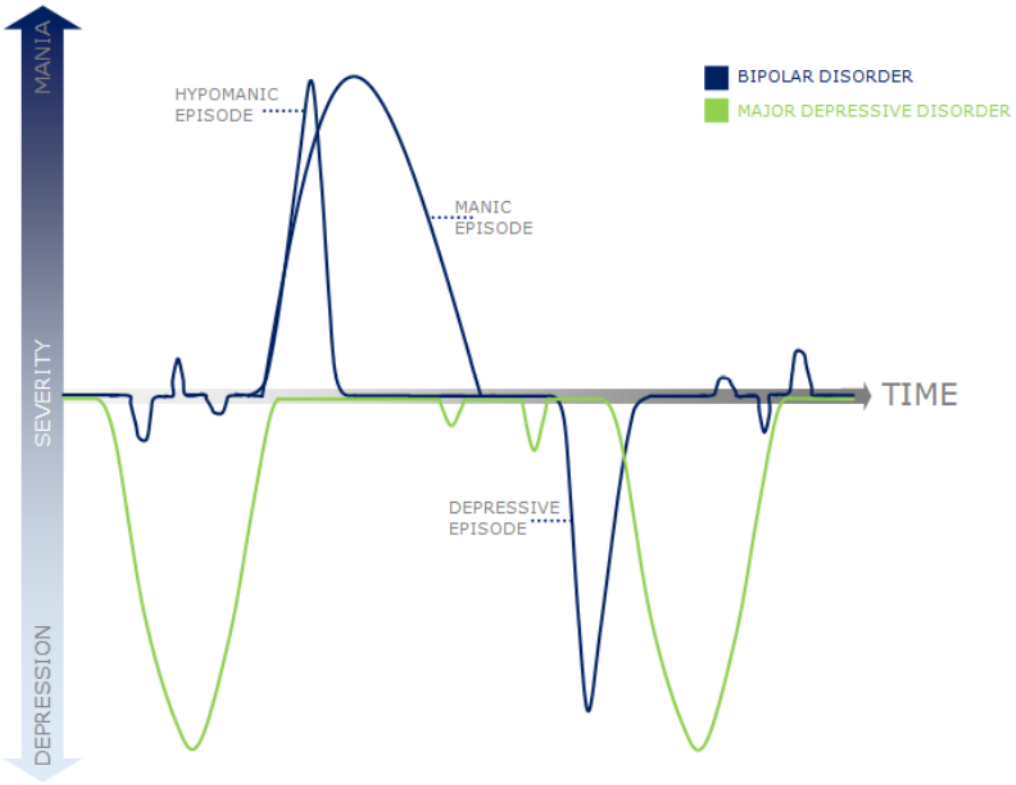


Figure 3 *- Mood episodes differences between BD type I, BD type II and MDD (unipolar depression).*

Therefore, BD can be classified, together with major depressive disorder, along a continuum defined by the duration and the severity of mood alterations. If MDD patients are characterized

by the recurrence of depressive episodes of a different entity, proceeding on the spectrum, the occurrence of hypomanic and manic episodes define BD type II and BD type I.

The main difference between bipolar disorder and major depression lies in the fact that people with bipolar disorder must have at least one manic or hypomanic episode, while people with major depression do not experience such high feelings.

1.3 Risk factors and genetics

Both type I and type II bipolar disorder are associated with genetic, environmental, and physiological risk factors. One of the strongest risk factors seems to be, in particular, a family history of bipolar disorders. BD have one of the highest heritability rates (0.79 – 0.90) among all psychiatric disorders (Hanford, Nazarov et al., 2016), suggesting a fundamental role of both genes and related biological factors. Meta-analyses seem to enlighten a small contribution from a few candidate genes, specifically the serotonin transporter, SLC6A4, d-amino acid oxidase (DAOA), and brain-derived neurotrophic factor (BDNF). Finally, the most successful strategy to identify a reliable association comes from the GWAS. More than 20 GWAS performed until today converge on three significant genes: ANK3, encoding ankyrin B protein involved in axonal myelination; CACNA1C, encoding an L-type voltage-gated ion channel implicated in neuronal development; TRANK1, encoding a large uncharacterized protein expressed in several tissues that seem to be involved in the homeostasis of the blood-brain barrier (BBB) (Gordovez & McMahon, 2020). Even if two Copy number variants (CNVs) were recently associated with BD, it seems that CNVs play a small role in BD (Grozeva, Kirov et al., 2010). Finally, with the introduction of the next-generation sequencing (NGS) technology, several studies are ongoing to look for rare single nucleotide and small insertion/deletion variants. Still, they need more significant sample sizes than GWAS (Wang, Li et al., 2010).

Despite the strong genetic influence, other environmental factors can influence the course of the disease, for example, negative perinatal events are associated with an increased risk of bipolar disorders. Alcohol and drug abuse in adolescence could also lead to an early onset of the disease and a more severe course. In reality, it remains to be clarified whether substance abuse is the cause or consequence of BD, but certainly, the association between the two pathologies determines an increase in affective changes, greater prevalence of physical disorders and suicide attempts and worse adherence to treatments. Other medical factors, such as hyperthyroidism, cardiovascular disease, diabetes and obesity, and seasonal could increase the likelihood of developing the disease.

There are currently several etiopathogenetic hypotheses to explain the close association between DB and weight gain and metabolic alterations. On the one hand, patients suffering from DB take psychopharmacological therapies for a long time, in particular atypical antipsychotics and mood stabilizers, which are associated with weight gain and the development of dyslipidemia[2] and diabetes, on the other hand they adopt harmful lifestyles such as excessive caloric intake, poor physical activity, tobacco smoking and alcohol consumption, which can increase cardiovascular risk.

On the biological level, the possibility that mood disorders in general, and depression in particular, are a cardiovascular risk factors is supported by numerous theories that have found ample clinical and experimental evidence, with the identification of alterations in platelet function, neurovegetative regulation of heart rhythm and inflammatory mediators, which would constitute the link between depression and cardiovascular risk.

[2] Alteration of blood lipids

1.4 Drug treatment and therapy

A meta-analysis conducted in 1999 on 315 controlled clinical trials lasting at least six weeks, evaluated the efficacy and tolerability of antidepressant drugs in the treatment of depression (Snow et al., 2000; Williams et al., 2000). The antidepressants tested, the main ones being Tricyclic Antidepressants (TCAs), Monoamine Oxidase Inhibitors (MAOIs), and Selective Serotonin Reuptake Inhibitors (SSRIs), were generally more effective than placebo and no significant differences in efficacy were found between the various classes of antidepressant, nor between the various compounds within the same class.

BD treatment can now count on a rich range of therapeutic strategies, approved for one or more phases of treatment but, although many points remain to be clarified, it is essential to know the medical implications, interactions and possible adverse events of the molecules used in the treatment of this disorder.

In the literature, there is a multitude of research aimed at studying the metabolic consequences related to the use of atypical antipsychotics that have shown that most of these drugs are associated with a significant increase in body weight, dyslipidemia and diabetes mellitus. Such complications, as we have seen previously, can entail not only serious risks from a medical point of view but also a negative impact on mental health.

Among the different factors that can contribute to weight gain and the aforementioned metabolic consequences we find, in addition to environmental factors, insulin sensitivity, gonadal steroids, genetic factors and alterations in the monoaminergic, cholinergic and histaminergic systems.

The differences observed in metabolic effects between different antipsychotics are due both to individual differences in molecular structure and to the different effects, they have on the secretion of leptin, a hormone derived from adipocytes that regulate insulin secretion and energy metabolism. The effectiveness of olanzapine, both in the acute phase and in the

maintenance phase of BD, has been widely documented for years, but the molecule is responsible for high metabolic risks, especially in long-term therapies.

Newcomer et al. (2002) performed glucose tolerance testing on a group of healthy controls and non-diabetic schizophrenic subjects treated with clozapine, olanzapine, risperidone, or typical antipsychotics, respectively, noting that patients treated with clozapine and olanzapine were the ones who had the most marked increase in blood sugar.

Clozapine, sometimes used in the treatment of resistant bipolar patients, also exposes patients to a high risk of diabetes and dyslipidemia.

Studies in patients switching from other atypical antipsychotics to aripiprazole have shown a reduction in body weight and improvement in other metabolic parameters.

Considered for many years the gold standard in the treatment of bipolar disorder, lithium has many studies that confirm its effectiveness both in the acute phase and in the maintenance therapy of mania. This indispensable molecule, however, is not free, in addition to the known possible toxic effects on renal and thyroid function, from a weight gain, observed from 30% to 65% of patients taking this drug.

Although the mechanisms by which lithium induces weight gain have not yet been clarified, various hypotheses have been suggested, including edema, a direct effect on carbohydrates and fat metabolism, lithium-induced hypothyroidism, and increased appetite.

The major problem related to the treatments available is that the efficacy is modest and has a delayed effect with several side effects (Kleindienst, Engel et al., 2005). Moreover, despite a correct pharmacological treatment set-up, with lithium as the gold standard, a 50% relapse rate over one year remains (Perry, Tarrier et al., 1999).

Many studies also highlight the usefulness of being able to combine pharmacotherapy with psychotherapeutic treatments, whose effectiveness is proven. It seems that the combination of treatments, where strategically chosen and planned, increases the magnitude of the response,

both in the sense of increasing the probability of response and in the sense of increasing the amplitude of the response itself. This result can be explained by at least two reasons. First of all, often psychotherapeutic work increases the acceptance of the pharmacological prescription, making the patient more collaborative and active in their own care process, because they are also more informed about the side effects, which they can learn to tolerate more. Secondly, different treatments have been shown to act on different aspects of major depressive disorder and on different depressive disorders.

It is within an integrated approach that psychotherapy has proved particularly useful to deal with the personal and relational difficulties caused by the disorder, positively affecting self-esteem and in general the quality of life of the patient suffering from bipolar disorder. All forms of psychotherapy can potentially be effective in the integrated approach to bipolar disorder. Only for some of them, come studies have highlighted and proven its effectiveness, including cognitive behavioral psychotherapy (CBT), family treatment called family focus treatment (F.F.T.) and interpersonal psychotherapy and social rhythms (I.P.S.R.T.).

Thus, for example, medications can quickly and effectively reduce vegetative symptoms in moderate and severe depression, and CBT can effectively work on subjective symptoms, which make the patient so vulnerable to relapse.

Furthermore, interpersonal therapy can effectively work on the quality of relationships even when the patient has severe difficulties with concentration and attention, and CBT can do so even when the patient has severe social deficits and concomitant personality disorders. It, therefore, appears that CBT and interpersonal therapy, alone and in combination with drugs, have given good results in different populations for the treatment of the acute phase and, above all, CBT, for the reduction of recurrence.

1.5 Inflammation and mood disorders

Current research has also focused on the involvement of the immune system in BD as a possible pathological mechanism underlying the disorder. The dysfunction of the immune system that leads to neuroinflammation has been increasingly implicated in the pathophysiology of numerous psychiatric disorders. Currently, numerous pieces of evidence strongly support the hypothesis that alterations of the immune-inflammatory system are fundamental for the pathophysiology of BD: Grosse, Drexhag and colleagues (2011) underline the role of altered regulation of T lymphocytes in the etiopathogenesis of BD: Since helper T cells[3] play a key role in mediating the immune response by secreting cytokines, several studies have been interested in changes in cytokine concentrations that patients with BD may experience. A greater number of active T cells and various citokines are found in bipolar subjects at every stage of the disorder, suggesting that the T lymphocyte system is always active, especially in manic subjects (Barbosa et al., 2014). During the depressive phase, however, an increase in C-reactive protein (CRP), cholesterol level and pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α was observed. As for chemokines, higher plasma levels of CCL11, CCL4 and CXCL10 and lower levels of CXCL8 have been reported (Misiak et al., 2020). It should be noted that treatment with mood stabilizers reduced IL-6 levels in patients with BD after 6 weeks of treatment, suggesting an immunoregulatory effect of mood stabilizers on specific cytokines.

In addition, it has been observed that concentrations of TNF-α, hsCRP, sCD4+OL, IL1Ra and STNFRI are linked to cognitive decline, which is decisive in the progression of bipolar disorder. Inflammatory markers such as CRP are associated with high comorbidity between BD and increased rates of obesity and inflammation.

[3] T-helper cells recognize foreign antigens and release cytokines that can activate other cells of the immune system, such as B cells — so they can produce antibodies — or other cells such as T cells and macrophages.

There is also a link between stress, suicidal risk, inflammatory genes, and pro- and anti-inflammatory cytokines during the different stages of BD (Dome et al., 2019): it is possible that there is an anti-inflammatory pattern associated with the risk of suicidal acts and that there are different genotypes and distributions of alleles, as well as different polymorphisms of TNF-α, IFN-γ and IL-10 in patients with bipolar disorder. There is also an increase in gene expression levels of cytokines such as IL-6 and CCL3 in lymphocytes of patients with BD (Pandey et al., 2015). Vares et al. (2020) sought to understand whether the inflammation that accompanies BD is a marker of disease status or trait[4]: a sample of patients in euthymic status, compared with a control group, did not present major differences in the levels of cytokines IL-1β, TNF-α and sIL-6R, except for the cytokine IL-1β that correlated with depressive symptoms in BD.

Finding no differences in cytokine concentration compared to controls suggests that this pro-inflammatory activation is closely related to the stage of the disease. IL-1β may therefore be an early indicator of the recurrence of depressive symptoms in subjects in the euthymic phase.

Cytokines appear to be related to the pro-inflammatory state present in manic or depressive episodes and not to the euthymic state. In the euthymic phase, patients tend to report higher levels of cytokines IL-33, IL-6 and osteoprotegerin (OPG) which inhibits the transcription factor NF-kB, involved in the regulation of inflammation, immunity and cell differentiation (Hope et al., 2010).

Alterations have also been found at the level of chemokines (Misiak et al., 2020), with a greater presence of CXCL8 and CCL2 chemokines, both equipped with receptors widely expressed in the CNS therefore responsible for neuronal differentiation, migration and proliferation of microglia, as well as modulation of the response of the HPA axis. It has also

[4] A trait marker is a factor that plays an important role in susceptibility to getting sick, while a status marker represents the most transient characteristics of the patient.

been seen that there is a positive association between the presence of these chemokines and cortical thickness in bipolar patients. These differences seem to diminish as the disease progresses.

Thus, inflammation in bipolar disorder is present not only at the level of the peripheral response – resulting in the production of proinflammatory cytokines – but also in the activation of microglia, considered the most important immune cells. Such inflammation also appears to be a genetic marker of the disease, causing dysregulation of the immune response. The proinflammatory cytokines most involved in inflammation are IL-1β, IL-6 and TNF-α, as well as C-reactive protein.

Immune dysfunction has therefore been identified as a new target of BD treatment with numerous clinical trials of anti-inflammatory agents.

As we have seen, BD is associated with significantly high rates of several medical comorbidities. The factors contributing to the aforementioned increase in the rate of medical comorbidity are likely multidimensional however, immune dysfunction has been proposed as a significant factor: many of the medical comorbidities of BD are inflammatory.

Inflammatory comorbidities that have been associated with BD include arthritis, type II diabetes mellitus, systemic lupus erythematosus, autoimmune thyroid, psoriasis, autoimmune hepatitis, multiple sclerosis, and obesity (Rosenblat and McIntyre, 2017). Aside from diagnosed inflammatory medical comorbidities, many other factors can result in inflammation in BD, including histories of early childhood adversity, chronic oxidative stress, and systematic idiopathic inflammation.

The direction of causality has yet to be established. However, inflammation can be a common cause of both BD and medical comorbidity.

Summing up, BD could in a sense be considered as a multisystem inflammatory disease, characterized by an imbalance between proinflammatory and anti-inflammatory cytokines,

secretion of TNF-a, IL-6 and IL-1β and abnormalities of chemokine receptors even in quiescent stages of disease (Benedetti et al., 2020). BD is characterized by the presence of inflammatory alterations (Savanna et al., 2017) that can affect both the progression of the disease and the nature of the episode under investigation (depressive, euthymic and manic). Cytokines can affect both the neurotransmitter and the neuropeptide system, as well as alter the balance of the HPA axis. It has been observed that, during the manic phase, higher levels of TNF-α and IL-4 emerge, as well as a high presence of IL-6 and IL-18 in correspondence with manic and hypomanic states, suggesting that these cytokines may be valid markers of manic episodes (Siwek et al., 2017). Higher levels of C-reactive protein – produced by hepatic hepatocytes in response to the cytokines IL-6, IL-1 and TNF-α – have also been observed in correspondence with the manic states of the disorder (Evers et al., 2019). In addition, activation of the acute phase protein is also associated with an increased risk of contracting inflammatory diseases, such as coronary heart disease.

In MDD cytokines have been found to influence almost every pathway involved in the pathogenesis of depression including alterations to the expression of neurotransmitters, neuroendocrine

function, synaptic plasticity and basal ganglia. The similarities between cytokine-induced sickness behaviour and MDD further support a role of inflammation in depression as well as the anti-inflammatory effects of successful antidepressant treatment.

1.5.1 White matter and neural correlates of bipolar disorder

The white matter (WM) lies beneath the grey matter of the cortex and comprises more than half of the human brain. It is composed of electrically insulated neuronal fibres called myelin, and its main components are the axons – myelinical and amyelinical – of glial cells and blood vessels. The integrity of white matter is the basis of effective communication at both the

neuronal and cortical levels. The most used tool to investigate, in vivo, white matter condition is the Diffusor Tensor Imaging (DTI) (Alexander et al., 2007) (see chapter 4: Methods).

Since structural alterations of both white and grey matter have been proposed as possible endophenotypes of bipolar disorder, recent studies have wondered whether these abnormalities in brain structure may be related to an inflammatory condition (Benedetti et al., 2020).

Neuroimaging studies have been widely performed on psychiatric disorders due to the unique feature of MRI techniques to investigate in a non-invasively way the brain structure and function. Structural investigation firstly reported, more than a decade ago, several WM alterations involving the limbic tracts (Houenou, Wessa et al., 2007, Wang, Jackowski et al., 2008), then followed by several studies that showed a more widespread WM modification, including other brain locations and related tracts (Cui, Chen et al., 2011, Leow, Ajilore et al., 2013, Repple, Meinert et al., 2017, Yurgelun-Todd, Silveri et al., 2007). All these DTI studies on WM shown microstructural alterations that, in general, involve both a reduction of those measures of WM integrity, specifically fractional anisotropy (FA) and axial diffusivity (AD), together with increased radial (RD) and mean diffusivity (MD), representative of disruption and loss of WM coherence (Abramovic, Boks et al., 2018, Bellani, Boschello et al., 2016, Benedetti & Bollettini, 2014, Benedetti, Yeh et al., 2011, Duarte, de Araujo et al., 2016). Other MRI techniques, such as voxel-based morphometry (VBM), broaden the brain alterations related to BD, including also a general volumetric WM reduction (Lee, Lee et al., 2020, Pezzoli, Emsell et al., 2018), that are detectable even at an early stage of the disorder (Duarte, Massuda et al., 2018)

Studies centered on WM alterations in bipolar disorder have proposed microglia activation as a possible mechanism of action underlying microstructural changes in white matter tracts. Microglia are in close contact with nearby neurons to perform beneficial functions, such as

synaptic remodelling, secretion of neurotrophic factors, and maintenance of tissue homeostasis. In the presence of external pathogens, microglia are activated and can respond both by enhancing the elimination of triggers and by producing inflammatory mediators. In the first case, the so-called "M2-like" activation, activated microglia perform functions of liquidation and repair through phagocytosis and the production of neurotrophic factors, leading to the resolution of inflammation without neuronal damage. In a condition of chronic inflammation, microglia switch to "M1-like" activation, which causes dysfunction and death of neural cells due to increased expression of nitric oxide, increased release of proinflammatory cytokines, chemokines and reduced production of neurotrophic factors (Heneka et al., 2014).

The evidence supporting a shift from M2 to M1 microglia activation in bipolar patients comes from the work of Brambilla et al. (2014): comparing gene expression of cytokines, chemokines, chemokine receptors and T cell markers, in individuals with BD, schizophrenia and healthy controls, higher levels of myocardial infarction markers and reduced expression of M2 markers were found in patients with BD compared to both schizophrenic patients and controls, indicating a peculiar form of myocardial infarction in bipolar patients linked to an inflammatory process that occurs in SNC.

The first study that investigated the association between a pro-inflammatory state and microstructural damage in the WM fibers of BD patients was the work of Benedetti et al., (2016): correlating serum levels of different pro-inflammatory cytokines and DTI measures in BD patients in course of a depressive episode, the authors reported a significant association between TNF-α, IL-8, and INF-γ concentrations and a decrease in FA together with increased RD and MD in key traits involved in brain functional integrity. These traits were the corpus callosum, the girdle, the upper and lower longitudinal bundles, the inferior fronto-occipital bundle, the hooked fasciculus, the radiated crown, the thalamic radiation and the inner capsule.

Since RD is an index of demyelination, the authors hypothesized that the association of higher RD and MD with lower FA indicates that cytokines exert their detrimental effects on white matter integrity probably exerting a detrimental effect on myelin sheaths, not inducing with a direct axonal damage.

Given that mood disorders have been associated with alterations in the activation and expression of T lymphocytes, a study by Poletti et al. (2017) investigated whether Thl and Th17 cells, related to proinflammatory activity, are associated with microstructural damage and whether T cell expression correlates with both DTI measures and neural responses to a moral valence attribution fMRI task in both depressed patients with BD and healthy controls (HC). The results showed that the rate of Th17 cells was positively correlated with FA values in the interhemispheric, fronto-occipital and temporo-occipital traits, involved in the emotional and cognitive functions of both bipolar patients and healthy controls. In addition, regulatory T cell levels were positively associated with RD and MD values but only in BD patients. Regarding the effects of T cells on brain function, in the BD group concentrations of T regulatory cells correlated with a decreased neural response to stimuli with negative valence compared to positive ones in the right dorsolateral prefrontal cortex. In light of these findings, it has been suggested that while higher levels of Th17 cells contribute to maintaining WM integrity, increased concentrations of regulatory T cells could promote structural disruption and functional deterioration. The balance between Th17 and T regulatory cells not only plays a crucial role in immune homeostasis, but also in the structural and functional integrity of the brain. Exploring the correlation between WM alterations and concentrations of T cells and cytokines in the different affective states that characterize BD, it was shown that mania is distinguished by an increase in IL-6 levels and CD4+ T lymphocytes, probably with T helper function, and a decrease in lymphocytes CD8+.

In addition, only mania was characterized by an increase in RD, particularly in the corpus callosum and the left upper radiated crown.

Recently, several studies have brought evidence of a relationship between inflammation and oxidative stress in BD, with increases in pro-inflammatory cytokine concentrations associated with a reduction in the production of oxidative elements (Niu et al., 2019). In particular, it has been suggested that, given the lipid content of myelin sheaths, lipid oxidative stress may induce damage in the WM microstructure, leading to the structural abnormalities observed in BD. To test this hypothesis, Versace et al. (2014) correlated serum lipid perioxidation with DTI indices in BD patients and HC. Since peripheral lipid hydroperoxides were significantly correlated with RD and FA in both groups, it would appear that oxidative stress may contribute to white matter damage in key mood traits, altered in BD patients.

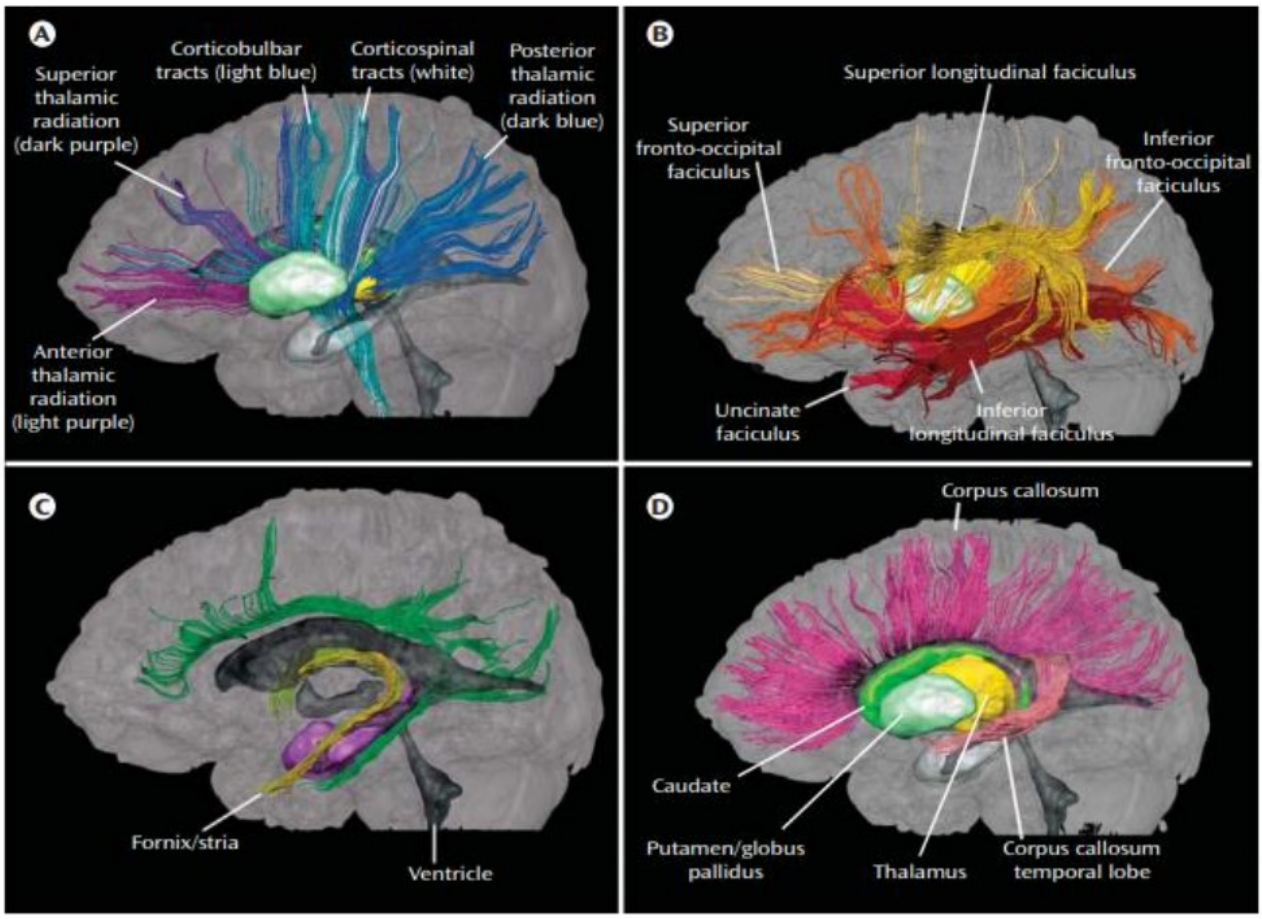


Figure 4 *- Left lateral views from three-dimensional depictions of major white matter fiber pathways, which were developed from normal DTI image (Mori et al., 2005)*

1.5.2 Inflammatory profiles in bipolar disorder and unipolar disorder: possible biomarkers

Existing studies state that alterations in immune system functions play a key role in mood disorders, but in different ways depending on the nature of the specific disorder. The study of inflammatory markers could therefore help in the differential diagnosis between bipolar disorder and major depression in particular. Few studies so far have directly compared unipolar and bipolar patients to identify immunological biomarkers that distinguish the two disorders. Bai et al. (2015) reported higher levels of inflammatory cytokines in bipolar patients

than in patients with major depression, suggesting more severe immunological dysfunction in bipolar disorder than in major depression.

Mota et al. (2013) instead focused on IL-1β to detect possible changes in its concentrations between patients with MDD and BD and showed higher IL-1β levels in MDD patients than in both BD patients and healthy controls. This increase in IL-1β concentrations was specific to unipolar depression but not to the depressant state of BD, suggesting that depressive states in the two different diagnoses are distinguished by different immunological profiles. To further study the differences in cytokine levels in unipolar and bipolar depression, Mao et al. (2018) evaluated pro and anti-inflammatory cytokine levels in patients with MDD and BD in a depressive state both at T0 and 12 weeks after treatment. Results showed higher levels of TNF-α and IL-13 in the MDD group than in the T0 bipolar groups. After 12 weeks of treatment, patients with major depression showed a significant reduction in IL-4, IL-12 and TNF-α concentrations regardless of response to treatment, while only bipolar patients who responded to treatment experienced an increase in IL-4 and TNF-α. These results confirm the idea that drugs may induce variations in cytokine concentration that may facilitate the prediction of response to treatment, especially in bipolar depression.

Patients with Bipolar II disorder were also shown to have higher concentrations of CRP than both patients with major depression and HC. At the end of the treatment period, the BD-II group showed no significant changes in CRP levels compared to T0, but patients with MDD showed an increase in CRP concentrations. Several studies agree that CRP can be considered a stable biomarker of bipolarity and increasing its concentration could favour the risk of mood shifting from depression to mania.

Other studies have investigated the differences in T cell populations between bipolar and unipolar depression as a possible biomarker of the disease: Wu et al. (2017) focused on immune control inhibitors, such as T-cell immunoglobulin, TIM-3 and programmed cell death

protein 1 (PD-1), which play an important role in regulating the immune response. Bipolar patients possessed a lower proportion of cytotoxic T cells, along with reduced expression of PD-L2, a PD-1 ligand, and high expression of TIM-3 on cytotoxic T cells, while both groups – bipolar and unipolar – showed an increase in IL-6 concentrations compared to healthy ones. These findings suggest that not only does bipolar and unipolar depression differ in cytokine production, but also in the number of T cells and the expression of immune control inhibitors.

Chapter 2: Childhood stress and adverse events

Sexual, physical and emotional abuse, as well as emotional abandonment, at an early age, leads to a very significant increase in the risk of developing mood disorders and anxiety disorders, substance or alcohol abuse and other medical disorders, in adulthood.

In 2012, the U.S. Department of Health and Human Services documented 3.4 million referrals to child protection services, representing 686,000 children. About 80% of the mistreatment was perpetuated by one or both parents. In this report, early stress was composed of neglect (78.3%), physical abuse (18.3%) and sexual abuse (9.3%). Negligence is often defined as the inability of a parent or other person with responsibility for the child to provide food, clothing, shelter, medical care or supervision necessary to the extent that the health, safety and well-being of the child are threatened. All authorities agree, however, that the vast majority of cases of child abuse and neglect go unreported. It is also important to note that some forms of abuse, especially sexual abuse, occur mainly in the younger age group. In 2000, 70% of all sexual assaults in the United States were committed against children.

A series of studies conducted since 1990 have focused precisely on the relationship between adverse childhood experiences (ACEs) and development. In particular, in the period of development our brain is subject to neuronal plasticity, that is, it can to modify its connections,

eliminating some of them and creating new ones. This is why the child's brain is extremely sensitive and goes through what is called a "critical period"[5].

Specifically, we can identify two types of brain plasticity: synaptic and cellular. The first represents a change in the junctions between neurons, called synapses: synaptic plasticity lasts a lifetime, unlike cellular plasticity that occurs only in the first months of life. During synaptic pruning the brain eliminates the extra synapses: with growth, then, our experiences strengthen the most relevant circuits while the others weaken and vanish.

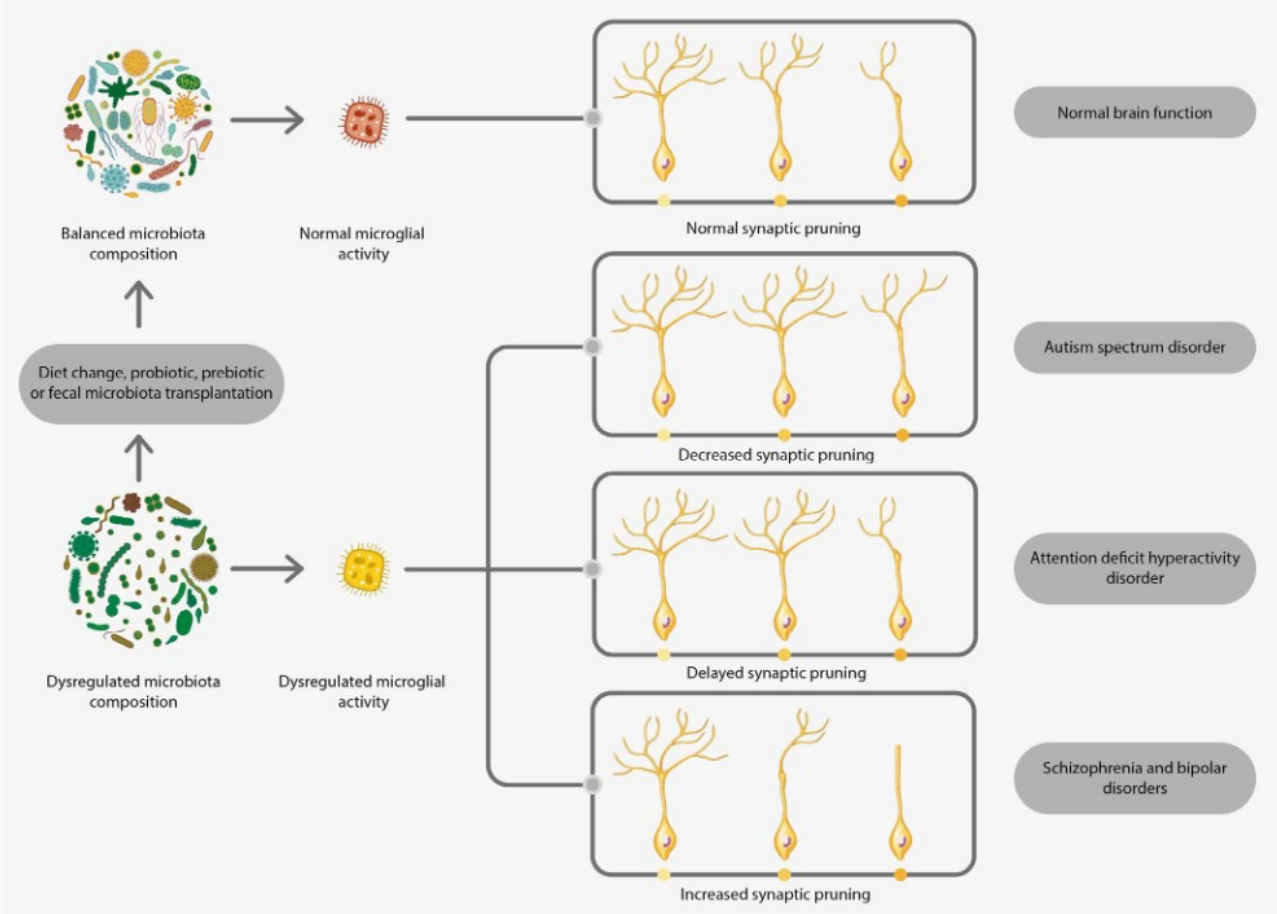


Figure 5 *- Different patterns of dysregulated synaptic pruning have been linked to various neuropsychiatric phenotypes including autism spectrum disorder, attention deficit hyperactivity disorder, schizophrenia, and bipolar disorder (Elthoki et al., 2019)*

[5] A critical period is defined as a period during development in which experiences lead to irreversible changes.
A sensitive period is defined when the brain is particularly sensitive to an environmental stimulus.

Situations of chronic stress can lead to progressive wear and tear of the body, determining the so-called allostatic load[6]. When a threat to the balance of the organism emerges, there is an activation of the immune system in inflammatory terms, followed by activation of the CNS. As a result, there is also activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (HPA).

Even the immune system is still at a primitive stage so it can be modified by the external and maternal environment. For this reason, any external stimulus can have a profound impact on a developing immune system, triggering an inflammatory response and altering microglia activation and neuroendocrine function.

Therefore, when we talk about ACE, we mean any dysfunctional environment capable of influencing, in one way or another, the development of the child, victim or witness (Fellitti et al., 1998).

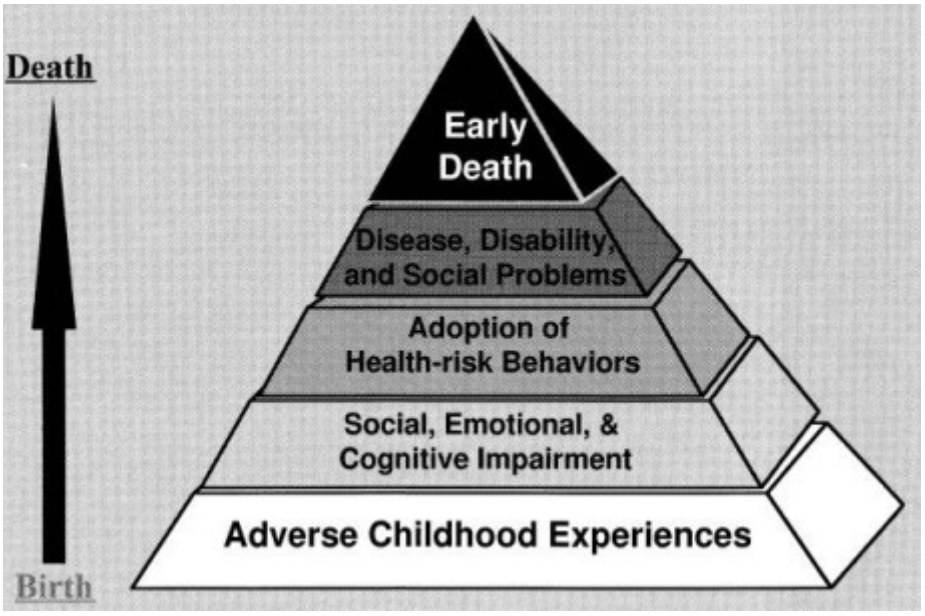


Figure 6 *- The Adverse Childhood Experiences Study (Fellitti et al., 1998).*

[6] Allostasis refers to the changes that the organism makes in relation to its internal environment.

There seems to be a significant link between ACE and mental and physical illnesses that can even lead to premature death in adulthood. The long-term effects of dysfunctional dynamics in the home can be assessed by focusing on the presence of health problems, quality of life and the use of health systems. These experiences can become risk factors for smoking/substance abuse, obesity, depression, alcoholism, and sexual promiscuity, but also heart problems, cancer, bronchitis, diabetes and hepatitis. The literature on this subject is flourishing: among a group of psychiatric patients with variable diagnoses in France and Italy (n = 587), a significant correlation between childhood trauma and suicidal behavior has been observed (Sarchiapone et al., 2009). In a study of 1,488 military and veterans, after controlling for the effects of combat exposure and post-traumatic stress disorder (PTSD), exposure to childhood trauma was associated with both depressive symptoms and suicidal ideation (Youssef et al., 2013). There is also evidence that child abuse and neglect are associated with an increased risk of bulimia (Sanci et al., 2008), obesity (Noll et al., 2007) and unintentional teenage pregnancy (Bellis et al., 2014). There are many studies attesting that childhood trauma is associated with a more severe course of depression, including chronicity (Wiersma et al., 2009), the characteristics of atypical depression (hypersomnia, sensitivity to interpersonal rejection, increased appetite, paralysis of the limbs) (Withers et al., 2013) and, above all, a poor outcome of psychopharmacology and psychotherapy treatment (Nanni et al., 2012).
These consequences and in particular the abuse of alcohol, derived from ACE, are associated with a dysregulation of emotions and poor self-control, precisely because of the dysfunctional environment in which the subjects grow up and because of the lack of adaptation to certain situations.

From the neurological point of view, it has been observed that subjects exposed to ACE shown a reduced activity of the prefrontal cortex, in particular of the orbito-frontal cortex and a

decrease in N-acetylcysteine[7], the lack of which is associated with neuronal damage or even death (Kolb et al., 2012). There was also an increase in the volume of the amygdala which in adulthood no longer seems to be present. Some data also report abnormalities in the hippocampus.

From a neuroinflammatory perspective, on the other hand, children who have suffered maltreatment report an increase in the activation of pro-inflammatory cytokines, an alteration in cortisol production and greater reactivity to stress in psychosocial tests such as the Trier Social Stress Test (De Bellis et al., 2014).

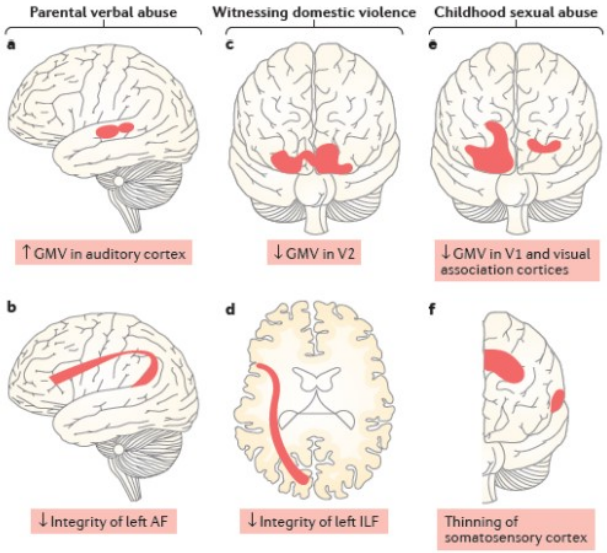


Figure 7 *– Abuse-type specifiic effect on the developing brain (Teicher et al., 20016)*

It is therefore essential to study the role of ACE not only as a possible risk factor with regard to the onset of mental and physical disorders but also concerning the impact on the evolutionary trajectory of the individual. The greater sensitivity to environmental stress can exert both functional and structural influences, modifying the volume of some areas and the integrity of the traits that guarantee their connections.

[7] Reducing agent with antioxidant properties

2.1 Inflammation and adverse childhood experiences

Due to the prevalent role of the HPA axis in regulating the stress response of mammals, this endocrine system has received more attention than other systems that could potentially be affected by an early life trauma. This is an extraordinarily complex area with a considerable number of studies documenting both the increase and decrease in HPA axis activity as a result of child abuse and neglect.

In particular, the mother-child separation during the first postnatal phase is one of the factors that most leads to childhood stress, associated with an increase in glucocorticoid secretion (GC), greater endocrine reactivity and disruption of the homeostatic mechanisms that regulate the activity of the HPA axis (all factors considered pregnant in mood disorders). The control of glucocorticoid secretion affects, in particular, two hypothalamic neuropeptides: corticotropin-releasing hormone (CRH) and vasopressin (AVP). Together they stimulate the secretion from the pituitary gland of adrenocorticotropin (ACTH), which in turn promotes the synthesis and release of glucorticoids from the adrenal glands.

Many studies link vasopressin and the corticotropin-releasing hormone to mood and related disorders, making their receptors targets of psychopharmacological agents (Holmes et al., 2003).

AVP, in addition to having an important role in the postnatal development and functional maturation of the pituitary-adrenal hypothalamic axis, also enhances the actions of CRH when prolonged activation of the pituitary and adrenal glands is required (Engelman et al., 2004).

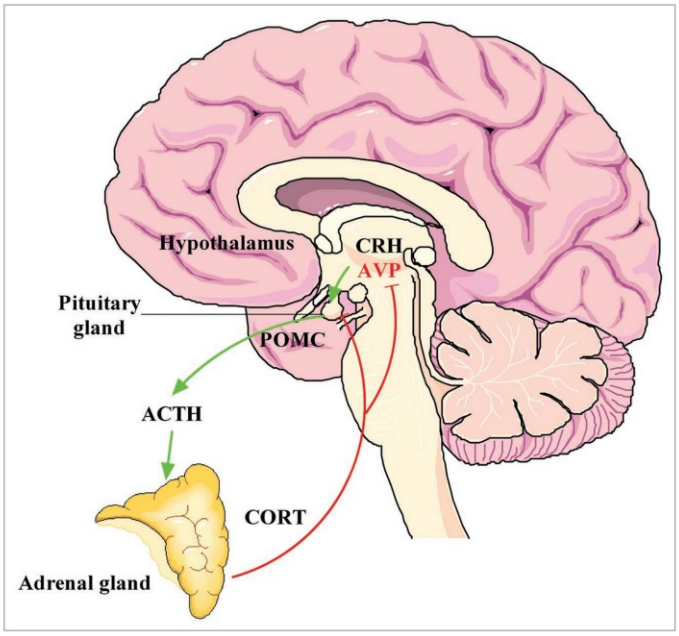


Figure 8 *– HPA axis: CRH and AVP are expressed in the parvocellular neurons of the hypothalamic nucleus paraventricularis. The joint release of CRH and AVP into the portal blood vessels leads to potent stimulation of anterior pituitary ACTH secretion and POMC transcription. ACTH is derived from the POMC precursor mRNA and stimulates in turn secretion and synthesis of the stress hormone corticosterone by the adrenal glands. The activational effects of the HPA axis are counteracted by the inhibitory effects of corticosterone on the hypothalamus and pituitary and serve to attenuate and determinate the stress response (Murgatroyd et al., 2010)*

Studying the relationship between negative events in childhood, the appearance of an inflammatory phenotype in adulthood and certain risk factors - such as socio-economic status, depression or stress - it was observed that more than 10% of subjects who reported high levels of CRP experienced maltreatment in childhood (Iob et al., 2020). CRP is produced by the liver in response to systemic inflammation, such as the release of pro-inflammatory cytokines. Danese et al., (2007) have shown that acute stressors can induce the activation of the transcription of the NF-kB factor, therefore the secretion of pro-inflammatory cytokines that would impact glucocorticoids: NK-kB is an intracellular protein with an important role in cell survival and proliferation, cytokine expression, apoptosis and CNS efficiency. Coelho et al. (2014) noted that maltreatment is associated with an increase in C-reactive protein, fibrogen and pro-inflammatory cytokines: this condition can give rise to mental disorders, diabetes,

obesity, asthma, neurodegeneration and coronary problems. All united by an inflammatory mechanism. The existence of this inflammatory process has therefore been hypothesized to be the mediator of the relationship between child abuse and health problems in adulthood.

Childhood traumas can cause alterations not only at the level of the HPA axis, but also at the metabolic level: there is a high risk of developing obesity, due to an accumulation of adipose tissue linked to the dysfunction of the HPA axis and the alteration of the immune response. A relationship between childhood maltreatment and inflammation was observed, confirmed by high levels of CRP, as well as an association with body mass index (BMI) and food abuse as a coping mechanism. There are also gender differences regarding the type of trauma reported: women mainly report sexual abuse while men report trauma independent of each other, especially physical and emotional neglect.

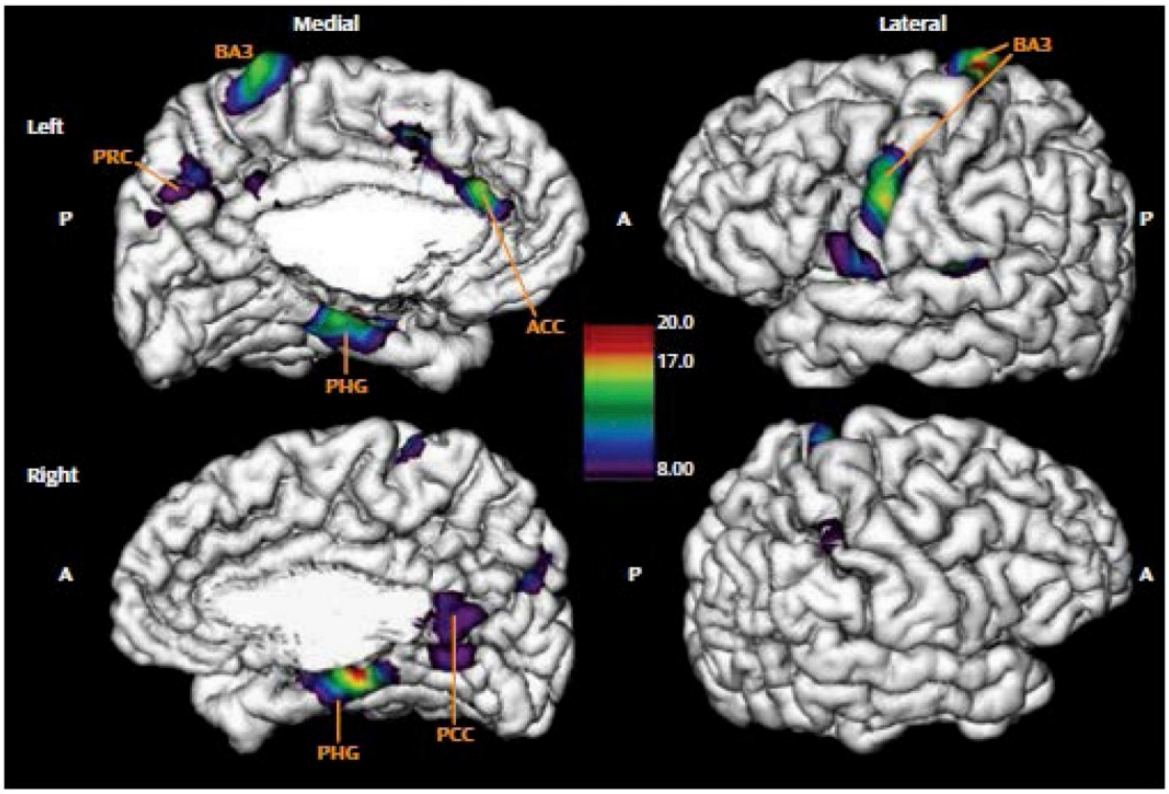


Figure 9 *- Effects of childhood trauma - analyzed by CTQ - on cortical thickness in women with childhood sexual abuse. Main effects are seen in the somatosensory cortex in the female genital and mouth area on the left, the parahippocampal gyrus (PHG) bilaterally, the left anterior cingulate cortex (ACC), and the precuneus (PRC) bilaterally (Heim et al., 2013).*

Women also report a greater link between high levels of CRP and BMI, while men show a more significant link between BMI and coping mechanisms. This suggests that there are several mechanisms underlying stress regulation, with a greater predisposition for women to accumulate adipose tissue and therefore to have a higher BMI due to states that cause inflammatory reactions. Evidence shows that obesity is related to systemic inflammation, due to increased levels of pro-inflammatory cytokines. Danese, Pariente and their colleagues (Danese et al., 2007, 2008) using the approximately 1,000 subjects of the Dunedin Multidisciplinary Health and Development Study reported that maltreated children show a significant and gradual increase in CRP 20 years later; moreover, this effect was independent of the most recent stressors of life, from the health and behavior of adults.

In a meta-analysis of 24 studies conducted on 48,801 subjects, ACE was also associated with neurological and musculoskeletal problems, as well as gastrointestinal, respiratory, cardiovascular and metabolic disorders (Wegman and Stetler, 2009).

A study by Murgatroyd et al., (2009) showed that mice subjected to early stress, such as separation from the mother for 3 hours in the first 10 days of life, caused hypomethylation in a key region for the regulation of the AVP gene in the paraventricular hypothalamus. The stress-induced endocrine phenotype in early childhood lasted for at least a year after the initial adverse event. In addition, these mice showed a reduced ability to cope with stress, combined with memory deficits: these neuroendocrine and behavioral alterations are frequent features in depression.

Further studies revealed long-term consequences of maternal separation in adult male rats, including elevated cerebrospinal fluid corticotropin (CRF) concentrations, as well as increased expression of CRF mRNA and CRF concentrations in the paraventricular nucleus (PVN), central nucleus of the amygdala, terminal stria bed nucleus and locus coeruleus (Plotsky et al., 2005). Effects of maternal deprivation on the response of neuronal activity of

the locus coeruleus to CRF and dendritic morphology have also been observed (Swinny et al., 2010). Yang et al. (2015) recently observed marked effects of repeated exposure to stress during the first postnatal week on dendritic development in the dorsal agranular cingulate cortex and prelimbic cortex in neonatal mice, an effect mediated by CRF1 receptors.

It is possible, therefore, that living in a dysfunctional environment may lead to the appearance of a given inflammatory pattern (Miller & Chen, 2010). In a sample of adolescent girls raised in at-risk families, an increase in IL-6 activity and a progressive desensitization of glucocorticoid receptors was observed. An increase in the pro-inflammatory cytokine IL-6 is therefore linked to a dysfunctional environment, as well as an increase in TNF-α which seems positively associated with the number of traumatic events experienced in childhood.

Pro-inflammatory cytokines such as IL-13 and IL-5 are associated with higher levels of asthma in subjects abused as children, while CRP is associated with negative events in life. In addition, adiponectin, a protein from the cytokine family that plays an anti-inflammatory role, is found to be present at lower levels in individuals with a history of childhood maltreatment.

In longitudinal studies carried out by Danese & Lewis (2017) it was shown that ACEcauses an increase in inflammatory biomarkers - such as CRP, fibrinogen and white blood cells, and impairment of acquired immunity, with an increase in levels of IL-6 and NF-kB.
Particularly relevant to the role of inflammation in the course of medical disorders is the report of Crosswell et al. (2014), which found a strong effect of ACE on IL-6 levels in breast cancer survivors, even after controlling for several potential covariates such as cancer treatment, age, BMI, ethnicity and alcohol use.

Importantly, there is a relative scarcity of knowledge regarding the effects of ACE on many other endocrine axes including the gonad-hypothalamic-pituitary axis and its target hormones

(estrogen, progesterone and testosterone), the hypothalamic-pituitary-thyroid axis, and the secretions of growth hormone and prolactin.

Among the tools most used to assess the severity of childhood trauma and understand the neural basis of ACE, childhood trauma questionaire (CTQ[8]) scores are often used, both with healthy subjects and with patients suffering from mood disorders, to investigate the relationship between the scores obtained and the response to stimuli in adulthood. In particular, the response to emotional/affective stimuli activates multiple areas including the anterior cingulate gyrus, the amygdala, the hippocampus and the prefrontal areas. The left medial temporal lobe, in particular, is activated only by negative affective stimuli and there is a negative correlation between CTQ scores and activation of the left upper parietal lobule and hippocampus.

2.1.1 The role of genetics in ACE

As far as genetics are concerned, the modulating role of the FKBP5 genotype and CRH receptor gene polymorphisms has already been demonstrated. In a review that attempts to reconcile the wide range of findings in this area (Struber et al., 2014) a two-way model was suggested to explain the hyperactivity of the HPA axis after interactions invoking ACE of the glucocorticoid system with oxytocin pathways and the serotonergic system.

The last decade has seen a notable increase in understanding of the seminal role of oxytocin, a neuropeptide present in the CNS and posterior pituitary gland, in mediating social affiliation, attachments, maternal behavior, intimacy and trust, as well as its well-established role in childbirth and breastfeeding. Not surprisingly, then, it has recently been examined as a neural system that could be affected by ACE.

[8] See Chapter 4 - Methods

Inflammation and other factors have also been shown to reduce telomere length, which has been associated with early cell shortening and increased mortality of age-related diseases. Telomeres are DNA-protein agglomerates placed on the ends of chromosomes and have the function of covering and protecting the genome from damage. Tyrka et al. (2009) studied 31 individuals without any diagnosis of past or current psychiatric disorder and found that childhood maltreatment was associated with a significant increase in telomeres. Shalev et al. (2013) instead studied 236 children who lived in at-risk environments: those who had been exposed to violence showed greater erosion of telomeres between the ages of 5 and 10 years. Another study by Chen et al. (2014) compared 20 depressed subjects with 20 healthy controls: it was seen that increased exposure to ACE reduced telomere length in healthy subjects without affecting telomerase, while in the pathological group a change in telomere length was not observed but an increase in telomerase activity.

In a pioneering work by Caspi and colleagues that used 1037 subjects evaluated multiple times from 3 years to adulthood, he showed that those who possessed one or two copies of the short allele of serotonin transporter promoter polymorphism had higher rates of depression and suicide likelihood, when exposed to ACE, compared to homozygotes with long alleles and equal exposure to childhood adverse events.

In addition to the FKBP5 gene described above, the polymorphism of the CRHR1 gene has also been extensively studied thanks to evidence that this CNS circuit is hyperactive in MDD. Bradley et al. (2008) reported in two separate cohorts of 422 and 199 subjects that specific CRHR1 polymorphisms interact with child abuse to predict depressive symptoms in adults. These findings have been confirmed and extended to include the risk of suicide attempts (Ben-Efraim et al., 2011).

2.3 Alterations of white matter in abused subjects

Having experienced ACEs implies consequences also in brain structures. ACEs were positively associated with reduced volumes in GM and WM in several brain areas, both in psychiatric and healthy populations with a history of maltreatment and/or abuse (Nemeroff, 2016). A recent study on MDD patients, found a negative association between ACE and WM integrity indexes (Poletti, Aggio et al., 2018). Interestingly, in healthy volunteers, ACEs were associated with widespread of WM reduction in the prefrontal cortex, hippocampus and parahippocampus, orbitofrontal cortex and stratum (Lim, Radua et al., 2014).

Another study sought to investigate the relationship between alteration of the HPA axis (via Cortisol Awakening Response - CAR) and atrophy in different brain regions including the hippocampus, medial prefrontal and anterior cingulate cortex, in subjects who suffered ACE. A significant relationship has been identified between the amount of cortisol secreted and the volume reduction at the level of the right medial gyrus. In addition, CAR was related to the CTQ score, thus highlighting a relationship between exposure to trauma and cortisol levels. Reduction of the right middle cingulate gyrus was also inversely related to ACE.

Analyzing a sample of individuals who experienced child maltreatment versus non-abused controls, Lim et al. (2020) found significant abnormalities in several WM tracts. In particular, in subjects exposed to maltreatment, the most compromised tracts with lower FA were thalamic radiation, fornix, optical radiation, corpus callosum and the lower longitudinal fasciculus. These results highlight the broad involvement of the callous, corticolimbic, fronto-striated and occipital visual pathway which can therefore lead to alterations in emotional and sensory functioning.

A TBSS study of WM integrity related to childhood exposure to verbal abuse (Choi et al., 2009) reports a reduction in FA values in the arcuate fasciculus and the fornix: the first is important for the verbal and auditory understanding of information; the second is fundamental

for the processing of emotions as well as the modulation of emotional reactions; finally, the fornix plays an important role in the mediation of anxiety, through its connection with the hippocampal system. It should be noted that fornix sends serotonin fibers to the hippocampus: a reduced FA therefore, together with a high verbal abuse by the parents, can lead to an alteration of the serotonergic system.

Frazier et al. (2007) saw that children with BD have reduced FA values in the right and left upper frontal tracts, including the longitudinal fasciculus and cingulate gyrus, as well as in the left orbito-frontal tract and corpus callosum. The orbito-frontal tract has a significant role in decision-making, therefore in cognitive modulation that can allow different choices to be made. The corpus callosum, on the other hand, is the most important WM bundle concerning integrity and communication between the areas located in both hemispheres. A significant decrease in corpus callosum volume is significantly related to BD (Bucker et al., 2014). It is possible to notice, therefore, how WM can represent a relevant indicator of the progression of BD, early detectable during childhood.

It has also been seen that the integrity of WM bundles is an important marker of disease, particularly in determining the onset, course and severity of certain pathologies. Benedetti et al. (2014) saw that an association between ACE and the development of fibres that transmit information to frontal, temporal or limbic regions could contribute to the pathophysiology of BD by hindering structural connectivity in the most critical cortico-limbic networks. It has been shown that the more bundles are compromised, the greater the alterations of the phenotypic manifestations of the patient's behavior.

Finally, another study report a decrease in the TBSS FA index in the corpus callosum and corona radiata (Stevelink et al., 2018). Child maltreatment has therefore been linked to lower FA and psychopathology through a mediation model: in it child abuse seems to be a possible

risk factor for the development of BD and therefore FA is also associated with the history of the disease.

Child abuse, rather than neglect, appears to be more related to reduced WM integrity, especially among people vulnerable to the development of psychic disorders. Finally, childhood abuse can affect WM integrity in patients with BD. Therefore, the presence of childhood trauma – the sooner the onset, the more severe the consequences – interferes with the formation of some connections within brain areas, altering WM integrity. Thus, the ACE experience can modulate the relationship between WM integrity and the onset of BD.

2.4 ACE and mood disorders

Elevated inflammatory levels have been associated with an increased risk of developing depression and psychosis in adulthood. High levels of inflammation were also observed in depressed subjects exposed to ACE compared to unexposed subjects, suggesting that inflammation is also modulated by early exposure to ACE.

Child maltreatment, therefore, seems to play a role in the progression of the disease: several mood disorders seem to be related to ACE. BD is considered the fourth psychopathology associated with exposure to trauma, after PTSD, ADHD, and suicide attempt. Patients with mood disorders, in particular, report more physical and sexual abuse than people with other psychiatric illnesses, such as schizophrenia (Lu et al., 2008).

It is important to keep in mind that it is not possible to deduce the role of ACEs in promoting the onset and progression of BD, probably underestimating the extent of the relationship. Thus, to determine whether ACE have played a mediator and role concerning the development of a given disorder, further investigation is needed.

Van der Kolk et al. (1991) showed that ACE, particularly sexual abuse and neglect, were a predictors in the implementation of self-injurious behaviors and suicidal instincts in a clinical population suffering from type II BD and borderline personality disorder.

BD patients have also been studied to investigate a possible association between ACE and the onset of psychotic symptoms (Etain et al., 2017). The extent of the trauma was assessed using CTQ, while psychotic symptoms were detected through the Peters Delusion Inventory (POI): the POI score was significantly correlated with the CTQ score, and the frequency of reported abuse in childhood were related to delusional beliefs.

Agnew-Blais and Danese (2016) showed that ACE exposure worsens the progression of BD in terms of the number of depressive and manic episodes, rapid cyclicity and difficult remission. In addition, the presence of ACE correlates with the onset of some psychopathological manifestations such as substance abuse, anxiety disorders and self-injurious behaviors, as well as with medical pathologies such as allergies, arthritis, asthma, hypotension, fibromyalgia etc. (Hosang et al., 2017).

To conclude, BD could be associated with ACE, which negatively affects the course of the disease and WM integrity in adulthood.

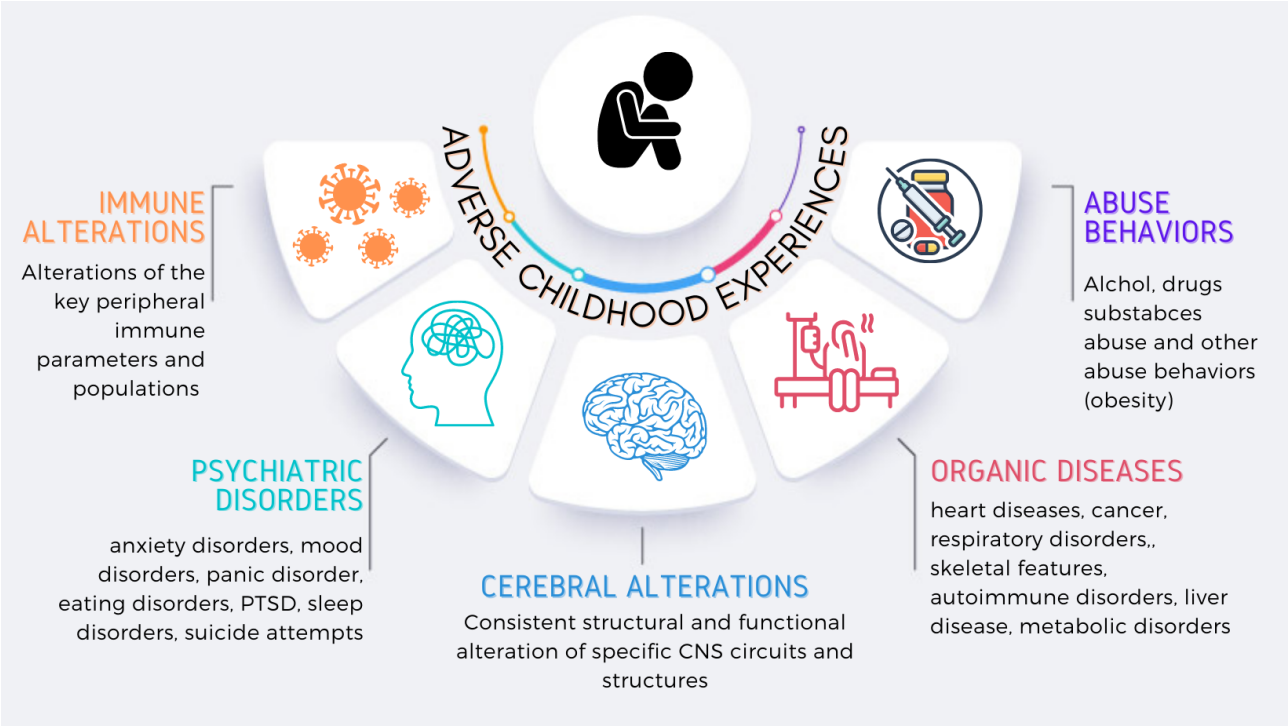


Figure 10 *- Effects of childhood adverse events*

Chapter 3: The immune system

The main function of our immune system is to defend the body from external pathogens and inhibit substances that can cause inflammatory processes or tissue damage. Immune cells are distributed throughout the body, especially in the blood and lymphatic tissue and are characterized by the function of defence, cleansing and surveillance.

The immune system can be divided into innate or adaptive – also called cell-mediated immunity and humoral immunity. The innate immune system is the first line of defence against pathogens and consists, in particular, of monocytes, dendritic cells and macrophages derived from monocytes, neutrophils, natural killer (NK) cells, mast cells and various cytokines and chemokines, such as interferon (IFN)-y, interleukin (IL) 1, IL-6 and Tumor Necrosis Factor-α (TNF-α) (Fearon & Locksley, 1996).

Adaptive immunity, on the other hand, occurs after a latent phase and is activated mainly by antigen-presenting cells, which include macrophages and dendritic cells. The adaptive

immune response, which is activated by antigen 1 recognition receptors on the surface of T lymphocytes called pattern recognition receptors, is mediated by T (CD4 and CD8) and B lymphocytes. CD8 T lymphocytes show cytotoxic activity, and are responsible for the destruction of intracellular pathogens, while CD4 T lymphocytes (also called T helper lymphocytes) play an important role in immunomodulation, thanks to the secretion of signal cytokines involved in the activation and recruitment of immune cells.

There are four main CD4 T cells: Th1, Th2, Th17 and Treg. Th1 cells release pro-inflammatory cytokines, such as IFN γ, TNF and IL-2; Th2 cells release IL-4, IL-5 and IL-10, which act as anti-inflammatory cytokines and are responsible for the activation of humoral immunity thanks to the activation, in turn, of B cells through IL-4. Th17 and Treg play an important role in the pathogenesis of autoimmunity. Cytokines and T cells are therefore an important component of cell-mediated immunity. A constant balance between anti-inflammatory and pro-inflammatory cytokines is necessary to ensure the optimal functioning of the immune response.

B lymphocytes, on the other hand, play an important role in humoral immunity, through the production of antibodies against foreign antigens.

3.1 Neuroinflammation and microglia

Neuroinflammation is the result of an immune response of the CNS that involves a complex interaction between the innate immune system, blood-brain barrier (BBB) and peripheral immune system. Microglial cells are the first line of defence of the CNS: neuronal damage, infection or ischemia can activate microglia through pattern recognition receptors, which are divided into three large families: Toll-like receptors (TLR), Nod-like receptors (NLR) and RIG1-like receptors (Van Rossum and Hanisch, 2004).

TLR and NLR are capable to recognize molecular patterns that are associated either with pathogens (PAMPs), during an infection, or with damage (DAMPs) – such as intracellular

proteins or nucleic acids during neuronal damage. TLR activates transcription factors, such as NF-kB, which lead to the release of inflammatory cytokines (IL-1B, TNF-α, IL-6) and chemokines. So, despite the activation of microglia acts as a protector of the CNS, in some cases its hyperactivation can lead to neurotoxicity and neurodegeneration.

The cytokine IL-1B increases the NMDA receptor that triggers inflammation and cell death and can reactivate microglia to initiate a forward feedback loop (Kaindl et al., 2012).

IL-6 and TNF-α activate the HPA axis, increasing cortisol levels that can be neurotoxic and cause depressive symptoms and cognitive deficits (Daban et al., 2005): too high cortisol, in fact, reduces the postsynaptic serotonin receptors and its reactivity, influencing the regulation of neurotransmitters.

Microglia also interact with astrocytes and oligodendrocytes, which play a relevant role in neural, cognitive and behavioral functions: the former maintain the integrity of BBB, regulating synaptic transmission, express TLR and can amplify the innate immune response of the CNS by releasing chemokines; oligodendrocytes, on the other hand, play an important role in the myelination process and can reduce the inflammatory response of microglia by secreting anti-inflammatory cytokines (Edgar & Sibille., 2012).

In the CNS, inflammation causes the production of inflammatory mediators such as arachidonic acid (AA), TNF-α, IL-1B and IL-6, which can increase the permeability of BBB (Shalev et al., 2009).

In summary, the activation of microglia causes the activation of an inflammatory cascade that produces a series of downstream mediators that can cause oligodendrocyte apoptosis, neuronal damage, BBB alterations and mitochondrial dysfunction.

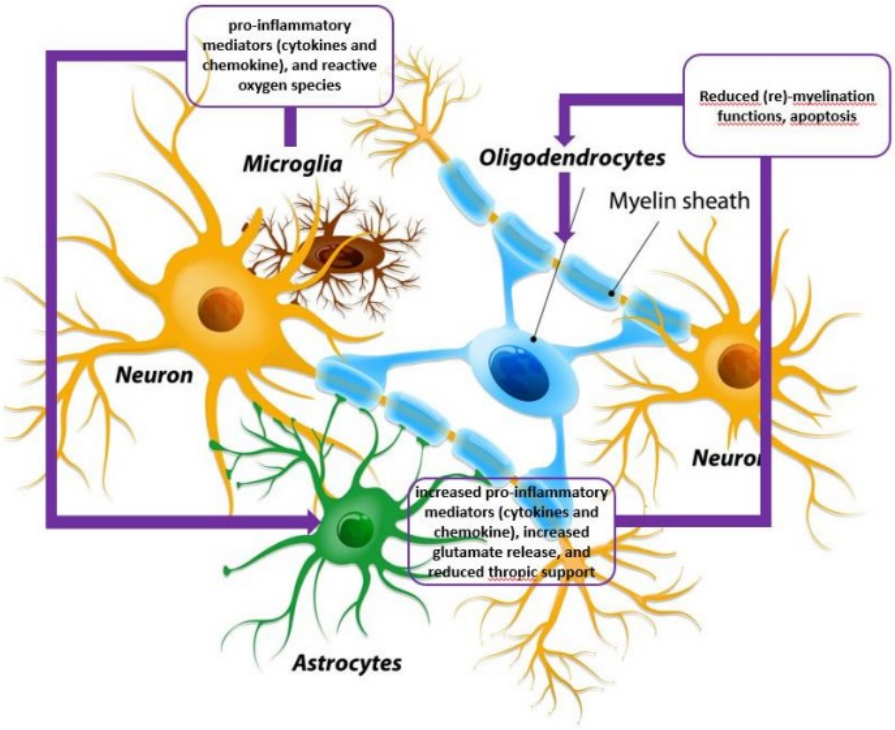


Figure 11 *- Inflammatory process that occurs after microglia and activation of astrocytes.*

3.1.1 Cytokine levels and cognitive dysfunction of bipolar disorder

The most used and easy way to investigate immune alterations, is to quantify the levels of cytokines and chemokines in the peripheral blood.

Cytokine levels have been extensively studied to determine the strength of the association between inflammation and BD. In particular, several researchers focused on peripheral cytokines while few investigated pro inflammatory analytes levels in the CNS through cerebrospinal fluis (CSF).

Nertheless, peripheral cytokines can cross the BBB through the choroid plexus and via active transport. Lymphatic vessels in the CNS are also a potential route for cytokine transport to and from the brain. Taken together, peripheral cytokine levels can strongly affect central cytokine levels and represent a good marker of neuroinflammation.

Some post-mortem studies in BD brain have highlighted the presence of excitotoxic and neuroinflammation markers within the frontal cortex with an activation of the IL-1 receptor (Rao et al., 2010). Moreover, IL-1B indicative of activated microglia, is elevated within the

cerebrospinal fluid of BD patients in euthymic phase who have recently had a manic or hypomanic episode (Soderlund et al., 2011). Zanetti et al. (2009), in a DTI study, report disruptions in ventromedial prefronto-limbic-striated WM in bipolar depressed patients.

Changes in cytokine levels can therefore provide a deeper insight into the mechanistic basis of how immune dysfunction can affect brain function and the pathophysiology of mood disorder.

Many studies have shown elevated levels of pro-inflammatory cytokines during periods of depression, mania and euthymia (Barbosa et al., 2014; Modabbernia et al., 2013; Brietzke et al., 2009; O'Brien SM et al., 2007).

In particular, most studies on bipolar disorder have focused on cell-mediated immunity, measuring inflammatory cytokines and markers: Munkholm et al. (2013) in a meta-analysis conducted on bipolar patients found elevated levels of TNF-α, soluble tumor necrosis factor receptor type 1 (STNF-R1) and soluble interleukin receptor 2 (sIL-2R) in manic patients, compared to healthy, control subjects, and subjects with euthymia. In addition to the presence of high proteins, such as PCR and haptoglobin, high levels of C3C and C4 have been found in bipolar disorder (Maes et al., 1997; O'Brien et al., 2006; Wadee et al., 2002).

There appears to be a significant difference between the cytokine levels of manic bipolar patients, who show high levels of TNF-α, IL-2 and IL-4, compared to controls (Ortiz-Dominguez et al., 2007).

There is also evidence of cytokine abnormalities related to the stage of the disease: all interleukins and TNF-α are elevated in the early stages of the disease, while in the later stages TNF-α and IL-6 continue to be elevated, but not IL-10. During periods of euthymia, however, sTNF-R1 is the only constantly altered inflammatory marker, while during manic episodes serum levels of IL-6, TNF-α, sTNF-R1, IL-RA, CXCL10, CXCL11 and IL-4 are increasing. During depressive episodes, however, we find increased sTNF-R1 and CXCL10.

Some studies that have evaluated patients during a major depressive episode have shown increased serum levels of TNF-α, IL-6 and IL-18 (Dantzer et al., 2008; Felger & Lotrich, 2013).

In fact, due to the transversal nature of BD (euthymia alternating with days of mania), cytokine levels could be misleading: if a patient is euthymic on the day of blood sampling, but few days before was in a manic phase, cytokine levels can still be affected by the levels of the previous episode.

Thus, these cytokines may represent important markers of the progression of BD (Kauer-Sant'Anna et al., 2009), which appears to be associated with a pro-inflammatory state and innate immune system dysfunction.

BD patientis in euthymia also show abnormalities in chemokine levels that induce chemotaxis (migration of leukocytes to sites of inflammation), indicating the presence of inflammation in a quiescent state of the disease (Brietke et al., 2009).

IGF-1, the insulin-like growth factor, has also been implicated in the pathogenesis of bipolar disorder and may also be a possible marker of interest (Squassina et al., 2013).

Today, the cognitive dysfunction associated with BD has become a therapeutic target, since it is a strong determinant of functional deterioration. In addition, cognitive dysfunction is often present even in periods of remission and is often affected by mood stabilizers.

Several studies have shown that elevated levels of pro-inflammatory cytokines in BD are associated with poorer cognitive function (Lotrich et al., 2014; Barbosa et al., 2012). Such results indicate that cognitive dysfunction is highly associated with increased levels of pro-inflammatory markers such as YKL40, IL-6, sCD4+OL, IL-1Ra, CPR and TNF-α. Therefore, innate immune system dysfunction may be associated with a progressive worsening of cognitive function in BD.

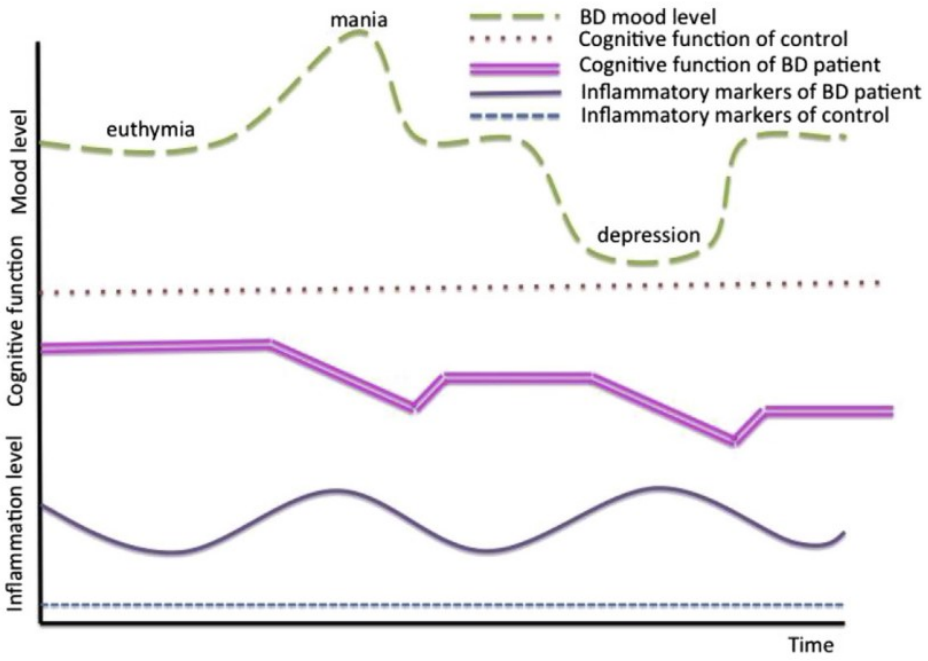


Figure 12 *- Possible simultaneous changes in cognitive function, mood levels and inflammatory cytokines (Rosenblant et al., 2018)*

3.2 Peripheral autoimmunity and bipolar disorder

Autoimmunity refers to the process by which an immune response is directed against the body's autoantigens. An autoimmune disease occurs when the immune reaction is higher than the normal tolerance level and occurs in the absence of an offensive antigen (Goodnow, 2007).

The characteristic feature of autoimmune diseases is the presence of autoantibodies, important markers for the diagnosis of autoimmune diseases. Tissue damage derived from autoimmune conditions can be cell-mediated through the activity of T, B, or both cells. It has been seen that TLRs on the surface of T and B lymphocytes play an important role in the early stage and progression of autoimmune disease, through the recognition of exogenous and endogenous antigens (Marshak-Rothstein, 2006).

The DAMPs that are released during mitochondrial apoptosis bind to NLRs, activating the inflammatory response. Tissue damage, which is mediated by T lymphocytes, occurs through

the release of cytotoxic cytokines. Subjects with BD possess a propensity for a Th1 pro-inflammatory profile together with a lower number of Treg cells, as well as higher levels of CD8 and CD25 T cells. Alterations in TLR agonist-mediated activity have been identified in BD patients (McKernan et al., 2011): following recognition of the TLR-mediated antigen, GSK3 plays a crucial role in the immune response and lithium is a GSK3 inhibitor that can reduce inflammation (Beurel et al., 2010). Therefore, abnormalities in T lymphocytes and cytokines may represent the underlying autoimmunity of T and B cells.

Immunity mediated by B lymphocytes occurs mainly through antibodies. For example, IL-4, released by Th2 cells, activates B cells to produce antibodies. In some individuals, B lymphocytes can produce autoreactive antibodies, which are the hallmark of autoimmune disease. Autoreactive antibodies can cause tissue damage. Moreover, several studies report that thyroid autoimmunity is a risk factor for BD and may act as an endophenotype for BD (Vonk et al., 2007).

Cell damage in the CNS can result in the release of DAMPs that can become immunogenic, initiating an autoimmune response in T cells against autoantigens (Harris & Fabry, 2012). Extracellular mitochondrial DNA is known to be extremely immunogenic and can directly initiate an autoimmune response (Zhang et al., 2010). Kapadia and Sakic (2011) integrated the mechanisms of the innate and adaptive immune system of the CNS, proposing two different phases, an inflammatory one, dominated by innate immunity leading to functional damage, followed by an autoimmune phase, responsible for neurodegeneration and atrophy.

3.3 Immunity and Major Depressive Disorder

MDD is accompanied by an immune dysregulation, characterized by an activation of the inflammatory response system. In particular, the latter has been demonstrated by the increased production of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IFN, TNF, IL-6R and IL-

1RA (the IL-1 receptor antagonist). They would induce neuroendocrine changes and central stress-reactive neurotransmitters that would lead to depression. In fact, the association between cytokine activation and the inflammatory response system is not significant for all of the cytokines mentioned above: a meta-analysis conducted by Dowlati et al. in 2009 reported significantly higher concentrations of TNF and IL-6 cytokines in depressed subjects compared to healthy controls.

Within the CNS, proinflammatory cytokines play a crucial role in the stress response system and in the regulation of adult neurogenesis. A candidate mechanism to cause the effects of cytokines on mood is their ability to modulate the neurogenesis of the hippocampus, implicated as a key mechanism in the pathophysiology and treatment of depression.

Antidepressant treatment, particularly with selective serotonin reuptake inhibitors (SSRIs), has also been shown to reduce some increases in cytokines associated with MDD.

3.4 Summarizing: a proposed pathophysiological mechanism

Immune system dysfunction can have deleterious effects on the mood and cognitive abilities of people with affective disorders, through different pathophysiological pathways. Moreover, as shown before these alterations may provide biologically plausible mechanisms by which neuroinflammation can affect also the structure and function modification reported in different brain regions underlying mood and cognition in both BD and MDD. We have seen that the pro-inflammatory cytokines TNF-α, IL-2 and IL-6 directly alter monoamine levels. IL-2 and interferon, in particular, increase the enzymatic activity of indolamine 2,3-dioxygenase, augmenting the breakdown of tryptophan into depressogenic catabolites. The depletion of tryptophan leads to a decrease in the levels of production and release of serotonin and for this reason it seems to be a potential mechanism underlying affective and cognitive dysfunction. Taken together, pro-inflammatory cytokines can induce both mood alteration and cognitive

dysfunction, promoting tryptophan depletion, serotonin degradation, and the production of tryptophan depressogenic catabolites.

Another key mechanism proposed is the hyperactivation of microglia. It plays an important role in neuroplasticity, facilitating synaptic pruning through apoptosis of neurons and neural pathways that are not used. In hyperinflammatory and pathological conditions, microglia can be overactive, abnormally destroying important neural pathways.

The microglial hypothesis related to mood disorders suggests that microglia may be overactivated in regions of the brain that regulate mood and cognition, such as the prefrontal cortex, hippocampus, anterior cingulate cortex, amygdala, and insula.

Overactive microglia also release cytokines that further increase inflammation: the release of cytokines, especially TNF-α, accentuates monoamine changes that can alter mood and cognition. Hyperactivation of microglia also increases local oxidative stress, damaging the neural circuits of key brain regions.

Another mechanism by which inflammation can induce mood dysfunction in BD and MDD is the dysregulation of the HPA axis. High levels of proinflammatory cytokines such as interferon, TNF-α and IL-6, upregulate the activity of the HPA axis, increasing cortisol levels, which, in turn, powerfully alters mood. Finally, high levels of inflammatory cytokines reduce the synthesis, transport and sensitivity of glucocorticoid receptors within the pituitary and hypothalamus. The reduction in cortisol suppression has long been recognized as a strong predictor of mood disorders (Cowen, 2010).

3.5 The role of stress and its interaction with the immune system

The link between stress and mood disorders is well recognized, with the corticotropin-releasing hormone playing an important role in pathophysiology (Stout & Nameroff, 1994).

Psychosocial stressors activate the corticotropin-releasing hormone, raising the level of NF-kB in inflammatory cells and causing the release of pro-inflammatory cytokines, which induce the release of cortisol, which should reduce the inflammatory response. There is wide evidence to support the idea that naturalistic stressors, such as a university exam, enhance a Th2 response, while chronic stress reduces both Th1 and Th2 responses (Plotnikoff, 2007).

To understand the relationship between stressors and the immune system, it is good to divide immunity into natural and specific. The natural immune response is mammalian-specific, and the cells involved in that response provide no defence against specific pathogens. Instead, they are all-purpose cells, which can attack different pathogens in a very short amount of time. The group of cells involved in this response are granulocytes, which include neutrophils and macrophages: these are phagocytic cells that destroy their targets by generating inflammation. During this phase, neutrophils and macrophages attach to the site of infection releasing toxic substances such as oxygen radicals, which damage pathogens and engulf both invaders and damaged tissues. In particular, macrophages release proinflammatory cytokines such as IL-1, IL-6 and TNF.

Another cell-type involved in natural immune rest is the natural killer cells, which are important in containing the early stages of viral infections attacking malignant cells.

Specific immunity, on the other hand, is characterized by greater specificity and lower speed than natural immunity. These antigen-specific cells, called lymphocytes, have receptor sites on the cell surface and respond to one and only one type of invader. When activated, they divide to create a population of cells with the same antigenic specification in a process called *clonal proliferation* or *proliferative response*.

The three types of lymphocytes that mediate specific immunity are T-helper cells, cytotoxic T cells and B cells: the former has the function of creating cytokines capable of amplifying the immune response; the latter recognize the antigen expressed by cells infected with viruses

and compromised and lyse these cells; B lymphocytes, finally, produce soluble proteins, antibodies, able to neutralize bacterial toxins.

Specific immunity in humans composes the cellular and humoral responses seen above. While cellular immune responses act against intracellular pathogens, such as viruses, and are coordinated by a subset of helper T lymphocytes called Th1, humoral immune responses act against extracellular pathogens, such as parasites and bacteria, and are coordinated by a subset of helper T lymphocytes called Th2.

In the Th1 response, the T-helper cell produces IL-2 and IFN-γ, which activate cytotoxic T cells and natural killer (NK) cells.

In the Th2 response, the T-helper cell produces IL-4 and IL-10, which activate B lymphocytes and mast cells to fight extracellular pathogens.

For both antibodies and cytokines, increased protein production would represent a more robust immune response that can provide protection against disease. An exception is represented by the case of pro-inflammatory cytokines (IL-1, IL-6, TNF) and antibodies against a latent virus: proinflammatory cytokines are increased with systemic inflammation, which represents a risk factor for heart disease, diabetes mellitus and osteoporosis (Ershel & Keller, 2000; Luster, 1998). The production of antibodies against a latent virus, on the other hand, occurs when viral replication activates the immune system to produce antibodies in an attempt to contain the infection. A higher level of antibodies against latent viruses may indicate poorer immune control over the virus.

3.5.1 The path of stress to the immune system

The sympathetic fibers that descend from the brain to the primary and secondary lymphoid tissues release a variety of substances that affect our body's immune responses, binding to

receptors on white blood cells. The differential density and sensitivity of adrenergic receptors on lymphocytes may affect the stress response between various cell subgroups. For example, NK cells have both very high densities and affinities, B lymphocytes have high density but low affinity, and T lymphocytes have lower densities (Anstead et al., 1998).

In addition, the HPA axis, the sympathetic-adrenal-medullary axis and the hypothalamic-pituitary-ovary axis secrete adrenal hormones such as epinephrine, norepinephrine and cortisol, the pituitary hormones prolactin and growth hormone, and the brain peptides melatonin and endorphins. All these substances bind to specific receptors on white blood cells and affect their functions and distributions in various ways.

Finally, people's behaviors to deal with stressful events such as alcohol or drug abuse could also change the processes of the immune system.

Over the years, numerous studies and models have conceptualized the relationship between stress and the immune system, starting from Selye who first, in 1975, supported the idea that stress is essentially immunosuppressive. Early human studies supported this hypothesis, reporting that chronic forms of stress were accompanied by reduced cytotoxicity of natural killer cells, as well as suppression of lymphocyte proliferative responses and attenuated humoral responses.

The large decreases in immune function predicted by the global immunosuppression model would not be adaptive in dangerous life circumstances, which is why Dhabhar and Mc Ewen (1997, 2001) carried out a new experiment on mice in which they saw that during acute stress T lymphocytes selectively redistributed into the skin, where they contributed to the improvement of the immune response; in contrast, during chronic stress, T cells moved away from the skin. They concluded proposing a biphasic model according to which acute stress improves the immune response, while chronic stress suppress it.

In fact, short-term changes are unlikely to occur in all-natural and specific components of the immune system, because otherwise, we would be spending too much energy to be adaptive in life-threatening circumstances. Instead, stress should activate natural processes and decrease specific processes. Natural immune responses would therefore be better suited to managing the potential complications of life-threatening situations than specific immune responses, both because they develop more quickly and because they are subject to fewer inhibitory constraints and require less energy.

Some researchers, rejecting both the immunosuppression and biphasic models, hypothesized that chronic stress elicits the simultaneous improvement and suppression of the immune response, altering the secretion of cytokines (Marshall et al., 1998). Th1 cytokines, which activate cellular immunity to ensure defence against infections and neoplastic diseases, are suppressed. Such suppression facilitates the production of Th2 cytokines, which activate humoral immunity.

Such a change can occur through the effects of hormones on stress such as cortisol: the shift from Th1 to Th2 changes the balance of the immune response without changing the level of activation or function within the system. Since a reduced cellular immune response mediated by Th1 could increase vulnerability to infectious and neoplastic diseases, while a Th2-mediated humoral immune response could increase vulnerability to autoimmune diseases, this cytokine displacement model appears to be able to reconcile patterns of stress and immune change (Marshall et al., 1998).

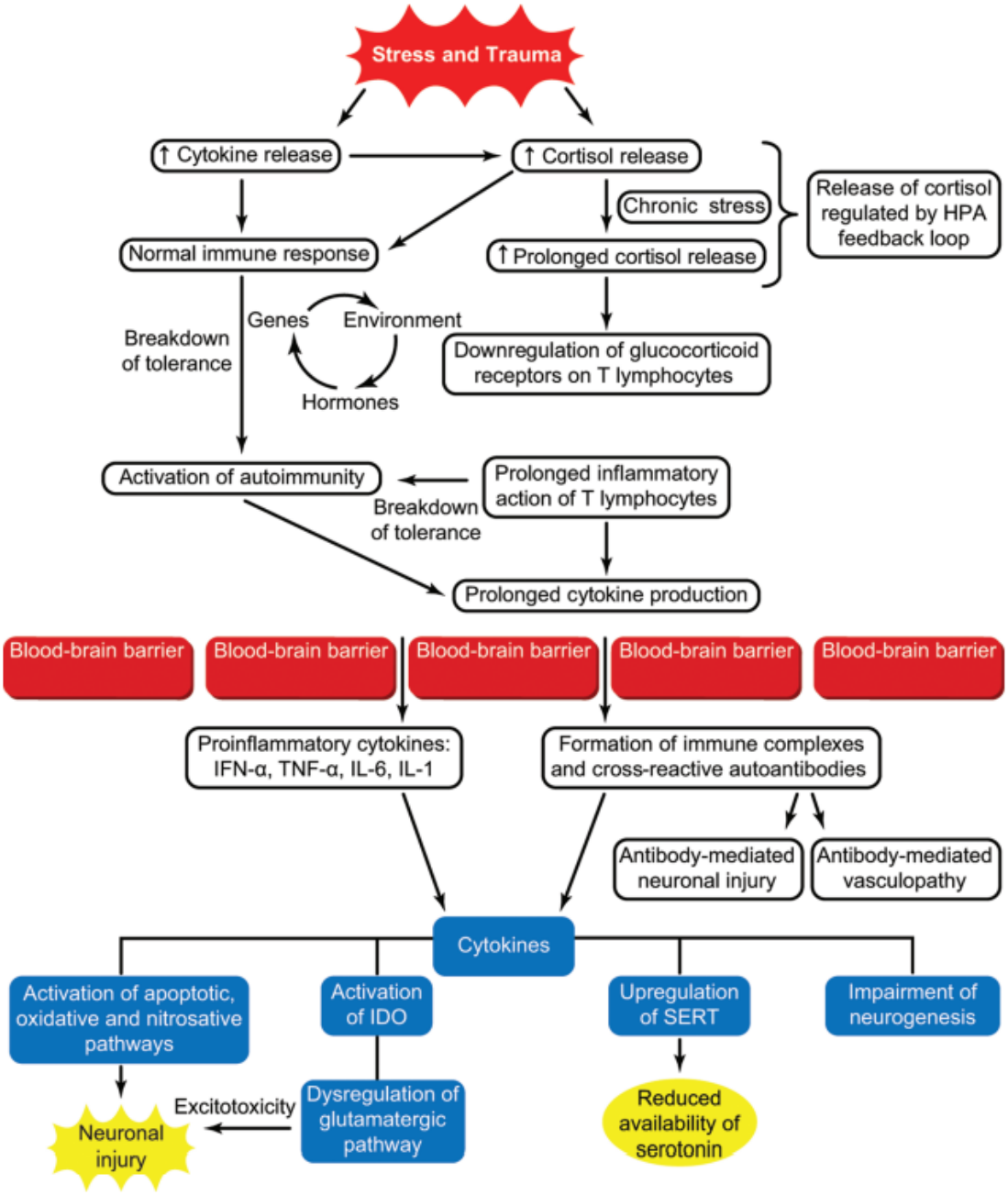


Figure 13 *- The role of stress and its interaction with the immune system (Rege et Hodgkinson, 2013)*

3.5.2 ACE and the immune system in mood disorders

Evidence suggests that the immune system plays an important role in the pathophysiology of affective disorders (Aas et al., 2017). It has been shown that there are alterations of the acquired immunity in these patients and that some genes related to immune regulation are more susceptible to modifications. In addition, the presence of environmental risk factors can

only aggravate the process of immune dysregulation and inflammation, increasing the probability of developing a psychopathology. Genetic overlap between inflammation and stress markers has been observed, particularly among those who have experienced maltreatment in childhood, with increased inflammatory response (increased levels of CRP, TNF-α and IL-6) resulting in a decrease in glycoprotein (gp130), an antagonist of pro-inflammatory cytokine IL-6. In addition, the role of the BMI has been investigated, since it has been observed that in BD patients there could be metabolic abnormalities and a consequent weight gain; in turn, inflammatory mediators can be produced by fat tissue cells, increasing the activation of the immune system. What has been seen is that BMI could act as a mediator between ACE and the activation of inflammatory mediators (Felitti et al., 1998). There also seems to be long-term effects on the physical health of the individual – both as a child and in adulthood – contributing to the emergence of metabolic and vascular problems, including obesity. Finally, traumatic experiences – regardless of type – can lead to an increase in immune system activation.

High levels of pro-inflammatory cytokines were reported also in psychotic disorders (Quidè et al. 2019), as well as in the case of subjects exposed to childhood trauma. The authors investigated the relationship between different types of trauma exposure and levels of peripheral inflammatory markers in schizophrenic and bipolar patients, compared to a control sample. It has been seen that schizophrenic (SZ) and BD patients shown increased level of IL-6. In addition, only in the schizophrenic patients group, TNF-a levels were higher. As a result, different types of childhood trauma can induce a chronic state of inflammation in both SZ and BD patients, increasing levels of pro-inflammatory cytokines and therefore suggesting a potential role for early trauma exposure in the regulation of both the immune and stress systems in psychotic and mood disorders.

As previously described, the exposure to ACE can induce a condition of chronic inflammation, which can affect the central nervous system by modulating neurotransmission, metabolism, oxidative stress and mitochondrial function (Ridout et al., 2018). In addition, being exposed to ACE turns out to be a possible risk factor for the emergency of cardiovascular diseases, type 2 diabetes and other medical conditions.

Another possible effect of being exposed to ACE is a change in glutamate levels (Poletti et al., 2016), particularly in structures sensitive to high stress levels, such as the hippocampus. Glutamate is a fundamental excitatory neurotransmitter in the brain, particularly because it guarantees synaptic function and plasticity. Alterations in ionotropic and metabotropic glutamatergic receptors – AMPA and NMDA respectively – lead to dysregulation due to excessive release of glutamate in response to chronic stress. While the release of glutamate appears to be adaptive in acute stress, in a condition of chronic stress can becomes harmful to the body. Since glutamatergic alteration is one of the underlying mechanisms of different mental disorders, an attempt has been made to examine the interaction between ACE and mood disorders in modulating glutamate levels of the hippocampus. Single proton magnetic resonance spectroscopy (1H-MRS) was used, a non-invasive brain imaging technique that can detect alterations in brain biochemistry.

First, a significant diagnosis-ACE interaction was found. Therefore, it was seen that patients with mood disorders presented lower levels of glutamate. Glutamate is fundamental for maintaining brain signalling and therefore a decrease in the glutamateglutamine cycle might compromise it and lead to glia reductions. Accordingly, stress seem to increase vulnerability to depression, while treatments or conditions that enhance synaptogenesis have antidepressant actions and reduce vulnerability. Finally, ACE has been associated with hippocampal volume reductions both in the bipolar and control groups.

Two emerging theories regarding the development of mood disorders, involve excessive activation of inflammatory pathways and alterations in glutamate metabolism as possible underlying biological machanisms (Dantzer et al, 2008; Haroon et al., 2017). Recent evidence indicates that these two pathways converge at the level of the glia inducing behavioral alterations in patients with mood disorders. Data indicates that inflammatory mediators might regulate extracellular glutamate concentrations by exerting profound effects on the functioning of glial cells including astrocytes, oligodendrocytes, and microglia that regulate glutamate levels under both physiological and pathological conditions.

Finally, another important factor that has been widely studied in mood disorders and seems to be modulated by ACE exposure is brain-derived neurotrophic factor (BDNF). BDNF, a protein fundamental for developing, belongs to the family of neurotrophins and participate in the regulation of neuronal development, neurogenesis and plasticity (Bathina & Das, 2015). BDNF protects the brain from the toxic effects of glucocorticoids and supports neurogenesis. It is produced by different types of cells and it is influenced by several environmental factors, such as exercise, diet and stress. Reduced BDNF levels was reported in mood disorders, such as MDD and BD and also in schizophrenic patients.

Moreover, it has been observed that ACE exposure are associated with lower levels of BDNF (Benedetti et al., 2017). Moreover, reduced BDNF levels was also associated with the short-allele variant of the polymorphism concerning the serotonin transporter. Specifically, people carriers of the short allele variant are more prone to manifest depressive symptoms; moreover, the depressive symptomatology was even more severe if they experienced ACE. In fact, it cannot be concluded that only genes are decisive in giving rise to this phenotype, but rather an interaction between genetic and environmental factors takes place.

Another study by Aguilera and colleagues analyzed the relationship between the short version of the 5-HTTLPR, the concentration of BDNF and the impact of ACEs on a sample of BD

patients (Aguilera et al., 2009). Exposure to trauma was found to be correlated to BDNF levels. Regarding the link between 5-HTTLPR and ACE, the relationship is based on an overactivation of the stress axis that leads to a more significant reaction and a more obvious impairment.

Related to BDNF implication in mood disorder, the neurotrophic hypothesis suggests that pathological changes in brain areas caused by inflammatory processes can be associated to mood disorders and are closely related to BDNF functional down-regulation. One of the main factors of inflammatory activation is Nuclear Factor-Kappa B (NF-κB), a transcription factor that induces the expression of several pro- and anti-apoptotic genes, including BDNF (Calabrese et al, 2014). Furthermore, chronic stress and depression conditions decrease BDNF expression, increased apoptosis and decrease regeneration of neurons in the hippocampus.

Moreover, given the role of BDNF as an important mediator of neuroplasticity and neurogenesis - in contrast to the detrimental effect of pro-inflammatory cytokines – its modulation may be involved in one of the mechanisms by which inflammation may affect brain function. Several in vivo studies demonstrated that inflammation clearly affects the expression of BDNF within the brain. In particular, it has been reported that the administration of pro-inflammatory cytokines (Raetz and Whitfield, 2002) causes a significant reduction of BDNF gene expression. Indeed, BDNF has a well-recognized role in the aetiology as well as in the treatment response of patients affected by different psychiatric disorders, supporting the possibility that inflammation contributes to the development of mood disorders by compromising neuroplasticity via the reduction of BDNF.

As mentioned previously, long-term neurobiological changes in BDNF concentrations have emerged in subjects exposed to ACEs (Aas et al., 2019). In a study on schizophrenic and bipolar patients, a dose-relationship was observed between total ACE and lower BDNF levels. Sexual abuse was the most significant experience related to a dramatic decrease in BDNF

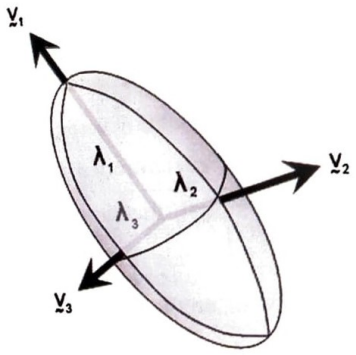
levels. Furthermore, lower peripheral BDNF levels were also associated with more depressive episodes, leading to a more severe illness. On the other hand, patients currently in remission presented higher levels of BDNF, suggesting its role as a marker of the ongoing disease. To conclude, BDNF levels should be considered as a potential biomarker for clinical staging in patients and it could be useful to be considered when treatment took place. Treatment designed to increase BDNF levels – such as physical activity and a zinc-based diet – should be considered.

Chapter 4: Method: Tract-Based Spatial Statistical

4.1 DTI

Diffusion Tensor Imaging (DTI) allows the in vivo study of the anatomy of white matter and has the advantage of being able to be exploited also for comparative studies to compare the organization of connections in animals and in the human brain. DTI uses Magnetic Resonance Imaging (MR) to visualize beams of fibers: MR imaging is based on the property of protons (hydrogen atoms) to align their axis of rotation with the magnetic field. Obviously you need a powerful magnetic field like the one generated by the MR scanner. A radiofrequency will then perturb the proton system and release energy that will be absorbed by the protons who will exploit it to change the orientation of their axis of rotation. The scanner will then measure the energy released by the protons that will be functional to the characteristics of the tissues in which the water will be present (of which our brain is rich). Tractography allows to evaluate not only the properties of the biological medium in which water is located but, through the breakdown of the brain into small units of space, it is able to evaluate the direction and extent of the flow of water within the tissues.

Myelin fibers within the brain impose an anisotropy, that is, a direction of diffusion of water flow: tractography allows us to know the direction of this flow and then to study in vivo the microstructural architecture of the brain even in pathological conditions.



Diffusion can be quantified by the coefficient of apparent diffusion (ACD) which is greater when the diffusion of water is not hindered, that is, in the ventricles and demyelinated white matter. This diffusion can be represented graphically by an ellipsoid. In MR, by applying different diffusion-sensitive gradients in different directions and then comparing the decay rate of the MR signal with that observed in a reference scan without any diffusion weighting, a set of information on the surface of the ellipsoid can be obtained to define its size, shape and orientation for each voxel.

The mathematical construct used to characterize anisotropic diffusion is the diffusion tensor. From the diffusion tensor estimated in each voxel, the amplitude and direction of diffusion can be estimated by evaluating the eigenvectors and eigenvalues of the tensor. The eigenvectors of the diffusion tensor identify the three axes of the ellipsoid. The three eigenvalues (λ_i) represent the diffusivity along each of these three directions. The eigenvector corresponding to the largest eigenvalue (λ_1), which represents the longest axis of the ellipsoid, is called the main diffusion direction or axial diffusivity (AD). The mean of the other two eigenvalues corresponds to radial diffusivity (RD).

$$RD = \frac{\lambda_2 + \lambda_3}{2}$$

The mean of the three eigenvalues represents the mean diffusivity (MD), i.e. the average molecular diffusion.

$$\lambda = MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

It is possible to determine the degree to which diffusion is directionally constrained by fractional anisotropy (FA).

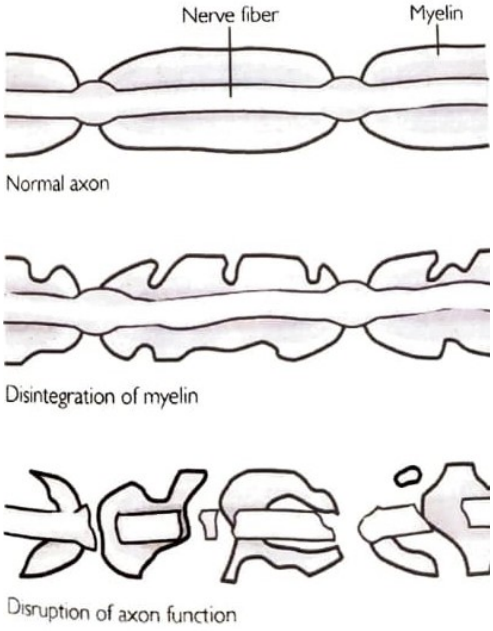
$$FA = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \lambda)^2 + (\lambda_2 - \lambda)^2 + (\lambda_3 - \lambda)^2}{\lambda_1 + \lambda_2 + \lambda_3}}$$

FA varies from 0 to 1, where 0 represents a preferred direction, therefore the isotropic diffusion, while 1 represents the unidirectional movement, that is, the anisotropic direction.

The water molecules of the brain's cerebrospinal fluid spread equally in all directions, while in the white matter movement occurs along the stretches of the fibers. Neural axons, in fact, have insulating sheets of myelin that envelop them and facilitate the movement of the molecules contained in the axon in a particular direction, reducing their possibility of escaping from the axonal walls. So DTI tunes in to how randomly scattered water molecules prefer to go in a specific direction, as opposed to all directions in each voxel.

Low AD reflects axon damage in both ischemic and chemically induced lesions, so it is a possible indicator of alterations in axonal integrity.

High levels of RD, on the other hand, have been associated with incomplete myelination in mice (Song, Sun et al., 2002), drug-induced demyelination, and loss of myelin following axonal damage. It then provides information about possible alterations in myelin integrity.



FA reflects the density of the fibers within a voxel so a reduced FA reflects the loss of integrity of the fibers.

Finally, high levels of MD have been observed in conditions of reduced membrane density, such as tissue degeneration after injury: it can therefore be a sensitive indicator of overall developmental changes in brain tissue (Pierpaoli et al., 1996).

Through these diffusivity measurements it is possible to characterize the macroscopic anatomy of white matter. Therefore, diffusion anisotropy is considered a marker for white matter integrity, therefore often used for the diagnosis of diseases and studies on the progression, development and aging of normal brain function (Horsfield & Jones, 2002; Pagani, Filippi et al., 2005).

4.1.1 Analysis of multi-subject diffusion data

Smith and colleagues (2006) proposed a new approach, called Tract-Based Spatial Statistics (TBSS), which performs localized statistical tests of FA and other diffusion-related data. This approach projects each subject's FA data into a common space, in a way that does not depend on the aforementioned non-linear recording. This is achieved by an initial approximate nonlinear recording, followed by projection onto a representation of the alignment invariant

stroke, called *mean_FA_skeleton*. No spatial leveling is required in image processing (Smith et al., 2006).

4.1.2 Tractography

Diffusion tensor imaging tractography is a method that allows the in vivo study of white matter fiber pathways, both under clinical and health conditions. It allows to reconstruct bundles of fibers and perform comparisons between FA subjects only for the voxels included in the identified traits. This procedure requires that an operator manually define the regions of interest that will be the starting point for tracing the bundles of faber in the diffusion image of each subject. An average stretch from the average of the images that will be used as a mask is then calculated. Finally, the alignment affine to the standard space will precede the voxelwise statistics inside the mask.

Tractography is based on the accuracy of alignment. Although some researchers have developed more sophisticated methods (Jones, Catani et al., 2006), to compare the variation in FA values along the bundles of fibers derived from tractography, directly between subjects, it is not based on a perfect alignment between subjects. In addition, the problem of user intervention in defining the areas of interest is still unresolved. Therefore, this is a major limitation of this method.

Another limitation is the possibility of evaluating FA only in those traits that have been specifically analyzed, drawing by hand the areas of interest.

Some investigations have tried to solve such problems but, before the advent of TBSS, "it is not safe to assume that recording can align FA data well enough between subjects to allow simple and unambiguous interpretations of voxelwise statistics" (Smith, Jenkinson et al., 2006).

4.2 TBSS

The optimal methods that use FA images in voxelwise statistical analysis in order to locate brain changes are compromised by the use of standard recording algorithms. There was no satisfactory solution to the question of how to align fa images of multiple subjects, so as to draw valid conclusions from the subsequent voxelwise analysis.

TBSS aims to solve these problems through: a) an accurately tuned nonlinear recording, followed by b) projection onto a representation of the alignment invariant trait, the *mean_FA_skeleton* (Smith, Jenkinson et al., 2006).

In gray matter density studies, in which VBM (Voxel-based morphometry) is used, the strengths of this technique is that it is fully automated, simple to apply, studies the entire brain and does not require you to manually define the regions of interest. The limitations of this technique imply only the inaccuracies of alignment.

Tractography-based approaches have complementary advantages and disadvantages: they can overcome alignment problems, however they do not allow the entire brain to be investigated and require human intervention to define regions of interest.

TBSS attempts to bring together the strengths of these two approaches, seeking to improve the sensitivity, objectivity and interpretability of the analysis of multi-subject diffusion imaging studies.

Now let's see in detail the steps of TBSS:

- *Pre-processing*: in the pre-processing phase, the images are corrected for eddy currents and head movements. The brain is separated from the non-brain and a diffusion tensor is mounted for each voxel of each subject.

- *Identification of the target for alignment*: the first step after pre-processing is the identification of the most suitable FA image to act as a target for all non-linear recordings: it could be, in particular, a predefined objective or be the "most typical" subject of the study. You can also align each FA image with all the others, identify the most representative one, and use it as the target image, after alignment in MNI152.
- *Nonlinear alignment*: Aligns all FA images to the chosen model with a nonlinear recording. To align the images well enough for the next step to work properly, while keeping the overall structure of the stroke intact, a nonlinear alignment with intermediate degrees of freedom is applied. After alignment, the entire aligned dataset is similarly transformed into 1 x 1 x 1 nm^3 in MNI152.
- *Creation of the media FA image and its skeleton*: the media FA image, obtained from the mediated FA images is thinned to obtain the skeleton of the WM traits shared by all individual subjects. The resulting middle FA skeleton represents the center of the WM strokes, with a bundle of tubular fibers as the center line and larger surfaces. The center of the stroke is represented by the voxels perpendicular to the orientation of the beam with the highest FA values.
- *Single-subject FA projection into the skeleton*: to account for residual misalignments between subjects, the maximum FA value within the perpendicular tract direction voxels is assigned to the closest skeleton voxel of the same tract, resulting in a skeleton distance map for each subject's FA image. In this way, the skeleton's alignment and the subject's FA image are resolved without a perfect nonlinear pre-registration.
- *Statistics and thresholding*: Voxel-wise cross-subjects analysis are performed on the 4D-"skeletonized" image resulting from the merging of each subject's (where the fourth dimension is the subject itself). Given the Gaussianity of the cross-subject null distribution of FA values for any given voxel, Smith demonstrates it is possible to apply the General Linear Model (GLM, e.g. multiple regressions) for each skeleton

voxel independently across subjects, performing simple parametric regression and inference. Multiple-comparison correction is the last troublesome choice to perform. The most suitable approach for TBSS data is the permutation-based one (Nichols & Holmes, 2002), where an appropriate statistic test (e.g., cluster size) is tested against the null distribution, resulting from multiple random permutations of subjects, of maximum values of the statistic test. This approach allows controlling family-wise errors while searching for significant effect regions within the whole FA skeleton. Permutation-statistical nonparametric inference of voxelwise statistical analysis across-subjects was performed using the FSL Randomise program (Nichols & Holmes, 2002).

4.3 ComBat

ComBat is a feature that allows the removal of batch effects within neuroimaging studies. The characteristics of MRI scanners – such as subject positioning, field strength, gradient nonlinearity – are considered factors that increase bias and variance when measuring brain volume, cortical thickness, structural images, and voxel-based morphometry. Such unwanted sources are then confused as variables and included in data analyses. In particular, the term scanner effects is used to refer to unwanted variations that may be, in addition to being of a biological nature, also of a non-biological nature, that is, associated with differential scanning equipment or parameter configurations. Because different imaging sites use different physical scanners, site effects can be considered scanner effects.

ComBat has proven to be an effective harmonization technique that removes unwanted variations associated with the site and preserves biological associations in the data. It is then used as a suitable tool for modelling and removing site effects in DTI studies.

The ComBat harmonization model creates a unique linear model of position and scales for each feature, assuming that scanners or sites have an additive and multiplicative effect on the

data. The model assumes that the expected values of imaging feature measurements can be modeled as a linear combination of biological variables and site effects, whose error term is modulated by additional site-specific factors.

The ComBat model was formulated by Fortin et al. (2017) precisely for the harmonization of scalar maps of DTI data.

In our study we use ComBat to harmonize DTI images obtained from different scans, as the considered sample has pre and post measurements 2016, the year in which a new MRI scanner was introduced.

4.5 Childhood Trauma Questionnaire

Childhood trauma was measured using the Childhood Trauma Questionnaire (CTQ), consisting of 38 items (Bernstein, 1998). It measures the occurrence of five types of trauma during childhood, specifically: emotional abuse, physical abuse, sexual abuse, emotional abuse, physical neglect and emotional neglect.

The authors defined sexual abuse as a sexual contact or conduct between a child under the age of 18 and an adult or elderly person.

Physical abuse as bodily assaults on a child by an adult or elderly person that pose a risk or result in injury.

Emotional abuse such as “verbal aggression to a child's sense of worth or well-being or any humiliating behavior directed at a child by an adult or elderly person”.

Physical abandonment as “the inability of guardians to provide for a child's basic physical needs, including food, housing, clothing, security, and health care”.

Finally, emotional abandonment has been defined as “the inability of guardians to meet children's basic emotional and psychological needs, including love, belonging, education, and support”. CTQ is widely used in clinical research and general population samples (Janiri et al., 2015) and has good psychometric properties (Bernstein et al., 2003). Applications are

referred to the period between 5 and 15 years of age. The answers are quantified on a 5-point Likert scale based on how often the experiences occurred, between 1 = "never" and 5 = "very often". A cut-off score of 35 is used to classify patients into high and low childhood trauma exposure.

Chapter 5: The experimental study

5.1 Background

In recent years, many efforts have been made to understand the neurobiological basis of mood disorders (Muneer et al., 2018; Carlson et al., 2006). Among them, bipolar disorders (BD) is ranked as the 17th leading source of disability among all diseases worldwide due to its chronic course and early onset, typically before the age of 30 (Vieta et al., 2018; Carvalho et al., 2020). According to the World Mental Health Survey Initiative (Merikanga et al., 2010), lifetime and 12-month prevalence estimates for BD are around 2.4% and 1.5% respectively. In the last decades, an increasing amount of data has suggested a prominent role of inflammation and immune system dysregulation in mood disorder (Jones et al., 2013; Rosenblat, 2019). According to the inflammatory theory of depression (Maes, 2011; Gałecki & Talarowska, 2018) - which states the importance of neuroinflammation as a valid etiopathological mechanism underlying mood disorders - higher production of pro-inflammatory cytokines was observed in BD and MDD patients, and therefore considered as one possible biomarker of mood disorder.

Adverse childhood experiences (ACE) are defined by different forms of abuse, neglect, and household dysfunction (Fellitti et al., 1998), usually occurring before the age of 18 and are considered a risk factor for the pathogenesis of BD (Daruy-Filho et al., 2011). Adverse childhood experiences, has been linked also to the low grande inflammatory condition which characterize mood disorders (Jaworska-Andryszewska & Rybakowski, 2019). It has been

observed that the association between mood disorders and ACE can worsen the lifetime course of illness, leading to functional and structural changes later in life (Benedetti et al., 2014; Poletti et al., 2016; Park et al., 2020). Signs of widespread alteration of white matter (WM) integrity, indeed, have been associated with both markers of inflammation and BD, suggesting that inflammation may mediate the impact of ACE on brain structural integrity (Chen & Lacey, 2018; Lacey et al., 2020).

5.2 Experimental hypothesis

The present study investigates the impact of ACE and inflammation on WM integrity in a sample of BD and MDD patients. The relationship between CTQ questionnaire measures, - which retrospectively measures the severity of childhood stress and trauma - WM integrity and inflammation (through the analysis of cytokines peripheral levels as neuroinflammatory markers) has been studied through Tract-Based Spatial Statistics (TBSS) analysis on DTI MRI acquisitions. The aim of the present study is to investigate the role of the immune and inflammatory pathways in BD and MDD as moderator of the impact of ACE on brain integrity. Previous results led us to hypothesize that the alterations of WM microstructure observed in patients with mood disorders could be differently associated with peripheral inflammatory markers in patients exposed to high or low levels of ACE. In the present study we tested these hypotheses in a sample of BD and MDD patients.

5.3 Materials and methods

5.3.1 Participants and data collection

The study sample consisted of 200 depressed patients, of whom 100 were diagnosed with MDD and 100 diagnosed with BD, aged between 18 and 56 years. Patients were recruited within the psychiatric ward of San Raffaele Hospital. The exclusion criteria in the present

study protocol were: the current diagnosis of further psychiatric disorders (including alcohol or substance use and abuse), pregnancy, intellectual disability, severe medical and neurological disorders, and medical conditions affecting the immune system. An informed written consent was obtained following a complete description of the study protocol for each enrolled patients. All research activities have been approved by the Local Ethics Committee. Each patients underwent magnetic resonance imaging (MRI) at the C.E.R.M.A.C (Centro d'Eccellenza Risonanza Magnetica ad Alto Campo), and 130 subjects (75 MDD and 55BD) also underwent venous blood sampling, between 7:00 and 9:00 am in the morning. Among BD 44 patients had been taking lithium for at least 6 months. Other treatments involved taking antidepressants, atypical antipsychotics, and epileptic drugs

5.3.2 Adverse childhood events measurement

The 28 items of the Childhood Trauma Questionnaire (Bernstein, 1998) were used to measure the occurrence of early adverse events. Specifically, the subscales of physical, sexual, and emotional abuse were added together to have a total "abuse" score. The subscales of physical and emotional neglect were added together to obtain a total score of "neglect".

5.3.3 Laboratory determinants

Plasma concentrations of the following immune analytes were determined using the Bio-Plex Pro Human Cytokine 27-plex with the bead-based Luminex system (Bio-Rad Laboratory, Hercules, CA, USA): Interleukin (IL)-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Interferon (IFN)γ, Tumor Necrosis Factor (TNF)α, C-C motif ligand 1 (CCL2), CCL3, CCL4, CCL5, CCL11,; C-X-C motif chemokine (CXCL)10, fibroblast growth factor (FGF) basic, Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte Macrophage Colony Stimulating Factor (GM-

CSF), Platelet-Derived Growth Factor Beta (PDGF-B), Vascular Endothelial Growth Factor (VEGF).

Assays were performed on Luminex 200 system. Samples were analyzed according to manufacturer's instructions. The intra-assay coefficient of variation was 2.3–4.8 %, interassay coefficient of variation was 4.9–28.2 %

5.3.4 Image Acquisition

As we said in section 4.5, we used two different scanners for image capture. DTI was acquired using SE Eco-planar imaging (EPI) and the following parameters for the first scanner TR/TE=8753.89/58 ms, FoV (mm) 231.43 (ap), 126.50 (fh), 240.00 (rl); acquisition matrix 2.14×2.71×2.31; 55 contiguous, 2.3mm thick axial slices reconstructed with in-plane pixel size 1.88×1.87 mm; SENSE acceleration factor=2; 1 b0 and 35 non-collinear directions of the diffusion gradients; b value=900 s/mm^2; and the following for the second Philips scanner TR/TE=5900/78 ms, FoV (mm) 240 (ap), 129 (fh), 232 (rl); acquisition matrix 2.14×2.73×2.30; 56 contiguous, 2.3mm thick axial slices reconstructed with in-plane pixel size 1.88×1.88x2.30 mm; SENSE acceleration factor=2; 1 b0 and 40 non-collinear directions of the diffusion gradients; b value=1000 s/mm^2. Fat saturation was performed to avoid chemical shift artefacts.

5.3.5 *DT-MRI data preprocessing*

DTI analysis and tensor calculations were carried out using the "Oxford Center for Functional Magnetic Resonance Imaging of the Brain Software Library" (FSL 6.0; www.fmrib.ox.ac.uk/fsl/index.html) (Smith, Jenkinson et al. 2004, Woolrich, Jbabdi et al. 2009). First, each DTI volumes was affine registered to the T2-weighted b=0 volume

using FLIRT (FMRIB's Linear Image Registration Tool) (Jenkinson and Smith 2001). Then, correction for motion between scans and residual eddy-current distortions present in the diffusion-weighted images was performed. After removal of nonbrain tissue (Smith 2002), least-square fits were performed to estimate the fractional anisotropy (FA), eigenvector, and eigenvalue maps. Mean diffusivity (MD) was defined as the mean of all three eigenvalues $(\lambda_1 + \lambda_2 + \lambda_3)/3$, axial diffusivity (AD) as the principal diffusion eigenvalue (λ_1), and radial diffusivity (RD) as the mean of the second and third eigenvalues $(\lambda_2 + \lambda_3)/2$ (Stone, Laughren et al. 2009).

Next, all individuals' volumes were skeletonized and transformed into a common space as used in Tract-Based Spatial Statistics (Smith, Jenkinson et al. 2006, Smith, Johansen-Berg et al. 2007). Briefly, all volumes were nonlinearly warped to the FMRIB58_FA template supplied with FSL (http://www.fmrib.ox.ac.uk/fsl/tbss/FMRIB58_FA.html) and normalized to the Montreal Neurological Institute (MNI) space, by use of local deformation procedures performed by FMRIB's Non-Linear Image Registration Tool (FNIRT) (Rueckert, Sonoda et al. 1999, Westlye, Walhovd et al. 2010). Next, a mean FA volume of all subjects was generated and thinned to create a mean FA skeleton representing the centers of all common tracts. We thresholded and binarized the mean skeleton at $FA > 0.20$ to reduce the likelihood of partial voluming in the borders between tissue classes. Individual FA values were warped onto this mean skeleton mask. The resulting tract invariant skeletons for each participant were fed into voxelwise permutation-based cross-subject statistics. Similar warping and analyses were used on MD, AD, and RD data.

Voxelwise DTI analyses were performed using nonparametric permutation-based testing (Nichols and Holmes 2002) as implemented in Randomise in functional MRI of the brain software library. We tested for linear effects of metabolic markers on FA, MD, AD, and RD across the WM skeleton using general linear models. We accounted for the effects of nuisance

covariates which could influence WM structure such as: age (Kochunov, Thompson et al. 2007), gender (Herting, Maxwell et al. 2012), duration of illness (Poletti, Bollettini et al. 2015), lithium treatment (Benedetti, Bollettini et al. 2013), BMI (Mazza, Poletti et al. 2017) and equivalents of imipramine. Threshold-free cluster enhancement (TFCE) (Smith and Nichols 2009)was used to avoid defining arbitrary cluster-forming thresholds and smoothing levels. TFCE is particularly useful when the spatial correlation length of signal exceeds that of noise, as it is expected when studying WM tracts. It can be seen as a generalization of the cluster mass statistics (Bullmore Suckling et al., 1999), using spatial neighborhood information in a nonlinear image processing to increase sensitivity and boost the height of spatially distributed signals, without changing the location of their maxima. Voxelwise levels of significance, corrected for multiple comparisons, were then calculated with a standard permutation testing by building up the null distribution (across permutation of the input data) of the maximum (across voxels) TFCE scores, and then using the 95th percentile of the null distribution to threshold signals at a corrected $p<.05$. The data were tested against an empirical null distribution generated by 5000 permutations for each contrast, thus providing statistical maps fully corrected for multiple comparisons across space. Corrected $p<.05$ in a minimum cluster size of $k = 100$ was considered significant.

5.4 Statistical Analyses

Statistical analyses were performed using STATISTICA (StatSoft Statistica 11, Tulsa, OK, USA). Similarly to previous studies, only cytokines with non-detected (missing) values < 20% were included in the analyses (Wolf, Delgado et al. 1998).

Baseline clinical-demographic differences between BD and MDD groups were performed using t-test analysis and chi-square analyses for dichotomous variables, with $p < 0.05$. In those analyses comparing variables associated with an inflammatory response we employed non

parametric Mann-Whitney test to account for the distributional characteristics of the data and the heterogeneity of variance. In order to control for multiple comparisons p-values were corrected through False Discovery Rate procedure (obtaining q values) (Benjamini and Hochberg 1995).

To analyze the effect of stress on WM microstructure and the mediating/moderating role of inflammation we performed the following analyses separately for CTQ and PSS scores:

1) To investigate the effect of stress on peripheral levels of immune/inflammatory markers we performed separate MANCOVAs in MDD and BD, with immune/inflammatory markers as dependent variables and stress as factor. We accounted for the effects of nuisance covariates which could influence peripheral levels of immune/inflammatory markers: age, sex, duration of illness, BMI, imipramine equivalents and lithium treatment.

2) To investigate the effect of stress and inflammation on WM microstructure we performed voxelwise DTI analyses using nonparametric permutation-based testing (Nichols and Holmes 2002) as implemented in Randomise in FSL. We tested for linear effects of stress on FA, MD, AD, and RD across the WM skeleton with general linear models (GLM) separately in MDD and BD patients. Difference in DTI parameters between the two groups was assessed through t-test, and a separate slopes analysis was performed to investigate the different relationship between stress and WM in the two groups. Finally, we tested for linear effects of immune/inflammatory markers associated with stress.

3) We investigated whether immune/inflammatory markers mediated the association between stress and WM microstructure.

5.5 Results

BD patients had an earlier onset and a longer duration of illness compared to MDD patients and a lower dosage of imipramine equivalents.

The investigation of the effect of stress on immune/inflammatory markers showed a main effect of childhood trauma (λ=0.333, F=2.472, p=0.014) only in the BD group. Analyses of coefficients showed that higher levels of childhood trauma were associated with higher peripheral levels of IL-2 (β=0.387, p=0.003), IL-17 (β=0.298, p=0.027), bFGF (β=0.313, p=0.026), IFN-γ (β=0.392, p=0.002), TNF-α (β=0.263, p=0.049), CCL3 (β=0.270, p=0.046), CCL4 (β=0.340, p=0.014), and CCL5 (β=0.294, p=0.029), and PDGF-BB (β=0.265, p=0.044).

Within the neuroimaging analysis, the comparison between diagnosis showed that MDD patients had higher AD compared to BD patients (Figure 14, Table 1).

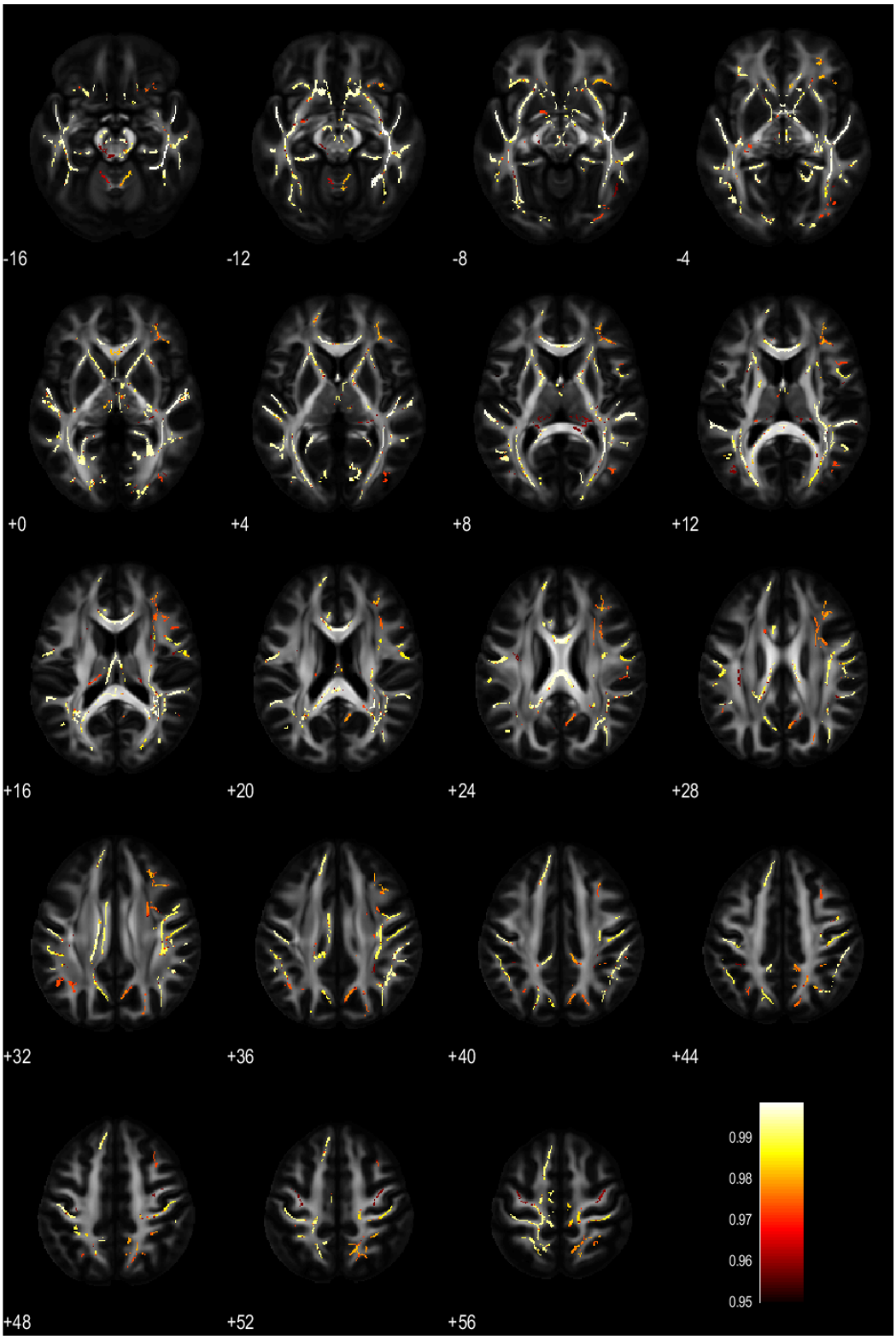


Figure 14 - *WM areas where MDD patients showed higher AD compared to BD patients. Significant voxels are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

	N° Voxels & Signal Peaks (x,y,z)	WM tracts
ALL M = 0.001140 SD = 0.000240 BD M = 0.001119 SD = 0.000239 MDD M = 0.001160 SD = 0.000242	50820 36 -18 -28 R Inferior Fronto-Occipital Fasciculus	Splenium, Body and Genu of Corpus Callosum R/L Forceps Minor and Major Cingulum (cingulate gyrus) L Inferior Fronto-occipital fasciculus L/R L/R Inferior Longitudinal Fasciculus L/R Superior Longitudinal Fasciculus L/R Anterior Thalamic Radiation L/R Uncinate Fasciculus L/R Corticospinal Tract L /R Inferior, Middle and Superior Cerebellar Peduncle L/R Anterior and Posterior Limb, and Retrolenticular part of Internal Capsule L Fornix (column and body of fornix, cres) / stria terminalis L Posterior Corona Radiata L R Anterior Corona Radiata R/L Sagittal Stratum (include Inferior Longitudinal Fasciculus and Inferior Fronto-Occipital Fasciculus) R/L Cerebral Peduncle L/R External capsule R/L Posterior Thalamic Radiation (include optic radiation) R/L Medial Lemniscus L Cingulum (Hippocampus)
	143 -29 28 15 L Inferior fronto-occipital fasciculus	L Inferior Fronto-occipital Fasciculus L Superior Longitudinal Fasciculus L Anterior Thalamic Radiation L Uncinate Fasciculus L Inferior Fronto-occipital Fasciculus L Superior Longitudinal Fasciculus (temporal part)

Table 1. Areas where MDD patients show increased AD compared to BD patients. In the second column, dimension of clusters (number of voxels, mm3) and localization (WM tracts and MNI coordinates) are given for regions showing maximal effects at TBSS (signal peaks). The third column lists the other WM tracts included in the clusters and the lateralization (Right or Left).

Moreover, a continuous covariate interaction model, performed on the whole sample (BD and MDD patients) showed a significant difference in the linear relationship between FA and CTQ

total scores between the two groups. Further, a similar effect was observed for neglect scores on FA and AD, and for abuse scores on FA (Figure 15), RD (Figure 16) and MD (Figure 17).

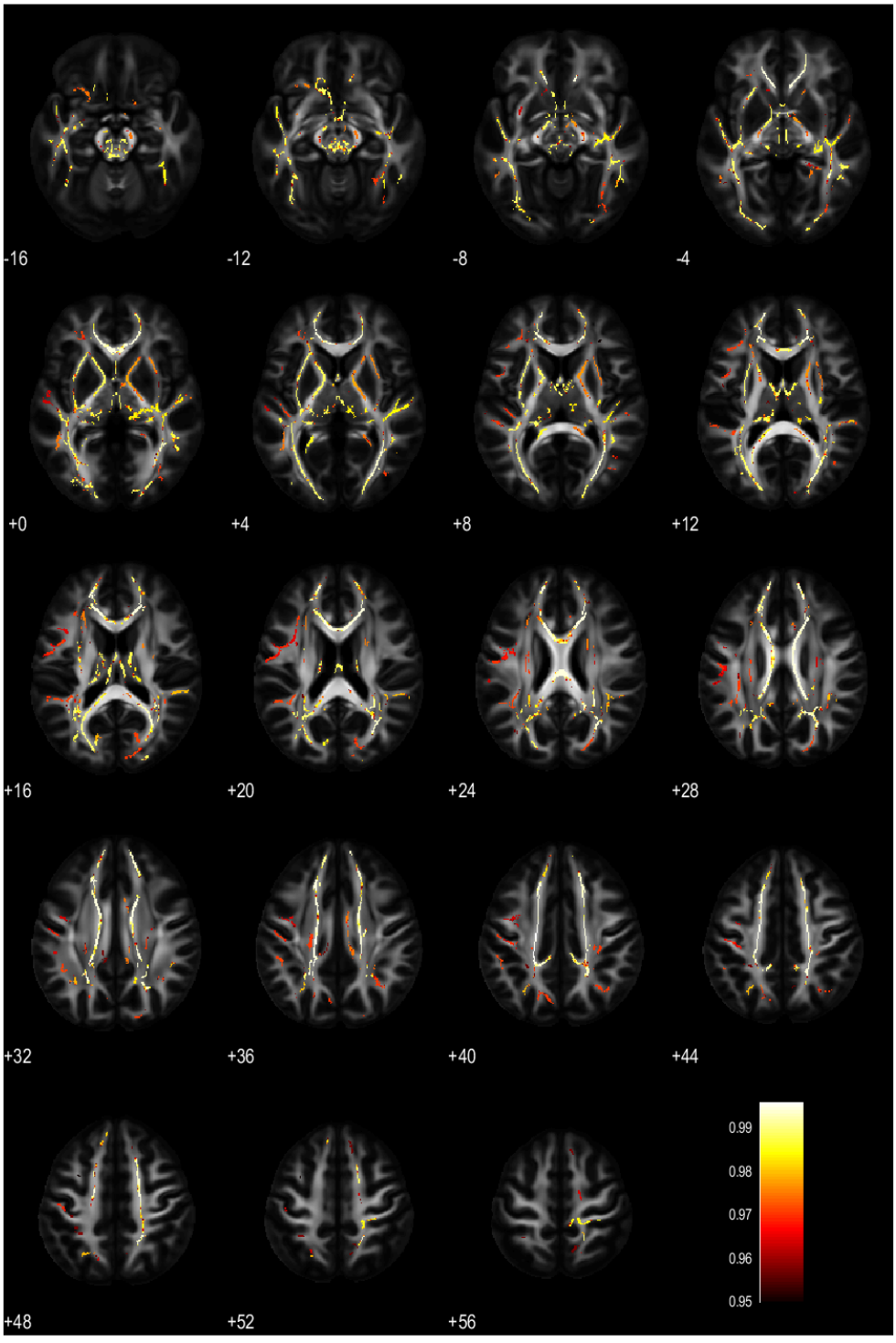


Figure 15 – *WM areas of the significant effect of abuse score on FA. Significant voxels are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

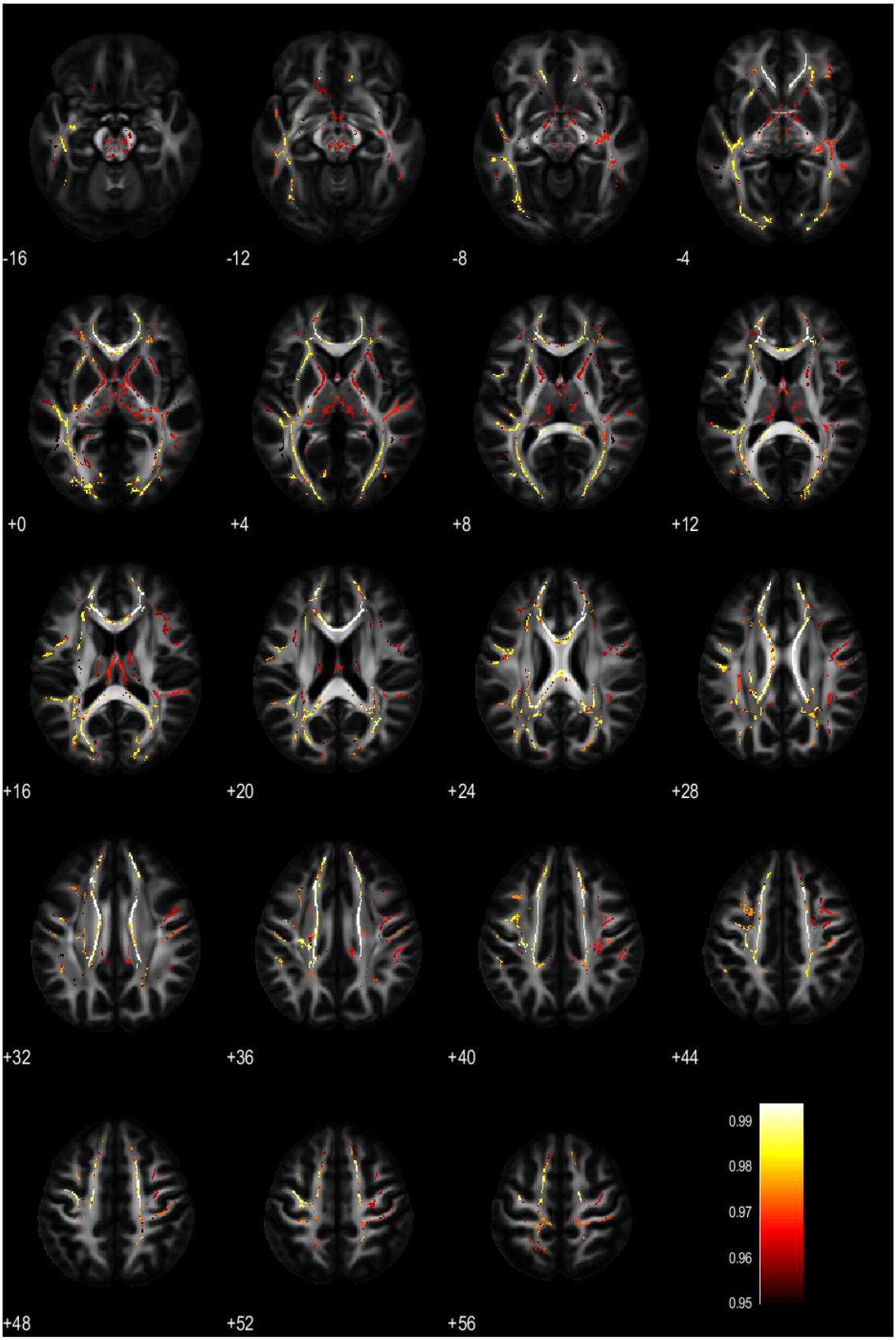


Figure 16 – *WM areas of the significant effect of abuse score on RD. Significant voxels are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

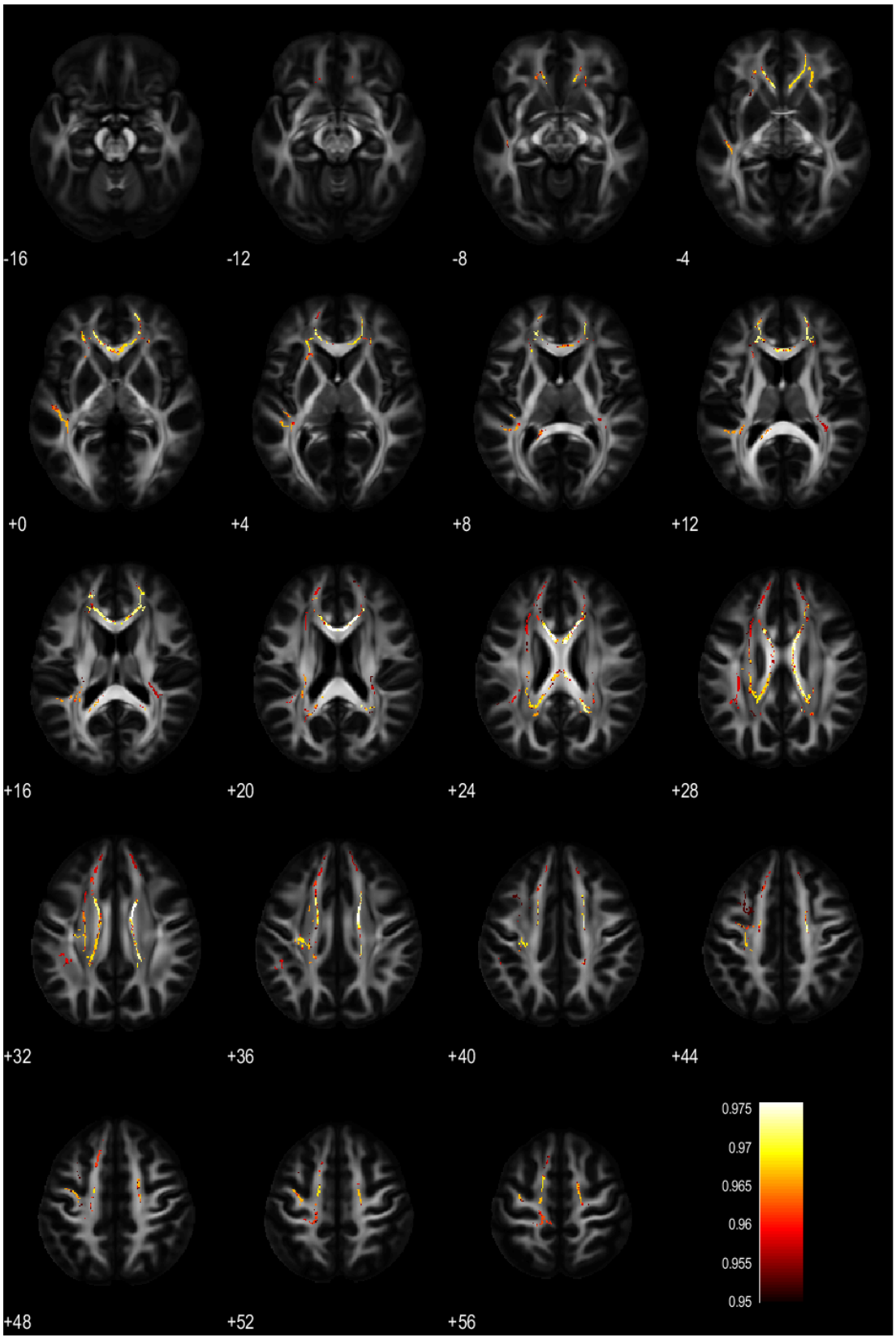


Figure 17 *- WM areas of the significant effect of abuse score on MD. Significant voxels are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

Simple regression analyses showed that CTQ total scores negatively associated with FA (Figure 18, Table 2) and AD (Figure 19) in BD patients in several WM tracts encompassing anterior and posterior thalamic radiation, anterior, posterior, and superior corona radiata, inferior and superior longitudinal fasciculus, inferior fronto-occipital fasciuculus, internal and external capsule, cingulum bundle, uncinate fasciculus, forceps minor and major, and body and splenium of corpus callosum.

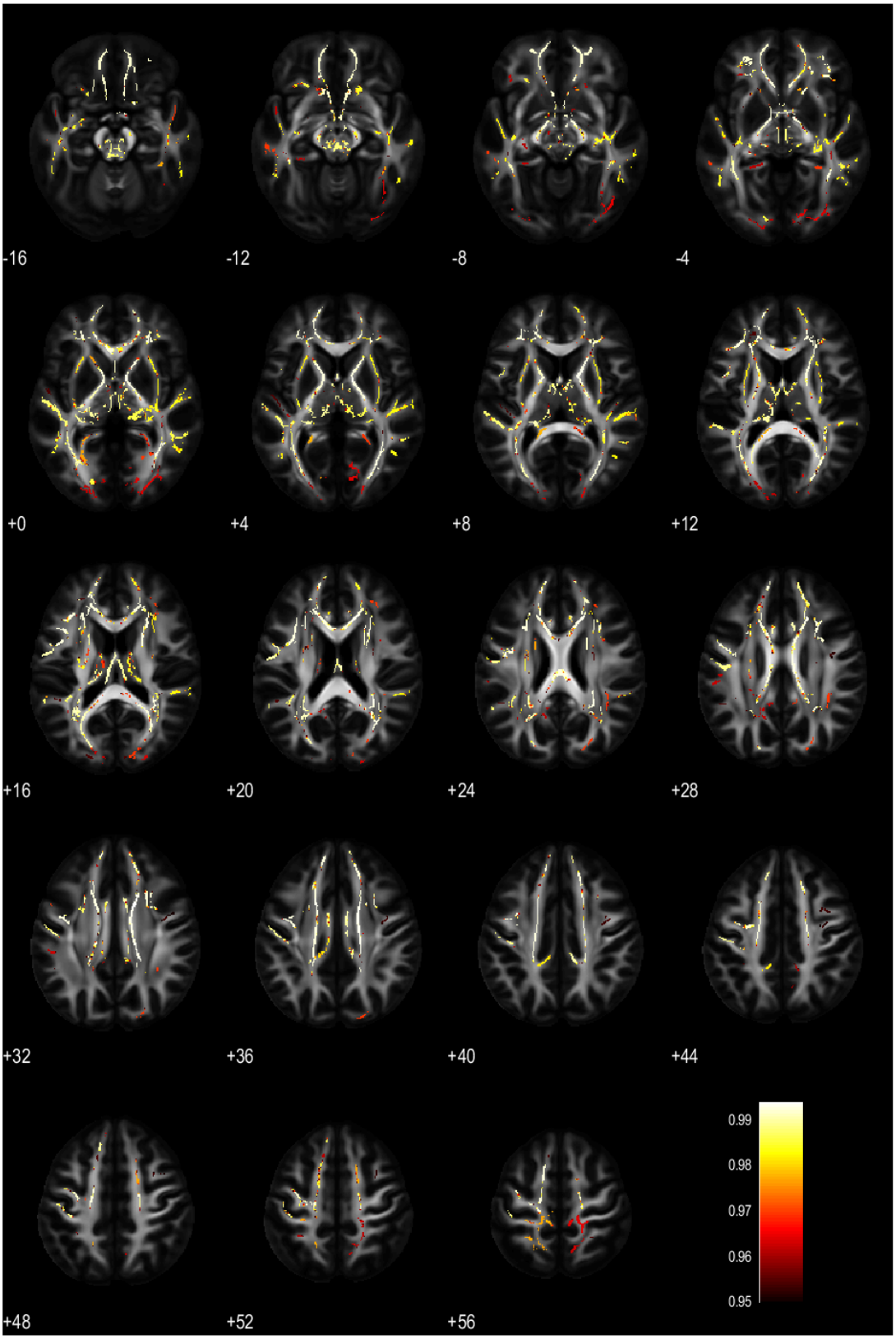


Figure 18 - *WM areas where CTQ total score negatively associated with FA. Voxels of significant negative correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

	N° Voxels & Signal Peaks (x,y,z)	WM tracts
FA M = 0.371834 SD = 0.206604	52292 14 4 31 Body of Corpus Callosum	R/L Inferior Longitudinal Fasciculus R/L Pontine Crossing Tract (a part of MCP) R/L Anterior Thalamic Radiation Genu, Body and Splenium of Corpus Callosum R/L Forceps Major and Minor L/R Superior and Posterior Corona Radiata R Fornix (column and body of fornix) R Corticospinal Tract R/L Superior Longitudinal and Fronto-occipital Fasciculus R/L Inferior Longitudinal Fasciculus R/L Sagittal Stratum (include Inferior Longitidinal Fasciculus and Inferior Fronto-occipital Fasciculus) R Superior Longitudinal Fasciculus (Temporal part) L/R Cerebral Peduncle L/R Corticospinal Tract L/R Anterior, Posterior Limb and Retrolenticular Part of Internal Capsule L External Capsule R/L Cingulum (cingulate gyrus) R L Superior Cerebellar Peduncle R/L Cingulum (hippocampus) R Posterior thalamic radiation (include optic radiation) R Tapetum R Superior cerebellar peduncle R Fornix (cres) / Stria terminalis (can not be resolved with current resolution) R/L Cerebral Peduncle R L/R Uncinate Fasciculus
	510 39 -6 38 R Superior Longitudinal Fasciculus	R Superior Longitudinal Fasciculus R Superior Longitudinal Fasciculus (temporal part)

Table 2. Areas where BD patients show a negative association between CTQ total scores and FA. In the second column, dimension of clusters (number of voxels, mm3) and localization (WM tracts and MNI coordinates) are given for regions showing maximal effects at TBSS (signal peaks). The third column lists the other WM tracts included in the clusters and the lateralization (Right or Left).

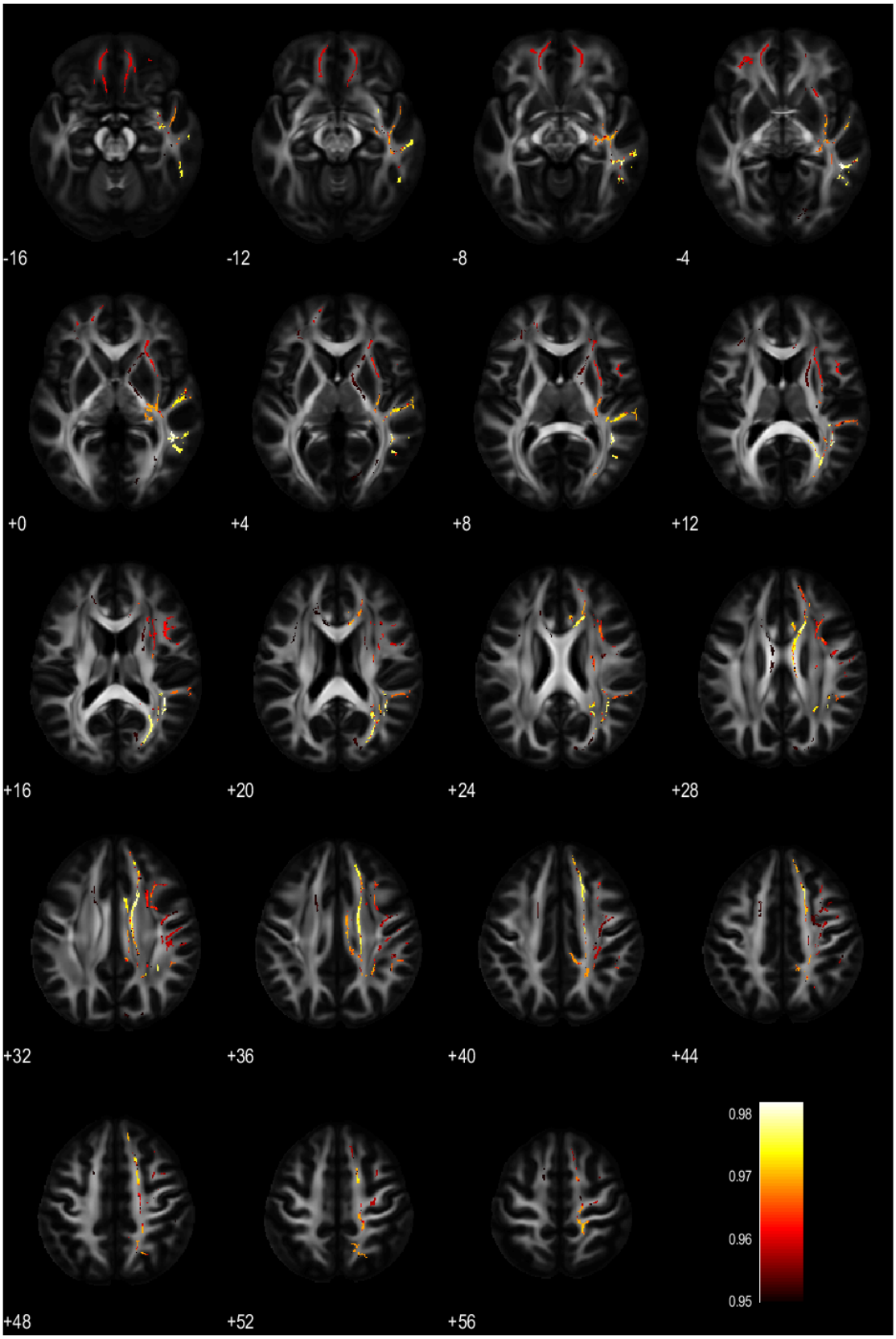


Figure 19 - *WM areas where CTQ total score negatively associated with AD in BD patients. Voxels of significant negative correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

CTQ scores, on the other hand, had no effect on DTI parameters in MDD patients. Abuse scores had a negative association with FA (Figure 20) and a positive one with RD (Figure 21) in BD subjects, while they exhibited a negative association with MD in unipolar patients. Neglect scores had a negative association with AD in bipolar patients, while it had no effect in MDD patients.

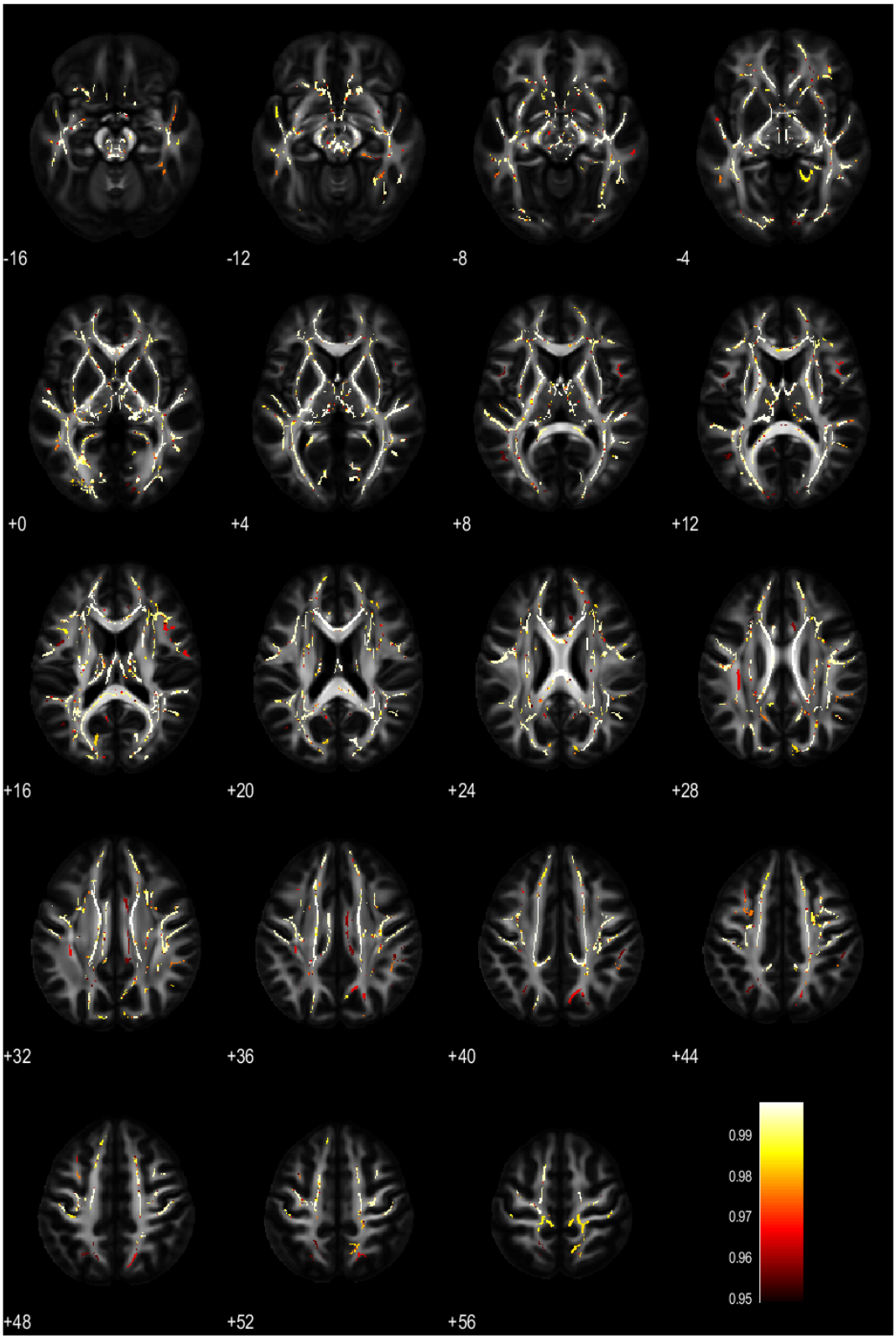


Figure 20 – *WM areas where abuse scores negatively associated with FA in BD patients. Voxels of significant negative correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

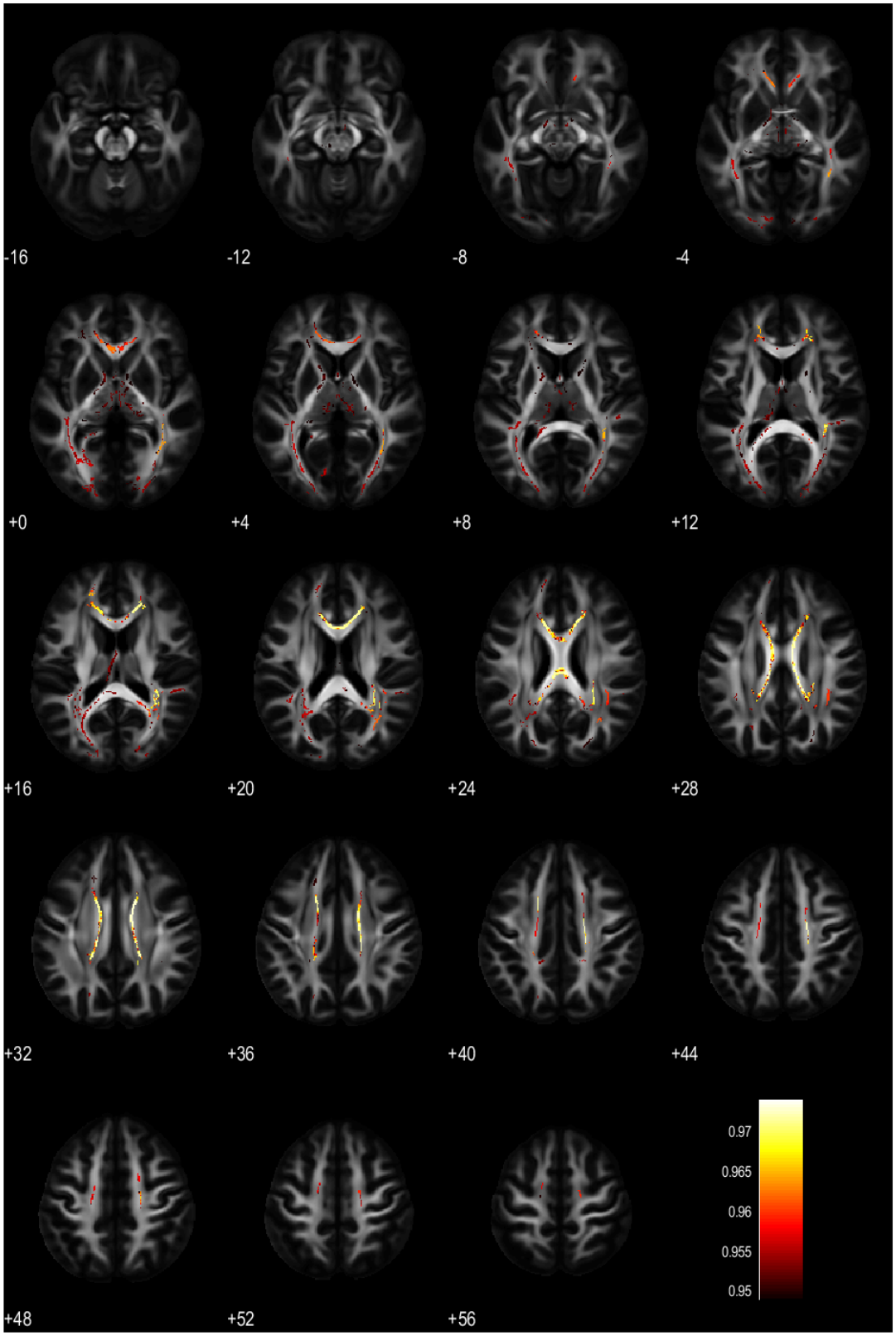


Figure 21 – *WM areas where abuse scores positively associated with RD in BD patients. Voxels of significant positive correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

Finally, among the immune-inflammatory markers associated with childhood trauma, we observed a negative association between both CCL3 (Figure 22, Table 3) and IL2 (Figure 23, Table 4) with FA in several WM tracts encompassing anterior thalamic radiation, anterior, posterior, and superior corona radiata, superior longitudinal fasciculus, inferior fronto-occipital fasciuculus, cingulum bundle, cortico-spinal tract, uncinate fasciculus, forceps major, and body and splenium of corpus callosum.

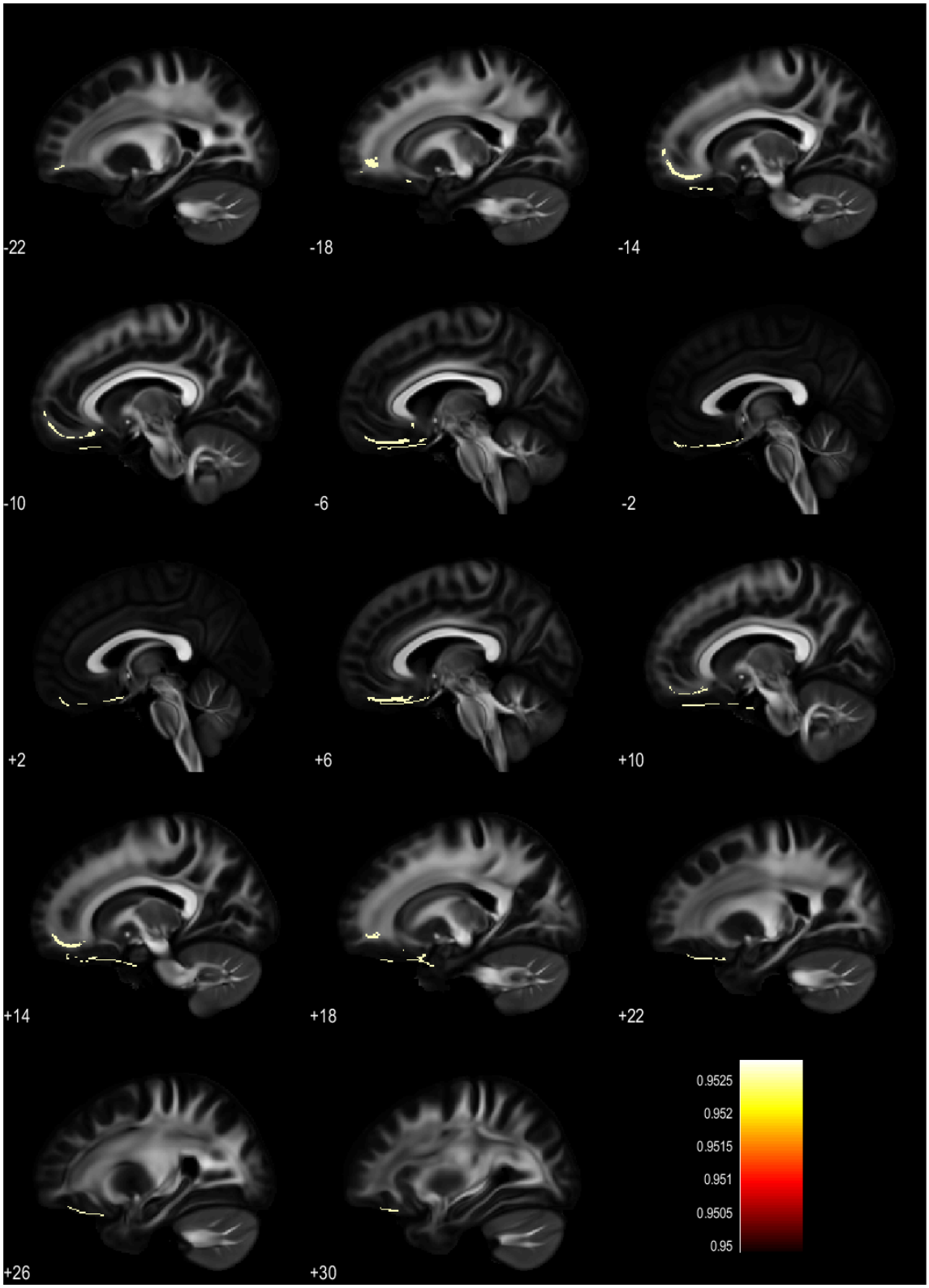


Figure 22 – *WM areas where CCL3 negatively associated with FA. Voxels of significant negative correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

Clusters of negative correlation between CCL3 and FA values		
N° Voxels & Signal Peaks (x,y,z)		WM tracts
FA M = 0.019093 SD = 0.088863	3555 18 43 -6 R Uncinate Fasciculus	R/L Forceps Minor R/L Uncinate Fasciculus R/L Inferior Fronto-Occipital Fasciculus L Anterior Corona Radiata R/L Anterior Thalamic Radiation

Table 2 - Clusters of negative correlation between CCL3 and FA. In the second column, dimension of clusters (number of voxels, mm3) and localization (WM tracts and MNI coordinates) are given for regions showing maximal effects at TBSS (signal peaks). The third column lists the other WM tracts included in the clusters and the lateralization (Right or Left).

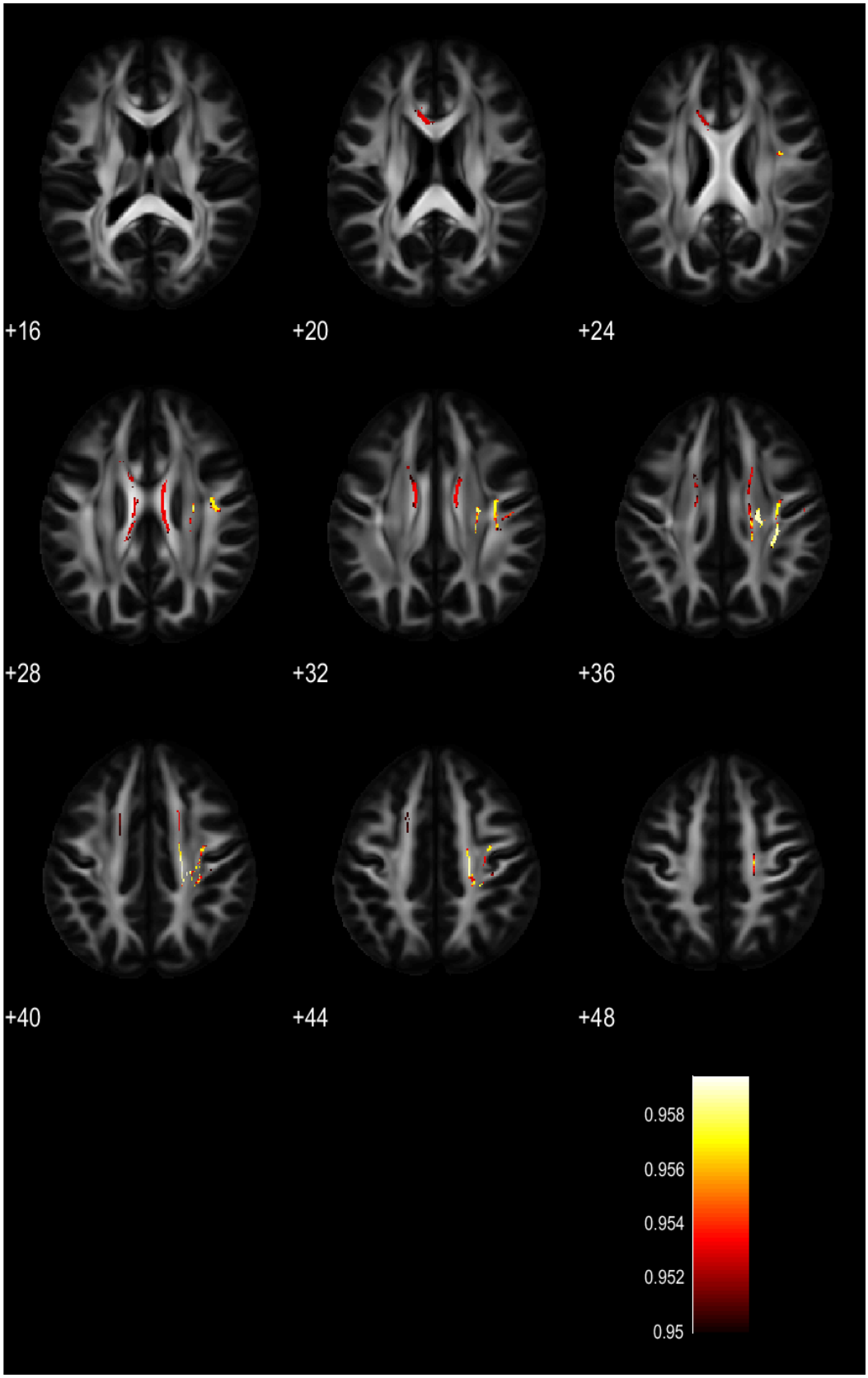


Figure 23 - *WM areas where IL2 negatively associated with FA. Voxels of significant negative correlation are mapped on the mean FA template of the studied sample. The colour-bar refers to 1-p values for the observed differences. Numbers are z coordinates in the standard MNI space.*

N° Voxels & Signal Peaks (x,y,z)		WM tracts
FA M = 0.516159 SD = 0.114122	2875 23 -23 37 R Corticospinal Tract	Body, Genu and Splenium of Corpus Callosum R/L Superior Corona Radiata L Superior, Posterior and Anterior Corona Radiata R Corticospinal Tract R Forceps Minor R Superior Longitudinal Fasciculus R L Cingulum (cingulate gyrus)

Table 3 - Clusters of negative correlation between IL2 and FA. In the first column, dimension of clusters (number of voxels, mm3) and localization (WM tracts and MNI coordinates) are given for regions showing maximal effects at TBSS (signal peaks). The second column lists the other WM tracts included in the clusters and the lateralization (Right or Left).

Chapter 6 – Discussion

Early adversities can have a long-lasting impact on mental health (Padgett & Glaser, 2003). Childhood trauma can cause immune disregulation and a higher production of pro-inflammatory cytokines (Quidé et al., 2019) that - once in the brain - can affect brain development, leading to alterations in brain structure and function; for this reason, inflammation is considered one of the possible biological mechanisms underlying the pathogenesis of mental illness (Baumeister et al., 2016).

6.1 The role of ACE

6.1.1 ACE and inflammatory markers

In our study, an effect of ACE on inflammation was found, showing that higher levels of childhood trauma measured by the CTQ are associated with higher peripheral levels of inflammatory markers, especially with CCL3, CCL4 and CCL5. ACE may disrupt the adaptive response to stress, thus reducing the ability of glucocorticoid signaling to control the HPA axis (Kielcot-Glaser et al., 2011; Danese et al., 2017; De Bellis et al., 2014). As a consequence, childhood maltreatment might cause an increase in the levels of inflammation in adulthood (Coelho et al., 2013; Danese et al., 2007; Slopen et al., 2012). Chemoattractant

cytokines known as chemokines are key immune mediators in leukocyte trafficking in both inflammatory and normal conditions (Stuart et al., 2015). Chemokines have also additional functions, such as inducing the release of pro-inflammatory mediators and control of T-helper (Th)-1/Th-2 phenotypic polarization (Cyster,1999). Some evidence suggests their role in the modulation of neurotransmitter system and neuroinflammatory responses (Taub & Oppenheim, 1994; Hughes & Nibbs, 2018). Moreover, recent studies have shown that CCL3, CCL4 and CCL5 are involved not only in microglia chemotaxis under chronic inflammatory states but also in low-grade inflammation underlying psychiatric disorders (Stuart & Baune, 2014). Chemokines may have a role also in brain development, with some evidence suggesting a role for CCL3 in the regulation of neural stem/progenitor cells (NSC/NPCs) migration in both the hippocampus and subventricular zone (Widera et al., 2004). CCL3, CCl4, and CCL5 exert various chemotactic functions in the inflamed CNS, including actions on monocytes, microglia, and neutrophils via their receptors CCR1, CCR3, and CCR5 (Johnson et al., 2011). An increase of chemokines activity has been reported in children exposed to serious life events (Carlsson et al., 2014), therefore, it is possible that those who experienced major stressors early in life may be more vulnerable to immune dysregulation in adulthood, which in turn could lead to the development of psychopatology (Leigthon et al., 2018). Our finding is in line with previous researches reporting the involvement of chemokines in BD, showing that chemokines discriminate BD patients from MDD patients and that chemokine alterations appear mainly in depressive BD patients (Poletti et al., 2021; Misiak et al., 2020). Moreover, pharmacological treatment – such as lithium administration – may impact chemokines levels in BD, interacting with their signalling pathways and having an impact on cortical thickness (Barbosa et al., 2014; Poletti et al., 2019).

6.1.2 ACE and WM microstructure

We observed a negative correlation between childhood trauma exposure and WM integrity in BD patients. These patients that have experienced trauma in early childhood and scored higher on the CTQ questionnaire reported lower FA values, especially in two clusters, peaking at the left uncinate fasciculus (UF) and the left superior longitudinal fasciculus (SLF). FA reductions has been previously explained has related to a condition of lower axonal density, demyelination or axonal injury (Jones, D. K., & Leemans, 2011). Moreover, FA reflect the degree of diffusion directionality of water – which can be influenced by structural properties such as axonal density, organization and myelinization - and can be considered an overall indicator of white matter integrity. These neuroimaging results are in line with previous literature in this area, showing how childhood adversity can affect WM microstructure both in healthy (Ugwu et al., 2015; McCarthy-Jones et al., 2018) and psychiatric populations (Benedetti et al., 2014; Stevelink et al., 2018; Poletti et al., 2019; Meinert et al., 2019). The uncinate fasciculus (UF) is part of the connections between the amygdala and the prefrontal cortex within the limbic system, thereby involved in emotion processing and memory functions (Catani & De Schotten, 2008). It is also part of the temporo-amygdalo orbitofrontal circuit, extending from the anterior component of the temporal lobe to the hippocampal gyrus, to the amygdala, up to the polar and frontal cortex. (McCarthy-Jones et al., 2018). Reduced structural integrity of the UF, as indicated by lower FA, may result in a diminished prefrontal inhibition of responses to negatively valenced stimuli (Hanson et al., 2015) and can be caused by previous traumatic experiences (Koch et al., 2017). Our findings are consistent with earlier work reporting decreased FA values of the UF in children experiencing childhood trauma such as environmental deprivation and socio-economic problems (Eluvathingal et al., 2006). However, our study investigated trauma retrospectively and focused on different types of trauma, such as emotional and physical ones as assessed by the CTQ, a sensitive and valid screening questionnaire for childhood trauma also for psychiatric samples (Bernstein et al., 1997). The superior longitudinal fasciculus (SLF) is a huge association fibre tract connecting

cortical areas of the frontal, parietal, temporal and occipital lobes, involved in a wide range of functions including executive functioning and emotional regulation, in addition to language processing thanks to its connections to Broca and Wernicke areas (Mori et al., 2017; Forkel et al., 2020). The SLF is also associated with sustained attention performance independently of age (Klarborg et al., 2013). WM integrity in the SLF has been consistently reported to be reduced in BD (Mamah et al, 2019; Benedetti et al., 2014) with lower FA in the frontal-parietal WM cluster of the SLF (Chaddock et al., 2009; Frazier et al., 2007; Zanetti et al., 2009), further suggesting its role in the pathophysiology of the disorder. Other WM tracts whose integrity was associated with ACE in our study include the anterior thalamic radiation, the external capsule and the splenium of the corpus callosum, implying widespread white matter alterations. The corpus callosum (CC) could be a valid index of interhemispheric connectivity thanks to its large fiber tract connecting the left and the right hemispheres (Bellani et al., 2009), whereas the anterior thalamic radiation (ATR) is associated with executive control-related functions such as cognitive control, executive attention and working memory, supporting multiple forms of information processing (Kelly et al., 2017). Association between childhood trauma exposure and aberrant structural connectivity both in the CC and the ATR have been previously reported (Teicher et al., 2004; Bremner, 2002; Cyprien et al., 2011, Lim et al., 2020; Tendolkar et al., 2018). Our findings are consistent with previous observations that have identified the CC as most vulnerable to the effects of stress, all reporting decreased FA values after childhood trauma exposure (Paul et al., 2008; Rinne-Albers et al., 2016; Daniels et al., 2013; Lim et al., 2020) and to a previous report in a healthy sample of young adults showing significant reductions in FA values among these structures as a consequence of the exposure to early life stressors (Lu et al., 2013). Moreover, CC damage was proposed to be differently involved in the pathophysiology of several mental disorders, including BD (Piras et al., 2021). As a matter of fact, reduced FA in the CC has emerged as the most robust finding in several meta-analyses in individuals with BD (Yang et al., 2018; Dong et al., 2017).

Furthermore, lower FA in CC has been related to suicidal ideation in BD patients (Zhang et al., 2019) and both to the euthymic (Macritchie & Lloyd, 2010) and manic state of the disorder (Matsuoka et al., 2016; Wang et al., 2008).

Regarding ATR, this bundle also receives information from the hippocampus and projects to the anterior cingulate gyrus and into the limbic system. Previous DTI studies have demonstrated that a reduced integrity in the ATR is linked with ACE exposure, childhood trauma (Lim et al., 2020; Tendolkar et al., 2018) and also with PTSD diagnosis (Yoshii, 2021). It also appears to be compromised in BD: significantly lower average FA values of ATR in BD compared with healthy controls have been reported (Lin et al., 2011; Niida et al., 2018). The external capsule and the inferior fronto-occipital fasciculus (IFO) are association fiber connecting different cortical areas. The external capsule is a route for cholinergic fibers from the basal forebrain to the cerebral cortex and this complex is the neuroanatomical backbone of perceptual and motor functions and other higher cognitive functions (Schmahmann et al., 2008). The IFO is a direct pathway between the posterior temporal, occipital and orbitofrontal areas, also linked to emotion appraisal and visual perception (Ashtari, 2012). It has been seen in previous studies how this bundle is linked to exposure to physical neglect in healthy young males (Tendolkar et al., 2017) and is associated to a range of neurocognitive functions, including attention, language, visual processing, and emotional empathy (Catani & Thiebaut de Schotten, 2008; Duffau, Herbet, & Moritz-Gasser, 2013; Parkinson & Wheatley, 2014). Finally, the corticospinal tract (CST) plays a major role in cortical control of spinal cord activity, in particular, is the principal motor pathway for voluntary movements (Welniarz et al., 2017). FA reduction in CST has been associated with PTSD and depressive disorder (Guo et al., 2012), while in BD decreased white matter integrity of this tract has been linked with psychotic symptoms (Ji et al., 2017). Therefore, a reduction of FA in WM tracts underlying emotion regulation, cognitive functions and mood could partially explain the clinical

characteristics of the BD. These results seems to suggest that ACE could play an important role in the onset and maintenance of depressive and cognitive symptoms of BD and MDD.

6.2 Inflammatory markers and WM integrity

Concerning the relationship between inflammatory markers and WM integrity, we found that IL-2 negatively associated with AD, whereas CCL3 negatively associated with FA. In agreement with previous literature linking IL-2 activity with white matter integrity (Bettcher et al., 2014). Higher levels of IL-2 correlated with lower AD in three main clusters with peak in the left SLF, right IFO and CST tracts, and encompassing also the corona radiata, UF, internal and external capsule, and anterior thalamic radiation. AD indicate the magnitude of the diffusion parallel to fiber tracts; a decrease in AD may therefore suggests axonal injury (Song et al., 2002), reduced axonal caliber, or less coherent axons orientation.

IL-2 is known to be a pro-inflammatory cytokine, eliciting the acute phase response of inflammation; at the same time however, it can display regenerative effects by stimulating remyelination and homeostasis; these different kinds of activity are related to two distinct types of signalling. Classical IL-2 signalling mediates regulatory effects, while IL-2 transsignalling is responsible for pro-inflammatory effects (Maes et Carvalho, 2018). Previous researches indicate that even moderate systemic inflammation may alter WM development (Favrais et al., 2011) and, specifically, IL-2 activity was already been linked to WM integrity (Bettcher et al., 2014). Accordingly, animal models showed a link between WM damage in inflammatory diseases of the brain and levels of cytokines (Martino et al., 2000).

The mediation analysis showed that CCL3 mediates the effect of childhood trauma on FA in the superior longitudinal fasciculus. CCL3 is a chemokine involved in the acute inflammatory state in the recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4 and CCR5 (Schaller et al., 2017).CCL3, also known as

macrophage inflammatory protein 1-alpha (MIP-1-alpha), is considered a neutrophil chemoattractant (Ghoryani et al., 2019). CCL3 has been shown to be responsible for the induction of astrocyte activation and/or sustenance of microgliosis contributing to WM lesions in multiple sclerosis (Buschmann et al., 2012). Further, animal studies suggest that chemokines and, in particular, CCL3 may play a role in the regulation of oligodendroglial cells (Nguyen et al., 2003).

The mediation analysis also suggests that CCL3 may be responsible for the negative effects exerted by early stress on WM microstructure.

Generally, our findings are in line with previous researches which linked a chronic inflammatory condition and ACE exposure to mood disorders onset and maintenance (cita articlo Benedetti Aggio Neuroinflammation). Despite that, this is the first study to differentiate the effect of ACE and inflammatory markers in two different mood disorders: MDD and BD. The difference here reported between the two diagnosis suggest the need a deeper investigation into the differences between immune profiles of the disorder, in order to improve early diagnosis and better tailored treatment.

6.3 Limitations of the study

Future work employing longitudinal design could be able to mitigate the shortcomings associated to using retrospective reports to measure childhood adversity. Secondly, the lack of a healthy control group limits generalizability of the results for the general population. Third, issues such as generalizability, possible undetected past comorbidities should be considered. Moreover, the use and reporting of medications varied between patients and it was very difficult to rule out the potentially negative effect. Likewise, drug treatments administered during the course of the illness could have also influenced DTI measures. Recruitment was in a single center and in a single ethnic group, thus raising the possibility of

limiting the generalizability of the current findings. In this study the total CTQ score was used. While the specific contribution of any subtype of maltreatment could not be replicated, previous studies indicated that different types of maltreatment lead to different results in brain structure. Hence, the different subtypes should be focused on and disentangled in future studies using DTI. Further, a retrospective self-report questionnaire to estimate childhood maltreatment was used, which might have been influenced by a negative recall bias in BD patients, even though recall of childhood experiences was shown to provide reliable information in previous studies.

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