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Early-life stress and inflammatory cytokines in psychoses: from bench to community-based research

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Master's Dissertation presented to the Graduate Program in Medicine (Neurology) at Ribeirão Preto Medical School – University of São Paulo, Brazil to obtain the Master of Science Degree.

Concentration area: Neuroscience
Supervisor: Profa. Dra. Cristina Marta Del-Ben

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I dedicate this work to my beloved family, for their unconditional love and support.

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"Just because it's "all in your head" doesn't make it any less real;

(...)

What matters is precisely this; the unspoken at the edge of the spoken"

(VIRGINIA WOOLF)

ABSTRACT

CORSI ZUELLI, FMG. **Early-life stress and inflammatory cytokines in psychoses: from bench to community-based research.** 2018. 187 f. Dissertation (Master of Science in Neuroscience) – Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 2018.

Epidemiological studies suggest an interaction between biological and environmental factors in the development of psychoses and early-life stress constitutes an important risk factor. Recently, significant attention has been given to the inflammatory hypothesis of psychoses; yet, investigations on the relationship between early-life stress, inflammation and psychoses are scarce. We aimed to investigate associations between early-life stress and inflammatory cytokines in a preclinical model of schizophrenia (post-weaning social isolation), as well as in a clinical study of first-episode psychosis (FEP) patients, unaffected siblings and community-based controls. i) Preclinical study: male *Wistar* rats (n=20) were submitted to post-weaning social isolation for 10 weeks. After that, rats were assessed for locomotion in the open field (20 minutes) and euthanised for cytokines measurement. Cytokines protein and gene expression (IL-6, TNF-α, IL-10) were measured in the peripheral blood as well as in the prefrontal cortex and hippocampus. Social isolated rats had decreased IL-10 protein and gene expression in the blood and decreased IL-10 protein in the hippocampus. We also observed reduced IL-6 protein and gene expression in the prefrontal cortex. IL-10 hippocampal levels were negatively correlated with hyperlocomotion in the open field. Although the unexpected decrease in IL-6 and unchanged TNF-α levels contrast to the expected pro-inflammatory phenotype, this may suggest that reduced anti-inflammatory signalling may be critical for eliciting abnormal behaviour in adulthood. Altogether, these results suggest that prolonged early-life adverse events reduce the anti-inflammatory cytokine IL-10 and this is translated from blood-to-brain. ii) Clinical study: we recruited 114 first-episode psychosis (FEP) patients, 57 unaffected siblings of FEP patients, and 251 community-based controls. Cytokines plasma levels (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) were measured and differences across the three groups analysed after controlling for age, gender, body mass index and tobacco smoking. Childhood maltreatment was measured by the Childhood Trauma Questionnaire and plasma cytokines by multiplex. FEP had significantly higher levels of IL-6, TNF-α, IL-10 and TGF-β when compared with controls, and also higher levels of IL-1β, IL-6, TNF-α, and IL-10 when

compared with their siblings. Siblings presented decreased IL-1β when compared with controls. Physical childhood abuse was associated with increased levels of TGF-β in FEP. Experience of childhood maltreatment, specifically physical abuse, may contribute as a long-term immune priming for the TGF-β pathway in both patients and community-based controls. Normal or reduced levels of cytokines in siblings represent possibly a protective factor. In conclusion, the results from our preclinical and clinical study do not support associations between enhanced pro-inflammatory cytokines and early-life stress in psychoses. The blunted inflammatory profile found in chronic pwSI may represent stress-exposure during the latter stages of the disorder, contrasting the high inflammatory profile during earlier stages. The type and duration of adverse experiences may impact differently on the levels of inflammatory markers across different populations. Moreover, it is highly possible that the inflammatory profile reported in our clinical population arise from cumulative risk factors that will need to be explored in future investigations.

Keywords: Early-life stress. First-episode psychosis. Post-weaning social isolation. Inflammation. Cytokines.

RESUMO

CORSI ZUELLI, FMG. **Estresse precoce e inflamação nas psicoses: da bancada à pesquisa de base populacional**. 2018. 187 f. Dissertação (Mestrado em Neurociências) – Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2018.

Estudos epidemiológicos sugerem interações entre fatores biológicos e ambientais no desenvolvimento dos transtornos psicóticos, e o estresse precoce constitui-se um fator de risco importante. Atualmente tem-se dado grande atenção à hipótese inflamatória nas psicoses. Há uma escassez, todavia, de investigações que se concentrem na relação da tríade: estresse precoce, perfil inflamatório e psicoses. O objetivo do nosso estudo foi avaliar associações entre estresse precoce e citocinas inflamatórias em um estudo pré-clínico de esquizofrenia e em um estudo clínico envolvendo pacientes em primeiro episódio psicótico (PEP), seus irmãos e controles de base populacional. i) Estudo pré-clínico: Ratos *Wistar* machos (n=20) foram submetidos ao isolamento social a partir do desmame por 10 semanas. Após, os animais foram testados no campo aberto para avaliação da atividade locomotora (20 minutos) e eutanasiados para a mensuração das citocinas. A expressão proteica e gênica das citocinas (IL-6, TNF-α, IL-10) foi mensurada no sangue periférico, no córtex pré-frontal e hipocampo. Ratos isolados apresentaram redução da expressão proteica e gênica de IL-10 no sangue, bem como redução proteica desta citocina no hipocampo. No córtex pré-frontal, houve redução da expressão proteica e gênica de IL-6. Correlação negativa foi observada entre os níveis de IL-10 no hipocampo com a atividade locomotora dos animais isolados. Embora reduções de IL-6 e ausência de alterações de TNF-α contrastem o perfil pró-inflamatório esperado, nossos resultados sugerem que a redução de IL-10 pode ser um fator crítico para a ocorrência de alterações comportamentais na vida adulta. ii) Estudo clínico: Foram recrutados 114 pacientes, 57 irmãos e 251 controles de base populacional residentes na região de Ribeirão Preto. As citcocinas (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) foram mensuradas no plasma e todos os participantes responderam ao *Childhood Trauma Questionnaire* para investigar a ocorrência de traumas na infância. Todas as análises foram controladas para variáveis de confusão (idade, sexo, índice de massa corpórea e uso de tabaco). Pacientes apresentaram elevados níveis de IL-6, TNF-α, IL-10 e TGF-β quando comparados aos controles, e altos níveis de IL-1β, IL-6, TNF-α, e IL-10 quando comparados com seus irmãos. Os irmãos apresentaram

níveis reduzidos de IL-1β quando comparados com os controles. O abuso físico foi associado com altos níveis de TGF-β nos pacientes. Experiências traumáticas na infância, em especial o abuso físico, parece modular os níveis de TGF-β entre os diferentes diagnósticos. A ausência de alterações ou redução de citocinas nos irmãos sugere um efeito protetor. Em conclusão, os resultados dos nossos estudos pré-clínico e clínico não suportam associações entre citocinas pró-inflamatórias e estresse precoce no contexto das psicoses. O perfil inflamatório observado no modelo pré-clínico parece representar fases tardias da psicose, em contraste ao perfil inflamatório observado durante os estágios iniciais do transtorno. O tipo e a duração da exposição aos eventos adversos parecem modular de maneira distinta as concentrações de citocinas entre as diferentes populações estudadas. No entanto, é provável que o perfil inflamatório observado em nossa população clínica advenha de fatores de risco cumulativos que precisam ser explorados em futuras investigações.

Palavras-chave: Estresse precoce. Primeiro episódio psicótico. Isolamento social a partir do desmame. Inflamação. Citocinas.

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LIST OF ABBREVIATIONS

*A*ESOP: Aetiology and Ethnicity in Schizophrenia and Other Psychoses study

BD: Bipolar disorder

BDNF: Brain-derived neurotrophic factor

BLs: Type B lymphocytes

BMI: Body mass index

CNS: Central nervous system

CSF: Cerebrospinal fluid

CTLs: Cytolytic or cytotoxic CD8+ T lymphocytes

CTQ: Childhood Trauma Questionnaire

DA: Dopamine

DAMPS: Damage-associated molecular patterns

DALYs: Disability Adjusted Life Years

DNA: Deoxyribonucleic acid

DRS: Regional Department of Health

DSM: Diagnostic and Statistical Manual of Mental Disorders

D2-R: Dopamine receptor type 2

ELS: Early-life stress

ERK: Extracellular signalling regulated kinase

EU-GEI: European Network of National Schizophrenia Networks Studying Gene-Environment Interactions

FEP: First-episode psychosis

GABA: Gamma-AminoButyric Acid

GR: Glucocorticoid receptor

GxE interactions: Gene x Environment interactions

GWAS: Genome-wide association studies

HIPPO: Hippocampus

HPA: hypothalamic-pituitary-adrenal axis

IDO: Indolamine 2,3 dioxygenase

IFN: Interferon

IL: Interleukins

IL1-RA: IL-1 receptor antagonist

JNK: c-Jun protein kinase N-terminal

KYNA: Kynurenic acid

MAPKS: Mitogen-activated protein kinase family

MDD: Major depressive disorder

MHC: Major histocompatibility complex

MIA: Maternal immune activation

M0: Quiescent macrophages

M1: Macrophages producing pro-inflammatory cytokines

M2: Macrophages producing anti-inflammatory cytokines

NF-kB: nuclear factor kappa-B

NLRP3: NACHT, LRR and PYD domains-containing protein 3

NMDA: N-methyl-D-Aspartate

PAMPS: Pathogen-associated molecular patterns

PET: Positron emission tomography

PFC: Prefrontal cortex

pwSI: post-weaning social isolation

p38: protein kinase 38

QA: Quinolinic acid

sIL-2R: Soluble IL-2 receptor

TGF: Transforming growth factor

Th: T-helper lymphocytes

Th1: T-helper lymphocytes producing pro-inflammatory cytokines

Th2: T-helper lymphocytes producing anti-inflammatory cytokines

TLs: T lymphocytes

TNF: tumour necrosis factor

Treg: T regulatory lymphocytes

TSPO: 18kDa translocating protein

VTA: Ventral tegmental area

WHO: World Health Organization

YLDs: Years Lived with Disability

YLLs: Years Lost to Premature Mortality

5-HT_{2A}: Serotonin 2A receptor

3-HK: 3-hydroxyquinurenine

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1. Introduction

1. INTRODUCTION

1.1 PSYCHOSES: EPIDEMIOLOGICAL DATA AND CLINICAL FEATURES

Mental disorders are highly prevalent worldwide, reach the top ten public health problems and are responsible for significant socioeconomic burden (KESSLER et al., 2009; LINDEN, 2011; VOS et al., 2015; WHITEFORD et al., 2015).

According to the "Global Burden of Disease Study 2010" (WHITEFORD et al., 2015), mental, neurological and substance use disorders were together responsible for 10.4% of the *Disability Adjusted Life Years* (DALYs concept), 2.3% of the *Years Lost to Premature Mortality* (YLLs concept), and 28.5% of the *Years Lived with Disability* (YLDs concept) in the year 2010. When grouped, these disorders were considered the third leaders in the rank of incapacities, preceded only by cardiovascular disorders and common infectious diseases. In the DALYs concept, from the three disorders aforementioned, mental disorders were the ones that contributed the most (56.7%), demonstrating, therefore, their prominent role in public health problems (WHITEFORD et al., 2015).

Supporting the previous statement, data extracted from the "Global Burden of Disease Study 2013", confirm the leading position of mental disorders in the disability rates (YLDs), emphasising a sharp increase of 45% in these proportions from 1990 to 2013. It is also noteworthy that the great impact attributed to disabilities in the context of mental disorders occurs at the beginning of adult life, a critical time for socioeconomic contributions (VOS et al., 2015). Despite the importance of mental disorders, the development of actions to prevent their occurrence are still largely neglected, especially in undeveloped or emerging countries (VOS et al., 2015; WHITEFORD et al., 2015).

Two international classification systems are currently used for clinical diagnostic and research purposes on mental disorders – the 10th edition of the International Classification of Diseases of the World Health Organization (ICD-10, WHO, 1993) and the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM) of The American Psychiatric Association (APA, 2013). Even with the most recent DSM-V publication, most research in psychiatry was conducted using the diagnostic criteria of previous versions of the DSM, specially the 4th version – DSM-IV (APA, 1994) and the 4th version revised – DSM-IVR (APA, 2000).

Among the existing mental disorders are those of the diagnostic criteria designated as psychoses. The disorders included in the diagnostic criteria of psychoses are defined by abnormalities, according to the DSM-V, in one or more of the following five domains of the psychopathology: (i) delusions (fixed beliefs not amenable to change in light of conflicting evidence), including a variety of themes (persecutory, referential, somatic, religious, grandiose); (ii) hallucinations (involve the sensory system in the absence of an external stimulus); (iii) disorganised thinking/speech (loose and incoherence of associations, tangentiality); (iv) grossly disorganised or catatonic motor behaviour (difficulties in performing daily-life activities; catatonic behaviour is usually associated to decrease in reactivity to the environment, ranging from rigidity to a complete lack or excessive motor response; v) negative symptoms. The first four domains are examples of positive psychotic symptoms – an excess or distortion of normal functions – whereas the negative symptoms are characterised by reduction or absence of normal functions, such as diminished emotional expression, avolition, alogia, anhedonia, and lack of sociability. In addition to the symptomatology, the DSM also considers factors such as duration of symptoms, possible functional impairment caused by the disorder, as well as disorders and/or conditions that should be excluded (APA, 2013).

Although psychotic symptoms are an essential feature of psychoses, this symptomatology is also present in mood disorders. In this context, psychoses can be grouped into two main clusters: non-affective psychoses and affective psychoses. Non-affective psychoses include: schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, and brief psychotic disorder. Affective psychoses include: bipolar disorder (BD) with psychotic features and major depressive disorder (MDD) with psychotic features (VAN OS; KAPUR, 2009).

Psychoses are considered serious public health problems, contributing significantly to high rates of morbidity and mortality. From the non-affective psychoses described before, schizophrenia should be highlighted, as this is possibly the most debilitating disorder among the existing psychotic disorders (VOS et al., 2015; WHITEFORD et al., 2015).

Data from the World Health Organization (WHO) inform that schizophrenia affects more than 21 million people worldwide, and typically begins at the end of adolescence or early adulthood (WHO, 2016). Disability rates associated with schizophrenia increased by 52.1% from 1990 to 2013, as demonstrated by a recent epidemiological study involving 188 countries, including Brazil (VOS et al., 2015). Besides that, it is estimated that the mean incidence rate of this psychotic disorder varies according to the geographic location. About 90% of people with

untreated schizophrenia live in undeveloped or emerging countries. Among all mental disorders, schizophrenia occupies the third place in terms of incapacitation, but it is the main cause of disability among the existing psychoses (VOS et al., 2015). Regarding mortality rates, the presence of schizophrenia increases the risk of death by two to three times, and this rate has increased considerably in recent decades and may be related to factors such as cardiovascular, metabolic and infectious diseases (MCGRATH et al., 2008; WHO, 2016).

Schizophrenia is more common among males (12 million) than females (9 million), and it usually starts earlier among men (WHO, 2016). More specifically related to the first-episode psychosis (FEP), the mean age of onset is usually between the beginning and the middle of 20 years for the males, and the end of 20 years for females. The early onset is usually associated as a predictor of poor prognosis. FEP can be abrupt or insidious, but most individuals present a slower and more gradual manifestation (APA, 2013). Although the highest rates of disability in most mental disorders are related to women, in disorders such as psychoses men are those who stand out (WHITEFORD et al., 2015).

In relation to mood disorders, these are described with high prevalence, both in the community and institutional sectors (VIGO; THORNICROFT; ATUN, 2015). It is estimated that MDD and BD affect approximately 350 million and 60 million people worldwide, respectively (WHO, 2016). Different from schizophrenia, higher rates of depression are described for women, and similar rates for both sexes are described for BD (VOS et al., 2015).

MDD occupies a prominent position as a public health problem since the 1990s, ranking as the second leader among the 20 most important health problems in the world and the first among the existing mental disorders (VIGO; THORNICROFT; ATUN, 2015; VOS et al., 2015; WHITEFORD et al., 2015). Recent data show that among all mental disorders, MDD is the leader in the YLDs concept (53.4%), schizophrenia the second (52.1%) and BD the third (49.2%) (WHITEFORD et al., 2015). When unidentified and uncontrolled, psychoses, whether affective or non-affective, can lead not only to major disabilities but also to suicide (WHO, 2016).

In Brazil, the first FEP study conducted in the city of São Paulo over a 3-year period revealed a psychosis incidence rate of 15.8/100,000 person-years at risk (95% CI: 14.3 – 17.6). Approximately 40% of the cases met criteria for schizophrenia or schizophreniform disorder, whereas for other non-affective psychoses 24% of the cases fulfilled the criteria. In relation to affective psychoses, approximately 23% met criteria for BD with psychotic features, and around 13% of the cases met criteria for MDD with psychotic features. For non-affective psychoses,

higher rates were attributed to young men, with a sharp decline in the FEP rate in more advanced ages (MENEZES et al., 2007).

The incidence rate for affective and non-affective psychoses discussed in the previous investigation are in line with those found in other studies, such as those conducted in Nottingham and Bristol (in the United Kingdom, U.K.), but differs drastically for those rates found in southeast London, according to the study *A*ESOP (*The Aetiology and Ethnicity in Schizophrenia and Other Psychoses* study) (KIRKBRIDE et al., 2006). Interestingly, higher rates among men, especially in non-affective psychoses, and similar rates for both sexes in affective psychoses, found in the São Paulo study, were also highlighted in other studies around the world, such as those conducted in the U.K. (KIRKBRIDE et al., 2006), Ireland (SCULLY et al., 2001), Australia (WELHAM; THOMIS; MCGRATH, 2004) and in a systematic review investigating the incidence of schizophrenia around 33 countries (MCGRATH et al., 2004). Regarding the mean age for the first contact with a mental health service, men usually display lower rates than women (before 35 years for men), both for affective and non-affective psychoses, confirming a higher frequency of psychoses in young adults both in Brazil and developed countries (KIRKBRIDE et al., 2006; MCGRATH et al., 2004; MENEZES; SCAZUFCA, 2007; SCULLY et al., 2001; WELHAM; THOMIS; MCGRATH, 2004).

The most recent FEP incidence study was carried out by the European Network of National Schizophrenia Networks Studying Gene-Environment Interactions (EU-GEI) involving Brazil and five European countries (England, France, the Netherlands, Spain and Italy) during the years 2010 – 2015. In Brazil, more specifically, having the coordinators of the present research project as members, the survey was carried out between 2012 and 2015 and covered the 26 municipalities of the Regional Department of Health XIII (XIII Departamento Regional de Saúde de Ribeirão Preto – DRS XIII), of which Ribeirão Preto is headquartered. In this multicentre study 2,774 cases (1,578 men and 1,196 women) were identified, indicating an incidence rate of 21.4% – the highest proportion attributed to non-affective psychoses (78.7%). In Ribeirão Preto catchment area, 565 cases (68.9% non-affective psychoses) were identified, indicating an incidence rate of 21.5% for all types of psychoses. Similar to other studies, a higher incidence rate was reported in young men aged 18 to 24 years; it also revealed higher incidence rates in ethnic minorities and in areas with a lower percentage of houses occupied by their owners (JONGSMA et al., 2017).

The aforementioned study emphasised the existence of an important variation in the incidence rate among the studied regions, pointing out that the determinant factors for such

variation are not only genetic; differently, the participation of a variety of environmental factors is highlighted, given rise to the need to explore such environmental influences in future investigations.

1.2 CONSIDERATIONS ABOUT THE ETIOPATHOGENESIS OF PSYCHOSES

1.2.1 Dopaminergic and glutamatergic hypotheses

Currently, the aetiology of mental disorders (including psychoses), is considered multifactorial, with individual, psychosocial, biological and environmental factors interacting in a complex way to generate vulnerabilities in their development (VAN OS; KENIS; RUTTEN, 2010).

Among the proposed hypotheses on the neurobiology of psychoses, and more specifically schizophrenia, important focus has been given to the role of neurotransmitters. Two hypotheses of great influence involve the dopaminergic and glutamatergic neurotransmission (HOWES; MCCUTCHEON; STONE, 2015). Overall, it is highlighted increased dopamine (DA) neurotransmission in the mesolimbic pathway and a reduction of this same neurotransmitter in the mesocortical pathway. Importantly, this imbalance is usually associated to hypofunction of the N-methyl-D-Aspartate (NMDA) glutamate receptors in these pathways (BRISCH, 2014; HOWES; MCCUTCHEON; STONE, 2015; RUBEŠA; GUDELJ; KUBINSKA, 2011).

The so-called mesolimbic pathway refers to dopaminergic projections that depart from the ventral tegmental area (VTA) and are directed to the ventral striatum (especially the nucleus accumbens) and related limbic areas (cingulate cortex, amygdala, hippocampus, parahippocampal girus, and the medial-orbital portion of the prefrontal cortex). In the mesocortical pathway, dopaminergic projections from the VTA are directed to the prefrontal cortex (PFC) (ELERT, 2014; IVERSEN; IVERSEN, 2007).

Increased DA neurotransmission in the mesolimbic pathway would be responsible for the positive symptoms, whereas reduction of DA neurotransmission in the mesocortical pathway would be responsible for the negative symptoms. Glutamatergic projections to the mesolimbic pathway have an inhibitory effect on DA release in this pathway via the participation of inhibitory Gamma-AminoButyric Acid interneurons (GABAergic interneurons). Thus, the action of glutamate on its NMDA receptors located in GABAergic interneurons leads to increased release of the inhibitory neurotransmitter GABA, which in turn

inhibits DA release in the mesolimbic pathway. In this sense, hypofunction of the NMDA receptors residing in GABAergic interneurons leads to an increased concentration of DA in the mesolimbic pathway. In the mesocortical pathway, however, communications between glutamatergic and dopaminergic neurons are free from GABA interneurons. In this case, glutamate increases DA release in the mesocortical pathway. As a result, hypofunction of NMDA receptors in the mesocortical pathway leads to reduction of the DA concentration in this pathway (LARUELLE, 2014).

The implication of DA in the neurobiology of schizophrenia is supported by pharmacological therapies involving conventional or typical antipsychotics (DA receptor type 2 antagonists, D2-R), in which an improvement in positive symptoms is observed. However, typical antipsychotics are known to generate significant side effects, such as exacerbation of negative symptoms and extrapyramidal symptoms, due to a sharp decreased of DA in the mesocortical and nigrostriatal pathways, respectively (ELERT, 2014; GOBIRA et al., 2013). Atypical (or second generation) antipsychotics have been developed to ameliorate the side effects observed with the use of typical antipsychotics, especially extrapyramidal effects and the negative symptoms. These drugs present antipsychotic action with lower extrapyramidal effects because they are partial antagonists of the D2-R; besides that, they slightly benefit the negative symptoms through antagonistic action in 5-HT_{2A} receptors (COHEN et al., 2015; ELERT, 2014; GOBIRA et al., 2013; HOWES; MCCUTCHEON; STONE, 2015; LARUELLE, 2014).

Although second generation antipsychotics attenuate the side effects related to the mesocortical and nigrostriatal pathways, atypical antipsychotics are diversely implicated in metabolic disorders with high risk of cardiovascular morbidity and mortality (GARDNER-SOOD et al., 2015). In this sense, the search for new approaches to the understanding and treatment of schizophrenia is necessary.

1.2.2 Gene x Environment (GxE) interaction and the multiple hits hypothesis

Despite the significant contribution of the classical hypotheses on the understanding of psychoses aetiology, as well as in the progress of therapeutic drugs, the consideration of multifactorial factors in the development of these disorders is poorly addressed in such hypotheses.

Taking into account the multifactorial nature of psychoses, the development of gene-environment interactions (GxE) studies has been an important strategy. The GxE approach differs from considerations that postulate a direct causal role of genes or environmental factors (as isolated factors) in the development of the disorder; on the contrary, the effect would be conditioned by the co-participation of both, so that the exposure to one or another isolated factor would not lead to the outcome in question, but the joint exposure would in fact (HOWES; MURRAY, 2014; KARL; ARNOLD, 2014; OWEN; SAWA; MORTENSEN, 2016; VAN OS; KENIS; RUTTEN, 2010).

In this sense, the multiple hits hypothesis has been proposed, given the consideration of genetic factors and their interaction with multiple environmental risk factors (*hits*), which occurrence during critical periods of the neurodevelopment would generate vulnerabilities for the development of the disorder (DAVIS et al., 2016).

The multiple hits hypothesis is supported by studies demonstrating that adopted and genetically high risk individuals are significantly more sensitive to adverse events present in the adoptive family context than those also adopted, but genetically considered as low risk individuals (TIENARI et al., 2004). In addition, the concordance rate for the development of schizophrenia in dizygotic twins is 15%, while in monozygotic twins the risk is around 50% (GOTTESMAN, 1991), which reinforces the presence of environmental components in the development of this disorder. In support to that, genome-wide association studies (GWAS) have until now failed to identify major candidate genes that could have direct associations with schizophrenia (SANDERS et al., 2008). Therefore, it is believed that genes can influence the development of mental disorders in an indirect way. As an illustration, several studies grouped in a review suggest a synergistic effect among several genes and risk factors for the development of psychoses, including migration, urbanity, obstetric complications, cannabis use, stress, childhood trauma, infections, among others. In most studies, genetic and environmental factors had little significance when analysed alone, being the most part mediated by GxE interaction (VAN OS; KENIS; RUTTEN, 2010).

Among the biological risk factors, great attention has recently been paid to the role of the immune system and inflammation in the development of mental disorders, including psychoses (MEYER; SCHWARZ; MÜLLER, 2011a; MILLER; RAISON, 2016; MÜLLER et al., 2015). GWAS investigations addressed to patients with schizophrenia strongly support this hypothesis, based on evidence of genetic factors related to polymorphisms in the major histocompatibility complex (MHC) located on chromosome 6, a region that contains more than

140 genes related to the immune function (RIPKE et al., 2014). Not only that, polymorphisms are also observed for several inflammatory cytokines (MEYER; SCHWARZ; MÜLLER, 2011).

In view of these great findings, several hypotheses relating the immune system and psychoses were postulated and will be discussed below.

1.3 IMMUNE AND INFLAMMATORY RESPONSE IN PSYCHOSES

1.3.1 General properties of the immune system

Didactically, the immune system is subdivided into two major arms: the innate and the adaptive immune system. In general, the physiological function of the immune system is the defence against pathogens or tissue damage. (HIRAHARA; NAKAYAMA, 2016; STERNBERG, 2006; TURNER et al., 2014).

The innate immune system (also known as natural or native) is the first line of defence, composed mainly by physical and chemical barriers (epithelium), phagocytes (neutrophils, dendritic cells, monocytes/macrophages, natural killer cells), and by inflammatory mediators proteins (the complement system and cytokines). The innate immunity is nonspecific and consists of cellular and biochemical defence mechanisms programmed to respond rapidly, which already exist prior to an infection/tissue damage (WARRINGTON et al., 2011).

On the other hand, the adaptive (or acquired) immunity, different from the innate immunity, is highly specific and characterised by memory. The acquired immunity is composed by the humoral immunity, in which the main components are the antibodies produced by type B lymphocytes (BLs), and the cellular immunity, in which the main components are the cytokines produced by T lymphocytes (TLs). TLs consist of functionally distinct populations, among which deserves attention: CD4+ T-helper lymphocytes (CD4+ Th), responsible for mediating the immune response through the production of various cytokines; the cytolytic or cytotoxic CD8+ TL (cTLs), which destroy infected cells; and the regulatory TL (T_{reg}), which are responsible for the suppression of the immune response (HIRAHARA; NAKAYAMA, 2016; STERNBERG, 2006; TURNER et al., 2014). Together, the innate and adaptive immune system form an integrated system, communicating directly to promote the host defence.

1.3.2 General properties of the inflammatory response

The inflammatory response consists of four important components, described as inducers, sensors, mediators and effectors. Inducers are the signals that initiate the inflammatory response, which can be grouped in exogenous (microbial or non-microbial) or endogenous (stressed, damaged or malfunctioning tissues). Inducers activate specialised sensors (such as receptors), which in turn culminates in the production of numerous inflammatory mediators that alter the functional state of effectors (Illustration 1) (MEDZHITOV, 2008).

Illustration 1: Components of the inflammatory response

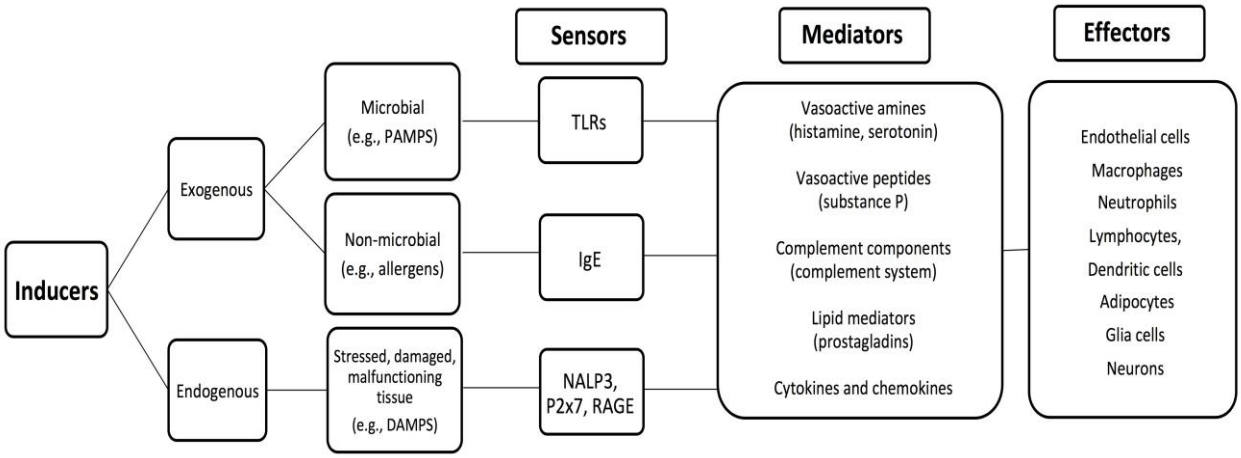


Illustration adapted from MEDZHITOV, 2008. We provide only well-known examples for each component, although a broader variety of elements exist. PAMPS: pathogen-associated molecular patterns; DAMPS: Damage-associated molecular patterns; TLRs: Toll-like receptors; Ig: immunoglobulin; NALP3: NACHT-, leucine-rich-repeat-and pyrin-domain-containing protein; RAGE: advanced glycation end-product-specific-receptor.

In fact, inflammation is a complex reaction characterised by vascular and cellular events and it is the first defence reaction in response to infection or tissue damage. Vascular events of inflammation include the production of vasoactive amines (histamine and serotonin) produced by degranulated mast cells/platelets, vasoactive peptides (substance P) released by sensory neurons, the complement system and prostaglandins, which all together contribute to vasodilation with increased blood flow, followed by extravasation and deposition of plasma and proteins in the interstitium. The cellular events are characterised by the recruitment, accumulation and activation of leukocytes, as well as activation of the endothelium. These

events are promoted by several mediators, including prostaglandins, leukotrienes, cytokines, and chemokines, among others. Fundamentally, inflammation is a defence mechanism, which the ultimate goal is to eliminate the initial cause. Without inflammation, infections would develop wildly and the tissue repair process would not occur. However, when exacerbated, inflammation is potentially harmful and causes tissue damage. Historically, inflammation is characterised by its cardinal signs, known as heat, redness, pain, swelling, and loss of function (ALLAN; ROTHWELL, 2001; DEVERMAN; PATTERSON, 2009; RIVEST, 2009; STERNBERG, 2006; STOLP, 2013).

Cytokines, the components of both innate and adaptive immunity, coordinate the immune system and the inflammatory response, and are important in establishing the communication between the two types of immunity. Cytokines are low molecular weight proteins produced by different cell types, which regulate the intensity and duration of the immune and inflammatory response, acting as important mediators in cellular communication, both at the peripheral and central levels, where they stimulate cell recruitment, growth, differentiation and activation (MEDZHITOV, 2008).

Classically, cytokine production occurs after stimulation of pattern recognition receptors, such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs), which can be activated by pathogen-associated molecular patterns (PAMPS) present in pathogens, or by damage-associated molecular patterns (DAMPS), respectively. Cytokines exert their functions by binding to specific receptors. The receptors to which the cytokines bind are called α- and β-type receptors – while the former are not involved in signal transduction, the latter are. Thus, the bind to their respective α-type receptors triggers the formation of a complex, which binds to the β-receptor, culminating in signal transduction. Signal transduction involves the activation of pathways that regulate gene expression, such as the activation pathway of the mitogen-activated protein kinase family (MAPKs), including the extracellular signalling regulated kinase (ERK), the c-Jun protein kinase N-terminal (JNK) and the protein kinase 38 (p38), which results in the activation of several transcription factors. Another pathway involves the activation of the nuclear factor kappa-B (NF-kB), which translocates to the nucleus and acts in the deoxyribonucleic acid (DNA) promoter regions. Activation of these pathways is involved in the regulation of various cellular activities, such as cell differentiation, programmed killing, and the regulation of the expression of several genes, including the production of cytokines (ALLAN; ROTHWELL, 2001; STERNBERG, 2006; TURNER et al., 2014).

Although there are numerous cytokines, functionally and didactically, cytokines have been classified into two large groups: pro-inflammatory or anti-inflammatory cytokines. Classic examples are the various interleukins (IL), the tumour necrosis factor (TNF), the interferon (IFN) and the transforming growth factor (TGF). Nonetheless, it is known that cytokines may influence the synthesis and actions of other cytokines, and during this complex interaction, the same cytokine can act as pro-inflammatory in one context, and as anti-inflammatory in another context, for instance IL-6. Thus, the innate immunity is able to determine the type of adaptive immune response through the action of the various existing cytokines (HIRAHARA; NAKAYAMA, 2016; STERNBERG, 2006; TURNER et al., 2014).

The balance between the pro- and anti-inflammatory cytokines is important for the establishment of homeostasis (O'SHEA; MA; LIPSKY, 2002). In response to various physical (severe disturbances triggering responses of highest magnitude; e.g., infection, tissue injury) or psychological stressors (social and environmental circumstances that challenge the adaptive capability and resources of an organism), innate immunity macrophages are polarized, resulting in a phenotypic and functional change from quiescent (M_0) to macrophages producing pro-inflammatory cytokines (M_1). Cytokines released by M_1 macrophages, especially IFN-gamma (IFN-γ) and IL-12, stimulate naïve CD4+ Th differentiation from the adaptive immunity to Th_1, also producers of pro-inflammatory cytokines. In this interaction, the major inflammatory agents released are IL-1β, IL-2, IL-12, TNF-alpha (TNF-α) and IFN-γ. Cytokines play an important role in cell growth and differentiation and are necessary for the elimination of pathogens and control of tissue damage. However, excessive production of inflammatory cytokines can lead to an exacerbated inflammatory process, which can culminate in tissue lesion and death (CALCIA et al., 2016; WALKER; NILSSON; JONES, 2013).

In contrast, M_2-type macrophages of the innate immunity are responsible for the production of anti-inflammatory cytokines, and through the production of IL-4 and IL-2 they stimulate the differentiation of naïve CD4+ Th of the acquired immunity into Th_2, which are also producers of anti-inflammatory cytokines (CALCIA et al., 2016; WALKER; NILSSON; JONES, 2013). In this case, the major cytokines released are IL-4, IL-5, IL-6, IL-10 and IL-13, which inhibit pro-inflammatory cytokine-related responses and are involved with neuroplasticity mechanisms.

In addition to the classics Th_1 and Th_2, other subpopulations of lymphocytes also exist, including Th_9, Th_{17}, Th_{22}, Th-follicular, and T-reg. It is worth highlighting the action of T-reg, essential for the regulation of the immune response. This subpopulation produce anti-

inflammatory and immunosuppressive cytokines, such as IL-4, IL-10 and TGF-beta (TGF-β) (HIRAHARA; NAKAYAMA, 2016; PATEL, 2013). Overall, the cellular response to most cytokines consists of alterations in the target cell gene expression.

Due to the broad variety of cytokines, a summary of the selected cytokines for the present study, including information about the main cellular source and major biological role, can be find in Chart 1.

1.3.3 Neuroimmune interactions

In addition to the important role of cytokines in the peripheral system, these mediators have also a prominent role in the central nervous system (CNS). Despite the traditional view of the CNS as an immunologically privileged site, currently it is known that cytokines also act in the CNS, where cytokine receptors have been described in neurons and glia cells (microglia, astrocytes and oligodendrocytes) (MEYER; SCHWARZ; MÜLLER, 2011b). In fact, cytokines mediate the communication between the peripheral immune system and the CNS (KHANDAKER; DANTZER, 2015). In the brain, cytokines participate in neurodevelopment and neurogenesis processes and their physiological functions include neural differentiation, proliferation and migration; synaptic maturation and plasticity; as well as the synthesis, release and reuptake of neurotransmitters (BORSINI et al., 2015; MEYER, 2011a).

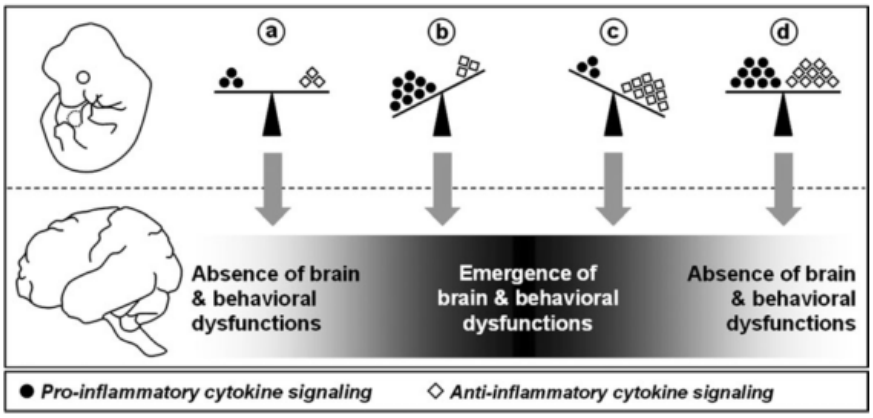
For the establishment of the CNS homeostasis, there must be a balance between pro- and anti-inflammatory cytokines (BORSINI et al., 2015; KHANDAKER; DANTZER, 2015; MEYER; SCHWARZ; MÜLLER, 2011a). It is postulated that the imbalance between pro- and anti-inflammatory cytokines during critical periods adversely affects neurodevelopment; consequently, the resulting abnormal behavioural would increase the risk for the development of mental disorders in adult life (Illustration 2). In fact, cytokine imbalance during critical periods of the neurodevelopment has been pointed out as an important mechanism for the pathophysiology of psychoses (KNUESEL et al., 2014; MEYER, 2011a, 2014; MEYER; FELDON; YEE, 2009; MEYER; SCHWARZ; MÜLLER, 2011b; SMITH et al., 2007).

Chart 1: Major cellular source and biological activities of selected cytokines

Cytokine	Main cellular source	Major biological role
IL-1β	Activated monocytes/macrophages and microglia; endothelial cells; dendritic cells, adipocytes.	Endogenous pyrogen (fever); stimulation of other pro-inflammatory cytokines; induction of acute-phase proteins; stimulation of the HPA axis; activation of T-, B- and endothelial cells; microglial and macrophage polarization towards pro-inflammatory state.
IL-6	Activated monocytes/macrophages; T cells (Th2 and Th17); hepatocytes; osteoclasts; fibroblasts; astrocytes, adipocytes.	Pleiotropic cytokine; i) pro-inflammatory actions: neutrophil/monocyte/lymphocyte recruitment; in combination with TGF-β promotes T-cell differentiation into Th17 and away from T-regulatory; endogenous pyrogen (fever); induction of acute-phase proteins; stimulation of the HPA axis; ii) anti-inflammatory actions: promotes wound healing and tissue regeneration; Th2 and T-regulatory polarization, increases sIL1-RA, sTNFR. It also induces late B-cell differentiation with stimulation of immunoglobulin-G production.
TNF-α	Activated monocytes/macrophages and microglia; T cells (Th1), natural killer cells; endothelial cells, adipocytes.	Endogenous pyrogen (fever), promotion of sepsis; cytotoxic effects (apoptosis); activation of monocytes, lymphocytes and endothelial cells; microglial and macrophage polarization towards pro-inflammatory state.
IFN-γ	Th1, natural killer cells	Anti-viral; macrophage activation; increases neutrophil and monocyte function; inhibits IL-4 production by Th2 cells; microglial and microphage polarization towards pro-inflammatory state.
IL-4	Th2 cells	Promotes Th2 polarization producing IL-4, IL-5, IL-6, IL-10, IL-13; inhibits IFN-γ production by Th1 cells; stimulates activation, proliferation and differentiation of B cells with synthesis of Immunoglobulin-E; microglial and microphage polarization towards anti-inflammatory state.
IL-10	Activated monocytes/macrophages; T cells (Th2, T-regulatory).	Inhibition of pro-inflammatory cytokines; macrophage suppression; inhibits TNF-α/IFN-γ production by Th1 cells; stimulates Th2 cells; control of sepsis; promotes humoral immune response involving antibody production; microglial and microphage polarization towards anti-inflammatory state.
TGF-β	T cells (T-regulatory).	Pleiotropic cytokine; Inhibition of pro-inflammatory cytokines; inhibition of natural killer cell activity and growth of T- and B-cells; microglial and microphage polarization towards anti-inflammatory state; promotes wound healing; in combination with IL-6 promotes Th17 polarization.

IL: interleukin; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; TGF-β: transforming growth factor-β; Th: T-helper cell; HPA: hypothalamic pituitary adrenal; sTNFR: soluble TNF receptor; IL-1RA: IL-1 receptor antagonist

Illustration 2: Cytokines and CNS homeostasis



Imbalance between pro- and anti-inflammatory cytokines during critical periods adversely affects neurodevelopment, contributing to the emergence of brain and behavioural dysfunctions (MEYER; FELDON; YEE, 2009).

Cytokine imbalance might be a consequence of physical or psychological stressors, since both participate in peripheral and central immune activation (CALCIA et al., 2016; WALKER; NILSSON; JONES, 2013). Nowadays, it is known about the existence of an intrinsic innate immune system in the CNS and several studies have demonstrated the existence of pathways that facilitate neuroimmune interaction, allowing the communication between peripheral and central cytokines. In the context of an immune disturbance, this mechanism would facilitate behavioural, cognitive and emotional changes that could increase the risk for mental disorders, including psychoses (DANTZER et al., 2008; KHANDAKER; DANTZER, 2015).

Five pathways that facilitate neuroimmune interactions have been identified so far (ASPELUND et al., 2015; DANTZER et al., 2000; DESBONNET et al., 2015; KHANDAKER; DANTZER, 2015; LOUVEAU et al., 2015; MAIER et al., 1998; MILLER; RAISON, 2016): a) the humoral pathway: peripheral cytokines diffuse into the CNS through circumventricular organs and structures lacking the blood-brain-barrier; b) the cellular pathway: involves the activation of the sympathetic nervous system, culminating in the production of catecholamines (such as noradrenaline), which stimulate the bone marrow, the release of leukocytes in the peripheral blood and the recruitment of immune cells from the periphery into the CNS through chemoattractant mediators (chemokines) (KHANDAKER; DANTZER, 2015; MILLER; RAISON, 2016); c) the microbiota-gut-brain-axis: the microbiota-gut can transmit signals to the brain via the vagus nerve thereby altering neurotransmission in the CNS (DESBONNET et

al., 2015); d) the recently discovered central lymphatic pathway or the glymphatic system: mediated by functional lymphatic vessels in the CNS. In this pathway, extracellular fluids (the cerebrospinal fluid and interstitial fluid) draining from the brain parenchyma to the cervical and lumbar lymph nodes facilitate the traffic of antigens and immune cells affecting peripheral and central inflammation (ASPELUND et al., 2015; LOUVEAU et al., 2015); e) the neural pathway: the afferent vagus nerve detects peripheral inflammatory cytokines and transmits signals to the nucleus tractus solitarius and hypothalamus (CORSI-ZUELLI et al., 2017).

Although the aforementioned pathways facilitate neuroimmune interactions, an abnormal production of inflammatory cytokines would contribute to changes in brain neurotransmission, as it will be discussed in details.

1.3.4 Cytokines and neurotransmitters

A classic example on how neuroimmune interactions can facilitate neurotransmission and behavioural dysfunction is based on observations that one-third of patients receiving interferon-α treatment for hepatitis C evolves with depressive symptoms (DANTZER et al., 2008). This observation stimulated the search for mechanisms that could explain the impact of cytokines on the synthesis of neurotransmitters.

One of the most studied pathways involving cytokines in the modulation of neurotransmitter synthesis is known as the kynurenine pathway. Physiologically, the kynurenine pathway involves the activation of the tryptophan dioxygenase enzyme located in the liver, which results in around 95% degradation of the tryptophan acquired from the diet, being only the smallest part of this precursor used for serotonin synthesis. On the other hand, the extra-hepatic pathway of tryptophan degradation involves the activation of the enzyme indolamine 2,3 dioxygenase (IDO), located in peripheral and central immune system cells (DANTZER et al., 2008; MEYER; SCHWARZ; MÜLLER, 2011).

The exacerbated production of pro-inflammatory cytokines, especially TNF-α and IFN-γ, elicits a high expression and activation of the IDO enzyme. This enzyme uses the serotonin precursor, tryptophan, to produce a metabolite known as kynurenine. Cytokines, therefore, divert the serotonin pathway to the kynurenine pathway, reducing the serotonin bioavailability in the CNS (CAMPBELL et al., 2014).

Kynurenine produced during inflammatory reactions is rapidly metabolised by glial cells to generate other active metabolites. For example, kynurenine can be degraded to generate the metabolites 3-hydroxyquinurenine (3-HK), quinolinic acid (QA) or kynurenic acid

(KYNA). The 3-HK metabolite results in the generation of free radicals, which are involved in the processes of oxidative stress and lipid peroxidation, whereas the metabolite QA is a neurotoxic compound acting as an NMDA receptor agonist. In this sense, 3-HK and QA metabolites would be involved with neurodegenerative and cytotoxic events, including those related to loss of grey and white matter, and therefore, with diverse cognitive deficits. In contrast, the metabolite KYNA acts as a potent endogenous NMDA and alpha (α)-7 nicotinic receptor antagonist, the latter involved in the cholinergic anti-inflammatory pathway and in several aspects of cognitive functioning (CAMPBELL et al., 2014). Together, these mechanisms are postulated to contribute to the occurrence of negative, positive, and cognitive deficits associated with psychoses (DANTZER et al., 2008; MEYER; SCHWARZ; MÜLLER, 2011).

1.3.5 The role of inflammation in psychiatric disorders: an evolutionary perspective

From an evolutionary point of view, it is believed that the communication between the immune and the CNS would facilitate behavioural responses related to avoidance and alarm, conferring an evolutionary advantage against many pathogens and predators during an ancestral environment. In such perspective, the inflammatory response and the "sickness behaviour" would increase host survival and reproduction in the highly pathogenic environment. Sickness behaviour refers to an adaptive response to illness, commonly associated to infections, which is characterized by depressed mood, social withdrawal, decreased appetite, lethargy, impaired concentration, irritability and pain. In fact, within an evolutionary approach, this syndrome would benefit the host by saving and directing energy to fight infection and to promote healing. Even psychological stress and its relation to the immune system was understood under the evolutionary perspective. For instance, during the ancestral times, psychological distress related to the risk of death due to pathogen invasion during hunting would contribute to the activation of the immune system. Thus, cytokines would act in the CNS to regulate motivation and motor activity (social avoidance and energy conservation), as well as arousal, anxiety and alarm (hypervigilance) (MILLER; RAISON, 2016).

In modern times, however, the dysfunctional communication between the neuro and immune systems would increase the risk of developing psychiatric disorders, as well as confer resistance to the current pharmacological therapy. The next sections present detailed information on the association between inflammation and psychoses.

1.3.6 Infection, inflammation and psychoses

Epidemiological studies support the role of inflammation in the pathophysiology of psychoses. A Danish population-based cohort study reported that previous history of autoimmune diseases increases the incidence of schizophrenia by 36%, and that this incidence rate can reach figures of up to 60% in patients with a previous history of infection or hospitalization. When combined, a significant increased dose-response is observed. Interestingly, in this cohort study, schizophrenia incidence rate was proportional to the number of severe infections (BENROS et al., 2011). Besides that, a recently published meta-analysis revealed positive association between non-neurological autoimmune disorders (pernicious anemia, pemphigoid, psoriasis, celiac disease and Graves' disease) and psychoses, suggesting the participations of multiple factors underling this association, including inflammation and shared genetic vulnerability (CULLEN et al., 2018).

In addition to that, other epidemiological investigations demonstrated an association between prenatal infection – followed by maternal immune activation – and subsequent risk for the development of mental disorders in children, including psychoses. In fact, this association seems to be related to neurodevelopmental impairment mediated by the aberrant inflammatory process (BROWN; DERKITS, 2010; KNUESEL et al., 2014).

The first reports on this association came from ecological studies carried out in Finland, Denmark and England, which reported higher rates of schizophrenia in infants born from infected mothers during the influenza epidemic when compared to those infants born during non-epidemic periods (MEDNICK et al., 1988). Subsequently, epidemiological studies of population-based birth cohorts were developed. Three of these studies stand out, namely: 1) "The Child Health and Development Studies", held in Alameda, California, composed by all children born during the years 1959-1967; 2) "The Collaborative Perinatal Project", conducted in different regions of the United States, composed of multiple birth cohorts between the years 1959 and 1967; and 3) the Danish national study, which included all those born in the country since 1981 (CANETTA; BROWN, 2012).

In all the three large epidemiological studies cited above, there was an increased risk of developing schizophrenia among those whose mothers were seropositive for a number of different pathogens during pregnancy. For example, exposure to influenza virus during the first trimester conferred a 7-fold increased risk for schizophrenia in the offspring. High rates were also associated for the protozoan *Toxoplasma gondii*, and also for cases involving rubella virus

infections or bacterial infections. Other cases involving herpes virus infections showed increased risk for both affective or non-affective psychoses (BROWN, 2006; BROWN; DERKITS, 2010; CANETTA; BROWN, 2012).

Overall, these epidemiological studies suggest that the risk for developing psychoses is not related to a specific pathogen but rather to the inflammatory process resulting from the infectious process. This hypothesis is supported by studies demonstrating that the exacerbated production of cytokines produced during pregnancy, especially TNF-α and IL-8, increases offspring's risk for developing schizophrenia in adult life, whereas anti-inflammatory cytokines (IL-4 , IL-5, IL-10, IL-13) would act as a protective factors (ALLSWEDE et al., 2016; BUKA et al., 2001; KNUESEL et al., 2014). Not only that, it is also suggested that the gestational inflammatory process would lead to an impaired foetal neurodevelopment (KNUESEL et al., 2014).

The "Development Insult and Brain Anomalies and Schizophrenia Study", demonstrated that a history of infection during the gestational period is associated with offspring's brain morphology alterations in areas of relevance for schizophrenia, such as the prefrontal cortex (PFC) and the hippocampus (HIPPO). These alterations were translated into deficits related to executive functions, memory and attention processes, as well as verbal and neuromotor deficits in those adults with schizophrenia who were born from the infected mothers (BROWN et al., 2009). Based on such findings from large studies, the inflammatory process has been widely investigated as the biological mechanism for psychoses.

Preclinical studies inspired by the results of epidemiological investigations are congruent with the inflammatory hypothesis proposed for psychoses. In the maternal immune activation (MIA) model, injection of compounds that mimic viral (PolyI:C) or bacterial (lipopolysaccharide) infections in pregnant dams elicits the following changes: i) behavioural (hyperlocomotion, prepulse inhibition deficits, diminished social interaction and deficits in the recognition of noval objects); ii) morphological (volumetric reduction in the PFC and HIPPO, as well as changes in the connectivity between these areas); iii) neurochemical/molecular (dopaminergic, GABAergic and glutamatergic alterations, especially in the PFC and HIPPO); iv) besides an increased sensibility to antipsychotics in adult offspring (MEYER, 2014; MEYER; FELDON; YEE, 2009; SMITH et al., 2007).

Furthermore, it is remarkable an increase in inflammatory cytokines, including IL-6 and TNF-α, and decreased IL-10, as a consequence of MIA, not only in maternal serum, but also in the amniotic fluid, placenta and in the offspring's CNS, including changes in the gene

expression (mRNA) of these cytokines (OSKVIG et al., 2012). Interestingly, many of the aforementioned behavioural, morphological, and neurochemical changes are abolished via overexpression of the anti-inflammatory cytokines (IL-10) in the macrophages of pregnant dam. However, the increased expression of this cytokine in the absence of a pro-inflammatory prenatal stimulus also elicit behavioural changes, emphasising the importance of the balance between anti- and pro-inflammatory cytokines during neurodevelopment (MEYER, 2014).

1.3.7 Inflammatory hypothesis of psychoses: some clinical insights

In view of the above observations, several inflammatory hypotheses of psychoses have been postulated.

Almost 30 years ago, Smith (1992) proposed the "Macrophage-T-Lymphocyte Theory of Schizophrenia" claiming that the complex interaction between these cells lineages would be the key biological mechanism of schizophrenia (SMITH, 1992). According to this theory, the prodromal phase of psychoses would be related to the macrophage-related cytokines (such as TNF-α, IL-β and IL-6). However, the transition from the prodromal to the active phase would be marked by the failure of chronic activated macrophages to proper control the synthesis of T-lymphocytes-related cytokines, resulting in a massive amount IL-2, IL2 receptors (IL-2Rs) and IFN-γ. The exaggerated amount of IL-2 and IL-2Rs would be responsible for the positive symptoms, whereas IFN-γ would act stimulating macrophages to produce their depressive-related cytokines.

Years after, Schwarz et al. (2001) proposed the "Th2-hypothesis of Schizophrenia", claiming about the existence of a subgroup of patients presenting with increased Th2 immune reactivity. Contrasting Smith (1992) theory, the authors believed that increased Th2-humoral immune reactivity could be related to prenatal viral infection and would be a characteristic of patients with more pronounced negative symptoms and treatment-resistance. The hypothesis was formulated based on observations of increased antibody (immunoglobulin, IgE, IgG), IL-6 (a potent antibody stimulator) and Th2-related cytokine (IL-4) production in patients with more pronounced negative symptoms and poor pharmacological response, whereas key Th1-related cytokines, such as IFN-γ and IL-2, would be decreased in these patients (SCHWARZ et al., 2001).

Schwarz et al. (2001) hypothesis was soon afterwards opposed by Kim et al. (2004), arguing for a shift away from Th2-produced IL-4 and towards Th1 produced IFN-γ (KIM et al., 2004), after demonstrating an increased Th1(IFN-γ)/Th2 (IL-4) ratio in untreated patients with

schizophrenia when compared with controls. Nevertheless, these patients were not marked by negative symptoms like in Schwarz et al. (2001) investigation. Besides, Kim et al. (2004) also hypothesised about increased TGF-β in schizophrenia, which are produced by T-reg/Th3 cell lineages. This cytokine was also pointed as a key regulator of the Th1/Th2 imbalance proposed for schizophrenia (BOROVCANIN et al., 2012), and higher T-reg cell was shown to be correlated with better clinical outcome (DREXHAGE et al., 2011).

Although contradictory, the hypotheses discussed above have in common the fact of postulating an inflammatory cytokines imbalance in psychoses, culminating in the existence of a low-grade inflammatory profile in these patients. However, it is known that this imbalance may result from confounding variables, such as pharmacological treatment, clinical status of the disease (first episode vs. chronic), age, sex, substance abuse, body mass index, besides methodological differences regarding cytokine measurement. Therefore, investigations aimed to uncover the inflammatory profile of psychoses unconfounded by such variables still deserves attention (GOLDSMITH; RAPAPORT; MILLER, 2016; MILLER et al., 2011; UPTHEGROVE; MANZANARES-TESON; BARNES, 2014).

1.3.8 Is there increased inflammation in psychoses? Findings from meta-analyses

The latest published meta-analysis pointed to important similarities regarding the imbalance of some cytokines in psychoses, bipolar disorder and depression when compared with controls (GOLDSMITH; RAPAPORT; MILLER, 2016). In particular, it is highlighted that elevated levels of IL-6 and TNF-α are the most replicated findings for the three aforementioned disorders. Meta-regression analysis also allowed the study of important covariates, and the results indicate that increased IL-6 and TNF-α in FEP were not related to confounding variables such as age, sex, disease duration, tobacco use, body mass index, or pharmacological treatment. For other cytokines, including anti-inflammatory cytokines, meta-regression analyses were not possible due to the low number of studies; therefore, future investigations are encouraged (GOLDSMITH; RAPAPORT; MILLER, 2016).

Specifically for psychoses, there is some variation in inflammatory cytokines and cytokine receptors according to the clinical status of the patients (FEP, acute exacerbation, or chronic schizophrenia). For example, levels of IL-6, TNF-α, IL-1β, and the soluble IL-2 receptor (sIL-2R) are increased in patients with FEP, acutely ill or chronic, whereas levels of IL-8, IL-12, TGF-β, IFN-γ and the IL-1 receptor antagonist (IL-1RA) are increased only in patients with FEP or acutely ill. Interestingly, the levels of IFN-γ are usually high in patients

with FEP or during acute exacerbation but decreased in chronic patients. This discrepancy is also observed for some cytokines with anti-inflammatory properties. For example, IL-4 levels are reduced in patients in FEP or acutely ill, while IL-10 levels appear to be increased in patients in FEP but reduced during acute exacerbation of chronic schizophrenia (DE WITTE et al., 2014; GOLDSMITH; RAPAPORT; MILLER, 2016; MILLER et al., 2011; UPTHEGROVE; MANZANARES-TESON; BARNES, 2014). A summary of these findings can be found in Chart 2.

Regarding the influence of antipsychotics on the inflammatory profile, the meta-analyses cited previously investigated the influence of antipsychotic treatment after an average of 53 days. The results allowed the classification of cytokines in the peripheral blood in two important groups: state and trait markers. The first refers to cytokines that are elevated (IL-1β, IL-6 and TGF-β) but normalised after pharmacological treatment with antipsychotics. In contrast, IL-12, IFN-γ, TNF-α and sIL-2R appear to act as trace markers, since their levels remain elevated in the acute phases of the disease and even after treatment with antipsychotics (MILLER et al., 2011). In another meta-analysis, however, 56 days of antipsychotic treatment yielded increased IL-12 and sIL-2R, while reduced plasma levels of IL-1β and IFN-γ (TOURJMAN et al., 2013).

Another meta-analysis investigating the effect of antipsychotics (4 weeks) in patients during FEP without previous antipsychotic treatment revealed that the reduction of cytokines IL-2, IL-6 and IL-1β occurred after a month, which did not occur for cytokines IL-17, IFN-γ, and TNF-α (CAPUZZI et al., 2017). In other studies, antipsychotic treatment has been shown to increase anti-inflammatory markers, such as IL-10, sIL-2R, IL-1RA, and sTNF-R (PETRIKIS et al., 2017; POTVIN et al., 2008; TOURJMAN et al., 2013). Taken together, it is believed that the ability of antipsychotics to normalise the levels of the state markers could provide information regarding the pharmacological efficacy in symptom relief. In this sense, it is suggested that refractory patients maintain elevated levels of the state markers described previously (MILLER et al., 2011). In accordance, pronounced cytokine aberrations have been found to predict non-response to pharmacological antipsychotic treatment and unfavourable long-term outcomes in FEP (MONDELLI et al., 2015). Specific information regarding the class of antipsychotics involved is still a limitation, given the use of mixed treatment in most studies.

Chart 2: Summary of meta-analyses reporting blood cytokines changes in psychosis versus controls, including information about clinical status

Meta-analyses (Author, year)	Clinical status (number of studies included)	Biomarker in the peripheral blood	Number of investigations	Result
Miller et al. (2011)	Acute relapse (10)	IL-6	6	↑
		TNF-α	4	↑
		IL-8	2	↑
		TGF-β	2	↑
		IL-1RA	2	↑
		IFN-γ	2	↑
		IL-10	2	↓
		IL-2	2	NS
		sIL-2R	2	NS
	Drug-naïve FEP (14)	IL-6	4	↑
		TNF-α	4	↑
		IL-1β	3	↑
		sIL-2R	3	↑
		IFN-γ	2	↑
		TGF-β	2	↑
		IL-12	2	↑
		IL-2	2	NS
	Chronic (3)	IL-6	3	NS
	Treatment-resistant psychosis (5)	sIL-2R	5	↑
		IL-6	5	NS
Upthegrove et al. (2014)	Drug-naïve FEP (14)	IL-6	5	↑
		TNF-α	3	↑
		IL-1β	3	↑
		sIL-2R	3	↑
		IL-2	3	NS
		IFN-γ	3	NS
		IL-4	2	NS

(Continues next page)

(Continuation)

Meta-analyses (Author, year)	Clinical status (number of studies included)	Biomarker in the peripheral blood	Number of investigations	Result
Goldsmiths et al. (2016)	Acute relapse (15)	IL-6	9	↑
		TNF-α	7	↑
		TGF-β	6	↑
		IL-4	5	↓
		IFN-γ	4	↑
		IL-1β	3	↑
		sIL-2R	3	↑
		IL-1RA	2	↑
		IL-10	2	↓
		IL-2	2	NS
	FEP Drug-naïve (26)	IL-6	11	↑
		TNF-α	9	↑
		IFN-γ	7	↑
		IL-1β	6	↑
		IL-2	5	NS
		IL-4	4	↓
		IL-10	4	↑
		TGF-β	3	↑
		IL-12	3	↑
		IL-18	3	NS
		sIL-2R	3	↑
		IL-17	2	NS
		IL-8	2	↑
		IL-1RA	2	↑
	Chronic (18)	IL-6	12	↑
		TNF-α	9	↑
		IL-2	6	NS
		IFN-γ	4	↓
		IL-1β	4	↑
		IL-10	4	NS
		sIL-2R	3	↑
		IL-4	2	NS

FEP: first-episode psychosis. Results indicate overall increased (↑), decreased (↓) or non-significant (NS) changes in cytokines levels; IL: interleukin; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; TGF-β: transforming growth factor-β; sIL-2R: soluble IL-2 receptor; IL-1RA: IL-1 receptor antagonist

Due to the limitation of conventional antipsychotics in relieving negative and cognitive symptoms, and considering the presence of inflammatory markers in the blood and CNS of patients with psychosis, adjuvant anti-inflammatory therapy has been explored. Studies have shown promising effects of anti-inflammatory agents, such as non-steroidal anti-inflammatory drugs, omega-3 fatty acids, the neurosteroid pregnenolone, tetracycline antibiotics, and antioxidant compounds in the treatment of schizophrenia. The results seem more prominent for the negative and cognitive symptoms, especially when administered in the early stages of the disease. These studies highlight the efficacy of the adjuvant therapy with anti-inflammatory agents when compared to the effects of antipsychotics in isolation (KELLER et al., 2013; NITTA et al., 2013; SOMMER et al., 2012, 2014).

The production of cytokines in the peripheral blood appears to involve the abnormal functioning of leukocytes in the blood of psychotic patients. In the latest meta-analysis, it was reported an increased CD3+ and CD4+ lymphocytes in drug naïve FEP. In acutely ill patients, increased CD4+ and *natural killer* cells were also apparent, the former being classified as state markers, and the second as trait markers, since the number of these cells are normalised after antipsychotic treatment for the former but not for the latter (MILLER et al., 2013).

In the lymphocytes of medication-free patients with recent schizophrenia, increased protein and gene expression of IL-6 and TNF-α but not IL-1β has been reported. In addition, increased protein and gene expression of the membrane receptors of these cytokines, namely, TNFR1, TNFR2, IL1-R1, but not IL-6R, gp130, IL1-R2 or IL1-RA, were also observed (PANDEY et al., 2015). Regarding data on monocytes, although these are still insufficient, existing studies point to an increased number of monocytes, as well as increased protein and gene expression of cytokines IL-1β, IL-6, TNF-α in these cells from newly diagnosed patients. Some studies report that the levels of these cytokines in these cells are reduced after one month of treatment with antipsychotics, and that monocytosis is associated with worsening of psychotic symptoms, whereas resolution of monocytosis is associated with improvement of symptoms (MILLER et al., 2013).

In addition to cytokine changes in the peripheral blood, there is also reports about increased cytokines in the cerebrospinal fluid (CSF), and these changes appear consistent for patients with schizophrenia, bipolar disorder and depression. For example, the latest meta-analysis reported that both patients with schizophrenia and bipolar disorder present increased

IL-1β and KYNA, while patients with depression, similar to those with schizophrenia, have increases in IL-6 and IL-8 when compared with controls (WANG; MILLER, 2017).

Specifically in schizophrenia, increased IL-1β, IL-6, IL-8, kynurenine and KYNA has been reported, whereas sIL-2R levels are decreased, suggesting an imbalance between pro- and anti-inflammatory mediators in the CNS. As in the peripheral blood, levels of IL-6 were the most replicated, whereas for TNF-α there are no reports. Meta-regression analysis showed that high levels of IL-6 are not related to sex or age, whereas for other confounding variables there is insufficient data (WANG; MILLER, 2017). A summary of these findings can be found in Chart 3.

Although the changes in the CSF resemble those reported in the peripheral blood for some mediators, the results should be interpreted with caution, since they come from independent studies. Conversely, in three identified studies investigating the levels of cytokines in the peripheral blood and CSF of the same subject, the results appeared to be very discrepant. For example, for the most replicated cytokine (IL-6), the studies reported no correlation between IL-6 levels in the blood and CSF of the same patient (KATILA et al., 1994; LINDQVIST et al., 2009; SASAYAMA et al., 2013). There are also discrepancies in relation to the soluble receptor of IL-6, which amplifies the pro-inflammatory actions of this cytokine, sometimes reported as increased in CSF but reduced in peripheral blood.

Chart 3: Summary of meta-analyses reporting cytokines changes in the cerebrospinal fluid of patients with psychotic versus controls

Meta-analyses (Author, year)	Clinical status (number of studies)	Biomarker in the cerebrospinal fluid	Number of investigations	Results
Miller et al. (2011)	Non-stratified (7)	IL-2	4	↑
		IL-12	2	↑
		IL-6	2	↑
		IL-1β	2	↓
Wang et al. (2017)	Non-stratified (16)	IL-6	7	↑
		IL-2	4	NS
		IL-1β	3	↑
		IL-8	3	↑
		IL-1α	2	NS
		sIL-2R	2	↓
		sTNF-R2	2	NS

Results indicate overall increased (↑), decreased (↓) or non-significant (NS) changes in cytokines levels; IL: interleukin; sTNF-α: soluble tumor necrosis factor receptor 2; sIL-2R: soluble IL-2 receptor.

In addition to changes reported in the peripheral blood and CSF, *post-mortem* studies point to changes in cytokines and other inflammatory markers in the brain of patients with schizophrenia, not only at the protein level but also at the level of gene expression. Some studies highlight increases in IL-6 and TNF-α, and reduction in IL-10, for example in the PFC (FILLMAN et al., 2013; PANDEY et al., 2017b) and increased TNFR1 receptor in this region and in the cingulate gyrus (DEAN et al., 2012). However, recent meta-analyses show that results are still very scarce and inconsistent, with the majority focusing on specific regions, such as the PFC, combined to the inexistence of robust studies investigating anti-inflammatory cytokines (TRÉPANIER et al., 2016; VAN KESTEREN et al., 2017).

Besides data on cytokines, a broad GWAS study had initially identified several genes related to immunological and inflammatory functions in the HIPPO of patients with schizophrenia (HWANG et al., 2013). These inflammatory changes seem to be accompanied by reduction in trophic factors, such as the brain-derived neurotrophic factor (BDNF), decreased viability of inhibitory interneurons (FILLMAN et al., 2013), cognitive deficits and volumetric reduction (FILLMAN et al., 2016).

The involvement of specific immune pathways was also replicated in other GWAS study (NETWORK, 2015). Nevertheless, even GWAS studies are contradictory. While there has been data showing the participation of the MHC region in schizophrenia, recent studies have failed to find variation in grouped immune genes outside of the MHC region (POUGET et al., 2016). Another recent study using a systematic genetic approach in schizophrenia *post-mortem* brain (dorsolateral PFC, HIPPO) also failed to confirm a primary role of immune pathways in these brain areas (BIRNBAUM et al., 2018). These negative results in the CNS contrast recent literature conducted on peripheral lymphoblastoid cell lines, which provided strong evidence of immune gene enrichment in schizophrenia (SANDERS et al., 2017). Adding to that, accumulating evidence of shared genetic risk for schizophrenia and a subset of immune-mediated diseases further support the participation of the immune system, at least in a certain proportion of patients (CULLEN et al., 2018).

With these contrasting observations, the role of the immune system in psychoses remains unclear; besides that, these conflicting results could indicate lack of synergism between blood-brain immune dysfunction in psychoses. In view of the current inconsistent literature, the meta-analyses discussed before (GOLDSMITH; RAPAPORT; MILLER, 2016) suggest that results should be replicated, considering the relative low number of investigations for each of the cytokines under consideration, especially anti-inflammatory cytokines. For example,

although IL-6 and TNF-α cytokines are the most replicated findings, an average of 11 studies are reported for IL-6, and 9 studies for TNF-α in drug-naïve FEP according to the latest meta-analysis. For anti-inflammatory cytokines, IL-10 and IL-4, an average of 4 studies are reported and for TGF-β 3 studies in the blood, whereas in the CSF and *post-mortem* tissue this number is even lower or inexistent.

In sharp contrast to the numerous discussions on the role of pro-inflammatory cytokines in psychoses, the role of anti-inflammatory cytokines has received less attention. This discrepancy is somehow surprising, considering the intimate interaction between these mediators. In addition, meta-regression analysis for confounding variables was only possible to be conducted in the blood and only for the two most replicated cytokines (IL-6 and TNF-α) (GOLDSMITH; RAPAPORT; MILLER, 2016).

In view of the above, it is suggested that cytokines or cytokine receptors, may indicate the existence of a subgroup of patients with a low-grade inflammatory profile. These mediators may act as markers of response to pharmacological treatment or as markers of disease exacerbation phases, at least in genetically susceptible patients. However, a significant limitation among the hypotheses discussed so far refers to the disregard of important confounding variables that could influence such markers (specially age, sex, body mass index, tobacco smoking). Not only that, another important limitation refers to the lack of studies investigating the role of stressors as potential moderators of the inflammatory process reported in psychoses. As a consequence, the discrepancy seen across studies could be related to such limitations.

1.3.9 Inflammatory hypothesis of psychoses: focus on environmental factors

Different from infection or tissue injury, which are known to trigger an inflammatory response of high magnitude, low-grade inflammation is thought to be generated during noxious conditions that facilitate tissue stress and malfunction in the absence of infection, and is typically described to have lower magnitude than the classic responses induced by infections or injury. It is suggested that this sustained malfunction might result from responses to environmental insults (MEDZHITOV, 2008).

Unlike the inflammatory hypotheses discussed before, Monji et al. (2009; 2013) proposed the microglial hypothesis of schizophrenia. In this hypothesis, the role of environmental risk factors in the hyperactivation of microglial cells is highlighted. More specifically, it is suggested that genetic vulnerability combined with the exposure to various

environmental risk factors during critical periods of the neurodevelopment is capable of inducing immune cells sensitisation. Afterwards, during the postnatal period, under the exposure to new stressors, the immune cells that were once sensitised would become easily re-activated, producing several inflammatory mediators. By means of the various neuroimmune crosstalk, microglial hyperactivation would also occur, contributing to the crosstalk between peripheral and central inflammation (MONJI et al., 2013; MONJI; KATO; KANBA, 2009). As a consequence, the generation of a low-grade inflammatory state with negative effects on the neurodevelopment is postulated; this inflammatory profile would persist and be evident during adulthood as well.

The immune system in the CNS is complex and still poorly elucidated. The major components are glial cells and macrophages (perivascular and invading macrophages). Microglial cells are usually considered the resident macrophages of the CNS, although they differ considerably from resident macrophages, mainly because they are derived from primitive progenitors that arise in the yolk sac during the early embryonic stages (PRINZ; PRILLER, 2014; WAKE et al., 2009).

Despite having a distinct embryogenic origin, microglial cells, similar to macrophages, have different activation states. When in its quiescent state, these cells present with a ramified shape that facilitates their phagocytic activity, playing therefore a crucial role in tissue surveillance (CALCIA et al., 2016; WALKER; NILSSON; JONES, 2013). However, environmental factors, whether physical (infections) (HÄUSLER et al., 2002) or psychological (such as early stressors), contribute to a phenotypic change from a ramified state to an amoeboid state (DE PABLOS et al., 2006; FRANK et al., 2007; GRACIA-RUBIO et al., 2016; JOHNSON et al., 2002; ROQUE; OCHOA-ZARZOSA; TORNER, 2016; WANG et al., 2017). In response to such stressors, microglial cells are polarised, resulting in a pro-inflammatory state. These cells release large concentrations of cytokines and other pro-inflammatory mediators facilitating the activation of astrocytes, which also have the ability to produce inflammatory mediators. Initially, this inflammatory response is necessary for the control of pathogens or damage. However, exacerbated and unregulated production results in damage to healthy tissues. Cytokines influence the synthesis, release, and uptake of neurotransmitters, facilitating neurotoxicity events when unbalanced. In contrast, when exposed to cytokines with anti-inflammatory properties (IL-4, IL-10, TGF-β), these microglial cells are polarised towards a neuroprotective state, essential for homeostasis. Thus, the imbalance between the different states of microglial activation – due to the exposure to environmental risk factors in critical

periods of neurodevelopment – seems to contribute to the development of psychiatric disorders, including schizophrenia (CALCIA et al., 2016; KHANDAKER; DANTZER, 2015; WALKER; NILSSON; JONES, 2013).

Increased microglial density and microglial activation has been observed not only in schizophrenia *post-mortem* studies (BAYER et al., 1999; BUSSE et al., 2012; RADEWICZ et al., 2000; STEINER et al., 2006) but also in vivo, through the positron emission tomography (PET scan) technique (DOORDUIN et al., 2009; VAN BERCKEL et al., 2008), mainly in the HIPPO and PFC of psychotic patients.

In this sense, it has been proposed that the efficacy of atypical antipsychotics may be due to microglial suppression, with subsequent neuroprotection (BIAN et al., 2008; KATO et al., 2007; SEKI et al., 2013). Atypical antipsychotics inhibit the production of TNF-α in IFN-γ-stimulated microglial cells, when compared to typical antipsychotics (KATO et al., 2007; SEKI et al., 2013). Minocycline, a tetracycline antibiotic, has a potent effect on microglial inhibition and is being suggested as a new approach for the treatment of psychoses (CHAUDHRY et al., 2012; SEKI et al., 2013), although recent research refutes this claim (DEAKIN et al., 2018).

However, even PET scan studies are conflicting. The first studies in the field used first generation radioligands, pointing to increased microglial density and activation in psychoses (DOORDUIN et al., 2009; VAN BERCKEL et al., 2008), which has not been fully replicated in more recent studies using the same technology (VAN DER DOEF et al., 2016). Other recent studies were performed using second generation radioligands, with some confirming the increase (BLOOMFIELD et al., 2016), and others failing to replicate such results (COUGHLIN et al., 2016; HAFIZI et al., 2016; KENK et al., 2015; TAKANO et al., 2010).

The reasons for such discrepancy may involve methodological differences, specially related to the type of radioligand used or even differences related to patients clinical status. For example, increased microglial activation and density have been reported in patients during recent stages (HOLMES et al., 2016; VAN BERCKEL et al., 2008), but not in chronic stages of the disease (KENK et al., 2015; TAKANO et al., 2010). In addition, increased microglia has been reported in recent-onset patients refractory to risperidone treatment (HOLMES et al., 2016), but not in recent-onset schizophrenia without previous antipsychotics treatment or in drug-naïve FEP (HAFIZI et al., 2016; HOLMES et al., 2016).

A recent meta-analysis on PET scan, however, has shown that patients with schizophrenia or FEP show reduced glia marker, the 18kDa translocating protein (TSPO), in

three important brain regions (frontal cortex, temporal cortex and hippocampus), and this effect is not modulated by antipsychotic treatment, strongly contrasting the hypothesis of high TSPO in these patients. Due to limited number of studies, exploration of different disease stage or exposure to stressful conditions was not addressed (PLAVÉN-SIGRAY et al., 2018), which could have significantly helped in the interpretation of the data.

In fact, some limited data suggest a positive association between stress measurements and microglial re-activation in a subsample of drug-naïve FEP patients (HAFIZI et al., 2017). The current contradictory findings may be, therefore, related to the lack of concern of stressors in these investigations, especially considering that stress stimulates increased protein expression in the mitochondria of microglial cells, including proteins commonly detected by PET scan technology, such as the TSPO. Noteworthy, in *post-mortem* investigations, some few data report an associations between microglial hyperactivation and stress-mediated suicide in patients with schizophrenia (STEINER et al., 2008). Thus, the identification of subgroups of patients with or without a history of early stress could help to clarify the association between psychosis and inflammation.

1.4 EARLY LIFE-STRESS, PSYCHOSES AND INFLAMMATION

1.4.1 Early life-stress: general and epidemiological aspects

Several environmental factors are associated with an increased risk for the development of psychotic disorders. A recent study analysed a total of 41 meta-analyses, identifying 98 possible associations. From this amount, four environmental risk factors were highlighted as highly suggestive: obstetric complications, stressful events during adulthood, history of childhood adversities, and cannabis use. Noteworthy, history of childhood adversities and cannabis use presented the most robust evidence after sensitivity analyses that included only prospective studies (BELBASIS et al., 2017).

Considering this robust association and taking into account the importance of stressful events in peripheral and central immune activation, the present study focuses on the participation of early-life stress (ELS), also designated as early trauma/adversities or childhood trauma/maltreatment, in the inflammatory hypothesis of psychoses. ELS is usually defined as acts of commission or omission occurring during childhood or adolescence that result in harm, potential harm or threat of a harm (WHO, 2002). It can be classified into forms of abuse,

including physical, emotional or sexual abuse, as well as into forms of neglect (emotional or physical) (Chart 4) (BERNSTEIN et al., 2003).

Other forms of ELS include unstable and dysfunctional relationships with family or caregivers, experience of accidents, physical illnesses, surgeries, natural disasters, as well as situations of poverty or terrorism (BERNSTEIN et al., 2003; MCGRATH et al., 2017).

Chart 4: Definitions of abuse and neglect according to BERNSTEIN et al., 2003

Emotional abuse	Verbal assaults on a child's sense of worth or well-being or any humiliating or demeaning behaviour directed toward a child by an adult or older person.
Physical abuse	Bodily assaults on a child by an adult or older person that posed a risk of or resulted in injury.
Sexual abuse	Sexual contact or conduct between a child younger than 18 years of age and an adult or older person.
Emotional neglect	The failure of caretakers to meet children's basic emotional and psychological needs, including love, belonging, nurturance and support.
Physical neglect	The failure of caretakers to provide for a child's basic physical needs, including food, shelter, clothing, safety and health care

Data from the World Mental Health Surveys has revealed that childhood maltreatment affects about one-third of the world's population (KESSLER et al., 2010). Approximately 57,000 homicide deaths were estimated among children under the age of 15 in 2000. Results vary according to the economic development and the regions. For example, in underdeveloped or emerging countries, the estimated mortality coefficient is 6.1/100,000 for boys under 5 years of age and 5.1/100,000 for girls, rates considered to be 2-3 times greater than for those estimated in developed countries (WHO, 2002).

According to a meta-analysis study, South America presents the highest estimates of childhood maltreatment in comparison to other continents, these being larger estimates related to physical and emotional neglect. More specifically, Brazil's estimates were particularly higher when compared to the other continents. Added to that is the inadequate investment in public policies against childhood maltreatment in the country (VIOLA et al., 2016). In Brazil, between 1999 and 2007, around 159,754 children were victims of maltreatment: 65,669 cases of negligence; 49,482 cases of physical abuse; 26,590 cases of emotional abuse; and 17,482 cases

of sexual abuse were reported. In 2016, an integrative review indicated that 98,115 medical consultations on maltreatment cases were registered for children and adolescents aged < 1 to 19 years old. Regarding the type of violence, neglect appears as the principal. It is also worth noting that despite the significant occurrence, maltreatment cases are masked by sub notification, and the current reports reflect only a small part of reality (NUNES; SALES, 2016).

In Ribeirão Preto, epidemiological data regarding the occurrence of ELS is still very scarce. However, a study based on data from the Municipal Health Department (Secretaria Municipal de Saúde) conducted between 2006 and 2008 revealed a total of 498 cases of violence against children between 0 and 9 years old. The majority of victims were females (56.4%) and the most frequent type of aggressions was physical abuse (59.2%), with the highest occurrence being at the family's home (75.5%) (FARIAS et al., 2016). Another study carried out in Ribeirão Preto estimated the prevalence of child maltreatment in children aged zero to ten years enrolled in public and private educational institutions. Results pointed a prevalence rate of 5.7% - much higher than the estimated prevalence of 0.3% expected by the Tutelary Councils of the city. It is worth noting the occurrence of sub notifications in the reported estimates, which emphases the importance of detection, notification and early intervention (FALEIROS; MATIAS; BAZON, 2009).

A range of studies, including large population-based epidemiological studies controlling for confounding variables, point to a strong association between ELS and risk for the development of psychiatric disorders in adult life, including psychoses (BENDALL et al., 2008; MISIAK et al., 2017; MORGAN; FISHER, 2007; READ et al., 2005). In fact, it is suggested that ELS increases the risk of psychoses in a dose-dependent manner (SCHAFER; FISHER, 2011). Meta-analyses studies reveal that psychotic patients are three times more likely to have been exposed to traumatic events in childhood when compared to healthy controls (OR = 2.78, 95% CI = 2.34-3.31). In addition, the prevention of traumatic experience could reduce the incidence of psychoses by 33%, and these results are independent of the methodology adopted by studies (VARESE et al., 2012).

The type and number of traumatic events seems have a great importance for the association between psychoses and childhood trauma. In the latest meta-analysis of the World Report on Mental Health conducted across 17 countries, including Brazil, it was found that the prevalence of childhood trauma in subjects with psychotic experience is 59.8% compared to 36.6% in those without psychotic experience. In addition, in those reporting psychotic experience, it is observed a dose-response increase between the number of stressors and the risk

of psychotic experience, with the prevalence of three or more childhood traumatic events reaching 19.7% compared to 6.9 % in those without psychotic experience (MCGRATH et al., 2017).

In the same report cited above, it was pointed out that the exposure to any traumatic event in infancy increases the risk of psychotic experiences by 31%, twice as high as in those not exposed (OR = 2.3, 95% CI 1.9 - 2.6). Events related to abuse, neglect and family maladjustment present the strongest association for the development of psychosis throughout life, representing 24% of the risk for the development of psychotic experiences, being the greater risks observed for sexual and physical abuse. More specifically, sexual abuse is considered to be the strongest association for the development of psychotic experiences during childhood (OR = 8.5; 95% CI 3.6 - 20.2), while physical abuse is associated with psychotic experiences during all stages of the development – from childhood to adulthood. In addition, the association between childhood adversity and psychotic experiences is independent to prior psychiatric comorbidities (MCGRATH et al., 2017).

In brief, such data highlight the importance of investigating the history of childhood traumatic events in the neurobiology of psychoses. Not only that, the prevention of traumatic events could reduce the development of psychoses in the population.

1.4.2 Early Stress: focus on inflammation and psychoses

Among several factors that could contribute to the proposed inflammatory imbalance, and therefore characterise psychoses as an inflammatory phenotype, ELS deserves attention. In fact, ELS is strongly associated with an increased risk of developing psychoses and also with immunological dysregulation in general population (MISIAK et al., 2017).

The neurobiology of trauma involves cognitive, social, emotional, and physiological abnormalities. Among the physiological changes, it is highlighted irregularities in the autonomic response to stress (involving the sympathetic nervous system) and changes in the functioning of the hypothalamic-pituitary-adrenal axis (HPA). The immune system is closely linked and modulated by the neuroendocrine system, especially the HPA axis. Pro-inflammatory cytokines participate in the activation of the HPA axis, whereas glucocorticoids act as immune suppressors by displaying important anti-inflammatory action, thus reducing the synthesis of inflammatory cytokines. The exacerbated production of inflammatory cytokines leads to the malfunctioning of the HPA axis, contributing to glucocorticoid resistance. Several

mechanisms are suggested, such as the reduced affinity of glucocorticoids to their receptors, the inhibition of the translocation of the complex to the nucleus or by promoting the expression of the inactive glucocorticoid receptor variant (GR-β) in detriment of the active variant (GR-α) (CARVALHO et al., 2014).

Another mechanism involves the inflammasome pathway. Psychological and physical stressors contribute to stimulation of the bone marrow with increased production and release of myeloid cells in the circulation through activation of the sympathetic nervous system. This culminates in increased production of catecholamines and DAMPS, which contributes to the production of inflammasomes (e.g., NLRP3). Inflammasomes are cytosolic proteins complexes that are produced in myeloid cells in response to pathogenic and non-pathogenic stressors. The inflammasome pathway leads to activation of caspase 1, which contributes to the production of inflammatory cytokines (IL-1β and IL-18). Also, NLRP3 upregulation and cleavage of the glucocorticoid receptor can lead to glucocorticoid resistance, whereas blockade of NLRP3 reverses stress-induced cytokine upregulation. The increased production of inflammatory cytokines can also activate the inflammatory pathway linked to the NF-kB, which will stimulate the release of other pro-inflammatory cytokines and contribute also to glucocorticoid resistance. Thus, glucocorticoid resistance accompanied by concomitant low-grade inflammation appear to be characteristic of exposure to ELS (MILLER; RAISON, 2016).

Even minor stressors, such as the trier social stress test (that requires participants to perform difficult mental tasks or to deliver a speech in front an intimating audience), increases the production of cytokines, such as IL-6 (QUINN et al., 2018), and this is higher in subjects with a history of early-life adversities (CARPENTER et al., 2010).

It is postulated that ELS negatively affects neurodevelopment. The consequences of childhood traumatic events appear to be long lasting and may trigger the FEP in biologically vulnerable individuals. In a long term, ELS can also facilitate not only the emergence of new episodes but also an increased risk for the development of other disorders, substance abuse, and alteration in the efficacy of pharmacological treatment (GRASSI-OLIVEIRA, 2016; MISIAK et al., 2017).

Systematic reviews and meta-analyses studies strongly indicate a relationship between ELS and changes in the inflammatory response contributing to a low-grade pro-inflammatory profile in adult life, which is independent of clinical comorbidities (BAUMEISTER et al., 2015; COELHO et al., 2014a). This association is highly significant for TNF-α, followed by IL-6 and C-reactive protein (CRP). It is also worth noting that this relationship may differ among the

different types of childhood trauma (BAUMEISTER et al., 2015; COELHO et al., 2014a), emphasizing the importance of discriminating the type of trauma in future research.

Although the relationship between ELS and inflammation is well described in the literature, and despite this relationship may increase the risk for the development of physical and mental illnesses, only few studies have investigated associations between inflammation and ELS in the context of psychoses. In fact the latest published meta-analysis (BAUMEISTER et al., 2015) revealed the existence of only two studies in this area, emphasising the need for more studies.

Limitations found in these studies include mainly the characteristics of the sample, especially regarding: i) the use of convenience sample, rather than population-based samples; ii) reduced sample size ($n < 50$); iii) non-inclusion or inclusion of a small number of controls with history of trauma; and iv) inclusion of patients suffering from chronic schizophrenia. None of the studies controlled for important confounding variables. It is also worth noting that the studies were conducted on the European continent, and until now there are no reports in the Southern Hemisphere.

In fact, the study of patients during their FEP is considered an important strategy in investigations that involve potential biological markers, since it helps in the reduction of possible confounding factors. More specifically, cytokine alterations found in patients with chronic schizophrenia may be related to factors such as natural disease progression, weight gain, metabolic disorders, obesity, as well as pharmacological treatment. FEP patients, on the other hand, besides being at the first manifestation of the disease, present a short period of exposure to antipsychotic drugs, and consequently, lower metabolic and immunological changes associated with the use of such drugs, which therefore brings advantages for a better understanding of the neurobiology of psychoses.

It is known, however, that a great challenge in clinical studies relates to limitations to access the CNS. In the context of the inflammatory hypothesis of schizophrenia, the study of inflammatory changes in the CNS is necessary, given the existence of several pathways that facilitate neuroimmune communication. In addition, considering the important role of cytokines in various aspects of the neurodevelopment, the study of encephalic structures closely related to the neurobiology of schizophrenia, such as PFC and HIPPO are of great relevance.

In this context, animal models might help in the understanding of possible biological mechanisms underlying early adversities and psychoses. In this perspective, it is highlighted in

the literature the pre-clinical model known as post-weaning social isolation (pwSI), proposed for the study of the neurobiology of schizophrenia and related disorders.

1.5 POST-WEANING SOCIAL ISOLATION

The complexity of psychoses makes difficult the establishment of preclinical models that can reliably replicate the clinical symptoms associated with the disorder, especially given the heterogeneity of such symptoms. However, preclinical studies are widely used as important tools for the understanding of the neurobiology of mental disorders. According to Jones, Watson and Fone (2010), available models of schizophrenia fit into four categories: i) neurodevelopmental; ii) drug-induced; iii) lesion; and iv) genetic manipulation (FONE; PORKESS, 2008a; JONES; WATSON; FONE, 2011).

pwSI is a non-pharmacological neurodevelopmental model widely used to study the neurobiology of schizophrenia. Considered an early and chronic affective stressor, this model elicits several long-term behavioural, morphological and neurochemical changes of great relevance to study the neurobiology of schizophrenia (FONE; PORKESS, 2008a; JONES; WATSON; FONE, 2011). Nevertheless, it is important to highlight that it would be inappropriate to characterise this or any other preclinical model as specific for schizophrenia or any other disorder, given the complexity of mental disorders.

In spite of that, Fone and Porkess (2008) highlight that pwSI presents with appropriate triad of validity for the study of the neurobiology of schizophrenia, including: i) face validity (homology for the neurobiology of the positive symptoms, resembled by hyperfunction of mesolimbic dopaminergic system; homology for the neurobiology of the negative symptoms, resembled by hypofunction of mesocortical dopaminergic neurotransmission and changes in the serotonergic neurotransmission; homology for cognitive deficits, characterised by several impairments in learning and memory); ii) construct validity (morphological and neurochemical changes similar to changes observed in clinical schizophrenia, especially in the PFC and HIPPO); and finally, iii) predictive validity (related to the pharmacological response to antipsychotics) (JONES; WATSON; FONE, 2011).

Among the behavioural alterations, changes in the exploratory behaviour when tested in a new environment is one of the most classic observations reported in rodents reared under social isolation from weaning, and such change seems to be independent of the sex. Hyperlocomotion induced by social isolation was described as a suitable marker to confirm the development of the "*isolation-induced stress syndrome*", strengthening the hypothesis of the

importance of environmental factors in the aetiology of schizophrenia (FONE; PORKESS, 2008a; JONES; WATSON; FONE, 2011).

Despite hyperlocomotion is described as the most classical change, other behavioural, cognitive, morphological and neurochemical impairments should also be mentioned. Besides hyperlocomotion, sensorimotor impairment is also observed (evidenced by impaired prepulse inhibition of acoustic startle, PPI), aggressive behaviour, anxiogenic behaviour when tested in the elevated plus maze, and various cognitive deficits (deficits in conditioned learning, in the recognition of novel objects, and in spatial memory) (FONE; PORKESS, 2008a; JONES; WATSON; FONE, 2011). Regarding morphological changes, it is highlighted impaired connectivity between the PFC and HIPPO, with marked reduced volume and impaired synaptic plasticity and dendritic arborization. At the neurochemical level, besides alterations in the dopaminergic system, changes in neurotrophic factors, including reduced BDNF, and in the glutamatergic system, such as reduced expression of NMDA receptor subunits are also reported (FONE; PORKESS, 2008a; JONES; WATSON; FONE, 2011).

Besides presenting a suitable constellation of changes that resembles the neurobiology of schizophrenia, pwSI does not involve any type of pharmacological or surgical intervention. This allows the investigation of neurodevelopmental changes provoked essentially by the environment at a significant low-cost, which opens opportunity for identification of potential related biomarkers.

pwSI protocol involves placing rodent puppies in individual boxes, from the first day of weaning (which corresponds from day 21st to 30th, for rats). From that time point, isolated animals should not be handled more than once a week. Visual, auditory or olfactory contact with other animals is not banned; however, no form of social contact is allowed. Fone and Porkess (2008) point out that the behavioural changes resulting from such protocol are only observed when the puppies are social isolated from the 21st to 30th day of life, considered a critical period for the neurodevelopment, but not when the same protocol is applied in rodents during their adulthood (FONE; PORKESS, 2008a).

In the context of the inflammatory hypotheses of psychoses, we highlight the scarcity of studies investigating peripheral and central inflammatory cytokines changes through this model. The study of cytokines abnormalities will help in the understanding of stress as a possible moderator of the inflammatory profile currently reported in patients with psychoses. This preclinical model will also help to clarify whether the potential inflammatory abnormalities will be independent of factors related to pharmacological therapy or other clinical

comorbidities. For the present project, we emphasise that this preclinical model was solely used to assess cytokine abnormalities simultaneously in the peripheral blood and CNS (PFC and HIPPO) of rodents – a limiting approach in clinical investigations – while mechanistic protocols were not taken into account in the present study.

2. Research Justification

2. RESEARCH JUSTIFICATION

According to epidemiological studies, childhood maltreatment is a strong environmental risk factor for many physical and psychiatric disorders, including psychoses. Imbalance in the production of inflammatory cytokines at critical periods of the neurodevelopment is being suggested as the biological basis for psychoses, and some recent investigations started to suggest that childhood adversity may underlie this relationship.

We justify our research proposal based on the observation of scarce number of clinical studies (n=2) investigating the relationship between childhood maltreatment and abnormal production of inflammatory cytokines in the context of psychoses, and several limitations can be addressed from the aforementioned studies. Firstly, studies were compromised by the characteristics of the sample, including a convenience sampling of small size (n<50), which are not representative of the target population. Secondly, there was not sufficient number or even absence of controls with history of childhood trauma in these studies, which again brings disadvantages to the interpretation of the results. Thirdly, one of these studies included a small sample of patients with chronic schizophrenia. This is very problematic considering the number of confounding variables, especially those related to the duration of the mental disorder, pharmacological treatment and metabolic disorders, which all are strictly related to changes in the immune response. Fourth, none of the studies were conducted in the Southern Hemisphere, where childhood trauma estimates are higher than those compared to Europe. Finally, the studies considered different types of abuse and neglect as one phenomenon. Whether cytokines abnormalities are specific to one or more types of childhood adversity in the context of psychoses remains unclear and still deserves attention.

In consideration to the problematic afore discussed, important methodological improvements are needed to better understand the relationship between childhood adversity, psychoses and inflammation. To the best of our knowledge, this will be the first study to investigate the influence of different types of childhood maltreatment on the low-grade inflammatory profile of a large population-based sample of patients during the first manifestation of the disease. The methodological aspects of our study are distinctive from the two previous investigations reported. We recruited a sizeable, epidemiologically based sample of first-episode of psychoses with reduced risk of selection bias. Besides, we have also included the siblings of these patients, a high-risk group that will help to understand the relationship

between environmental and biological factors, and the inclusion of a large community-based control group will surely favour the consolidation of the findings. In our study, we included patients during their first manifestation of the disease, which reduces possible confounding factors related to long disease duration or chronic treatment.

A major challenge in clinical investigations is, however, limitations related to the access of the CNS. For this reason, we included in our investigations a preclinical model of schizophrenia (pwSI), which is free from any pharmacological interventions and presents appropriate triad of validity to study the neurobiology of schizophrenia. Although clinical studies suggest an inflammatory imbalance in patients with psychoses, it should be noted that peripheral variations of these cytokines might not be reflected in the CNS. Considering that, we opted to perform a correlational preclinical study to investigate variations in cytokines in blood and brain.

The consideration of early-life stressors in psychosis will help in the investigation of blood-based inflammatory biomarkers and to tailor more specific treatment strategies for the disease. We also highlight the problematic of higher estimates of childhood maltreatment in South America; therefore, the outcomes from this epidemiologic-based study will be useful for the alarming need of establishing public policy actions in Brazil to prevent mental illness with early detection, and can aid health care practitioners and researchers on stress coping strategies to prevent stress-related disorders caused by early-life experience.

2.1 RESEARCH CLARIFICATION

By the time we formulated the present project, we initially proposed to study three cytokines: IL-6 and TNF-α, which are the most replicated findings in the literature; and IL-10, given the scarce information on anti-inflammatory cytokines in the context of psychoses.

Due to some methodological limitations, we started by investigating these three cytokines in our animal model and right after we performed the investigation in our clinical study. However, given the fact that none of the three cytokines yielded positive results in the association with childhood trauma, and considering that we had invested densely in the design of our epidemiological-based sample, we were motivated to make an effort to include extra cytokines in our clinical analysis. Therefore, we included the measurement of four extra cytokines – two cytokines with potent pro-inflammatory proprieties (IL-1β, INF-γ) and two cytokines with anti-inflammatory and regenerative proprieties (IL-4, TGF-β) – all described

recently to be involved in the pathogenesis of psychoses, major depressive disorder and bipolar disorder. Unfortunately, these extra cytokines were not possible to be analysed in our preclinical investigation.

In the following sections we present the aims and hypotheses of our study. Afterwards, we describe in details the outcomes of our research, which will be presented in the form of submitted manuscripts. We firstly describe the results of our preclinical model, which included the measurement of the cytokines initially proposed in our project (IL-6, TNF-α, IL-10), and this is followed by the outcomes of our clinical data, which included the initially proposed and the extra cytokines.

3. Research aim and hypotheses

3. RESEARCH AIM AND HYPOTHESES

To investigate associations between early-life adversities and inflammatory cytokines in an epidemiological-based sample of first-episode psychosis, unaffected siblings and population-based controls as well as in an animal model of the disease;

3.1 Preclinical study:

General aim:

To investigate inflammatory cytokine abnormalities in the blood and brain tissues of male *Wistar* rats reared isolated or grouped since weaning and possible correlation between cytokines and locomotor activity.

Specific aims:

a) To measure the locomotor activity in the open field (20min) of rats reared isolated or grouped since weaning;
b) To investigate protein and gene expression of pro- and anti-inflammatory cytokines (IL-6, TNF-α, IL-10) simultaneously at blood and brain tissues (prefrontal cortex and hippocampus) of male *Wistar* rats reared isolated or grouped since weaning;
c) To investigate correlations between cytokine abnormalities and locomotor activity in the open field in social isolated-animals.

Hypothesis:

We hypothesised that rats submitted to pwSI would present increased levels of both IL-6 and TNF-α, but decreased levels of IL-10 in blood and brain and that abnormal levels of these cytokines would correlate with increased locomotion in the open field.

3.2 Clinical study:

General aim:

To investigate inflammatory cytokine levels in an epidemiological-based sample of first-episode psychosis, unaffected siblings and population-based controls and the influence of early-life stress in the inflammatory profile.

Specific aims:

a) To investigate plasma cytokine levels (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) among patients, patients' siblings, and community-based controls, controlling for age, gender, body mass index and tobacco smoking;

b) To investigate the role of childhood maltreatment and subtypes (physical abuse, emotional abuse, sexual abuse, physical neglect, emotional neglect) in determining the differences in cytokine levels while controlling for confounding factors.

Hypothesis:

We hypothesized that: i) FEP patients would have increased levels of inflammatory cytokines (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) when compared with controls, and that unaffected siblings would act as an intermediate group; ii) reports of traumatic events would be associated with increased levels of inflammatory markers in all three groups; and iii) the subtypes of childhood trauma would impact differently on the levels of the inflammatory markers.

*4. Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain**

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4. PROLONGED PERIODS OF SOCIAL ISOLATION FROM WEANING REDUCE THE ANTI-INFLAMMATORY CYTOKINE IL-10 IN BLOOD AND BRAIN

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Abstract

Life stressors during critical periods are reported to trigger an immune dysfunction characterised by abnormal production of inflammatory cytokines. Despite the relationship between early stressors and schizophrenia is described, the evidence on inflammatory biomarkers remains limited. We aimed to investigate whether an imbalance between pro- and anti-inflammatory cytokines in the brain is reflected in the peripheral blood of rats submitted to post-weaning social isolation (pwSI), a model with validity to study schizophrenia. We evaluated pro- and anti-inflammatory cytokines (IL-6, TNF-α, IL-10) simultaneously at blood, prefrontal cortex and hippocampal tissues (Milliplex MAP; pg/mL), including the respective cytokines gene expression (mRNA) (qRT-PCR TaqMan mastermix). We also investigated whether abnormal cytokine production would correlate with hyperlocomotion in social isolated-animals. Male *Wistar* rats (n = 10/group) were kept isolated (n = 1/cage) or grouped (n = 3-4/cage) since weaning for 10 weeks. After this period, rats were assessed for locomotion and sacrificed for blood and brain cytokines measurements. Prolonged pwSI decreased IL-10 protein and mRNA in the blood, and IL-10 protein in the hippocampus, along with decreased IL-6 and its mRNA expression in the prefrontal cortex. IL-10 hippocampal levels were negatively correlated with hyperlocomotion in the open field. Although the unexpected decrease in IL-6 and unchanged TNF-α levels contrast to the expected pro-inflammatory phenotype, this may suggest that reduced anti-inflammatory signalling may be critical for eliciting abnormal behaviour in adulthood. Altogether, these results suggest that prolonged early-life adverse events reduce the ability to build proper anti-inflammatory cytokine that is translated from blood-to-brain.

Keywords: Anti-inflammatory cytokines; early stress; cytokines; inflammation; interleukin-10; post-weaning social isolation; schizophrenia; social isolation rearing.

4.1 INTRODUCTION

Rearing rat pups in isolation since weaning significantly interferes with the morphological and neurochemical development of the early postnatal brain, contributing to long-term maladaptive behaviours in adulthood (FONE; PORKESS, 2008b; JONES; WATSON; FONE, 2011). The behavioural, morphological and neurochemical alterations elicited by post-weaning social isolation (pwSI) have translational significance to several core features that seems to share the neurobiology implicated in the pathophysiology of schizophrenia. Classical and already validated neurochemical alterations include both hyper and hypofunctional dopaminergic neurotransmission in the mesolimbic and mesocortical pathways, respectively, (HALL et al., 2002; HEIDBREDER et al., 2000; HOWES et al., 2000; LAPIZ et al., 2003; TOUA et al., 2010) in agreement with the dopaminergic hypothesis suggested for schizophrenia (HOWES; MCCUTCHEON; STONE, 2015). On the behavioural level, isolated rats present impaired sensorimotor gating, cognitive deficits, increased aggressive behaviour, reduced social interaction and hyperlocomotion. Remarkably, hyperlocomotion was described before as a suitable marker to confirm the development of the "isolation-induced stress syndrome" before performing more complex behavioural analyses, due to consistent replicability across studies (FONE; PORKESS, 2008b).

Several lines of evidence suggest impaired prefrontal cortex-hippocampus connectivity as the neuroanatomical substrate involved in spontaneous hyperlocomotion in isolated reared rats. The prefrontal cortex and the hippocampus are critical brain areas of dysfunction in clinical schizophrenia (GLANTZ; LEWIS, 2000; LEWIS et al., 2003; PANTELIS et al., 2005; VITA; DE PERI, 2007), and lack of social stimulation would contribute to reduced volume, besides impaired neurogenesis, plasticity and connectivity in these two brain areas (BIRO et al., 2017; DAY-WILSON et al., 2006; HARTE et al., 2004; IBI et al., 2008; LU et al., 2003; PASCUAL et al., 2007; PEREDA-PÉREZ et al., 2013; QUAN et al., 2010; SCHUBERT et al., 2009; SILVA-GÓMEZ et al., 2003).

The immune system profoundly affects brain development and function, with cytokines participating in both neurodevelopment and neurogenesis processes (BORSINI et al., 2015). Even though the relationship between early-life stress and schizophrenia is widely described (SCHAFER; FISHER, 2011; VARESE et al., 2012), the biological mechanisms underlying this association remain to be elucidated. A current working hypothesis is that life stressors during critical periods of the neurodevelopment trigger an immune dysfunction characterized by

abnormal production of inflammatory cytokines (BAUMEISTER et al., 2015; KNUESEL et al., 2014; MONJI et al., 2013). An imbalance between inflammatory cytokines during early brain development results in abnormal neurotransmission in the central nervous system, affecting brain morphology (CORSI-ZUELLI et al., 2017; KHANDAKER; DANTZER, 2015; MEYER; SCHWARZ; MÜLLER, 2011a), and therefore, can increase the risk of psychiatric disorders in adulthood, including schizophrenia (KNUESEL et al., 2014; MEYER, 2011b, 2014; MEYER; SCHWARZ; MÜLLER, 2011a).

Crescent interest has been given upon enhanced pro-inflammatory cytokines in the pathophysiology of schizophrenia. In clinical trials, abnormal production of IL-6 and TNF-α in the peripheral blood of patients with schizophrenia and other psychotic disorders is the most replicated finding (GOLDSMITH; RAPAPORT; MILLER, 2016; MILLER et al., 2011; POTVIN et al., 2008; UPTHEGROVE; MANZANARES-TESON; BARNES, 2014), whereas the participation of anti-inflammatory cytokines, such as IL-10, remains poorly explored (GOLDSMITH; RAPAPORT; MILLER, 2016; MILLER et al., 2011; POTVIN et al., 2008). In the central nervous system, however, besides a pro-inflammatory state, there seem to exist a blunted anti-inflammatory activity, as demonstrated by reduced IL-10 levels in the cortex (PANDEY et al., 2017a). Nevertheless, it remains unclear whether this blunted anti-inflammatory activity is being translated into the blood.

Despite the pro-inflammatory state reported in schizophrenia, not all patients present with immune dysregulation (GOLDSMITH; RAPAPORT; MILLER, 2016). In fact, data on early-stress and inflammation in clinical studies are scarce but provide with clearly heterogenous results (COELHO et al., 2014a) that could be influenced by pharmacological treatment, substance abuse and other social and environmental factors. Rodent models provide the opportunity to investigate inflammatory cytokines simultaneously in the peripheral blood and in the brain in the absence of such influences. In the context of pwSI, however, the few existing studies have focused mainly on peripheral changes of pro-inflammatory cytokines (KO; LIU, 2016, 2015; MÖLLER et al., 2013a; WANG et al., 2017). From these, only one attempted to determine a concomitant change of cytokines (IL-6, IL-1β, TNF-α) at both the peripheral blood and the hippocampus, indicating a pro-inflammatory state (WANG et al., 2017); nevertheless, this study did not include the measurement of anti-inflammatory cytokines. Conversely, a recently published study found reduced IL-6 and IL-10, but unchanged TNF-α levels in the hippocampus of animals submitted to the pwSI model (DUNPHY et al., 2017). However, in this study blood cytokines were not investigated. To this end, whether the reduced

ability to produce anti-inflammatory cytokines is reflected in the peripheral blood remains to be explored, as no existing study included the measurement of this type of cytokine concomitantly at blood and brain in the pwSI model. The existing synergism in blood-brain cytokines would contribute as potential blood biomarkers in psychosis, at least in a subgroup of patients exposed to early-trauma.

Given the aforementioned, we evaluated a possible imbalance between protein and gene expression of pro- and anti-inflammatory cytokines (IL-6, TNF-α, IL-10) simultaneously at both blood and brain tissues (prefrontal cortex and hippocampus) of rats submitted to the pwSI model. We hypothesised that social-isolated animals would present imbalanced levels of both IL-6 and TNF-α together with reduced IL-10 in the brain, and that this deregulation of inflammatory molecules would be reflected in the peripheral blood as well. Additionally, since locomotor habituation depends on a functional prefrontal cortex-hippocampus connectivity (SILVA-GOMEZ et al., 2003), and given the participation of inflammatory cytokines in the neurodevelopment and neurogenesis of these brain sites (BORSINI et al., 2014), we have also hypothesised that the aberrant cytokine production would correlate with hyperlocomotion in social isolated-animals.

4.2 MATERIAL AND METHODS

4.2.1 Animals

Male *Wistar* rat pups (University of São Paulo, Ribeirão Preto *Campus*) were kept with their lactating dams until weaning. Right after weaning on postnatal day 21, the pups (40g) were separated from their mothers and randomly divided into two different rearing conditions for a period of 10 weeks: (A) group-housed (n = 10; 3-4 per cage); (B) isolation-reared (n = 10; housed individually). Handling was performed for cleaning purposes only and by suspending the rats by the tail (5 seconds) to allocate them into a clean cage. The same person performed all the handling procedure. Rats (grouped or isolated) were housed in the same animal facility room, kept in plastic cages (48.5 cm × 25.8 cm × 15.6 cm), in a temperature-controlled room (23 ± 1°C), under 12h light/dark cycle (lights on 06:30 a.m.) with free access to food and water. Isolated rats were always prevented from any form of contact with a conspecific, although rats could see, hear and smell their mates. The experiments were carried out according to the Brazilian COBEA (Colégio Brasileiro de Experimentação Animal) guidelines, which complies the National Institutes of Health guide for care and use of Laboratory animals (NIH Publications

No. 8023, revised 1978). This study was approved by the Ribeirão Preto School of Medicine local ethics committee (024/2016).

4.2.2. Open Field Test

On postnatal day 91, rats were tested in the open field. The apparatus consisted of a square arena (dimensions 40 cm x 72 cm x 72 cm), with the ground separated into 16 equal squares by black lines. All testing was performed in the light phase of the circadian cycle. The number of square crossings of each rat was videotaped for 20 min. Square crossings were evaluated at both the periphery and centre of the arena over the 20 min period divided into four time bins break (0-5; 5-10; 10-15; 15-20 min), using the EthoLog 2.2 software (OTTONI, 2000).

4.2.3 Sample processing

A week after the behavioural test, rats were decapitated using isoflurane as a pre-anaesthetic. Trunk blood was collected in 4 mL EDTA-containing tubes, which were immediately stored on ice for plasma and leucocytes preparation. For plasma collection, the tubes were centrifuged at 3,500 rpm at 4°C for 10 min. Peripheral blood leucocytes were collected right after the blood collection, by low-density gradient centrifugation via Ficoll-Paque PLUS (GE Healthcare), as previously described (DO PRADO et al., 2017). All the samples were stored at -80°C until the day of the assay procedure.

The whole brains were extracted, and the regions of interest (prefrontal cortex and the hippocampus) were manually dissected, frozen in isopentane and stored at -80°C before use. For cytokines protein quantification, the brain tissues were then placed in sterile phosphate-buffered saline (pH 7.4), homogenized, centrifuged and the supernatant was stored at -80°C until ready for cytokine measurement (PINHEIRO et al., 2015).

4.2.4 Multiplex assay

Cytokine measurement (IL-6, TNF-α IL-10) was performed in plasma and in tissue supernatant of brain areas collected (prefrontal cortex, hippocampus) from both social and isolated-reared rats. The three cytokines were simultaneously quantified from a single sample of each animal (25μL) using the Milliplex MAP Rat Cytokine/Chemokine magnetic bead panel

(#RECYTMAG-65K; EDM Millipore, Billerica, MA, USA; https://www.emdmillipore.com/US/en). The assay was performed in 96-well plates according to the manufacturer's instructions and the results were expressed in pg/mL. Briefly, each assay plate layout consisted of seven standards, two positive controls, two blank wells, all runned in duplicate, and up to 76 samples, as previously described (LU et al., 2017). Results were analysed on a Luminex-200 System (Luminex, Austin, TX, USA) and reported on the xPOTENT software version 3.1.

Sample wells with bead count < 50 were excluded from the analysis, according to manufacturer's instructions. Cytokines concentrations were calculated through the five-parameter logistic curve-fitting method using the median fluorescence intensity. All data were corrected using the Milliplex Analyst software.

4.2.5 Gene expression analysis

Gene expression analyses were conducted for *IL-6, TNF* and *IL-10* at prefrontal cortex, hippocampus, and peripheral leucocytes. Total RNA was extracted using the All prep DNA/RNA mini kit (Qiagen, Valencia). NanoDrop® ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) was used to determine the purity and quantity of the RNA samples. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Foster City, CA), by using approximately 400 ng of each RNA sample, and 100 ng were then diluted in H2O, mixed with TaqMan® Universal PCR Master Mix (Life Technologies) and disposed in a 96 well plate. The Real-Time quantitative PCR (RT-qPCR) primers and probes were chosen following the best coverage and mispriming absence according to manufacture's guarantee (http://www.thermofisher.com/br/en/home/life-science/pcr/real-time-pcr/real-time-pcr-assays.html, Applied Biosystems, USA). Probes and primers of 3 target genes (*IL6*: Rn01410330_m1; *TNF:* Rn01525859_g1 and *IL-10:* Rn01483988_g1) and two housekeeping genes (β-actin, *ACTB;* proteasome subunit beta type-2, *PSMB2*) were preloaded in the wells and the experiments were performed in accordance with the manufacturer's instructions using the ViiATM 7 Real-Time PCR System (Life Technologies). Gene expression was quantified using the Comparative threshold (Ct) method (ΔΔCt Method) (LIVAK; SCHMITTGEN, 2001; SCHMITTGEN; LIVAK, 2008), and the amount of target gene was normalized to the housekeeping genes and determined by $2^{-\Delta\Delta Ct}$, as previously described (JULIAN et al., 2014; WALDER et al., 2014), with relative expression levels reported as fold

change. Ct values higher than the cut-off of 35 were not considered as a reliable expression value, according to manufacturer's recommendations (Sigma Life Science, 2011), and therefore were excluded from the statistical analysis.

4.2.6 Data analysis

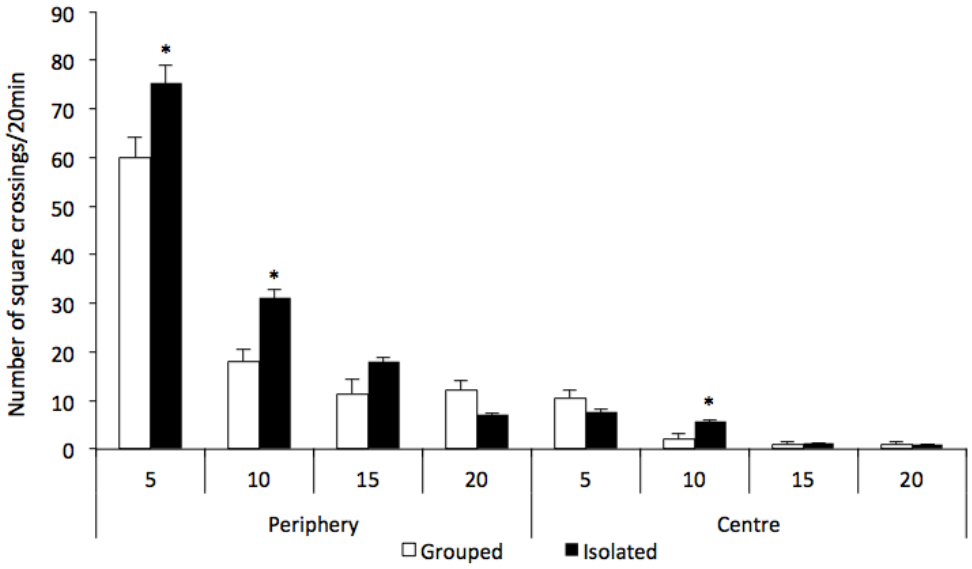
Behavioural data were analysed with repeated measures ANOVA (*Hotelling's Trace*) considering the experimental condition (grouped or isolated) as the between factor, and the number of crossings at the arena (periphery or centre) and time (0-5; 5-10; 10-15; 15-20 min) as within factors. Bonferroni was set as the *post-hoc* test. Cytokines measurements were logarithmic transformed for the statistical analysis with the purpose of normalizing the distribution errors and homogenizing variances, but figures are expressed as raw values. Significant differences between groups were determined by the *Student's* t test. Correlation analyses (*Spearman's rho*) were performed between cytokines measurements and total number of square crossings in the open field for isolated-reared animals. Statistical analysis was performed using the SPSS, version 24.0 (IBM Corp: Armonk, NY: USA). Values of $p \leq 0.05$ were considered significant.

4.3 RESULTS

4.3.1 Behavioural data

There were significant differences between groups regarding the locomotion in the open field test [group factor $F_{(1,15)} = 419.191$; $p = 0.030$; group & local & time interaction $F_{(3,13)} = 7.047$; $p = 0.005$] **(Figure 1)**. Isolated-reared rats habituated slowly, as the number of square crossings was significantly greater for this group when compared to controls [local factor $F_{(1,15)} = 294.402$; $p < 0.001$]. Rats reared isolated presented hyperlocomotion at the two first time bins (0-5 and 5-10 min) at periphery of the arena when compared to grouped [0-5 min: $F_{(1,15)} = 6.209$; $p = 0.025$; 5-10 min: $F_{(1,15)} = 14.272$; $p = 0.002$, respectively]. A higher number of crossings during the second time bin (5-10 min) at the centre of the arena was also detected in isolated reared animals when compared to grouped [5-10 min: $F_{(1,15)} = 6.452$, $p = 0.023$].

Figure 1: Effect of rearing condition (isolated vs. grouped) at spontaneous number of crossings at the open field (20 min)

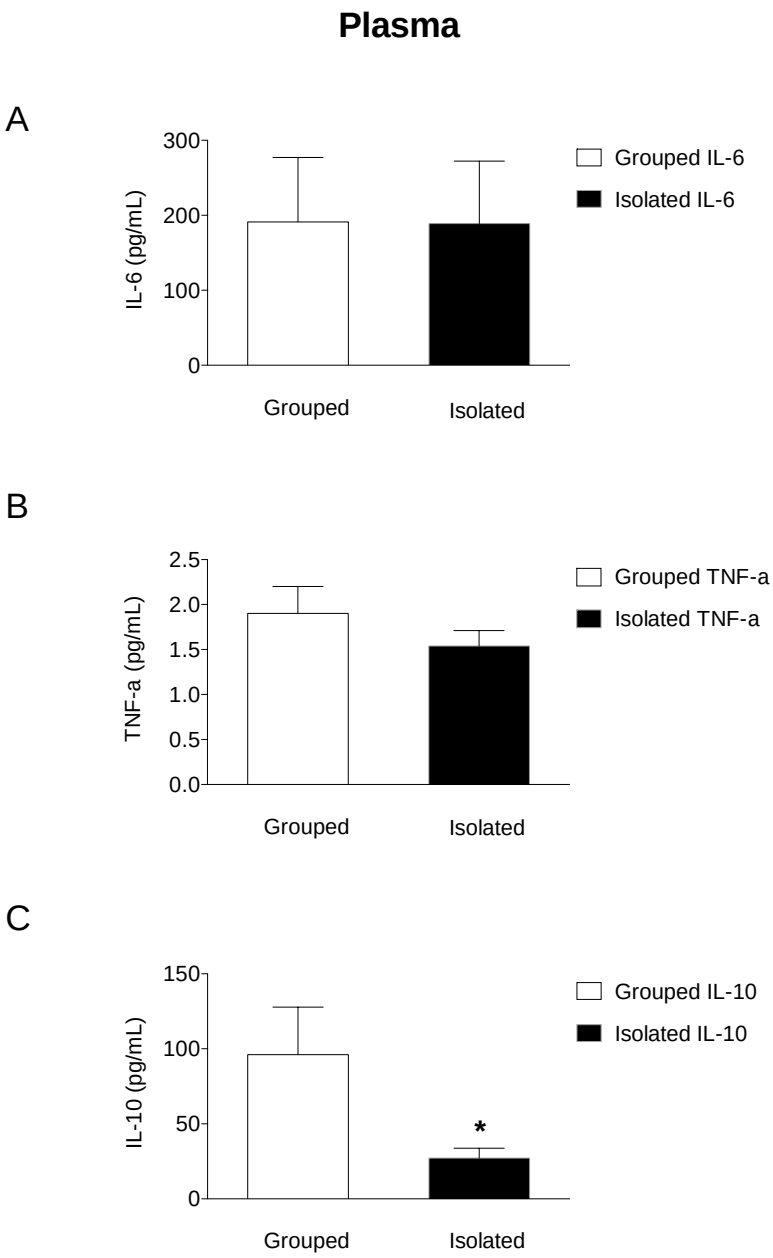


Number of crossings is represented as 5-min time bins over the 20 min of test. Repeated measures ANOVA showed a significant group & local & time interaction and *post-hoc* analysis revealed that rats reared isolated (n = 8) presented hyperlocomotion at the periphery of the arena at 5 and 10-min time bins, and at the centre of the arena at 10-min time bin when compared to grouped rats (n = 9). Data are expressed as mean ± S.E.M. * $p < 0.05$ compared to grouped rats.

4.3.2 Plasma cytokines concentrations

In the peripheral blood **(Figure 2A-C)** isolated-reared rats had lower c) IL-10 plasma levels when compared to grouped-housed animals ($t = 2.265$; d.f = 17; $p = 0.044$), whereas no significant differences between the two groups were found either for a) IL-6 ($t = 0.386$; d.f = 17; $p = 0.704$) or b) TNF-α ($t = 1.077$; d.f = 17; $p = 0.297$).

Figure 2: Effect of rearing condition (isolated vs. grouped) on cytokines plasma levels of rats exposed to 10 weeks of social isolation

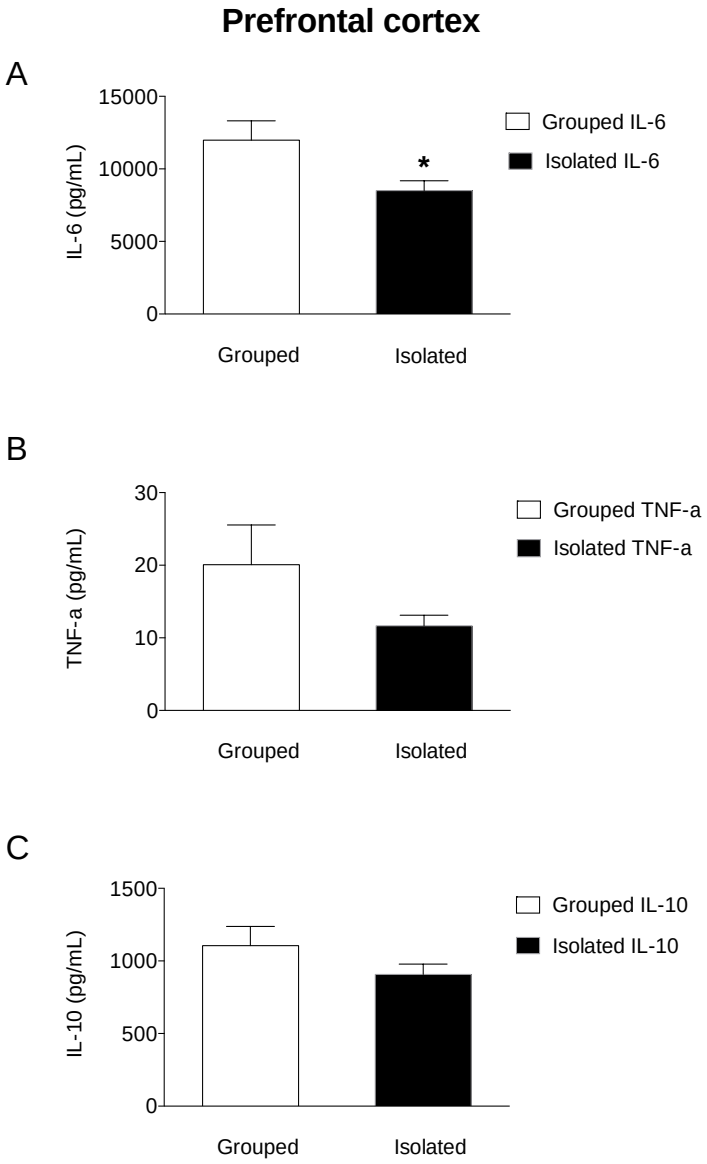


No difference was found for a) IL-6 or b) TNF-α plasma concentration in isolated rats (n = 10) when compared to grouped housed (n = 9). However, isolated rats (n = 10) showed lower b) IL-10 plasma concentration when compared to grouped-housed (n = 9). Data are expressed as mean ± S.E.M and given as pg/mL. *$p < 0.05$ (*Student's t test*) compared to grouped rats.

4.3.3 Brain cytokines concentrations

In the prefrontal cortex **(Figure 3A-C)**, isolated-reared rats had reduced levels of a) IL-6 ($t = 2.280$; d.f. = 16; $p = 0.037$), but not of b) TNF-α ($t = 1.476$; d.f. = 16; $p = 0.159$) or c) IL-10 ($t = 1.035$; d.f. = 16; $p = 0.316$) when compared to controls.

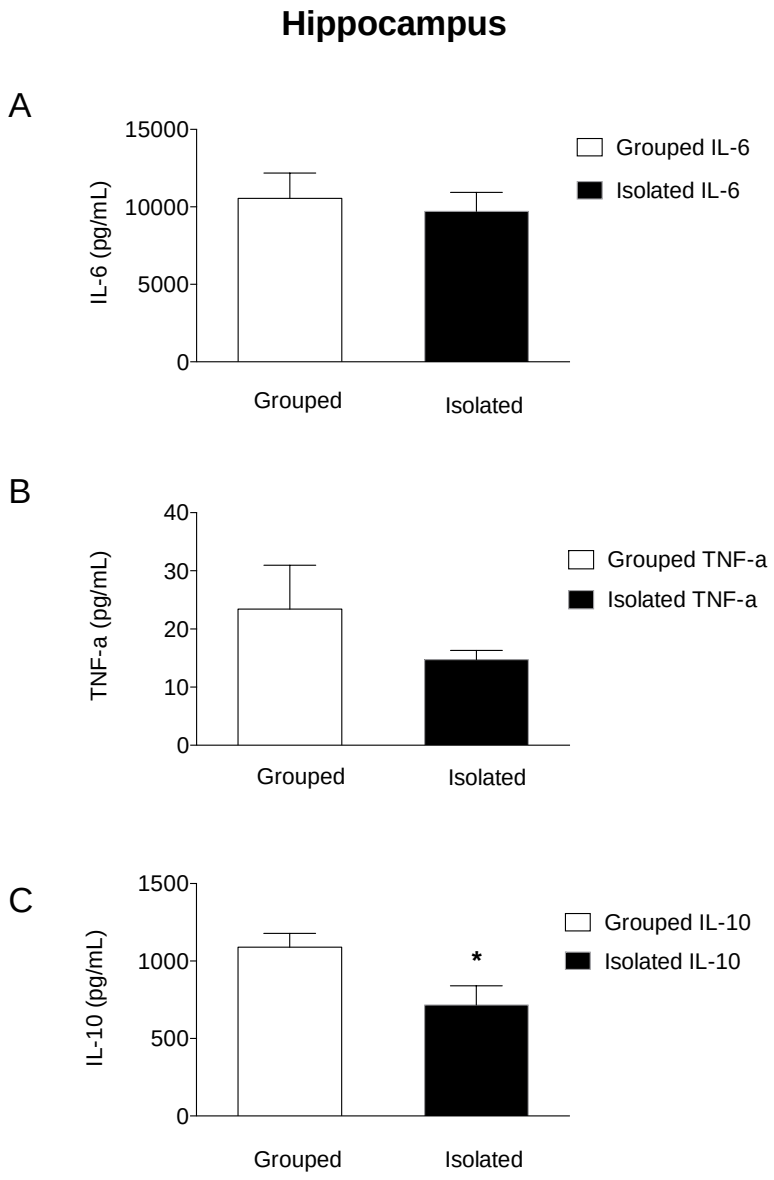
Figure 3: Effect of rearing condition (isolated vs. grouped) on cytokines in the prefrontal cortex of rats exposed to 10 weeks of social isolation



Isolated rats (n = 9) had reduced a) IL-6, but no difference was found for b) TNF-α or c) IL-10 in comparison to grouped rats (n = 9). Data are expressed as mean ± S.E.M and given as pg/mL. *$p < 0.05$ (*Student's t test*) compared to grouped rats.

In the hippocampus **(Figure 4A-C)**, the concentration of c) IL-10 was significantly lower than in group-housed rats ($t = 2.318$; $d.f = 15$; $p = 0.035$). Conversely, no difference was found for a) IL-6 ($t = 0.223$; $d.f. = 15$; $p = 0.827$) or b) TNF-α ($t = 0.745$; $d.f. = 15$; $p = 0.475$).

Figure 4: Effect of rearing condition (isolated vs. grouped) on cytokines in the hippocampus of rats exposed to 10 weeks of social isolation

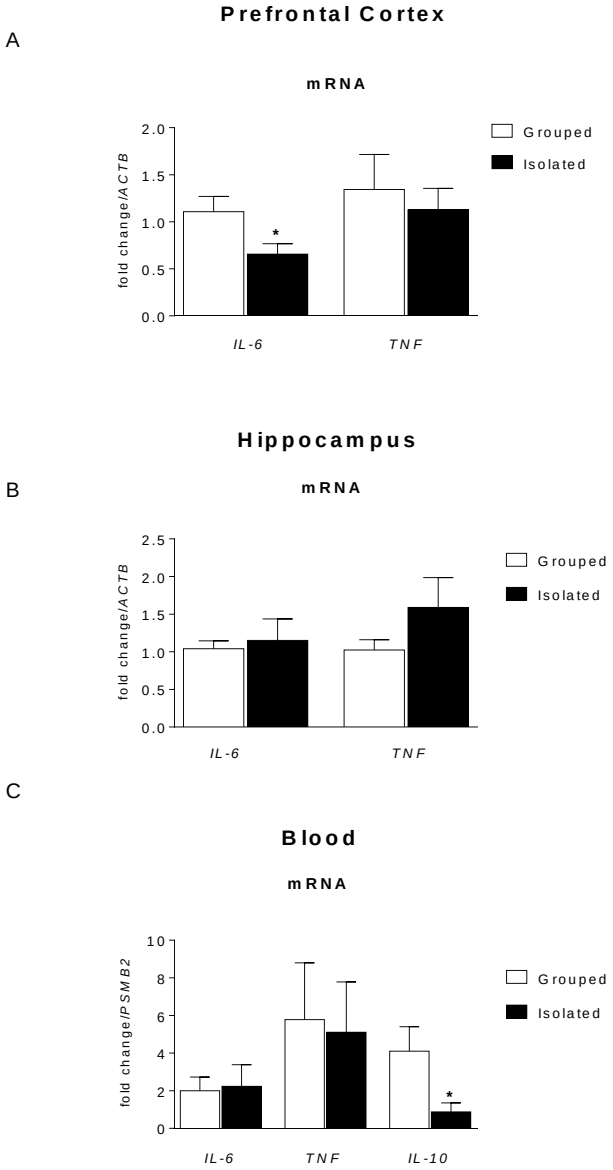


Rats reared isolated (n = 9) presented lower levels of c) IL-10, with no differences for b) TNF-α or a) IL-6 when compared to controls (n = 8). Data are expressed as mean ± S.E.M and given as pg/mL. *$p < 0.05$ (*Student's t test*) compared to grouped rats.

4.3.4 Cytokines gene expression

The RT-qPCR showed that *ACTB* and *PSMB2* were expressed at a stable level across all the samples. In the brain **(Figure 5 A-B)**, Ct values were higher than the cut-off of 35 for *IL-10,* which are considered below the detection limit of expression (data not shown). In the prefrontal cortex, isolated rats presented down regulated *IL-6* mRNA expression ($t = 2.234$; d.f. = 17; $p = 0.039$; Figure 5A, *left*), but not in the hippocampus ($t = 0.383$; d.f. = 17; $p = 0.707$; Figure 5B, *left*). No difference was found for *TNF* gene expression (prefrontal cortex: $t = 0.485$; d.f. = 17; $p = 0.634$; Figure 5A, *right*; hippocampus: $t = 1.407$; d.f. = 17; $p = 0.177$; Figure 5B, *right*). In the peripheral blood **(Figure 5 C)** *IL-10* mRNA was significantly down regulated in isolated-reared rats ($t = 2.342$; df = 12; $p = 0.049$), with no significant differences in IL-6 ($t = 0.172$; df = 12; $p = 0.867$) or TNF mRNA ($t = 0.166$; df = 12; $p = 0.871$).

Figure 5: Effect of rearing condition (isolated vs. grouped) on cytokines gene expression in the prefrontal cortex, hippocampus, and peripheral blood of rats after 10 weeks of social isolation

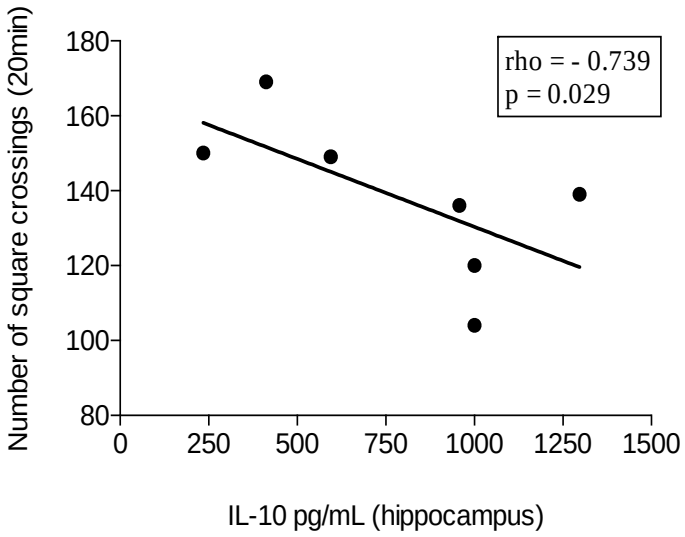


A) Prefrontal Cortex: Isolated-reared rats (n = 9) presented reduced *IL-6* mRNA in the prefrontal cortex when compared to controls (n = 10). **B) Hippocampus:** No difference was found for isolated (n = 9) or grouped-reared (n = 10) rats in *IL-6* or *TNF* mRNA. **C) Peripheral blood:** Isolated-reared rats (n = 7) had reduced IL-10 mRNA in peripheral blood leucocytes than controls (n = 7). Cytokine mRNA expression was measured by qRT-PCR. Data are expressed as mean fold change ± S.E.M in mRNA levels using the housekeeping genes (*ACTB, PSMB2*) as reference. *p < 0.05 (*Student's t test*) compared to grouped rats.

4.3.5 Behaviour, blood and brain cytokines correlations

Isolated-reared rats presented a significant negative correlation between the total number of square crossings and the IL-10 concentration in the hippocampus (rho: - 0.739; p = 0.029) **(Figure 6)**. No other significant correlations were found between locomotor behaviour and the remaining cytokines in the isolated-reared rats (p > 0.05 for all).

Figure 6: Correlation between hippocampal IL-10 and number of square crossings at the open field in isolated-reared rats



Isolated-reared rats presented a significant negative correlation between the total number of square crossings (20min) and the IL-10 concentration in the hippocampus (rho: - 0.739; p = 0.029; *Spearman correlation;* n = 7). Data are expressed as mean±S.E.M and given as pg/mL for cytokine IL-10 in the hippocampus.

4.4 DISCUSSION

The present study demonstrates, for the first time, that rats exposed to prolonged periods of social isolation since weaning have a set of peripheral and central inflammatory changes in adulthood, characterized by reduced IL-10 protein and gene expression in the blood and reduced IL-10 protein in the hippocampus, along with decreased IL-6 and its mRNA expression in the prefrontal cortex. Besides, we demonstrate that IL-10 hippocampal concentrations are negatively correlated with locomotion. Of particular note, these results suggest that prolonged

early-life adverse events reduce the anti-inflammatory cytokine IL-10 from blood-to-brain, which may contribute to the occurrence of abnormal behaviour in adulthood.

The pattern of spontaneous hyperlocomotion observed in our study is consistent with other investigations submitting *Wistar* rats to the same pwSI protocol (DOMENEY; FELDON, 1998; HEIDBREDER et al., 2000). Spontaneous hyperlocomotion is described as one of the earliest (appearing after two weeks of social isolation) and one of the most robust observation in isolated-reared rodents, and reflects an inability to habituate following placement in a novel environment (FONE; PORKESS, 2008b). Accordingly, in the present study, we confirm the robustness and existence of hyperlocomotion even after 10 weeks of social isolation.

Increased pro-inflammatory cytokines plasma concentration in chronic isolated rats was not confirmed in this study; instead, we observed reduced peripheral protein and gene expression of the anti-inflammatory cytokine IL-10, in the absence of any concomitant changes regarding IL-6 or TNF-α in isolated reared rats. From the existing studies investigating peripheral blood cytokines in rodents under pwSI, data are still conflicting, with some pointing to enhanced (KO AND LIU, 2016, 2015; MÖLLER et al., 2013; WANG et al., 2017), decreased or unchanged levels (DUNPHY et al., 2017; Möller et al., 2013), especially in IL-1β, IL-6, TNF-α, INF-γ, whereas changes in IL-10 are absent (KO; LIU, 2015) or not investigated (MÖLLER et al., 2013; WANG et al., 2017). Parallel to the observed reduced protein and gene expression of IL-10 in the peripheral blood, we have also observed the same direction of change of this cytokine in the hippocampus of isolated reared animals, again in the absence of modifications on the other two other cytokines investigated.

Congruent to our finds in the peripheral blood not showing enhanced pro-inflammatory activity, we also observed reduced IL-6 protein and gene expression in the prefrontal cortex of these animals. Although our results differ from a previous study, which detected enhanced IL-1β, IL-6 and TNF-α in the hippocampus of rats under social isolation (WANG et al., 2017), we are in agreement with a recent study reporting decreased levels of both IL-6 and IL-10 in the brain of isolated-reared rats (DUNPHY et al., 2017), in the absence of abnormalities of other pro-inflammatory markers.

These discrepancies could be due to methodological factors, including those related to the rat strain used, and probably more important, the length of social isolation period applied, as both can account for behavioural and inflammatory response discrepancies. Decreased inflammatory cytokines have been reported in chronic long-term social isolation protocols, consisting of 8-10 weeks of social isolation (CRUCES et al., 2014; DUNPHY et al., 2017;

MÖLLER et al., 2013a), while the enhanced pro-inflammatory cytokines have been observed in animals exposed to a shorter social isolation period (four to six weeks) (KO; LIU, 2015; KRÜGEL et al., 2014; WANG et al., 2017). In this respect, we herein speculate that the existence of an enhanced inflammatory profile tends to occur at the initial phases of the social isolation period, followed an exhaustion of the immune system as a possible compensatory mechanism under prolonged periods of stress.

Several lines of evidence might support our hypothesis of an impaired anti-inflammatory cytokine under prolonged periods of social isolation. For instance, no effect of rearing condition was seen on IL-10 plasma concentration of *Sprague-Dawley* rat pups exposed to four weeks of social isolation (KO; LIU, 2015); however, exposing these animals to eight-weeks social isolation decreases IL-4 plasma concentration, another potent anti-inflammatory cytokine (MÖLLER et al., 2013a). Similarly, IL-6 plasma elevation has been described in four weeks social isolated animals (KO; LIU, 2015), contrasting with the reduction of this cytokine after eight weeks of social isolation (MÖLLER et al., 2013a). Increased microglia activation is seen in the rats hippocampus under six weeks isolation period (WANG et al., 2017), contrasting with a reduced glial density in the prefrontal cortex after eight weeks of social isolation (BIRO et al., 2017).

Indeed, clinical studies of psychosis also support that changes in inflammatory markers may be variable. For instance, in the *postmortem* schizophrenia brain, pro-inflammatory cytokines concentrations seem to be inconsistent across studies, with some showing enhanced or decreased concentrations (TRÉPANIER et al., 2016; VAN KESTEREN et al., 2017), but the scarce data regarding anti-inflammatory factors point to reduced concentrations of the soluble IL-2 receptor in the cerebrospinal fluid (WANG; MILLER, 2017), and reduced levels of both IL-1 receptor antagonist (IL-1RA) (VAN KESTEREN et al., 2017) and IL-10 in the cortex (PANDEY et al., 2017a). *In vivo* studies using PET scan are in agreement, showing increased glia activation in the central nervous system of patients in the early stages (VAN BERCKEL et al., 2008), but not in chronic stages of psychosis (KENK et al., 2015; TAKANO et al., 2010). Besides that, systematic reviews of clinical studies on early-life adversities and inflammation point again to inconsistent findings, highlighting that different types and duration of early social adversities may promote changes in the inflammatory system in many diverse directions (COELHO et al., 2014a).

Interestingly, in our study, the reduced ability to produce the anti-inflammatory cytokine IL-10 was reflected at both the peripheral blood as well as in the brain. In isolated animals, we

found reduced blood and hippocampal IL-10 concentrations, and hippocampal IL-10 concentrations were negatively correlated with locomotion. In fact, IL-10 is a potent anti-inflammatory cytokine, produced mainly by M-2 type macrophages, Th-2 lymphocytes, T-regulatory lymphocytes and B regulatory cells. In the central nervous system, IL-10 is also produced by microglia, and astrocytes (LEDEBOER et al., 2002; PÉREZ-DE PUIG et al., 2013; ROQUE et al., 2009). There may be several pathways through which IL-10 influences behaviour. One indirect mechanism may have to do with its anti-inflammatory properties in dampen an aberrant production of pro-inflammatory cytokines. A more direct mechanism, however, involves its role in the central nervous system. IL-10 protects glial cells against lipopolysaccharide (LPS)/IFN-γ induced cytotoxicity (MOLINA-HOLGADO et al., 2001), and prevents glutamate-mediated neuronal death by blocking proapoptotic proteins and by enhancing anti-apoptotic factors (BACHIS et al., 2001). IL-10 also regulates neurogenesis processes, and polarizes macrophages and microglia cells towards a protective state (COUPER; BLOUNT; RILEY, 2008; KWILASZ et al., 2015; LEDEBOER et al., 2002; LOBO-SILVA et al., 2016; ROQUE et al., 2009; STRLE et al., 2001). The maternal immune activation model of psychosis leads to decreased IL-10 levels at both the maternal fluids and in the foetal brain, causing a set of psychotic-like behaviour disturbances, which are all prevented by IL-10 overexpression in the macrophages of pregnant dams (MEYER, 2014; OSKVIG et al., 2012; SMITH et al., 2007). In psychosis, reduced IL-10 levels are negatively correlated with negative and cognitive symptoms (XIU et al., 2014).

In addition, although commonly described as a pro-inflammatory cytokine, IL-6 is actually a multifunctioning cytokine, sometimes described as a neurotrophin factor due to its many anti-inflammatory properties. In the central nervous system, both neurons and glial cells express IL-6 and its membrane receptor (BURTON; SPARKMAN; JOHNSON, 2011). IL-6 enhances neurogenesis and gliogenesis, and both *in vitro* and *in vivo* studies demonstrate that IL-6 promotes survival of prosencephalic and septal cholinergic neurons, as well as mesencephalic catecholaminergic neurons (HAMA et al., 1989, 1991). This survival mechanisms may involve IL-6 inducing the expression of the neurotropic factor brain-derived neurotrophic factor (BDNF) (MURPHY et al., 2000). Akin to that, transgenic mice overexpressing IL-6 present faster healing and recovery following traumatic brain injury (SWARTZ et al., 2001), and microglia-induced IL-6 was shown to protect against neuronal loss induced by viruses (CHUCAIR-ELLIOTT et al., 2014). Differently, intracerebroventricular administration of IL-6 neutralizing antibody eliminates the protector effect of social housing

against cerebral stroke (KARELINA et al., 2009). Together, these evidences bring IL-6 as very important neuroprotective factor.

The fact that we failed to detect associations between hippocampus and prefrontal cortex in the cytokine concentrations is in agreement with studies reporting that cytokine expression typically occurs in an age and region-specific manner (GARAY et al., 2013). Moreover, the absence of associations between changes in blood and brain TNF-α or IL-6 may reflect the complexity reported in clinical studies. For instance, Wang and Miller (2017) identified four studies on blood and cerebrospinal fluid (CSF) cytokines in the same psychiatric patients. All these studies failed to report similar blood-to-CSF abnormalities for IL-6, TNF-α, IL-1β or IL-8 (KATILA et al., 1994; LINDQVIST et al., 2009; SASAYAMA et al., 2013; VAN KAMMEN et al., 1999).

In fact, we found an association between IL-10 protein and gene expression in the peripheral blood, but not in the brain, in which this association was found only for IL-6. However, dissociation between gene and protein expression are not uncommon (MAIER; GÜELL; SERRANO, 2009), and some hypothesis include biological factors related to transcriptional, translational or post-translational changes (PANDEY et al., 2017a). Cytokines tend to have a dynamic response presenting with a complex positive and negative feedback loop to regulate one-another expression (GARAY et al., 2013), and in order to minimize possible cytokine quantification errors, we used Luminex x-map technology for cytokine measurement, which provides the most consistent sensitivity for cytokines concentration using a minimal sample volume (25uL), enabling patterns of numerous cytokines to be examined, an approach that is not possible with conventional technologies (BELZEAUX et al., 2017). Besides, we employed TaqMan technology for RT-qPCR, which leads to less non-specific products and more reliable results (YIN et al., 2001), different from studies using SYBR Green.

A limitation of this study is the fact that the behavioural investigation was focused only on hyperlocomotion. However, there is evidence showing that a combination of multiple stressors influence cytokine response (LOVELOCK; DEAK, 2017), and consequently could mask the effect of the prolonged chronic social isolation on cytokine production. We selected hyperlocomotion because it is considered the most consistent response to social isolation, representing a suitable marker to confirm the development of the "*isolation-induced stress syndrome*" before performing more complex behavioural analyses (FONE; PORKESS, 2008b). Another limitation is that we were unable to include the measurement of other types of

cytokines; therefore, the investigation of other inflammatory markers would deserve further exploration in the pwSI model.

Our results bring the question to whether peripheral blood cytokines may reflect cytokines in the central nervous system. We also suggest that chronic stress may reduce anti-inflammatory cytokine in blood and brain. Despite it deserves more exploration, this synergism would open the opportunity to target potential blood biomarkers to identify subgroup of patients exposed to early trauma. Given the fact that pwSI represents a pure environmental and non-pharmacological preclinical model of schizophrenia with the appropriate triad of validity (FONE; PORKESS, 2008b), this study emphasizes the participation of early-traumatic environmental factors in the inflammatory hypothesis of schizophrenia.

4.5 CONCLUSION

Taken together, our study shows for the first time that exposing rats to prolonged periods of social isolation since weaning reduces the protein and gene expression of the anti-inflammatory cytokine IL-10, and this is translated from blood-to-brain. Although the unexpected decrease in IL-6 and unchanged TNF-α levels relative to controls contrast the expected pro-inflammatory phenotype suggested for schizophrenia, we are in accordance to reports suggesting immune dysregulation in many directions in individuals exposed to early trauma (COELHO et al., 2014a). This may be detrimental for altered synaptic and cortical connectivity and may contribute to the occurrence of abnormal behaviour in adulthood. We also highlight possible differences between shorter versus prolonged periods of social isolation. Future investigations on larger samples are needed to answer this question and would help to understand whether this represents an adaptive response. The results found herein may reflect the challenges in determining the temporal profile of blood biomarkers across the course of the clinical illness (prodrome, first-episode, acute relapse, chronic), empathizing perhaps the importance of considering disease course as a confounding variable in clinical trials. We also emphasize the need of more investigations focused on the association between early trauma and inflammation in schizophrenia. Despite it deserves more exploration, this synergism would open the opportunity to identify subgroup of patients exposed to early trauma.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

FC-Z designed the study, conducted the experimental procedures, analysed and interpreted the data and wrote the first draft of the manuscript. HF designed the study, obtained the approval from the ethical committee, wrote the protocol, was in charge of the data collection, including molecular experiments, and revised the manuscript. CL collected data and collaborated on the statistical analysis. RS helped on behavioural data analysis. GB collaborated with the protocol design and supervised cytokines measurements. SJ collaborated in the design of the study and supervised data collection. PM obtained funding and critically revised the manuscript. PL-J obtained funding, collaborated with data analyses and with the interpretation of the results, and critically revised the manuscript. CD-B conceived the study, obtained funding, obtained the approval from the ethical committee, supervised data analyses, participated in the interpretation of the results and critically revised the manuscript. All the authors approved the final version of the manuscript.

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*5. Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma**

**Submitted to Psychological Medicine*

5. CYTOKINE PROFILE IN FIRST-EPISODE PSYCHOSIS, UNAFFECTED SIBLINGS AND COMMUNITY-BASED CONTROLS: THE EFFECTS OF FAMILIAL LIABILITY AND CHILDHOOD TRAUMA

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4,457 words
Three Tables
One Figure

Abstract

Background. In this paper, we aim to investigate the role of familial liability, childhood trauma and recent stress in determining immune abnormalities at the onset of psychosis. **Methods.** We recruited 114 first-episode psychosis (FEP) patients, 57 unaffected siblings of FEP patients, and 251 community-based controls. Cytokines plasma levels (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) were measured and differences across the three groups analysed after controlling for age, gender, body mass index and tobacco smoking. Childhood maltreatment was measured by the Childhood Trauma Questionnaire, recent stress by the List of Threatening Experiences Questionnaire and plasma cytokines by multiplex. **Results.** FEP had significantly higher levels of IL-6, TNF-α, IL-10 and TGF-β when compared with controls, and also higher levels of IL-1β, IL-6, TNF-α, and IL-10 when compared with their siblings. Siblings presented decreased IL-1β when compared with controls. Physical childhood abuse was associated with increased levels of TGF-β in FEP but with decreased levels in controls. Other childhood trauma subtypes and recent stressors did not affect cytokines levels in none of the three groups. **Conclusions.** Experience of childhood maltreatment, specifically physical abuse, may contribute as a long-term immune priming for the TGF-β pathway in both patients and community-based controls. Normal or reduced levels of cytokines in siblings represent possibly a protective factor and suggest that the identified inflammatory profile in FEP can be a real pathophysiological component of psychosis.

Keywords: Anti-inflammatory cytokines; childhood maltreatment; cytokines; early-life stress; first-episode psychosis; inflammation; pro-inflammatory cytokines; schizophrenia; siblings; transforming growth factor-beta.

5.1 INTRODUCTION

Increasing body of evidence suggests a role of the immune system in the development of psychoses (BAUMEISTER et al., 2014; GOLDSMITH; RAPAPORT; MILLER, 2016). To date, it remains unclear whether the immune activation present at the onset of psychoses is mainly to be ascribed to genetic predisposition or to the exposure to environmental factors, such as childhood trauma, which is well-known to be associated with both onset of psychoses and increased inflammation (Baumeister *et al.* 2016; McGrath *et al.* 2017).

The presence of increased inflammation in patients with psychoses has been supported by a number of meta-analyses demonstrating enhanced levels of blood cytokines and cytokines receptors in patients with schizophrenia, as well as in drug-naïve patients in their first-episode of psychosis (FEP), with the most replicated findings for interlekin (IL)-6, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist (IL-1RA), and soluble IL-2 receptor (sIL-2R) (GOLDSMITH; RAPAPORT; MILLER, 2016; MILLER et al., 2011; POTVIN et al., 2008; UPTHEGROVE; MANZANARES-TESON; BARNES, 2014; ZAJKOWSKA; MONDELLI, 2014). Further evidence comes from reports of increased blood lymphocytes and monocytes in these patients (DREXHAGE et al., 2010; MILLER et al., 2013). In support of the contribution of genetic predisposition to immune activation present in psychoses, genome-wide association studies strongly suggest association between schizophrenia and the major histocompatibility complex region on chromosome 6p22.1, which is strictly linked to the immune system (RIPKE et al., 2014).

However, genetic predisposition may not be the only factor playing a role in increased immune activation in psychoses. Indeed, research into the aetiology of schizophrenia and other psychotic disorders suggest a complex interaction of genetic risk interfacing with environmental adversities occurring at critical periods of the neurodevelopment (OWEN; SAWA; MORTENSEN, 2016; VAN OS; KENIS; RUTTEN, 2010). There is now robust cross-national epidemiological evidence that childhood maltreatment increases the risk of psychotic experience across the life span in more than two-fold and in a dose-response fashion, with the highest association being attributed for sexual and physical abuse (MCGRATH et al., 2017). Furthermore, patients with psychosis are almost three times more likely to have been exposed to childhood trauma, and the prevention of traumatic experiences could reduce the incidence of psychosis by 33% (VARESE et al., 2012). Interestingly, one of the possible biological mechanisms underlying the association between early-life stress and psychoses is the trigger of an immune dysfunction characterized by abnormal production of inflammatory cytokines in

adulthood (in particular IL-6 and TNF-α) (COELHO et al. 2014; BAUMEISTER et al. 2016). Previous studies suggest that not all the types of trauma may affect the immune system in the same way and that physical and sexual abuse are associated with the strongest effects on the immune system (BAUMEISTER et al. 2016).

Few studies have attempted to investigate whether increased inflammation in psychosis could be attributed to the increased prevalence of early life adversities, and current evidence is limited and conflicting. Previous studies were limited by sample sizes ($n<50$) and lack of control for confounding factors related to age, gender, body mass index (BMI), tobacco smoking and pharmacological treatment. Peripheral inflammatory cytokines can be affected by these confounding factors, and therefore, controlling for these factors is needed to draw more reliable conclusions.

Recently, Aas et al. 2017 investigated inflammatory markers (high-sensitive C-reactive protein – hs-CRP), soluble TNF receptor type 1, and glycoprotein (gp) 130 and childhood abuse severity (combined as effects of sexual, emotional and physical abuse) in a large sample of patients with diagnoses of schizophrenia/bipolar disorder ($n=271$) and healthy controls ($n=212$), reporting lower levels of gp130 and higher levels of hs-CRP in patients with childhood abuse; however, when they controlled for BMI the effects of childhood maltreatment on hs-CRP disappeared (AAS et al., 2017). An important limitation of that study is the sample, composed by patients suffering with chronic schizophrenia, in whom the inflammatory markers could have been largely affected by other factors, most notably the effects of antipsychotic treatment on metabolic parameters or BMI (BAUMEISTER et al. 2016; CALEVRO et al. 2018).

In the present study, we investigated the association between childhood trauma and inflammation in psychoses in a large epidemiological sample of patients recruited in Brazil, where the estimates of childhood maltreatment can reach figures over 40% in the south-east part of the country (NUNES & SALES, 2016), and government's budget for childhood maltreatment prevention has been considered inadequate (ISPCAN, 2014). In order to investigate the possible role of familial liability to immune activation, we also recruited a large number of unaffected siblings of FEP patients. The inclusion of siblings as a high-risk group provides the advantage of controlling for possible effects of shared environmental and genetic risks in the context of inflammatory dysregulation that has not been explored elsewhere. We aimed to: i) investigate plasma cytokine levels among patients, patients' siblings, and community-based controls, controlling for age, gender, BMI and tobacco smoking, and ii) investigate the role of childhood maltreatment and recent stress in determining the differences

in cytokine levels shown among the above groups, controlling for confounding factors. We hypothesized that: i) FEP patients will have increased levels of inflammatory cytokines (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β) when compared with controls, and unaffected siblings will act as an intermediate group; ii) reports of traumatic events will be associated with increased levels of inflammatory markers in all three groups; and iii) the subtypes of childhood trauma will impact differently on the levels of the inflammatory markers.

5.2 MATERIAL AND METHODS

This case-sibling-control study is part of the epidemiological investigation named "STREAM" (Schizophrenia and Other Psychoses Translational Research: Environment and Molecular Biology) conducted in Ribeirão Preto catchment area (comprised by 26 counties with around 1.3 million inhabitants, located in the São Paulo state, Brazil) between 2012 and 2015 (DEL-BEN et. al., submitted). The STREAM study is also part of the multicentre "EU-GEI" (European Network of National Schizophrenia Networks Studying Gene-Environment Interactions; http://www.eu-gei.eu/), an incidence and case-sibling-control study investigating gene and environment interactions in psychosis (JONGSMA et al., 2017).

5.2.1 Participants

As previously described (LOUREIRO et al., 2018), we recruited patients in their first contact with mental health services due to psychotic symptoms during the study period. Any patients with psychotic symptoms originated from other medical condition or substance intoxication/withdrawal were excluded.

Patients' siblings were invited to participate in the study considering patients' agreement and the absence of lifetime history of psychotic symptoms.

Population-based controls were recruited considering the demographic characteristics of the Ribeirão Preto catchment area stratified by age and gender, according to the Brazilian Official Census Bureau 2010 (Instituto Brasileiro de Geografia e Estatística, IBGE, www.ibge.gov.br). Controls with lifetime history of psychotic symptoms were not included.

As part of the inclusion criteria, all the participants aged between 16-64 years old and were living in the Ribeirão Preto catchment area. This study was approved by the local research ethic committee.

Initially, we recruited 507 participants with blood collection (166 FEP, 76 siblings, 265 population-based controls). From these, we excluded 17 participants (8 patients, 1 sibling and 8 controls) presenting with any of the following: nephropathy, urinary tract infection, dengue fever, rheumatic fever, human immunodeficiency virus, syphilis, Crohn's disease, throat infection, pregnancy, corticosteroid treatment, multiple sclerosis, pneumonia, and hidradenitis suppurative. BMI data was missing in 42 patients, 18 siblings, and 6 controls, and two patients did not answer completely the childhood trauma questionnaire, and therefore were also excluded from the study.

5.2.2 Clinical Assessment

Diagnosis was obtained for all participants using the Structured Clinical Interview for DSM-IV, clinical version (SCID-CV) (DEL-BEN et al., 2001; FIRST et al., 1997). We used the Brief Psychiatric Rating Scale (BPRS) for the clinical assessment of symptom severity at the moment of blood collection (CRIPPA et al., 2001; OVERALL; GORHAM, 1962), and the Nottingham Onset Schedule (SINGH et al., 2005) to register psychosis onset date and the pharmacological treatment starting date. History of psychoactive substance use (lifetime and/or current) was assessed by The Cannabis Experience Questionnaire – Modified Version (CEQmv) (DI FORTI et al., 2009).

5.2.3 Stress measurements

We assessed the history of childhood maltreatment in our sample by using the Childhood Trauma Questionnaire (CTQ) (BERNSTEIN et al., 2003; GRASSI-OLIVEIRA; STEIN; PEZZI, 2006). The CTQ short form is a self-report questionnaire consisting of 25 items rated on a 5-point Likert scale (1 = never true; 5 = very often true) ranging from 5 to 25 points in order to assess the exposure to sexual, physical and emotional abuse, and physical and emotional neglect. The sum of values of the five scales generates the CTQ total score, which ranges from 25 to 125 points. In addition, 4 cut-off scores are provided for each scale: none to low; low to moderate; moderate to severe and severe to extreme. Subjects who scored in the "moderate to severe" cut-off scores ($\geq$ 13 for emotional abuse; $\geq$ 10 for physical abuse; $\geq$ 8 for sexual abuse; $\geq$ 15 for emotional neglect; and $\geq$ 10 for physical neglect) on at least one of the five subscales of the CTQ comprised the maltreated group (GRASSI-OLIVEIRA; STEIN; PEZZI, 2006).

The occurrence of adverse life events over the past 12 months was assessed by a questionnaire proposed by the EU-GEI consortium, which was based on the List of Threatening Experiences (BRUGHA et al., 1985). The translation and adaptation of this questionnaire to Portuguese was performed by the STREAM research team, and the final version was submitted for backtranslation by a bilingual researcher associated to the EU-GEI.

5.2.4 Cytokines measurements

Peripheral blood was collected after the diagnosis evaluation and the samples were processed as previously described (LOUREIRO et al., 2018). Cytokines were quantified in plasma (25µL) using the Milliplex MAP Human Cytokine/Chemokine magnetic bead panel (#HCYTOMAG-60K; #HTH17MAG-14K; #TGFBMAG-64K-01 EDM Millipore, Billerica, MA, USA; https://www.emdmillipore.com/US/en). The assay was performed in 96-well plates according to the manufacturer's instructions and the results were expressed in pg/mL. Briefly, each assay plate layout consisted of seven standards, two positive controls, two blank wells, all runned in duplicate, and up to 76 samples, as previously described (LU et al., 2017). Results were analysed on a Luminex-200 System (Luminex, Austin, TX, USA) and reported on the xPOTENT software version 3.1. Cytokines concentrations were calculated through the five-parameter logistic curve-fitting method using the median fluorescence intensity (MFI). All data were corrected using the Milliplex Analyst software.

5.2.5 Statistical analysis

Data were analysed using the SPSS version 24.0 (IBM Corp: Armonk, NY, USA). Demographic and clinical data were analysed using descriptive statistics. Statistical associations between categorical variables were analysed by Pearson's Chi-square tests with column proportions compared by the z-test (adjusted p values with Bonferroni method), and for continuous variables by analysis of variance with Bonferroni correction. Plasma cytokines were logarithmic transformed for the statistical analyses, while the raw values are provided (adjusted means±standard error mean). To test the overall difference among the three groups on the cytokine levels, we used two-way ANCOVA with multiple Bonferroni corrections, controlling for the effects of age, gender, BMI and tobacco smoking. The effects of early stress or recent stress were analysed by two-way ANCOVA within each group, controlling for the effects of confounders. We also explored whether the association between stress and cytokines could be

influenced by any other clinical variables (waist circumference, psychoactive substances or pharmacological treatment) when appropriate. In the patient group, significant associations between cytokines and stress measurements were also further explored by taking into account the trauma severity. Statistical significance was set at alpha < 0.05 (two-tailed).

5.3 RESULTS

5.3.1 Sample characteristics

The final sample was comprised by 114 FEP patients, 57 siblings and 251 controls (n=422) **(Table 1)**. Patients, siblings and controls did not significantly differ for age (p>0.05), but differed for gender; in particular, siblings had higher proportion of females when compared with FEP or controls (p<0.001). Patients had lower BMI than controls (p=0.026) but presented the highest frequency of cannabis use (n=56; 49.1%), tobacco smoking (n=42; 36.8%) and other psychoactive substances investigated (n=57; 50.0%), whereas siblings presented the lowest frequency, with significant differences among the three groups (p<0.001).

Clinical characteristics of the patients are presented in **Table 2.**

5.3.2 Stress measurements

Patients, siblings and controls differed in the proportion of experience of childhood maltreatment (p<0.001), with patients having the highest proportion (43.9%), followed by their unaffected siblings (35.1%), and controls (22.7%). Information regarding the different subtypes of childhood trauma is presented in **Table 1**.

Controls had significantly higher frequency of recent stress than patients (p=0.005) **(Table 1)**.

Table 1: Socio-demographic characteristics of the sample (n = 422)

	First Episode Psychosis (n = 114)	Siblings (n = 57)	Controls (n = 251)	Test and significance		
				Chi-square; F	df	p
Male, n (%)	73 (64.0)	18 (31.6)	129 (51.4)	16.176	2	<0.001 [a,c]
Mean age, (SD)	30.8 (12.5)	30.7 (10.5)	31.3 (11.0)	0.122	2,421	0.885
≥ 9 years of study, n (%)	49 (43.0)	42 (73.7)	193 (76.9)	42.182	2	<0.001 [a,b]
Stable Union, n (%)	32 (28.1)	34 (59.6)	136 (54.2)	25.087	2	<0.001 [a,b]
Body mass index (kg/m^2), mean (SD)	24.8 (5.1)	24.9 (4.9)	26.2 (5.3)	3.699	2,421	0.026 [b]
Waist circumference (cm), valid/missing	111/3	56/1	243/8			
Waist circumference (cm), mean (SD)	86.4 (13.3)	82.9 (14.6)	87.4 (14.9)	2.223	2,409	0.110
Cannabis						
Ever used (yes), n (%)	56 (49.1)	4 (7.0)	50 (19.9)	47.098	2	<0.001 [a,b]
Current use (yes), n (%)	11 (9.6)	-	7 (2.8)	11.971	2	<0.003 [a,b]
Other psychoactive substance, yes n (%)*	57 (50.0)	1 (1.8)	39 (15.5)	69.376	2	<0.001 [a,b,c]
Tobacco (yes), n (%)	42 (36.8)	10 (17.5)	42 (16.7)	19.162	2	<0.001 [a,b]
Childhood Trauma						
Total, n (%)	50 (43.9)	20 (35.1)	57 (22.7)	17.451	2	<0.001 [b]
Emotional abuse, n (%)	27 (23.7)	13 (22.8)	27 (10.8)	12.179	2	0.002 [b, c]
Physical abuse, n (%)	21 (18.4)	4 (7.0)	19 (7.6)	10.704	2	0.005 [b]
Sexual abuse, n (%)	7 (6.1)	5 (8.8)	8 (3.2)	3.888	2	0.143
Emotional neglect, n (%)	27 (23.7)	11 (19.3)	23 (9.2)	14.618	2	<0.001[b]
Physical neglect, n (%)	19 (16.7)	8 (14.0)	20 (8.0)	6.553	2	<0.038 [b]
Recent stress, n (%)	66 (57.9)	43 (75.4)	186 (74.1)	10.749	2	0.005 [b]

*Other psychoactive substance including the following, but excluding cannabis: alcohol, cocaine/crack, inhalants, sedatives, amphetamine, and hallucinogen.
Post-hoc analysis significance is reported as follow:
[a] First Episode Psychosis vs. Siblings
[b] First Episode Psychosis vs. Controls
[c] Siblings vs. Controls

Table 2: Clinical characteristics of the FEP sample (n = 114)

Variables	First Episode Psychosis
Psychosis onset age	
Mean (SD)	30.0 (12.5)
Median	26.0
DUP (in weeks)	
Mean (SD)	52.8 (151.3)
Median	11.0
BPRS (Total Score)	
Mean (SD)	8.6 (6.4)
Median	7.0
Duration of psychosis (in weeks)	
Mean (SD)	81.1 (154.7)
Median	35.5
Pharmacological treatment (in weeks)	
Mean (SD)	29.7 (43.3)
Median	12.5
Current treatment	
Antipsychotics (AP): n (%)	49 (43.0)
Antidepressants (AD): n (%)	1 (1.0)
Mood stabilizers (MS): n (%)	2 (1.8)
AP + AD: n (%)	23 (20.2)
AP + MS: n (%)	25 (22.0)
AP + AD + MS: n (%)	7 (6.0)
None: n (%)	7 (6.0)

FEP: first-episode psychosis
DUP: Duration of untreated psychosis
BPRS: Brief Psychiatric Rating Scale.

5.3.3 Cytokine levels in FEP patients, siblings and community-based controls

Table 3 shows the results of the plasma cytokines among the three groups controlling for age, gender, tobacco smoking and BMI. We found differences among the three groups in plasma concentrations of IL-1β, IL-6, TNF-α, IL-10 and TGF-β ($p<0.001$), but not IFN-γ ($p=0.991$) or IL-4 ($p=0.817$). FEP had significantly higher levels of IL-6, TNF-α, IL-10 and TGF-β when compared with controls ($p<0.001$). Patients also had higher levels of IL-1β, IL-6,

TNF-α, and IL-10 when compared with their siblings ($p<0.05$). Siblings presented decreased IL-1β when compared with controls and FEP patients ($p< 0.001$).

Table 3: Cytokines plasma levels in FEP, siblings and controls

Cytokines (pg/mL)	FEP (n = 114)	Siblings (n = 57)	Controls (n = 251)	Test and significance		
				F	df	p
IL-1β	3.0 (0.2)	2.4 (0.3)	3.3 (0.1)	7.458	2,421	< 0.001[a,c]
IL-6	2.4 (0.3)	1.8 (0.4)	1.8 (0.2)	15.246	2,421	< 0.001 [a,b]
TNF-α	6.2 (0.3)	5.1 (0.4)	4.4 (0.2)	24.837	2,421	< 0.001 [a,b]
IFN-γ	21.5 (2.2)	23.0 (3.0)	21.8 (1.4)	0.009	2,421	0.991
IL-4	0.2 (0.02)	0.2 (0.03)	0.2 (0.02)	0.202	2,421	0.817
IL-10	6.8 (0.5)	4.5 (0.7)	4.0 (0.3)	17.877	2,421	< 0.001 [a,b]
TGF-β	786.3 (58.1)	481.0 (84.0)	545.1 (37.3)	7.026	2,421	< 0.001[b]

ANCOVA analysis controlling for age, gender, body mass index, and tobacco smoking. Cytokines levels are presented as raw values (adjusted means±standard error mean) with statistics performed on the logarithmic transformed values.
FEP: First-episode psychosis.
Post-hoc analysis significance is reported as follow:
[a] First Episode Psychosis vs. Siblings
[b] First Episode Psychosis vs. Controls
[c] Siblings vs. Controls

5.3.4 Cytokines and history of childhood trauma

Cytokines found to be different among the three groups (IL-1β, IL-6, TNF-α, IL-10, TGF-β) were further tested to investigate the within-person association between the history of childhood trauma (global and subtypes) and inflammation in patients, siblings and controls. The main significant results were found for TGF-β and physical abuse **(Figure 1)**.

We found that FEP patients with physical abuse had higher levels of TGF-β when compared with FEP without physical abuse (1142.0±162.5 vs. 719.8±76.1; $F_{(1,113)}=6.587$; $p=0.012$). We further explored whether the higher TGF-β levels in FEP reporting physical abuse could be related to any other clinical variables. The descriptive statistics showed that the patients with physical abuse and patients without physical abuse did not differ in relation to waist circumference, past or recent history of cannabis/other psychoactive substances use ($p>0.05$); nevertheless, there was a longer duration of pharmacological treatment in weeks in

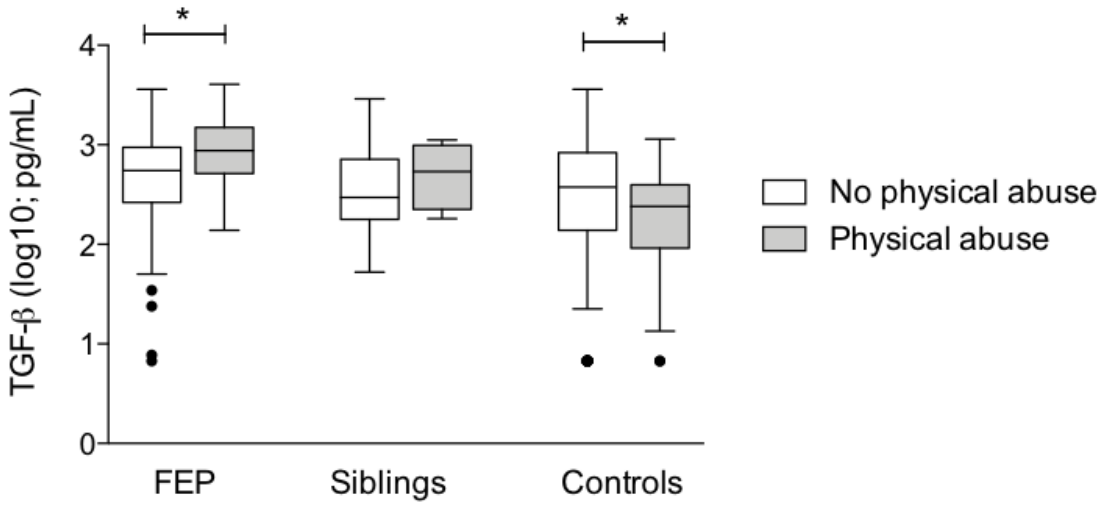
those with physical abuse (58.7±69.2 vs. 23.1±32.1; $F_{(1,113)}$=12.739;p=0.001). The results remained significant when including duration of treatment in the model ($F_{(1,113)}$=5.919;p=0.017). Furthermore, when we stratified physical abuse by different dimensions of severity [none to minimum (n=80), low to moderate (n=13), moderate to severe (n=13) or severe to extreme (n=8)], we found that the higher cut-offs of severity of physical abuse was associated with higher levels of TGF-β when controlling for the same confounders ($F_{(3,113)}$=3.510;p=0.018). More specifically, patients reporting the two highest cut-offs of severity had higher levels of TGF-β than patients reporting the low to moderate cut-off (moderate to severe: 984.3±196.5 vs. 449.6±201.0; severe to extreme: 1591.7±259.0 vs. 449.6±201.0).

Control subjects with experience of childhood trauma had lower TGF-β levels than controls without childhood trauma (409.8±72.3 vs. 576.1±38.8; $F_{(1,250)}$=6.822;p=0.010). The two groups did not differ for waist circumference or psychoactive substances other than cannabis (p>0.05); however, there was higher proportion of history of cannabis use (n=19, 33.3%) in controls with childhood maltreatment than those without childhood maltreatment (n=31,16.0%) (chi-square: 8.317; df=1; p=0.004). When including cannabis use in the model, the difference between the two groups remained significant ($F_{(1,250)}$=5.338;p=0.022). When focusing on individual subtypes of childhood trauma, controls with physical abuse showed lower TGF-β levels (314.5±126.0 vs. 556.5±35.4; $F_{(1,250)}$=5.501;p = 0.020) when compared to controls without physical abuse. No difference was found for waist circumference or psychoactive substance use (cannabis or other) in this group (p>0.05 for both).

There was no association between reports of childhood maltreatment and inflammatory cytokines in the sibling group.

The remaining cytokines IL-6, IL-1β, TNF-α, IL-10 were not associated with reports of childhood maltreatment in any of the three groups (p>0.05 for all).

Figure 7: TGF-β plasma levels in first-episode psychosis patients (n=114), siblings (n=57) and community-based controls (n=251) with and without physical childhood maltreatment



Plasma cytokines were logarithmic transformed and data are expressed as mean±S.E.M and given as pg/mL. *p < 0.05

5.3.5 Cytokines and history of recent stressors

Cytokine levels were not significantly different whether subjects experienced or not recent stressful events in none of the three groups (p>0.05).

5.4 DISCUSSION

The present study showed that FEP patients have a high pro- and anti-inflammatory cytokine profile (IL-1β, TNF-α, IL-6, IL-10 and TGF-β), whereas unaffected siblings have similar inflammatory profile to community-based controls. Whereas an association between general reports of childhood maltreatment and inflammation was not confirmed, different subtypes of childhood trauma may play a role. Specifically, in our sample, physical childhood abuse was associated with increased levels of TGF-β in patients but with decreased levels in controls.

The inflammatory profile reported in our study is not only in accordance but also add important information to the latest meta-analyses of peripheral cytokines in psychosis. For instance, whereas increased IL-6 and TNF-α are the most replicated findings in the literature, the participation of well-known anti-inflammatory cytokines are still vastly ignored

(GOLDSMITH; RAPAPORT; MILLER, 2016). In this sense, we demonstrate the co-existence of an up-regulation of anti-inflammatory cytokines in FEP. The existence of an anti-inflammatory profile complements previous findings in schizophrenia of enhanced levels of two cytokine receptors (the sIL-2R and the IL1-Ra) facilitating anti-inflammatory actions (GOLDSMITH; RAPAPORT; MILLER, 2016), and is also consistent with data showing higher percentages of both pro- and anti-inflammatory monocytes/T cells in recent-onset schizophrenia, including activation of CD4+CD25^{high}FoxP3+ T cells, which produce both IL-10 and TGF-β (DREXHAGE et al., 2011). In this sense, it could be that the concomitant up-regulation of pro- and anti-inflammatory cytokines may provide a compensatory response and may be favourable in preventing the detrimental effects of chronic inflammation.

It should be noted that FEP had increased levels of pro- and anti-inflammatory cytokines not only when compared with controls, but also when compared with their unaffected siblings. This could indicate that familial liability does not play a major role in determining the inflammatory profile found in FEP. Furthermore, given that the siblings not only did not show increased inflammatory profile when compared with controls, but they instead showed lower levels of IL-1β – one of the main pro-inflammatory cytokines – when compared with controls, we could speculate that the relative lack of immune activation in siblings may represent a protective factor in these individuals. Remarkably, the rate of childhood trauma exposure in our sample of siblings was intermediate between rates in patients and controls. This result is in accordance with a large case-sibling-control investigation reporting a significant dose-response relationship across the three groups, similar to the one we found in our sample, indicating that the experience of childhood trauma is higher not only in groups with illness but also in high vulnerability groups (HEINS et al., 2011). Therefore, although unaffected siblings would present with higher genetic risk and higher environmental risk, the findings that their immune profile is similar to controls may further support the role of immune dysfunction in the onset of psychosis.

One possible argument regarding the increased immune activation in our patients compared to both siblings and controls could be related to the exposure (although limited) of our FEP patients to antipsychotic treatment. The immunomodulatory effects of antipsychotic treatment are not always consistent across studies (BAUMEISTER; CIUFOLINI; MONDELLI, 2016) and large part of these effects have been recently suggested to be partly consequence of their metabolic side effects (BAUMEISTER et al. 2016; CALEVRO et al. 2018). Of note, in order to reduce the confounding effect of antipsychotic medication on the inflammatory

markers in our study, we focussed on the study of FEP patients, who had limited exposure to antipsychotic treatment, and we controlled all our analyses for important metabolic confounding factors, which have been discussed to contribute to cytokine abnormalities in general population, specially the effects of age, gender, BMI and smoking (GOLDSMITH; RAPAPORT; MILLER, 2016). It is important to point out that reports of peripheral cytokine alterations are not always consistent in the literature, and this inconsistence could reflect the contribution of many confounders or could indicate increased inflammatory markers in certain subgroups only. Nevertheless, we performed our data analysis controlling for such factors, which suggests that the identified inflammatory profile can be a real pathophysiological component of psychosis.

Early-life stress has been argued as an important factor in the immune activation reported in psychosis (BAUMEISTER *et al.* 2016), but our results do not support the findings of previous studies reporting enhanced TNF-α (DENNISON et al., 2012; DI NICOLA et al., 2013) and IL-6 (DENNISON et al., 2012) in psychotic patients exposed to childhood maltreatment. However, a large study in patients with chronic schizophrenia/bipolar disorder also failed to report the association of childhood trauma with the soluble TNF-1 receptor, although the authors found reduced gp130 (the IL-6 signal-transducing component) in patients compared to controls (AAS et al., 2017). In our study, we found that FEP patients with experience of childhood physical abuse had higher levels of TGF-β compared with patients without childhood physical abuse, and that the severity of childhood physical abuse was positively associated with the levels of TGF-β. The specificity for physical abuse, but not other subtypes of childhood maltreatment, in altering immune markers in FEP is intriguing is several ways and may suggest exposure-specific mechanisms. Indeed, physical abuse is the most prevalent subtype of childhood trauma in subjects reporting psychotic experience, and this subtype of traumatic experience is associated with the highest odds ratio for a subsequent psychotic episode in males (MCGRATH et al., 2017). This is also consistent with previous findings of a stronger association of physical and sexual childhood abuse, rather than other childhood trauma, with increased levels of inflammatory markers in adulthood (Baumeister *et al.* 2016).

Interestingly, we found an opposite effect in community-based controls, with low levels of TGF-β in those who reported general childhood maltreatment but also in the specific subtype of physical abuse. A possible explanation behind this would further support the role of the immune system in mediating the association between childhood maltreatment and psychosis,

with healthy controls showing opposite immune activation than controls as possible protective factor. Higher levels of TGF-β have been also reported in depressed patients with experience of childhood maltreatment (LU et al., 2013), possibly further suggesting a role of higher TGF-β in the link between childhood maltreatment and adulthood psychopathology. With regards to siblings, although we did not find a significant difference in TGF-β between those with and without physical abuse, the pattern of TGF-β in those exposed to physical abuse was similar to their FEP peers with TGF-β levels being higher in those abused. One possible explanation behind this finding is that siblings may present a similar genetic predisposition to increased TGF-β levels when exposed to abuse, but the fact that they remain at a lower threshold may represent a protective factor.

Increased TGF-β protein (BOROVCANIN et al., 2013; KIM et al., 2004) and TGF-β lymphocyte receptor (NUMATA et al., 2008) were described before in medication-free and FEP patients, although previous findings were not corrected for confounders as in this study. TGF-β is a cytokine with pleiotropic functions produced by both immune and non-immune cells. This regulatory cytokine presents with potent anti-inflammatory and neuroprotective functions, controlling Th1 and Th2 imbalance, besides mediating differentiation of naïve CD4+ T-cells towards T_{reg}-cells producing anti-inflammatory cytokines (IL-10, TGF-β), therefore controlling pro-inflammatory processes (CHEN et al., 2003). *In vivo*, the immune system is tightly regulated and the production of pro-inflammatory cytokines is usually counteracted by the release of anti-inflammatory cytokines. The elevated TGF-β levels may be therefore an epiphenomenon of an overall immune activation and of an attempt to re-establish a balance between the Th1 and Th2 cytokines, as suggested before (BOROVCANIN et al., 2013; KIM et al., 2004), with an attempt to limit ongoing inflammatory processes.

Lastly, our study does not support that recent stress (past 12 months) may have an effect on cytokines as this was not observed neither in patients nor in high risk groups or healthy controls. Moreover, it has been reported before that acute psychological stress may induce short-term systemic inflammations, whereas chronic stress may be associated with more permanent inflammatory changes (ROHLEDER, 2014). To illustrate that, a longitudinal study showed that childhood maltreatment but not recent stress accounted for the association between increased inflammation and depression in patients with cancer (ARCHER et al., 2012). We are also in accordance with a recent study reporting association between cytokines (IL-6, TNF-α) and childhood maltreatment but not recent stress in depression (GROSSE et al., 2016). If that is true, these findings may inform about sensitive periods in stress-induced immune changes.

5.5 STRENGTHS AND LIMITATIONS

In this study we attempted to overcome several methodological issues that were identified in previous investigations. First, this is the largest sample yet to test the association between childhood and recent trauma in FEP patients, siblings, and population-based controls controlling for well-known confounding effects of age, gender, BMI and smoking. Second, participants' recruitment in this study followed the National Census of Brazil for a representative sample, which is distinctive from the previous investigations including convenience samples sensitive to selection bias. Third, we also tested for each different subtypes of trauma, which were before considered as one phenomenon. Fourth, our cytokine profile was much broader, allowing to test both pro- and anti-inflammatory cytokines. Fifth, we included siblings in the study in order to test more specific confounding effects of shared environment and genetic risk than the classic case-control design. Besides, this also allowed us to somehow verify the validity of the patients' exposure reports. The results presented herein provide strong additional support for an association between early life stress and cytokine abnormalities in adulthood across the different diagnostic groups.

However, our study has some limitations that need to be addressed. First, we relied on retrospective self-reports questionnaires of stressful events, and therefore recall bias can be an issue. However, several investigations have shown the validity of retrospective self-reports in both patients and healthy controls, and that underestimation could be more likely to occur than overestimation (GRASSI-OLIVEIRA; STEIN; PEZZI, 2006). Besides that, siblings' reports acted towards validation of patients' reports. Second, the majority of patients in this study were not drug-naïve. However, inflammatory cytokine abnormalities have been reported in drug-naïve FEP (GOLDSMITH; RAPAPORT; MILLER, 2016), and therefore it is unlikely that the cytokine changes may be solely from medication effects. Moreover, we controlled for duration of antipsychotic treatment in the within-group analyses for the patients when looking at the effects of childhood trauma and we controlled for metabolic factors (contributing to the effects of antipsychotic on inflammatory markers) in all our analyses.

5.6 CONCLUSION

We found increased pro- and anti-inflammatory cytokines in FEP patients but not in unaffected siblings who, similarly to patients, were exposed to higher childhood trauma than

community-based controls, suggesting that the identified inflammatory profile can be a real pathophysiological component of psychosis and that normal or reduced immune activation may be protective in siblings.

Additionally, our study suggests that experience of childhood maltreatment, and more specifically physical abuse, may contribute as a long-term immune priming for the TGF-β pathway in both patients and community-based controls, although in opposite directions. Whether the opposite effects of childhood maltreatment on inflammatory markers reflect important biological mechanisms increasing risk or resilience to psychosis would need to be further explored in future studies.

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Conflict of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

6. Final Remarks

6. FINAL REMARKS

One of the most pressing interrogations in neuroscience and public health is how stress "gets under our skin" to bring about a myriad of adverse health problems, such as compromised brain development and consequent poor mental and physical health. Due to the wide heterogeneity of clinical symptoms and the current poor understanding of clinical disease etiopathogenesis, animal models of psychiatric disorders are challenging yet extremely relevant. Another great challenge refers to experimentally design interactions between multiple and varied biological and environmental factors that could result in the syndromic phenotype. A better understanding of the biological factors that influence brain resistance to environmental influences is in urgent need, and nonhuman models can help to disentangle such complicated interactions. Despite such difficulties, we attempted to investigate whether the exposure to early-life stress modulates the levels of inflammatory cytokines in a preclinical and clinical investigation of schizophrenia.

Through a pure environmental and non-pharmacological preclinical model of schizophrenia, we firstly tested the effects of early chronic social isolation stress in modulating the levels of three important cytokines (IL-6, TNF-α and IL-10) in the peripheral blood as well as in the brain. Different from our initial expectations, our preclinical model indicated that the exposure to chronic stress actually downregulates the expression of inflammatory cytokines. However, in accordance to our expectations, we observed reduced blood-to-brain expression of IL-10.

We then tested whether the aforementioned findings would be replicated in a large-epidemiological sample of FEP patients, unaffected siblings, and community-based controls exposed to early life stress. We found that FEP presented enhanced pro- and anti-inflammatory cytokines when compared to both siblings and controls. Nevertheless, when we looked the effects of early trauma, we failed to identify associations between IL-6, TNF-α or IL-10 and general reports of childhood maltreatment or any specific subtype of childhood trauma. By including extra cytokines in our analysis (IL-1β, TNF-α, IFN-γ, IL-6, IL-4, IL-10 and TGF-β), we did find that early-life stress modulated the levels of TGF-β. More specifically, FEP reporting physical abuse had increased TGF-β plasma levels. Due to methodological barriers, we were unable to test the effects of pwSI on TGF-β.

One of the most important questions regarding our study is how the results should be interpreted in the context of the current inflammatory hypothesis proposed for psychoses.

Regardless our methodological limitations, overall, the discrepant results between our animal and clinical data brings several important queries.

Firstly, even though a plentiful body of research has accumulated linking early life stress to immune abnormalities in general population, methods and results are very heterogeneous and nonoverlapping (BAUMEISTER et al., 2015; COELHO et al., 2014). When it comes to psychoses, studies linking childhood maltreatment with inflammation are rare and consist of very small sample sizes, running the risk that the significative pro-inflammatory results reported before were overestimated or due to the lack of control for important confounding variables, especially those related to age, gender, body mass index and tobacco smoking.

Secondly, unlike experimental studies where stress can be manipulated, stress exposure in humans is further complex. Even though we have controlled our all analyses for well-known confounding variables, disentangling the effects of early stress exposure from other potentially unhealthy behaviours associated with early victimization history is somehow impractical. It is now becoming widely accepted that the lack of translation between human and nonhuman research also extrapolates in terms of mechanistic research. For instance, current research is now strongly suggesting DNA methylation as a proposed mechanistic pathway through which early life stress could cause many adverse outcomes. Despite some positive results in preclinical and small clinical data, a recent large epidemiological-based study has failed to find changes in DNA methylation of victimized young people (MARZI et al., 2018). Such disappointing results indicate the need to explore the influence of cumulative risk factors instead of isolated social factors, which remains a great challenge in clinical research.

Thirdly, it is highly possible that our preclinical model of social isolation was inadequate to translate the complexity of childhood exposure in the context of psychoses. Not all types of victimization are alike; under the label of "early stress", research has focused on a variety of mixed exposures, ranging from early infections, parental loss, institutionalisation, child labour, childhood abuse/neglect besides many others complex situations that pose a great challenge when translating clinical into preclinical research. Supporting that, recent meta-analyses suggests that the type, duration and intensity of stress experience impact differently on the levels of the inflammatory markers across different clinical populations (BAUMEISTER et al., 2015; COELHO et al., 2014). Besides that, we relied on retrospective self-reports questionnaires of stressful events, which could pose a significant bias, although several investigations support the validity of retrospective self-reports (GRASSI-OLIVEIRA, 2016).

Because only TGF-β yielded positive association with childhood adversity in our clinical study, and considering that unfortunately we were unable to test this association in our preclinical model, we searched in the literature studies investigating associations between TGF-β and stress exposure in other preclinical models. We found that exposure to environmental insults other than social isolation, such as chronic restraint stress (21 days) in rats, increasead TGF-β in the hippocampus (GUO et al., 2014), while prenatal stress and maternal deprivation decreased the expression of this cytokine in rats' hippocampus and peripheral circulation, respectively (BREIVIK et al., 2015; CATTANEO et al., 2018). Alike suggested for clinical investigations, such findings reinforce perhaps the complexity of distinctive early stressors in modulating the inflammatory response in different ways.

Based on the aforementioned arguments, one could infer about the possibility that biological epidemiology is not well matched to experimental nonhuman models in uncovering the biological embedding of stress, at least in the context of inflammatory cytokines. Despite that, our study does not support associations between pro-inflammatory cytokines and early-life stress in psychoses. We believe that the findings from our comprehensive epidemiological and translational study reinforce that the type and duration of stress experience may impact differently on the levels of the inflammatory markers across different populations. Based on our results, future research should focus on cumulative risk factors or take into account the clinical features of the differential diagnoses to better understand the inflammatory profile reported in psychoses.

7. Conclusions

7. CONCLUSIONS

- Increased pro-inflammatory cytokines was not confirmed in chronic isolated rats; instead, we observed reduced circulating and hippocampal IL-10 expression, along with decreased IL-6 expression in the prefrontal cortex. Decreased anti-inflammatory cytokine in the brain may contribute to abnormal behaviour in adulthood;

- Prolonged periods of social isolation may account for the reduced cytokines observed, and possibly represent an exhaustion of the immune system as a compensatory mechanism under long-term periods of stress. This profile may be representing the heterogenous outcome and prognoses of schizophrenia, suggesting a blunted inflammatory response in latter stages of the disorder, contrasting the high inflammatory profile during earlier stages. Our study also brings the questions to whether peripheral blood cytokines may reflect cytokines in the central nervous system. Future investigations are needed to answer these questions;

- The inflammatory profile in FEP patients reported in our study are in accordance to previous investigations showing high pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) but we also demonstrate concomitant increased anti-inflammatory cytokines (IL-10 and TGF-β) unconfounded by age, sex, BMI or tobacco smoking. Increased pro- and anti-inflammatory cytokines was not observed in unaffected siblings who, similar to patients, were exposed to higher childhood trauma than community-based controls, suggesting the effect of protective factors in siblings;

- An association between general reports of childhood maltreatment and inflammation was not confirmed in our study; nevertheless, physical childhood abuse was associated with increased levels of TGF-β in patients but with decreased levels in controls. This is in accordance with previous research suggesting that the type of trauma may affect the immune system in different ways;

- The results from our preclinical and clinical study does not support associations between pro-inflammatory cytokines and early-life stress in psychosis. The type and duration of stress experience or the clinical features of the differential diagnoses may impact on the

levels of inflammatory markers across different populations. Moreover, it is highly possible that the inflammatory profile reported in our clinical population arise from cumulative risk factors. These factors should be explored in future investigations.

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9. Appendix

9. APPENDIX

BLOOD SAMPLE COLLECTION PROTOCOL

PROTOCOLO PARA COLETA DE SANGUE

PROJETO DE PESQUISA: Esquizofrenia e outros transtornos psicóticos: determinantes sociais e biológicos

Docente responsável: Paulo Louzada Junior
Pesquisadora responsável: Camila Marcelino Loureiro

- ✓ DATA DA COLETA:
- ✓ NOME – INICIAIS:
- ✓ NOME DA MÃE: NÚMERO STREAM:
- ✓ SEXO: F ( ) M ( ) IDADE:
- ✓ CASO: ( ) CONTROLE: ( ) IRMÃO (Ã): ( )
- ✓ RESIDÊNCIA ATUAL:

- ✓ TELEFONES DE CONTATO:

- ✓ DIAGNÓSTICO:

DATA DE INÍCIO DOS SINTOMAS PSICÓTICOS:
DATA DE PRIMEIRO CONTATO COM SERVIÇO DE SAÚDE MENTAL:
SERVIÇO DE SAÚDE MENTAL DE REFERÊNCIA:

COMORBIDADES: SIM ( ) NÃO ( )

- Se sim, descrever: _____

- ✓ FARMACOLOGIA UTILIZADA (TODAS):

Medicamento	Dose diária	Data de início	Regularidade de uso

- ✓ COLETA DAS CITOCINAS: A primeira amostra será coletada no momento em que o diagnóstico for estabelecido. Para todos os indivíduos será coletado uma amostra de 5 ml de sangue periférico, em tubo com EDTA, para centrifugação e separação do plasma para dosagem das citocinas. Os tubos serão mantidos no gelo e a centrifugação deverá ser realizada em até 2 horas após a coleta das amostras.

REGISTRO DA COLETA DE SANGUE

Data e hora da coleta	
Responsável pela coleta	
Data e hora da centrifugação	
Responsável pela centrifugação	
Data e hora de identificação e armazenamento	
Responsável pela identificação e armazenamento	

Aplicação da BPRS imediatamente antes das coletas de sangue – Escore Total: _____

SEÇÃO TABACO:

SIM ( ) NÃO ( )

- Se sim, descrever: _____

SEÇÃO ÁLCOOL:

SIM ( ) NÃO ( )

- Se sim, descrever: _____

SEÇÃO OUTRAS DROGRAS:

SIM ( ) NÃO ( )

Se sim, descrever: _____

10. Attachments

10. ATTACHMENTS

Attachment A – Nottingham Onset Schedule
(Cronograma de Início de Sintomas de Nottingham)

Versão modificada de duração de psicose não tratada (NOS-DUP)

Estudo: EU GEI	**Data de nascimento**
Número do participante: |__|__|__| - |__|__|__|__|	__|__|-|__|__|-|**1**|**9**|__|__|
Intervalo de tempo:	**Period – Replicat** |**0**|__|-|**0**|__|
Entrevistador: ……	**Data** |__|__|-|__|__|-|**2**|**0**|__|__|

Folha de definição

Por favor, registre a data mais precisa possível!
No caso da informação sobre o **ano** de início ser a única disponível, por favor, registre o dia **1 de julho** desse ano como data de início.
No caso da informação sobre o **mês** de início ser a única disponível, por favor, registre o **dia 15** daquele mês como data de início.

Data do início da psicose:

Primeiro dia do episódio psicótico.

Início do episódio psicótico (diagnóstico definitivo) é definido como:
Clara evidência de delírios, alucinações, sintomas de primeira ordem, sintomas catatônicos durante, pelo menos, uma semana (isto é, pontuação ≥ 4 nos itens P1, "delírios", P3 "comportamento alucinatório", P5 "Grandiosidade", P6 "Desconfiança" ou A9 "conteúdo incomum do pensamento" da PANSS).

Data do início do tratamento:

Primeiro dia do início do tratamento.

O início do tratamento é definido como:
Data inicial do tratamento com antipsicóticos mantido durante pelo menos **um mês** (pelo menos 75% aderente), ou até que tenha sido alcançada uma resposta significativa (isto é, "muito melhor" de acordo com o CGI), independentemente do que acontecer primeiro.

Duração da Psicose Não Tratada:

Data do início do tratamento - (menos) Data do início da Psicose

 (Nota para a entrada de dados: **NOS_GEN**)

Diagnóstico psiquiátrico atual (DSM IV): ☐☐☐ . ☐☐

Data de contato com serviço de saúde mental (dia/mês/ano): ☐☐ _☐☐ _☐☐☐☐

Data de início da psicose: ☐☐ _☐☐ _☐☐☐☐

Data de tratamento suficiente: ☐☐ _☐☐ _☐☐☐☐

Paciente usa antipsicóticos? 00 Não 01 Sim

Data do início do tratamento: ☐☐ _☐☐ _☐☐☐☐

 (Nota para a entrada de dados: **NOS_MED**)

Antipsicóticos	Dose total diária ou dose de depósito (mg)	Data de tomada regular (no mínimo 75% de adesão)	Código (a ser preenchido pelo investigador)*

DURAÇÃO DA PSICOSE NÃO TRATADA (DUP) em <u>semanas</u>: _____

*** veja a lista de medicações para incluir os códigos**

Attachment B – Brief Psychiatric Rating Scale (BPRS)
(Escala de Avaliação Psiquiátrica Breve)

BPRS	
Item	**Escore**
01. Preocupações Somáticas	
02. Ansiedade Psíquica	
03. Retraimento Emocional	
04. Desorganização Conceitual	
05. Sentimentos de Culpa	
06. Ansiedade	
07. Distúrbios Motores Específicos	
08. Auto-Estima Exagerada	
09. Humor Deprimido	
10. Hostilidade	
11. Desconfiança	
12. Alucinações	
13. Retardo Psicomotor	
14. Falta de Cooperação	
15. Conteúdo Incomum do Pensamento	
16. Afeto Embotado	
17. Agitação Psicomotora	
18. Desorientação e Confusão	
Escore Total BPRS	

Attachment C – Medication List (Present and Past)
Lista de Medicações (Presente e Passada)

(Note for DATA ENTRY: open **MED_PRES**) **O Participante usa medicação? O0 não O1 sim**

ESTUDO: EU GEI	**Data de Nascimento**
Número do participante: |__|__|__| - |__|__|__|__|	|__|__|-|__|__|-|1|9|__|__|
Intervalo de tempo: Atual	**Period – Replicat**|0|__|-|0|__|
Entrevistador:	**Data**|__|__|-|__|__|-|2|0|__|__|

No. **Nome** **Código** **Dose total diária – ou dose de depósito** **Dia**

01 ..... __ - __ - __ ____ , ___ ___

Motivo do uso: .....

Data de início: __ - __ - __

Unidade O1 mg O2 ml O3 mmol/l O4 microg
Horário diário: O1 8 O2 12 O3 18 O4 22
Depósito: O0 não O1 sim
Se necessário: O0 não O1 sim

02 ..... __ - __ - __ ____ , ___ ___

Motivo do uso: .....

Data de início: __ - __ - __

Unidade: O1 mg O2 ml O3 mmol/l O4 microg
Horário diário: O1 8 O2 12 O3 18 O4 22
Depósito: O0 não O1 sim
Se necessário: O0 não O1 sim

03 ..... __ - __ - __ ____ , ___ ___

Motivo do uso: .....

Data de início: __ - __ - __

Unidade: O1 mg O2 ml O3 mmol/l O4 microg
Horário diário: O1 8 O2 12 O3 18 O4 22
Depósito: O0 não O1 sim
Se necessário: O0 não O1 sim

Exemplos de Medicações para Classificação

B. SEDATIVOS (01-02-00):	Alprazolam	Lorazepam
	Clonazepam	Midazolam
	Diazepam	
D. ANTIPSICÓTICOS (01-04-00):	Clorpromazina	Paliperidona
	Clozapina	Quetiapina
	Haldol	Risperidona
	Olanzapina	Sulpirida
E. LÍTIO (01-05-00):	Lítio	
F. ANTIDEPRESSIVOS (01-06-00):	Amitriptilina	Mirtazapina
	Citalopram	Paroxetina
	Fluoxetina	Sertralina
	Imipramina	Venlafaxina
H. ANTIEPILÉPTICOS (02-01-00):	Ácido valpróico	Fenobarbital
	Carbamazepina	Topiramato
I. ANTICOLINÉRGICOS (02-02-10):	Biperideno	
J. ANTI-HISTAMÍNICOS (18-01-00):	Prometazina	
K. BETABLOQUEADORES (05-02-00):	Propanolol	

Attachment D – Childhood Trauma Questionnaire

Questionário de Trauma na Infância

(Nota para a entrada de dados: **EU_CTQ**)

ESTUDO: EU-GEI	**Data de Nascimento**
Número do Participante: |__|__|__| - |__|__|__|__|	|__|__|-|__|__|-|1|9|__|__|
Intervalo de tempo: tempo de vida	**Período – Replicat** |0|__|-|0|__|
Entrevistador:	**Data** |__|__|-|__|__|-|2|0|__|__|

"Para cada questão, marque a resposta que melhor descreve como se sente." [Todos os participante devem completar o questionári por conta própria. Assistência pode ser fornecida se a pessoa tiver dificuldade em ler as questões.]

Antes dos 17 anos:	**Nunca**	**Poucas vezes**	**Às vezes**	**Muitas vezes**	**Sempre**
1. Eu não tive o suficiente para comer.	O1	O2	O3	O4	O5
2. Eu sabia que havia alguém para me cuidar e proteger.	O1	O2	O3	O4	O5
3. As pessoas da minha família me chamaram de coisas como do tipo "estúpido(a)", "preguiçoso(a)", ou "feio(a)".	O1	O2	O3	O4	O5
4. Meus pais estiveram muito bêbados ou drogados para poder cuidar da família.	O1	O2	O3	O4	O5
5. Houve alguém na minha família que ajudou a me sentir especial e importante.	O1	O2	O3	O4	O5
6. Eu tive que usar roupas sujas.	O1	O2	O3	O4	O5
7. Eu me senti amado(a).	O1	O2	O3	O4	O5
8. Eu achei que meus pais preferiam que eu nunca tivesse nascido.	O1	O2	O3	O4	O5
9. Eu apanhei tanto de alguém da minha família que tive de ir ao hospital ou consultar um médico.	O1	O2	O3	O4	O5
10. Alguém da minha família me bateu tanto que me deixou com machucados roxos.	O1	O2	O3	O4	O5
11. Eu apanhei com cinto, vara, corda ou outras coisas que machucaram.	O1	O2	O3	O4	O5
12. As pessoas da minha família cuidavam umas das outras.	O1	O2	O3	O4	O5

Questionário de Trauma na Infância

	Nunca	Poucas vezes	Às vezes	Muitas vezes	Sempre
13. Pessoas da minha família disseram coisas que me machucaram ou me ofenderam.	O1	O2	O3	O4	O5
14. Eu acredito que fui maltratado(a) fisicamente.	O1	O2	O3	O4	O5
15. Eu apanhei tanto que um professor, vizinho ou médico chegou a notar.	O1	O2	O3	O4	O5
16. Eu senti que alguém da minha família me odiava.	O1	O2	O3	O4	O5
17. As pessoas da minha família se sentiam unidas.	O1	O2	O3	O4	O5
18. Tentaram me tocar ou me fizeram tocar de uma maneira sexual.	O1	O2	O3	O4	O5
19. Ameaçaram me machucar ou contar mentiras sobre mim se eu não fizesse algo sexual.	O1	O2	O3	O4	O5
20. Tentaram me forçar a fazer algo sexual ou assisitir coisas sobre sexo.	O1	O2	O3	O4	O5

Antes dos 17 anos:	**Nunca**	**Poucas vezes**	**Às vezes**	**Muitas vezes**	**Sempre**
21. Alguém me molestou.	O1	O2	O3	O4	O5
22. Eu acredito que fui maltratado(a) emocionalmente.	O1	O2	O3	O4	O5
23. Houve alguém para me levar ao médico quando eu precisei.	O1	O2	O3	O4	O5
24. Eu acredito que fui abusado(a) sexualmente.	O1	O2	O3	O4	O5
25. Minha família foi uma fonte de força e apoio.	O1	O2	O3	O4	O5

Attachment E – List of Threatening Events

Lista de Experiências Ameaçadoras

(Nota para a entrada de dados: **EU_LTE, list of threatening events**)

Data |__|__|-|__|__|-|**2**|**0**|__|__|

Gostaria de perguntar sobre o período de 12 meses antes [do início, a partir de NOS] **[Para não casos, considere os 12 meses antes da entrevista]**. Gostaria de fazer perguntas sobre coisas que podem ter acontecido com você ou com pessoas próximas a você (cônjuge, filhos, irmãos, pais, outros membros da família, amigos muito próximos). Durante este período de 12 meses, algumas das seguintes coisas aconteceram com você ou com pessoas próximas a você? Se sim, quando isso aconteceu (ou começou)? [Dê uma breve descrição.]

Período de tempo:

De 12 meses antes do início |__|__|-|__|__|-|__|__|__|__|

Até data do início |__|__|-|__|__|-|__|__|__|__|

	SIM	**NÃO**	**DATA**
1. Ferimento ou doença grave?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
2. Ferimento ou doença grave em um parente próximo?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
3. Morte de pais, filhos ou seu cônjuge (parceiro)?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
4 Morte de um amigo próximo da família ou outro parente?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
5. Nascimento de um filho?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
6. Separação do parceiro devido a dificuldades de relacionamento?	O1	O0	|__|__|-|__|__|-|__|__|__|__|

Lista de Experiências Ameaçadoras

	Sim	Não	Data
7. Término de namoro / Noivado?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
8. Revelação de alguma notícia chocante sobre parceiro ou filhos?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
9. Problemas graves e duradouros com o parceiro ou filhos?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
10. Problema sério com um amigo, vizinho ou parente?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
11. Problemas sérios no trabalho?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
12. Perdeu o emprego ou estava procurando emprego sem sucesso?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
13. Foi demitido ou despedido de seu trabalho?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
14. Crise financeira grave?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
15. Dificuldades financeiras e dívida (você/parceiro)?	O1	O0	|__|__|-|__|__|-|__|__|__|__|

Lista de Experiências Ameaçadoras

	Sim	Não	Data
16. Graves problemas de habitação, incluindo estar sem abrigo?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
17. Problemas legais ou com a polícia?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
18. Algo de valor foi perdido ou roubado?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
19. Vítima de assalto (inc. em casa), roubo ou furto?	O1	O0	|__|__|-|__|__|-|__|__|__|__|
20. Assistiu a uma agressão grave ou a outro evento traumático?	O1	O0	|__|__|-|__|__|-|__|__|__|__|

NOTAS:

Attachment F – Cannabis experience Questionnaire

Cannabis Experiences Questionnaire

(Nota para a entrada de dados: **EU_CEQ**)

ESTUDO: EU GEI	**Data de Nascimento**
Número do participante |__|**EU**|__|__| - |__|__|__|__|	|__|__|-|__|__|-|**1**|**9**|__|
Intervalo de Tempo:	**Period – Replicat** |**0**|__|-|**0**|__|
Entrevistador:	**Data** |__|__|-|__|__|-|**2**|**0**|__|__|

Instruções para o pesquisador: *Por favor, marque nos campos indicados as repostas do paciente.*

Por favor, tenha em mente que algumas questões permitem mais do que uma resposta.

15.1 Você já fumou/usou maconha alguma vez na vida? O1 Sim O0 Não
*Se a resposta for **NÃO**, vá para 15.17*

15.2 Quantos anos você tinha quando experimentou maconha pela primeira vez? ..... | | | Anos

15.3 Por que você experimentou maconha pela primeira vez? *(Você pode marcar mais de um item)*:

a) Os meus amigos usavam. O1 Sim O0 Não
b) Os meus familiares usavam. O1 Sim O0 Não
c) Para me sentir melhor (para obter alívio de algum desconforto físico ou psicológico) O1 Sim O0 Não
d) Outro motivo (por favor, explique) (a explicação não vai constar do banco de dados) O1 Sim O0 Não

Instruções para o pesquisador: *Por favor, considere como usuário atual todos os participantes que relatarem usar/fumar maconha com alguma frequência (incluindo pacientes que não fumaram enquanto estiveram internados ou presos ou pacientes que relatarem uso ocasional mesmo que seja apenas uma vez a cada dois anos, etc):*

15.4 Você usa maconha atualmente? O1 Sim O0 Não
Se **Sim**, *por favor, responda b, se* **Não**, *por favor responda c, e então vá para 15.7*

b. Se SIM, por que você continua a usar maconha? *(Você pode marcar mais de um item)*:

a) Eu gosto do efeito, dá-me um "barato" O1 Sim O0 Não
b) Faz com que eu me sinta relaxado(a) O1 Sim O0 Não
c) Faz com que eu me sinta menos nervoso(a) e ansioso(a) O1 Sim O0 Não
d) Faz com que eu me sinta mais sociável. O1 Sim O0 Não
e) Outro motivo *(por favor, explique)* O1 Sim O0 Não

Cannabis Experiences Questionnaire

15.5 Você gostaria de parar de usar maconha algum dia? O1 Sim O0 Não

b. Se sim, por favor explique *(resposta não vai constar no banco de dados):*

15.6 A maconha afeta ou já afetou a sua saúde de alguma maneira? O1 Sim O0 Não

b. Se sim, por favor explique *(resposta não vai constar no banco de dados)*:_____

15.7 Se atualmente você não é um usuário, há quanto tempo meses

b. Por que você parou de usar? *(a resposta não vai constar do banco de dados):*

15.8 Como usa/usava a maconha na maioria das vezes?

O1 Eu fumo/fumava misturado com cigarro comum com tabaco
O2 Eu fumo/fumava um baseado sem tabaco
O3 Eu fumo/fumava usando um cachimbo
O4 Ingerindo na comida ou bebida
O5 Outro (por favor, explique):_____

15.9 Com que frequência você usa/usava maconha?

O1 Diariamente/Todos os dias
O2 (Mais do que) uma vez por semana
O3 Poucas vezes por mês
O4 Poucas vezes por ano
O5 Apenas uma ou duas vezes na vida

15.10 Em qual período você mais usa/usava maconha?

O1 Durante o dia
O2 Durante a noite
O3 Durante o dia e à noite (dia todo)
O4 Aos finais de semana
O5 Aos finais de semana e dias da semana

15.11 Na maioria das vezes, você usa/usava maconha:

O1 Socialmente (com amigos)
O2 Sozinho(a)

Cannabis Experiences Questionnaire

15.12 Em média, quanto você gasta/gastava com maconha por semana?

O1 Menos de R$10.00

O2 R$10.00 – R$15.00

O3 R$16.00 – R$30.00

O4 R$31 – R$50.00

O5 R$51.00 – R$70.00

O6 Acima de R$70.00

15.13 Qual o tipo de maconha que você mais usa/usava?

O1 Haxixe (resina da cannabis)

O2 Erva (maconha comum)

O3 Sensimila (sem semente, planta fêmea plantada em casa; erva mais forte, com maior teor de THC)

O4 Skank

O5 Outro *(por favor, especifique)*:): _____

15.14. Por que você escolheu usar o tipo acima _____

Cannabis Experiences Questionnaire

15.15. Com que frequência você teve as seguintes experiências ao fumar maconha? *Por favor, assinale se a experiência foi boa, ruim ou neutra. Se você nunca ou raramente teve determinada experiência, ignore as três últimas colunas (boa, ruim ou neutra) e vá para a experiência seguinte.*

	Nunca ou raramente	Esporadi camente	às vezes	Mais vezes sim do que não	Quase sempre	Boa	Ruim	Neutra
a) Sensação de medo	O0	O1	O2	O3	O4	O1	O2	O0
b) Sensação de estar ficando louco	O0	O1	O2	O3	O4	O1	O2	O0
c) Nervosismo	O0	O1	O2	O3	O4	O1	O2	O0
d) Sensação de ficar desconfiado	O0	O1	O2	O3	O4	O1	O2	O0
e) Sensação de felicidade	O0	O1	O2	O3	O4	O1	O2	O0
f) Sensação de estar cheio de planos/ideias	O0	O1	O2	O3	O4	O1	O2	O0
g) Ouvir vozes	O0	O1	O2	O3	O4	O1	O2	O0
h) Capaz de entender melhor o mundo	O0	O1	O2	O3	O4	O1	O2	O0
i) Ter visões	O0	O1	O2	O3	O4	O1	O2	O0

Cannabis Experiences Questionnaire

15.16 Questionário sobre história de uso de Maconha ao longo da vida

Instruções para o pesquisador: Por favor, peça ao participante para completar esta seção. Explique-lhe como esta parte deve ser preenchida, usando como exemplo: *Se você fumasse maconha quando tinha 15 anos, você fumava 2-3 baseados por dia em média, fumava geralmente haxixe e você só fumava sozinho*.

a) FAIXA DE IDADE: 0-11

i. Você usou maconha entre os 0 e 11 anos de idade?	O1 Sim O0 Não
ii. Frequência	O1 Todo dia/diariamente O2 Mais do que uma vez por semana O3 Uma vez por semana O4 Uma ou duas vezes por mês O5 Algumas vezes por ano O6 Uma vez por ano O7 Só usei maconha uma ou duas vezes na vida
iii. Quantidade *(média de uso por dia)*	O1 1 baseado O2 2 ou 3 baseados O3 4 ou mais baseados
iv. Na maioria das vezes, os baseados eram compartilhados/divididos com outras pessoas?	O1 Sim O0 Não
v. Tipo	O1 Haxixe (resina da cannabis) O2 Erva (maconha comum) O3 Sensimila (sem semente, planta fêmea plantada em casa; erva mais forte, com maior teor de THC) O4 Skank O5 Outro *(por favor, especifique)*:_____
vi. Circunstância de uso	O1 Socialmente (com amigos) O2 Sozinho(a) O3 Ambos (às vezes socialmente, às vezes sozinho(a)

Cannabis Experiences Questionnaire

b) FAIXA DE IDADE: 12-16

i.	**Você usou maconha entre os 12 e 16 anos de idade?**	O1 Sim O0 Não
ii.	**Frequência**	O1 Todo dia/diariamente O2 Mais do que uma vez O3 Uma vez por semana O4 Uma ou duas vezes por O5 Algumas vezes por ano O6 Uma vez por ano O7 Só usei maconha uma
iii.	**Quantidade** *(média de uso por dia)*	O1 1 baseado O2 2 ou 3 baseados O3 4 ou mais baseados
iv.	**Na maioria das vezes, os baseados eram compartilhados/divididos com outras pessoas?**	O1 Sim O0 Não
v.	**Tipo**	O1 Haxixe (resina da cannabis) O2 Erva (maconha comum) O3 Sensimila (sem semente, planta fêmea plantada em casa; erva mais forte, com maior teor de THC) O4 Skank O5 Outro *(por favor, especifique)*:_____
vi.	**Circunstância de uso**	O1 Socialmente (com amigos) O2 Sozinho(a) O3 Ambos (às vezes socialmente, às vezes sozinho(a)

Cannabis Experiences Questionnaire

c) FAIXA DE IDADE: ACIMA DE 17 ANOS

i.	**Você usou maconha com 17 anos de idade ou mais?**	O1 Sim O0 Não
ii.	**Frequência**	O1 Todo dia/diariamente O2 Mais do que uma vez por semana O3 Uma vez por semana O4 Uma ou duas vezes por mês O5 Algumas vezes por ano O6 Uma vez por ano O7 Só usei maconha uma ou
iii.	**Quantidade** *(média de uso por dia)*	O1 1 baseado O2 2 ou 3 baseados O3 4 ou mais baseados
iv.	**Na maioria das vezes, os baseados eram compartilhados/divididos com outras pessoas**	O1 Sim O0 Não
v.	**Tipo**	O1 Haxixe (resina da cannabis) O2 Erva (maconha comum) O3 Sensimila (sem semente, planta fêmea plantada em casa; erva mais forte, com maior teor de THC) O4 Skank O5 Outro *(por favor, especifique)*:_____
vi.	**Circunstância de uso**	O1 Socialmente (com amigos) O2 Sozinho(a) O3 Ambos (às vezes socialmente, às vezes sozinho(a))

d) Se o seu padrão de uso de maconha mudou ao longo do tempo, por favor, explique porque.

(a resposta não vai constar do banco de dados)

_____

e) Rastreamento para dependência de maconha

Alguma vez você já teve 3 ou mais dos seguintes sintomas?

Cannabis Experiences Questionnaire

	Durante a vida		Últimos 12 meses	
1. Tolerância, definido por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
a. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar do conhecimento da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância	O1 Sim	O0 Não	O1 Sim	O0 Não

15.17 Instruções para o pesquisador: Por favor, para cada tipo de droga, pergunte: ***Alguma vez você já usou/experimentou*?** Se **SIM,** por favor continue com as questões sobre uso atual e passado. ***"Por favor, também avalie álcool e drogas quando for aplicável, (veja a folha Álcool & Nicotina em separado*).**

[a] **Rastreamento de dependência para todos os tipos de drogas:**

1. Tolerância, definida por qualquer um dos itens:
   a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.
   b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.
2. Abstinência, manifestada por qualquer um dos itens:
   a. A presença da síndrome de abstinência característica para a substância.
   b. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.

Cannabis Experiences Questionnaire

3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

(Nota para a entrada de dados: **EU_CEQ_DRUGS**, aberta para cada droga separadamente. Selecione o tipo correto de medicamentos na primeira pergunta!)

Data |__|__|-|__|__|-|2|0|__|__|

A. INALANTES[0], por ex: cola, gasolina, éter, benzina, lança-perfume.....

1. Uso atual (últimos 12 meses)

i.	**Usou?**	O1 Sim	O0 Não
ii.	**Se SIM: Usou durante a última semana?**	O1 Sim	O0 Não
iii.	**Quantas semanas nos últimos 12 meses?**	☐☐ Semanas	
iv.	**Frequência** *(isto é, uso médio nas semanas em que usou)*	O1 Diariamente O2 Uma vez por semana O3 Menos que uma vez por semana O0 Não se aplica	
v.	**Dependência** [a]	O1 Sim	O0 Não
vi.	**Abuso** [b]	O1 Sim	O0 Não

2. Ao longo da vida

i.	**Quantos anos você tinha quando usou inalantes pela primeira vez?**	☐☐ Anos	
ii.	**Houve uma época em que você usava frequentemente ?**	O1 Sim	O0 Não
iii.	**Quantos anos você tinha nesta época?**	**Dos:** ☐☐	**Aos** ☐☐
iv.	**Frequência no período de maior uso**	O1 Diariamente O2 Uma vez por semana O3 Menos que uma vez por semana	
v.	**Dependência ao longo da vida** [a]	O1 Sim	O0 Não
vi.	**Abuso ao longo da vida** [b]	O1 Sim	O0 Não

3. Se você não usa mais inalantes, quantos anos você tinha quando parou de usar? ☐☐ Anos

4. Por que você decidiu parar de usar?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

B. CRACK[1].....

1. **Uso atual (últimos 12 meses)**

i. **Usou?** O1 Sim O0 Não

ii. **Se SIM: Usou durante a última semana?** O1 Sim O0 Não

iii. **Quantas semanas durante os últimos 12 meses?** ☐☐ Semanas

iv. **Frequência** *(isto é, uso médio nas semanas em que usou)*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. **Dependência** [a] O1 Sim O0 Não

vi. **Abuso** [b] O1 Sim O0 Não

2. **Ao longo da vida**

i. **Quantos anos você tinha quando usou crack pela primeira vez?** ☐☐ Anos

ii. **Houve uma época em que você usava frequentemente?** O1 Sim O0 Não

iii. **Quantos anos você tinha nesta época?** **Dos** ☐☐ **Aos:** ☐☐

iv. **Frequencia no período de maior uso**
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

i. **Dependência ao longo da vida** [a] O1 Sim O0 Não

ii. **Abuso ao longo da vida** [b] O1 Sim O0 Não

3. **Se você não usa mais crack, quantos anos você tinha quando parou de usar** ☐☐ **Anos**

4. **Por que você decidiu parar de usar**?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

C. COCAÍNA[2]

1. Uso atual (últimos 12 meses)

i. Usou? O1 Sim O0 Não

ii. Se SIM: Usou durante a última semana? O1 Sim O0 Não

iii. Quantas semanas durante os últimos 12 meses? ☐☐ Semanas

iv. Frequência *(isto é, uso médio nas semanas em que usou)*

O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. Dependência [a] O1 Sim O0 Não

vi. Abuso [b] O1 Sim O0 Não

2. Ao longo da vida

i. Quantos anos você tinha quando usou cocaína pela primeira vez? ☐☐ Anos

ii. Já houve uma época em que você usava frequentemente? O1 Sim O0 Não

iii. Quantos anos você tinha nesta época? **Dos**: ☐☐ **Aos** ☐☐

iv. Frequência no período de maior uso

O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. Dependência ao longo da vida [a] O1 Sim O0 Não

vi. Abuso ao longo da vida [b] O1 Sim O0 Não

3. Se você não usa mais cocaína, quantos anos você tinha quando parou de usar ☐☐ Anos

4. Por que você decidiu parar de usar?

Cannabis Experiences Questionnaire

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[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

D. ANFETAMINAS/ESTIMULANTES[3], por ex: ecstasy, speed (bolinha)……

1. **Uso atual (últimos 12 meses)**

i. **Usou?** O1 Sim O0 Não

ii. **Se SIM: Usou durante a última semana?** O1 Sim O0 Não

iii. **Quantas semanas durante os últimos 12 meses?** ☐☐ Semanas

iv. **Frequência** *(isto é, uso médio nas semanas em que usou)*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. **Dependência** [a] O1 Sim O0 Não

vi. **Abuso** [b] O1 Sim O0 Não

2. **Ao longo da vida**

i. **Quantos anos você tinha quando usou anfetaminas/estimulantes pela primeira vez?** ☐☐ Anos

ii. **Houve uma época em que você usava frequentemente?** O1Sim O0 Não

iii. **Quantos anos você tinha nesta época?** **Dos** ☐☐ **Aos** ☐☐

iv. **Frequência no período de maior uso**
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. **Dependência ao longo da vida** [a] O1 Sim O0 Não

vi. **Abuso ao longo da vida** [b] O1 Sim O0 Não

3. **Se você não usa mais anfetaminas/estimulantes quantos anos você tinha quando parou de usar?** ☐☐ Anos

4. **Por que você decidiu parar de usar**?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

E. SEDATIVOS[4], por ex. calmantes, remédios para dormir, tarja preta, diazepam (uso sem prescrição médica) .....

1. Uso atual (últimos 12 meses)

i. Usou? O1 Sim O0 Não

ii. Se SIM: Usou durante a última semana? O1 Sim O0 Não

iii. Quantas semanas durante os últimos 12 meses? [ ][ ] Semanas

iv. Frequência *(isto é, uso médio nas semanas em que usou)*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. Dependência [a] O1 Sim O0 Não

vi. Abuso [b] O1 Sim O0 Não

2. Ao longo da vida

i. Quantos anos você tinha quando usou sedativos pela primeira vez? [ ][ ] Anos

ii. Houve uma época em que você usava frequentemente? O1 Sim O0 Não

iii. Quantos anos você tinha nesta época? **Dos** [ ][ ] **Aos** [ ][ ]

iv. Frequência no período de maior uso
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. Dependência ao longo da vida [a] O1 Sim O0 Não

vi. Abuso ao longo da vida [b] O1 Sim O0 Não

3. Se você não usa mais sedativos, quantos anos você tinha quando parou de usar?? [ ][ ] Anos

4. Por que você decidiu parar de usar?

_____

Cannabis Experiences Questionnaire

[a] **Rastreamento de dependência para todos os tipos de drogas:**

[a] **Rastreamento de dependência para *todos os tipos de drogas*:**

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] **Definição de 'Abuso'**

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

F. OPIÓIDES[5], por ex. heroína, morfina, metadona, codeína, dolantina, tramadol .....

1. Uso atual (últimos 12 meses)

i. **Usou?** O1 Sim O0 Não

ii. **Se SIM: Usou durante a última semana?** O1 Sim O0 Não

iii. **Quantas semanas durante os últimos 12 meses?** ☐☐ Semanas

iv. **Frequência** *(isto é, uso médio nas semanas em que usou)*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. **Dependência** [a] O1 Sim O0 Não

vi. **Abuso** [b] O1 Sim O0 Não

2. Ao longo da vida

i. **Quantos anos você tinha quando usou opióides pela primeira vez?** ☐☐ Anos

ii. **Houve uma época em que você usava frequentemente?** O1 Sim O0 Não

iii. **Quantos anos você tinha nesta época?** **Dos** ☐☐ **Aos** ☐☐

iv. **Frequência no período de maior uso**
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. **Dependência ao longo da vida** [a] O1 Sim O0 Não

vi. **Abuso ao longo da vida** [b] O1 Sim O0 Não

3. **Se você não usa mais opióides, quantos anos você tinha quando parou de usar?** ☐☐ Anos

4. **Por que você decidiu parar de usar**?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

G. ALUCINÓGENOS[6], por ex. LSD, cogumelos, PCP .....

1. **Uso atual (últimos 12 meses)**

i. **Usou?** O1 Sim O0 Não

ii. **Se SIM: Usou durante a última semana?** O1 Sim O0 Não

iii. **Quantas semanas durante os últimos 12 meses?** ☐☐ Semanas

iv. **Frequência** *(isto é, uso médio nas semanas em que usou)*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. **Dependência** [a] O1 Sim O0 Não

vi. **Abuso** [b] O1 Sim O0 Não

2. **Ao longo da vida**

i. **Quantos anos você tinha quando usou alucinogéneos pela primeira vez?** ☐☐ Anos

ii. **Houve uma época em que você usava frequentemente ?** O1 Sim O0 Não

iii. **Quantos anos você tinha nesta época?** **Dos**: ☐☐ **Aos** ☐☐

iv. **Frequência no período de maior uso**
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. **Dependência ao longo da vida** [a] O1 Sim O0 Não

vi. **Dependência ao longo da vida** [a] O1 Sim O0 Não

3. **Se você não usa mais alucinógenos, quantos anos você tinha quando parou de usar?** ☐☐ Anos

4. **Por que você decidiu parar de usar**?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

H. KETAMINA[7] (ou cetamina) .....

1. **Uso atual (últimos 12 meses)**

i. **Usou?** O1 Sim O0 Não

ii. **Se SIM: Usou durante a última semana?** O1 Sim O0 Não

iii. **Quantas semanas durante os últimos 12 meses?** ☐☐ Semanas

iv. **Frequência** *(isto é, uso médio nas semanas em que usou))*
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana
O0 Não se aplica

v. **Dependência** [a] O1 Sim O0 Não

vi. **Abuso** [b] O1 Sim O0 Não

2. **Ao longo da vida**

i. **Quantos anos você tinha quando usou ketaminas pela primeira vez?** ☐☐ Anos

ii. **Houve uma época em que você usava frequentemente?** O1 Sim O0 Não

iii. **Quantos anos você tinha nesta época?** **Dos**: ☐☐ **Aos:** ☐☐

iv. **Frequência no período de maior uso**
O1 Diariamente
O2 Uma vez por semana
O3 Menos que uma vez por semana

v. **Dependência ao longo da vida**[a] O1 Sim O0 Não

vi. **Abuso ao longo da vida**[b] O1 Sim O0 Não

3. **Se você não usa mais ketamina, quantos anos você tinha quando parou de usar?** ☐☐ Anos

4. **Por que você decidiu parar de usar**?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Cannabis Experiences Questionnaire

Data |__|__|-|__|__|-|2|0|__|__|

I. OUTRAS DROGAS[8]: Por favor, especifique:_____

1. Uso atual (últimos 12 meses)

i.	**Usou?**	O1 Sim	O0 Não
ii.	**Se SIM: Usou durante a última semana?**	O1 Sim	O0 Não
iii.	**Quantas semanas durante os últimos 12 meses?**	☐☐ semanas	
iv.	**Frequência** *(isto é, uso médio nas semanas em que usou)*	O1 Diariamente O2 Uma vez por semana O3 Menos que uma vez por semana O0 Não se aplica	
v.	**Dependência** [a]	O1 Sim	O0 Não
vi.	**Abuso** [b]	O1 Sim	O0 Não

2. Ao longo da vida

i.	**Quantos anos você tinha quando usou.....pela primeira vez?**	☐☐ Anos	
ii.	**Houve uma época em que você usava frequentemente ?**	O1 Sim	O0 Não
iii.	**Quantos anos você tinha nesta época?**	**Dos:** ☐☐	**Aos:** ☐☐
iv.	**Frequência no período de maior uso**	O1 Diariamente O2 Uma vez por semana O3 Menos que uma vez por semana	
v.	**Dependência ao longo da vida** [a]	O1 Sim	O0 Não
vi.	**Abuso ao longo da vida** [b]	O1 Sim	O0 Não

3. Se você não usa mais..... quantos anos você tinha quando parou de usar? ☐☐ Anos

4. Por que você decidiu parar de usar?

_____

Cannabis Experiences Questionnaire

[a] Rastreamento de dependência para todos os tipos de drogas:

[a] Rastreamento de dependência para *todos os tipos de drogas*:

	Passado		Presente	
1. Tolerância, definida por qualquer um dos itens:				
a. Necessidade de aumento significativo da quantidade da substância para ficar intoxicado ou obter os efeitos desejados.	O1 Sim	O0 Não	O1 Sim	O0 Não
b. Redução significativa dos efeitos após o uso crônico da mesma quantidade da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
2. Abstinência, manifestada por qualquer um dos itens:				
c. A presença da síndrome de abstinência característica para a substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
d. A mesma substância (ou substância semelhante) é utilizada para aliviar ou evitar os sintomas de abstinência.	O1 Sim	O0 Não	O1 Sim	O0 Não
3. A substância é geralmente usada em maiores quantidades ou por um período maior do que o planejado.	O1 Sim	O0 Não	O1 Sim	O0 Não
4. Existe um desejo persistente ou tentativas fracassadas de parar ou de reduzir o uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
5. Muito tempo é gasto em atividades necessárias para a obtenção e uso da substância, ou para recuperar-se dos seus efeitos.	O1 Sim	O0 Não	O1 Sim	O0 Não
6. Importantes atividades sociais, ocupacionais ou de lazer são deixadas de lado ou reduzidas por causa do uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não
7. O uso da substância é continuado, apesar da existência de problemas físicos ou psicológicos persistentes ou recorrentes que sejam provavelmente causados ou exacerbados pelo uso da substância.	O1 Sim	O0 Não	O1 Sim	O0 Não

[b] Definição de 'Abuso'

A. Um padrão mal adaptativo de uso de substância levando a prejuízo clínico significativo, manifestado por um (ou mais) dos seguintes itens, ocorrendo dentro de um período de 12 meses:

1. Uso recorrente da substância, resultando na falha em desempenhar obrigações importantes no trabalho, escola ou no ambiente doméstico.
2. Uso recorrente da substância em situações em que existe perigo físico.
3. Problemas legais recorrentes relacionados com o uso da substância.
4. Uso continuado da substância apesar de haver problemas interpessoais ou sociais persistentes ou recorrentes causados ou exacerbados pelos efeitos da substância.

B. Os sintomas nunca preencheram os critérios de Dependência de Substância para esta classe de substância.

Attachment G – Tobacco and alcohol

Tabaco e Álcool

(Nota para a entrada de dados: **EU_TAL)**

ESTUDO: EU GEI

Data |_|_|-|_|_|-|2|0|_|_|

Intervalo de tempo: 12 meses **Period – Replicat** |0|_|-|0|_|

Gostaria de lhe perguntar sobre o uso de tabaco.

Seção Tabaco

1. *Nos últimos 12 meses*, você fumou/usou diariamente por pelo menos um mês....
   a) Cigarros (comum ou cigarro de palha)? O1 Sim O0 Não
   b) charutos? O1 Sim O0 Não
   c) cachimbos? O1 Sim O0 Não
   d) tabaco cheirado ou mascado? O1 Sim O0 Não

2. *Nos últimos 12 meses, durante o período em que mais fumou/usou tabaco, quantos..... fumava/mascava por dia?*

	número/dia
a) cigarros (comum ou cigarro de palha)?	
b) charutos?	
c) cachimbo?	
d) tabaco cheirado ou mascado?	

Tabaco e Álcool

Seção Álcool

As próximas perguntas são sobre o seu uso de bebidas alcoólicas, incluindo cerveja, vinho e destilados.

1a. Bebeu pelo menos 12 ou mais doses de bebida alcoólica *nos últimos 12 meses? Se a resposta for NÃO: Mesmo quando considera bebidas durante o jantar, ocasiões especiais ou feriados?* O1 Sim O0 Não

Se a resposta for **NÃO**, vá para a questão 2a

1b. Quantos doses bebia em média em cada semana? ☐☐

2a. Dentro deste período de 12 meses, houve algum período de **pelo menos 2 semanas,** em que você bebeu mais? O1 Sim O0 Não

Se a resposta for NÃO, vá para a pergunta 3a

2b. Duração total deste(s) período(s) em semanas? ☐☐ semanas

2c. Quantas doses bebia em média em cada semana neste(s) período(s)? ☐☐

3a. Já houve, durante **os últimos 3 anos**, algum período (ou mais períodos) de pelo menos 4 semanas em que você bebia mais do que nos últimos 12 meses? O1 Sim O0 Não

Se a resposta for NÃO, você pode parar aqui.

3b. Duração total deste(s) período(s) em semanas. ☐☐ semanas

3c. Quantas doses você bebia, em média, a cada semana durante esse(s) período(s)? ☐☐

Attachment H – Ethics committee approval

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA
DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO

FMRP-USP
RIBEIRÃO PRETO

Ribeirão Preto, 22 de novembro de 2013

Ofício nº 4203/2013
CEP/MGV

PROCESSO HCRP nº 12606/2012

"ESQUIZOFRENIA E OUTROS TRANSTORNOS PSICÓTICOS – DETERMINANTES SOCIAIS E BIOLÓGICOS"

Prezadas Senhoras,

O Comitê de Ética em Pesquisa, em sua 378ª Reunião Ordinária, realizada em 18/11/2013, tomou ciência e aprovou as recomendações solicitadas pela CONEP e atendidas por Vossa Senhoria (conforme parecer anexo).

Atenciosamente.

DRª. MARCIA GUIMARÃES VILLANOVA
Coordenadora do Comitê de Ética
em Pesquisa do HCRP e da FMRP-USP

Ilustríssimas Senhoras
SILVIA HELENA GALLO TENAN
PROFª.DRª. CRISTINA MARTA DEL BEN
Depto. de Neurociências e Ciências do Comportamento

Campus Universitário – Monte Alegre
14048-900 Ribeirão Preto SP

Comitê de Ética em Pesquisa HCRP e FMRP-USP
FWA-00002733;IRB-00002186 e Registro PB/CONEP n 5440
(16) 3602-2228
cep@hcrp.usp.br

www.hcrp.usp.br

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA DE RIBEIRÃO PRETO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo para Uso de Animais em Experimentação **nº 024/2016**, sobre o projeto intitulado *"Estudo das alterações epigenéticas na neurotransmissão GABAérgica na esquizofrenia e outras psicoses e no modelo animal de isolamento social a partir do desmame"*, sob a responsabilidade da **Professora Doutora Cristina Marta Del Bem** está de acordo com os Princípios Éticos em Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi **APROVADO** em reunião de 25 de abril de 2016.

We certify that the protocol ***nº 024/2016,*** entitled ***"Study of epigenetic alterations in GABAergic neurotransmission in schizophrenia and other psychoses and in animals undergoing social isolation from weaning",*** is in accordance with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation (CONCEA) and was approved by the Local Animal Ethical Committee from the Ribeirão Preto Medical School of the University of São Paulo in *04/25/2016.*

Ribeirão Preto, 25 de abril de 2016.

Prof. Dr. Fernando Silva Ramalho
Presidente da CEUA – FMRP – USP

Attachment I – Informed Consent: Biological Samples

Termo de Consentimento para Guarda de Material Biológico

Eu, Cristina Marta Del-Ben, declaro ser responsável pelo banco de amostras STREAM criado no Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo com o objetivo de guardar amostras de sangue para estudos futuros sobre o papel dos genes e das reações inflamatórias nas causas de transtornos mentais. Este material é coletado logo após a conclusão das entrevistas previstas no projeto de pesquisa Esquizofrenia e outros transtornos psicóticos: determinantes sociais e biológicos, do qual você participa. Nós precisaremos colher amostras de sangue em três ocasiões (20 ml ao todo – equivalente a duas colheres de sopa). É importante lembrar que você poderá sentir dor durante a retirada do sangue e que pode ocorrer o aparecimento de manchas roxas no local. Após coletado, o material será guardado no Laboratório Multiusuário de Biologia Molecular em Neurociências da FMRP-USP.

Desta forma, gostaria de convida-lo(a) para guardar uma amostra do seu sangue para fins de pesquisa e análise científica. Sua participação é voluntária, tendo liberdade de aceitar ou não que sua amostra seja guardada, sem risco de qualquer penalização ou prejuízo no atendimento que lhe for prestado. O(A) Sr.(a) também tem o direito de retirar seu consentimento a qualquer momento.

Eu me comprometo a identificar as amostras e os dados coletados de modo que garanta o seu sigilo e a sua confidencialidade, para isso a sua amostra de sangue será identificada por meio de códigos criados especificamente para este banco de amostras. O (a) senhor(a) me passará todos os dados de como posso lhe encontrar e garanto fornecer as informações de seu interesse, além de receber eventuais benefícios provenientes do estudo com seu material biológico.

Declaro que toda nova pesquisa a ser feita com o seu material será buscado novamente seu consentimento específico, bem como será submetida ao Comitê de Ética em Pesquisa do Hospital das Clínicas e da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo.

Agradeço a colaboração, colocando-me à disposição para os esclarecimentos que se fizerem necessários.

Certificado de Consentimento

Tendo recebido as informações acima, aceito que minha amostra de material biológico seja armazenada no Hospital das Clínicas de Ribeirão Preto, sob a responsabilidade de **Cristina Marta Del-Ben**, para fins de pesquisa e análise científica.

Nome e documento de identificação do participante	Assinatura	Data
Nome e documento de identificação da testemunha imparcial	Assinatura	Data
Nome e documento de identificação do responsável legal	Assinatura	Data
Nome do pesquisador	Assinatura	Data

Attachment J – Informed Consent: Cases
Termo de Consentimento Livre e Esclarecido – Casos

ESQUIZOFRENIA E OUTROS TRANSTORNOS PSICÓTICOS: DETERMINANTES SOCIAIS E BIOLÓGICOS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – CASOS

Você está sendo convidado(a) a participar de um estudo chamado "Esquizofrenia e outros transtornos psicóticos: determinantes sociais e biológicos". Antes de você decidir sobre a sua participação é importante que você entenda porque esta pesquisa está sendo realizada e do que ela trata. Por favor, leia as seguintes informações com cuidado e fique à vontade para fazer perguntas, caso haja algo que não esteja claro para você ou se você precisar de mais detalhes.
Obrigado pelo seu interesse em nosso projeto.

Qual o objetivo deste estudo?

Nós estamos interessados em descobrir se o risco de desenvolver um transtorno psicótico, cuja principal característica são alterações do pensamento e da percepção dos estímulos da realidade, caracterizadas pela crença em situações que não existem (delírios) e por ver e/ou ouvir coisas que outras pessoas não estão vendo ou ouvindo (alucinações). É determinado por fatores biológicos (como a organização dos genes, alterações no sistema de proteção de seu organismo e alterações cerebrais) e por fatores ambientais (como pobreza, discriminação, isolamento social, uso de drogas e a ocorrência de eventos negativos durante a infância, como divórcio dos pais, humilhação ou abuso). Particularmente, estamos interessados em compreender se os efeitos destes fatores sociais no risco de psicose são diferentes, de acordo com os diferentes tipos de genes que as pessoas possuem.

Assim, pretendemos investigar a existência de variações nos casos novos de transtornos psicóticos considerando-se
a) a associação entre fatores de risco da própria pessoa, de seus familiares e das características da região onde o participante vive;
b) a existência de alterações no cérebro de pessoas com transtornos psicóticos, comparados com pessoas sem o mesmo diagnóstico (controles da comunidade e irmãos), através de um exame de Ressonância Nuclear Magnética;
c) a ocorrência de alterações na organização dos genes e no sistema de proteção do organismo em pessoas com transtornos psicóticos que apresentam alucinações e/ou delírios, comparando-as com pessoas sem o mesmo diagnóstico (controles da comunidade e irmãos).

Por que eu fui foi convidado?

Você foi convidado para participar do estudo porque você apresentou um primeiro episódio psicótico com a presença de alucinações e/ou delírios que desorganizaram seu comportamento e vive em uma das cidades pertencentes ao Décimo Terceiro Departamento Regional da Secretaria Estadual de Saúde (DRS XIII), cuja sede é Ribeirão Preto, onde nós estamos conduzindo o estudo.

Nós pretendemos convidar, em um período de três anos, 300 pessoas que apresentaram um primeiro episódio psicótico para participar do estudo, assim como 150 irmãos ou irmãs destes participantes e 300 pessoas que nunca tiveram episódio psicótico. O estudo faz parte de um grande estudo europeu que está sendo realizado em 15 centros europeus de 5 países. Você deve ter idade entre 16 e 64 anos para participar. Caso você tenha menos de 18 anos, seus pais ou outro responsável legal deverão concordar com a sua participação no estudo.

Eu sou obrigado a participar?

Esta é uma escolha sua. Antes que você concorde em participar, nós descreveremos o estudo ao longo desse termo de informação. Nós então pediremos que você assine um termo de consentimento para demonstrar que você concordou em participar. Você é livre para se retirar do estudo a qualquer momento, sem dar explicações. Essa escolha não irá afetar os cuidados de saúde que você recebe.

O que me pedirão para fazer?

Inicialmente, nós pediremos que você responda alguns questionários sobre o seu passado, sobre as suas condições de saúde atuais e circunstâncias sociais. Perguntaremos também sobre os seus sintomas e solicitaremos a sua permissão para ler o seu prontuário.

Nós precisaremos colher amostras de sangue em três ocasiões (20 ml ao todo – equivalente a duas colheres de sopa) para que nós possamos estudar a interação de genes e da capacidade de proteção de seu organismo com fatores sociais e experiências durante a vida. O sangue será colhido utilizando material descartável e este procedimento será realizado por profissionais experientes [médico(a) ou enfermeiro(a)]. É importante lembrar que você poderá sentir dor durante a retirada do sangue e que pode ocorrer o aparecimento de manchas roxas no local.

Você também será convidado a realizar de um exame de Ressonância Magnética. Esse exame será usado para investigarmos se existem diferenças no tamanho e nos níveis de algumas substâncias de algumas áreas do cérebro entre pacientes e controles.
Pediremos também a sua permissão para convidar seus irmãos e irmãs para participar do estudo.

Quanto tempo irá durar a coleta de dados?

Nós estimamos que precisaremos de cerca de 6 horas para completarmos todos os questionários e realizarmos as coletas de sangue. Esperamos completar todas as tarefas em 3 encontros, mas, se você preferir, podemos fazer outros arranjos. Você é livre para fazer pausas em qualquer momento que desejar ou pode escolher outro horário para retornar em outra ocasião para terminar a coleta caso se sinta cansado ou indisposto.

O exame de ressonância magnética será realizado em um dia previamente agendado e deve durar cerca de 40 minutos.

Eu receberei algum pagamento?

Nós iremos ressarcir seus gastos com transporte e alimentação.

Onde o estudo será realizado?

O estudo será realizado no Ambulatório do Hospital das Clínicas (HCFMRP-USP), nos dias em que você tiver retorno com seu médico. Se você preferir, nós poderemos ir até a sua casa para realizarmos as entrevistas, porém nós teremos que pedir que você vá até o Hospital das Clínicas para coletarmos a amostra de sangue e para a realização do exame de ressonância magnética.

O que acontecerá se eu optar por sair do estudo?

A participação no estudo é absolutamente voluntária. Se você optar por não participar, essa decisão não irá interferir no seu tratamento, ou no seu relacionamento com o seu médico ou outros profissionais de saúde. Você é livre para mudar de ideia a qualquer momento. Todas as informações pessoais serão destruídas. Esta situação também se aplica no caso de você se sentir indisposto para continuar participando do estudo.

Quais são os possíveis riscos e benefícios da participação?

Nós faremos preguntas sobre circunstâncias pessoais e do passado, o que algumas pessoas podem considerar angustiante. Todos os pesquisadores responsáveis pela coleta de dados são psicólogos treinados e experientes e oferecerão suporte se você precisar. Você também poderá achar o dia cansativo e então você poderá fazer uma pausa ou retornar em outra ocasião para completar as tarefas. Você não tem obrigação de responder nenhuma questão e você pode sair do estudo a qualquer momento. Um outro inconveniente pode ser um leve desconforto ao coletar a amostra de sangue e ao realizar o exame de Ressonância Magnética.

As entrevistas, assim como os exames de imagem e a coleta de sangue serão realizadas por profissionais treinados com os procedimentos e com experiência no manejo de problemas emocionais.

O exame de Ressonância Magnética de crânio é um exame seguro, não doloroso, não invasivo, sem emissão de radiação, e não será administrada anestesia ou contraste, portanto, não há risco de reações alérgicas. Você ficará deitado acordado enquanto o aparelho faz imagens do seu cérebro. Algumas pessoas podem se sentir desconfortáveis por ficarem deitadas em local estreito ou incomodadas com o barulho forte que a máquina de ressonância faz quando está funcionando. O exame será interrompido imediatamente, e você poderá sair do aparelho, se desejar, podendo fazer o exame em outro momento.

Você usará protetores de ouvido durante o exame para diminuir o desconforto do barulho. Terá também à mão uma campainha, que poderá acionar se precisar falar com o técnico que estará operando o aparelho. Um dos pesquisadores deste projeto estará presente durante todo o exame de Ressonância Magnética.

Ao participar do estudo você irá nos ajudar a entender mais sobre as diferenças entre pessoas com e sem psicose (presença de alucinações e delírios), o que pode ajudar a prevenir que outras pessoas venham a desenvolver transtornos psicóticos no futuro.

O que acontecerá com as minhas informações?

Sua confidencialidade será mantida em todos os momentos e as amostras de sangue, papéis e dados eletrônicos seguirão as práticas éticas e legais. Todas as informações sobre você serão manejadas com estrita confidencialidade. Você será identificado por um número, que será utilizado no lugar dos seus dados pessoais. Isso significa que toda informação que você nos der será efetivamente anônima. Informações identificáveis (como o seu nome) serão registradas em uma base de dados separada e protegida por senha, sendo acessível somente pelo coordenador do estudo. Nós só iremos passar informações suas em situações extremas como quando nós tivermos o dever de informar os seus cuidadores se nós acreditarmos que você está em risco de machucar a si ou a outras pessoas.

No final do estudo suas informações serão mantidas seguras por no mínimo 20 anos de acordo com as boas práticas de pesquisa e não serão utilizados com nenhum outro propósito além dos descritos no estudo. Se você decidir se retirar do estudo nos iremos destruir toda informação pessoal que nós temos de você, mas nós poderemos manter seus dados anonimamente para nossa pesquisa. Os resultados deste estudo serão publicados em jornais científicos em um nível grupal e não individual. Nós não iremos nunca revelar informações pessoais sobre você.

Como a quantidade de indivíduos que serão examinados neste estudo é muito grande, não será possível realizar todos os exames laboratoriais ao mesmo tempo. Para isso o material terá que ser estocado por algum tempo até a realização dos exames. Pedimos a você permissão para que o sangue seja guardado por tempo indeterminado, visto que este estudo e outros que têm sido feitos podem trazer novos conhecimentos sobre o assunto podendo haver necessidade

de realização de novos testes com o material guardado. No entanto, novos testes somente serão realizados após aprovação de um novo projeto de pesquisa pelo Comitê de Ética em Pesquisa do HCFMRP-USP e você deverá ser novamente consultado para autorizar os novos testes. Você deverá assinar um outro termo de consentimento a respeito do armazenamento de amostras de sangue.

Também pedimos sua autorização para enviar parte do sangue coletado para um laboratório na Inglaterra, para que as informações que estamos colhendo aqui no Brasil possam ser comparadas com as informações colhidas na Europa. É importante lembrar que você poderá solicitar o acesso aos resultados de seus exames de sangue caso tenha interesse. Se seu exame de sangue apresentar alterações que necessitam de intervenção seu médico o orientará sobre o que fazer e em caso de necessidade será feito acompanhamento clínico e/ou aconselhamento genético sem que isto lhe traga qualquer custo.

O que acontecerá se eu tiver algum dano em função do estudo?

Caso você tenha algum dano em relação a sua saúde em função da realização do estudo, lhe será oferecido acompanhamento clínico no Hospital das Clínicas com direito à assistência integral e a indenização, se for o caso.

Onde eu posso conseguir mais informação sobre o estudo?

Você pode entrar em contato com os responsáveis pelo estudo sobre qualquer dúvida que você tiver. Os contatos estão detalhados abaixo.

Onde eu posso fazer reclamações e/ou esclarecimentos sobre o estudo?

Se você está descontente ou precisar de esclarecimentos sobre como este estudo você pode fazer contato, a qualquer momento, com os responsáveis pelo estudo e com o Comitê de Ética em Pesquisa que garante as boas práticas de pesquisa além de zelar pelo cumprimento do é descrito neste termo de consentimento livre e esclarecido.

Caso seja necessário os contatos estão abaixo.

Contatos	**Página do estudo: www.eu-gei.eu**	**Comitê de Ética em Pesquisa-HCRP**
Coordenadora Profa. Dra. Cristina Marta Del-Ben CREMESP: 63638 Departamento de Neurociências e Ciências do Comportamento Faculdade de Medicina de Ribeirão Preto-USP Avenida Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-2607	**Pesquisadora responsável** Silvia Helena Gallo Tenan CRP: 06/49802-2 Departamento de Neurociências e Ciências do Comportamento Faculdade de Medicina de Ribeirão Preto-USP Avenida Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-1296 Email: stream@fmrp.usp.br	Avenida dos Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-2228 E-mail: cep@hcrp.usp.br Horário de funcionamento: Segunda à sexta das 8h às 17h.

Projeto de pesquisa

ESQUIZOFRENIA E OUTROS TRANSTORNOS PSICÓTICOS: DETERMINANTES SOCIAIS E BIOLÓGICOS

Coordenadora: Cristina Marta Del-Ben (CREMESP: 63638)
Pesquisadora responsável: Silvia Helena Gallo Tenan (CRP: 06/49802-2)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – CASOS

Antes de você concordar em participar deste estudo, é importante que você tenha lido e entendido o Termo de Consentimento Livre e Esclarecido, que é elaborado em duas vias sendo uma de propriedade do pesquisador e outra do participante. Todas as páginas, das duas vias do termo de consentimento deverão ser rubricadas pelo participante e pelo coordenador do estudo e estes deverão assiná-lo ao seu término. O termo de consentimento contêm informações importantes sobre a pesquisa e sobre o que será pedido para você fazer. Se você se sentir inseguro sobre o projeto ou tiver alguma dúvida, você pode fazer perguntas para qualquer membro da equipe de pesquisa. As declarações abaixo contêm informações importantes sobre a sua participação no estudo. Por favor, leia estas declarações e coloque as iniciais do seu nome no espaço apropriado.

	INICIAIS
Eu li e entendi o termo de consentimento livre e esclarecido e todas as minhas dúvidas foram respondidas satisfatoriamente.	
Eu entendo que a minha participação no estudo é voluntária e que eu posso mudar de ideia a qualquer momento, sem motivo ou qualquer prejuízo, e que isso não irá afetar meu tratamento atual e futuro ou meus direitos legais.	
Eu entendo que as minhas informações serão armazenadas confidencialmente e anonimamente e que não serão repassadas a terceiros ou usadas de outra forma a não ser para responder questões relevantes para os objetivos do estudo, exceto quando os pesquisadores tiverem obrigação de informar aos meus cuidadores se eu estiver em risco de causar danos a mim ou a outras pessoas.	
Eu entendo que as minhas informações podem ser utilizadas anonimamente, contribuindo para apresentações e artigos científicos.	
Eu entendo que será pedido que eu dê uma amostra de sangue, com a finalidade de análises genéticas e imunológicas.	
Eu entendo que serei convidado para a realização de exame de ressonância nuclear magnética do cérebro	
Eu dei permissão para que os pesquisadores envolvidos neste estudo acessem meu prontuário médico para finalidades da pesquisa.	

Por meio desta, concordo em participar do estudo EU-GEI e entendo que a minha participação é totalmente voluntária e que eu posso retirar o meu consentimento em qualquer momento, sem ter penalidades ou razões.

Nome e documento de identificação do participante	Assinatura	Data
Nome e documento de identificação da testemunha imparcial	Assinatura	Data
Nome e documento de identificação do responsável legal	Assinatura	Data
Nome do pesquisador	Assinatura	Data

Attachment k – Informed Consent: Controls
Termo de Consentimento Livre e Esclarecido – Controles

Projeto de pesquisa

ESQUIZOFRENIA E OUTROS TRANSTORNOS PSICÓTICOS: DETERMINANTES SOCIAIS E BIOLÓGICOS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - CONTROLES

Você está sendo convidado(a) a participar de um estudo chamado "Esquizofrenia e outros transtornos psicóticos: determinantes sociais e biológicos". Antes de você decidir sobre a sua participação é importante que você entenda porque esta pesquisa está sendo realizada e do que ela trata. Por favor, leia as seguintes informações com cuidado e fique à vontade para fazer perguntas, caso haja algo que não esteja claro para você ou se você precisar de mais detalhes.
Obrigado pelo seu interesse em nosso projeto.

Qual o objetivo deste estudo?

Nós estamos interessados em descobrir se o risco de desenvolver um transtorno psicótico, cuja principal característica são alterações do pensamento e da percepção dos estímulos da realidade caracterizadas pela crença em situações que não existem (delírios) e por ver e/ou ouvir coisas que outras pessoas não estão vendo ou ouvindo (alucinações), é determinado por fatores biológicos (como a organização dos genes, alterações no sistema de proteção do seu organismo e alterações cerebrais) e por fatores ambientais (como pobreza, discriminação, isolamento social, uso de drogas e a ocorrência de eventos negativos durante a infância, como divórcio dos pais, humilhação ou abuso). Particularmente, estamos interessados em compreender se os efeitos destes fatores sociais no risco de psicose são diferentes, de acordo com os diferentes tipos de genes que as pessoas possuem.

Assim, pretendemos investigar a existência de variações nos casos novos de transtornos psicóticos considerando-se:
a) a associação entre fatores de risco da própria pessoa, de seus familiares e das características da região onde o participante vive;
b) a existência de alterações no cérebro de pessoas com transtornos psicóticos, comparados com pessoas sem o mesmo diagnóstico (controles da comunidade e irmãos), através de exames de Ressonância Nuclear Magnética
c) a ocorrência de alterações na organização dos genes e no sistema de proteção do organismo em pessoas com transtornos psicóticos, comparando-as com pessoas sem o mesmo diagnóstico (controles da comunidade e irmãos).

Por que eu fui foi convidado?

Você foi convidado para participar do estudo porque você nunca apresentou um episódio psicótico com a presença de alucinações e/ou delírios que desorganizaram seu comportamento e vive em uma das cidades pertencentes ao Décimo Terceiro Departamento Regional da Secretaria Estadual de Saúde (DRS XIII), cuja sede é Ribeirão Preto, onde nós estamos conduzindo o estudo.

Nós pretendemos convidar, em um período de três anos, 300 pessoas que apresentaram um primeiro episódio psicótico para participar do estudo (casos), assim como 150 irmãos ou irmãs destes participantes e 300 pessoas que como você nunca tiveram episódio psicótico e que chamamos de controles. O estudo faz parte de um grande estudo europeu que está sendo

realizado em 15 centros europeus de 5 países. Você deve ter idade entre 16 e 64 anos para participar. Caso você tenha menos de 18 anos, seus pais ou outro responsável legal deverão concordar com a sua participação no estudo.

Eu sou obrigado a participar?

Esta é uma escolha sua. Antes que você concorde em participar, nós descreveremos o estudo ao longo desse termo de informação. Nós então pediremos que você assine um termo de consentimento para demonstrar que você concordou em participar. Você é livre para se retirar do estudo a qualquer momento, sem dar explicações. Essa escolha não irá afetar os cuidados de saúde que poderá a vir receber no Hospital das Clínicas FMRP-USP.

O que me pedirão para fazer?

Inicialmente, nós pediremos que você responda alguns questionários sobre o seu passado, sobre as suas condições de saúde atuais e circunstâncias sociais. Perguntaremos também sintomas psiquiátricos para caracterizar se você já experimentou manifestações relacionadas à alguma doença mental.

Nós precisaremos colher amostras de sangue em três ocasiões (20 ml ao todo – equivalente a duas colheres de sopa) para que nós possamos estudar a interação de genes e da capacidade de proteção de seu organismo como fatores sociais e experiências durante a vida. O sangue será colhido utilizando material descartável e este procedimento será realizado por profissionais experientes [médico(a) ou enfermeiro(a)]. É importante lembrar que você poderá sentir dor durante a retirada do sangue e que pode ocorrer o aparecimento de manchas roxas no local.

Você também será convidado a realizar de um exame de Ressonância Magnética. . Esse exame será usado para investigarmos se existem diferenças no tamanho e nos níveis de algumas substâncias de algumas áreas do cérebro entre pacientes e controles como você.

Quanto tempo irá durar a coleta de dados?

Nós estimamos que precisaremos de cerca de 6 horas para completarmos todos os questionários e realizarmos as coletas de sangue. Esperamos completar todas as tarefas em 3 encontros, mas, se você preferir, podemos fazer outros arranjos. Você é livre para fazer pausas em qualquer momento que desejar ou pode escolher outro horário para retornar em outra ocasião para terminar a coleta caso se sinta cansado ou indisposto.

O exame de ressonância magnética será realizado em um dia previamente agendado e deve durar cerca de 40 minutos.

Eu receberei algum pagamento?

Nós iremos ressarci-lo por gastos com transporte e alimentação.

Onde o estudo será realizado?

O estudo será realizado no Ambulatório do Hospital das Clínicas (HCFMRP-USP), nos dias em que você tiver disponibilidade para comparecer ao hospital. Se você preferir, nós poderemos ir até a sua casa para realizarmos as entrevistas, porém nós teremos que pedir que você vá até o Hospital das Clínicas para coletarmos a amostra de sangue e para a realização do exame de ressonância magnética.

O que acontecerá se eu optar por sair do estudo?

A participação no estudo é absolutamente voluntária. Você é livre para mudar de ideia a qualquer momento. Todas as informações pessoais serão destruídas. Esta situação também se aplica no caso de você se sentir indisposto para continuar participando do estudo.

Quais são os possíveis riscos e benefícios da participação?

Nós faremos preguntas sobre circunstâncias pessoais e do passado, o que algumas pessoas podem considerar angustiante. Todos os pesquisadores responsáveis pela coleta de dados são psicólogos treinados e experientes e oferecerão suporte se você precisar. Você também poderá achar o dia cansativo e então você poderá fazer uma pausa ou retornar em outra ocasião para completar as tarefas. Você não tem obrigação de responder nenhuma questão e você pode sair do estudo a qualquer momento. Um outro inconveniente pode ser um leve desconforto ao coletar a amostra de sangue e ao realizar o exame de Ressonância Magnética.

As entrevistas, assim como os exames de imagem e a coleta de sangue serão realizadas por profissionais treinados com os procedimentos e com experiência no manejo de problemas emocionais.

O exame de Ressonância Magnética de crânio é um exame seguro, não doloroso, não invasivo, sem emissão de radiação, e não será administrada anestesia ou contraste, portanto, não há risco de reações alérgicas. Você ficará deitado acordado enquanto o aparelho faz imagens do seu cérebro. Algumas pessoas podem se sentir desconfortáveis por ficarem deitadas em local estreito ou incomodadas com o barulho forte que a máquina de ressonância faz quando está funcionando. O exame será interrompido imediatamente, e você poderá sair do aparelho, se desejar, podendo fazer o exame em outro momento.

Você usará protetores de ouvido durante o exame para diminuir o desconforto do barulho. Terá também à mão uma campainha, que poderá acionar se precisar falar com o técnico que estará operando o aparelho. Um dos pesquisadores deste projeto estará presente durante todo o exame de Ressonância Magnética.

Ao participar do estudo você irá nos ajudar a entender mais sobre as diferenças entre pessoas com e sem psicose (presença de alucinações e delírios), o que pode ajudar a prevenir que outras pessoas venham a desenvolver transtornos psicóticos no futuro.

O que acontecerá com as minhas informações?

Sua confidencialidade será mantida em todos os momentos e as amostras de sangue, papéis e dados eletrônicos seguirão as práticas éticas e legais. Todas as informações sobre você serão manejadas com estrita confidencialidade. Você será identificado por um número, que será utilizado no lugar dos seus dados pessoais. Isso significa que toda informação que você nos der será efetivamente anônima. Informações identificáveis (como o seu nome) serão registradas em uma base de dados separada e protegida por senha, sendo acessível somente pelo coordenador do estudo.

No final do estudo suas informações serão mantidas seguras por no mínimo 20 anos de acordo com as boas práticas de pesquisa e não serão utilizados com nenhum outro propósito além dos descritos no estudo. Se você decidir se retirar do estudo nós iremos destruir toda informação pessoal que nós temos de você, mas nós poderemos manter seus dados anonimamente para nossa pesquisa. Os resultados deste estudo serão publicados em jornais científicos em um nível grupal e não individual. Nós não iremos nunca revelar informações pessoais sobre você.

Como a quantidade de indivíduos que serão examinados neste estudo é muito grande, não será possível realizar todos os exames laboratoriais ao mesmo tempo. Para isso o material terá que ser estocado por algum tempo até a realização dos exames. Pedimos a você permissão para que o sangue seja guardado por tempo indeterminado, visto que este estudo e outros que têm sido feitos podem trazer novos conhecimentos sobre o assunto podendo haver necessidade de realização de novos testes com o material guardado. No entanto, novos testes somente serão realizados após aprovação de um novo projeto de pesquisa pelo Comitê de Ética em Pesquisa do HCFMRP-USP e você deverá ser novamente consultado para autorizar os novos testes. Você

deverá assinar um outro termo de consentimento a respeito do armazenamento de amostras de sangue.

Também pedimos sua autorização para enviar parte do sangue coletado para um laboratório na Inglaterra, para que as informações que estamos colhendo aqui no Brasil possam ser comparadas com as informações colhidas na Europa. É importante lembrar que você poderá solicitar o acesso aos resultados de seus exames de sangue caso tenha interesse. Se seu exame de sangue apresentar alterações que necessitam de intervenção seu médico o orientará sobre o que fazer e em caso de necessidade será feito acompanhamento clínico e/ou aconselhamento genético sem que isto lhe traga qualquer custo.

O que acontecerá se eu tiver algum dano em função do estudo?

Caso você tenha algum dano em relação a sua saúde em função da realização do estudo, lhe será oferecido acompanhamento clínico no Hospital das Clínicas com direito à assistência integral e a indenização, se for o caso.

Onde eu posso conseguir mais informação sobre o estudo?

Você pode entrar em contato com os responsáveis pelo estudo sobre qualquer dúvida que você tiver. Os contatos estão detalhados abaixo.

Onde eu posso fazer reclamações e/ou esclarecimentos sobre o estudo?

Se você está descontente ou precisar de esclarecimentos sobre como este estudo você pode fazer contato, a qualquer momento, com os responsáveis pelo estudo e com o Comitê de Ética em Pesquisa que garante as boas práticas de pesquisa além de zelar pelo cumprimento do é descrito neste termo de consentimento livre e esclarecido.

Caso seja necessário os contatos estão abaixo.

Contatos	**Página do estudo: www.eu-gei.eu**	**Comitê de Ética em Pesquisa-HCRP**
Coordenadora Profa. Dra. Cristina Marta Del-Ben CREMESP: 63638 Departamento de Neurociências e Ciências do Comportamento Faculdade de Medicina de Ribeirão Preto-USP Avenida Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-2607	**Pesquisadora responsável** Silvia Helena Gallo Tenan CRP: 06/49802-2 Departamento de Neurociências e Ciências do Comportamento Faculdade de Medicina de Ribeirão Preto-USP Avenida Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-1296 Email: stream@fmrp.usp.br	Avenida dos Bandeirantes, 3900 CEP: 14049-900 Fone: 16 3602-2228 E-mail: cep@hcrp.usp.br Horário de funcionamento: Segunda à sexta das 8h às 17h.

Projeto de pesquisa

ESQUIZOFRENIA E OUTROS TRANSTORNOS PSICÓTICOS: DETERMINANTES SOCIAIS E BIOLÓGICOS

Coordenadora: Cristina Marta Del-Ben (CREMESP: 63638)
Pesquisadora responsável: Silvia Helena Gallo Tenan (CRP: 06/49802-2)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - CONTROLES

Antes de você concordar em participar deste estudo, é importante que você tenha lido e entendido o Termo de Consentimento Livre e Esclarecido, que é elaborado em duas vias sendo uma de propriedade do pesquisador e outra do participante. Todas as páginas, das duas vias do termo de consentimento deverão ser rubricadas pelo participante e pelo coordenador do estudo e estes deverão assiná-lo ao seu término. O termo de consentimento contêm informações importantes sobre a pesquisa e sobre o que será pedido para você fazer. Se você se sentir inseguro sobre o projeto ou tiver alguma dúvida, você pode fazer perguntas para qualquer membro da equipe de pesquisa. As declarações abaixo contêm informações importantes sobre a sua participação no estudo. Por favor, leia estas declarações e coloque as iniciais do seu nome no espaço apropriado.

	INICIAIS
Eu li e entendi o termo de consentimento livre e esclarecido e todas as minhas dúvidas foram respondidas satisfatoriamente.	
Eu entendo que a minha participação no estudo é voluntária e que eu posso mudar de ideia a qualquer momento, sem motivo ou qualquer prejuízo, e que isso não irá afetar meu tratamento atual e futuro ou meus direitos legais.	
Eu entendo que as minhas informações serão armazenadas confidencialmente e anonimamente e que não serão repassadas a terceiros ou usadas de outra forma a não ser para responder questões relevantes para os objetivos do estudo, exceto quando os pesquisadores tiverem obrigação de informar aos meus cuidadores se eu estiver em risco de causar danos a mim ou a outras pessoas.	
Eu entendo que as minhas informações podem ser utilizadas anonimamente, contribuindo para apresentações e artigos científicos.	
Eu entendo que será pedido que eu dê uma amostra de sangue, com a finalidade de análises genéticas e imunológicas.	
Eu entendo que serei convidado para a realização de exame de ressonância nuclear magnética do cérebro	
Eu dei permissão para que os pesquisadores envolvidos neste estudo acessem meu prontuário médico para finalidades da pesquisa.	

Por meio desta, concordo em participar do estudo EU-GEI e entendo que a minha participação é totalmente voluntária e que eu posso retirar o meu consentimento em qualquer momento, sem ter penalidades ou razões.

Nome e documento de identificação do participante	Assinatura	Data
Nome e documento de identificação da testemunha imparcial	Assinatura	Data
Nome e documento de identificação do responsável legal	Assinatura	Data
Nome do pesquisador	Assinatura	Data

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Professora Dra. Cristina Marta Del-Ben, Professora associada do Departamento de Neurociências e Ciências do Comportamento da Faculdade de Medicina de Ribeirão Preto, autorizo que aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a minha orientação, utilize em sua dissertação de mestrado o artigo científico intitulado **"Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain"** submetido ao jornal científico *Frontiers in Neuroscience*, e o artigo científico intitulado **"Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma"** submetido ao jornal científico *Psychological Medicine*. Declaro ainda que os artigos não serão apresentados em outros trabalhos de conclusão de curso.

Professora Dra. Cristina Marta Del-Bem
Professora associada. Divisão de Psiquiatria
Faculdade de Medicina de Ribeirão Preto
Departamento de Neurociências e Ciências do Comportamento
Universidade de São Paulo (USP) – Brasil

São Paulo, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Paulo Rossi Menezes, Professor Titular e Chefe do Departamento de Medicina Preventiva da Faculdade de Medicina da Universidade de São Paulo, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain**" submetido ao jornal científico *Frontiers in Neuroscience,* e o artigo científico intitulado **"Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma"** submetido ao jornal científico *Psychological Medicine*, dos quais sou co-autor. Declaro ainda que os artigos não serão apresentados em outros trabalhos de conclusão de curso.

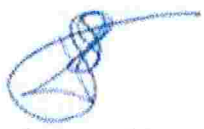
Prof. Dr. Paulo Rossi Menezes

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Paulo Louzada Junior, Professor Associado do Departamento de Clínica Médica – FMRP-USP, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain"** submetido ao jornal científico *Frontiers in Neuroscience*, e o artigo científico intitulado "Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma" submetido ao jornal científico "*Psychological Medicine*", dos quais sou co-autor. Declaro ainda que o artigo não será apresentado em outros trabalhos de conclusão de curso.



Prof. Paulo Louzada Junior
Professor Associado do Departamento de Clínica Médica
Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo
plouzada@fmrp.usp.br

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Sâmia R L Joca, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain**" submetido ao jornal científico *Frontiers in Neuroscience,* do qual sou co-autor. Declaro ainda que o artigo não será apresentado em outros trabalhos de conclusão de curso.

Sâmia R. L. Joca

Dr Valeria Mondelli

The Maurice Wohl Clinical
Neuroscience Institute, Room G.30.01
Cutcombe Road
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Tel: (+ 44) 0 20 7848 0353
e-mail: valeria.mondelli@kcl.ac.uk

London, 15th October 2018

To the kind attention of the Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

RE: Agreement to use the scientific manuscript for the purposes of MSc Dissertation

I am delighted to inform that I, Dr. Valeria Mondelli Senior Clinical Lecturer from the Psychological Medicine Department, Institute of Psychiatry, Psychology and Neuroscience, King's College London, U.K., agree that the MSc student Fabiana Maria das Graças Corsi Zuelli, USP ID 6809211, from the Programa Medicina (Neurologia), concentration area Neurociências, under the supervision of Professor Dra. Cristina Marta Del-Ben, uses in her dissertation the scientific manuscript entitled **"Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma"**, submitted to *Psychological Medicine*, from which I am co-author. I also declare that the aforementioned manuscript will not be part of any other completion work.

Should you have further questions, do not hesitate to contact me.

Yours Sincerely,

Dr Valeria Mondelli, MD, PhD
Senior Clinical Lecturer

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Helene Aparecida Fachim, pós-doutoranda do Departamento de Neurociências e Ciências do Comportamento da Faculdade de Medicina de Ribeirão Preto (FMRP-USP), Brasil e do Biomolecular Sciences Reseach Centre, Sheffield Hallam University, U.K, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado **"Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain"** submetido ao jornal científico *Frontiers in Neuroscience,* e o artigo científico intitulado **"Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma"** submetido ao jornal científico *Psychological Medicine*, dos quais sou co-autor. Declaro ainda que os artigos não serão apresentados em outros trabalhos de conclusão de curso.

Helene A. Fachim

PhD. Helene A. Fachim
Departamento de Neurociências e Ciências do Comportamento
Faculdade de Medicina de Ribeirão Preto (FMRP-USP), Brasil
Biomolecular Sciences Reseach Centre, Sheffield Hallam University, U.K

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, ROSANA SHUHAMA, doutora em ciências, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain**" submetido ao jornal científico *Frontiers in Neuroscience*, e o artigo científico intitulado **"Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma"** submetido ao jornal científico *Psychological Medicine*, dos quais sou co-autor. Declaro ainda que os artigos não serão apresentados em outros trabalhos de conclusão de curso.

Rosana Shuhama

Rosana Shuhama
Agente técnica de assistência em saúde
HCFMRPUSP

Ribeirão Preto, 15 de outubro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Camila Marcelino Loureiro, doutoranda do Programa de Pós-Graduação em Clínica Médica, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain**" submetido ao jornal científico *Frontiers in Neuroscience*, e o artigo científico intitulado "**Cytokine profile in first-episode psychosis, unaffected siblings and community-based controls: the effects of familial liability and childhood trauma**" submetido ao jornal científico *Psychological Medicine*, dos quais sou co-autor. Declaro ainda que os artigos não serão apresentados em outros trabalhos de conclusão de curso.

Camila M. Loureiro

Camila Marcelino Loureiro
Doutoranda do Programa de Pós-Graduação em Clínica Médica
Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo
camila.loureiro@usp.br

Ribeirão Preto, 05 de novembro de 2018

Ao Programa de Pós-Graduação em Neurologia e Neurociências da Faculdade de Medicina de Ribeirão Preto (FMRP – USP)

Assunto: Autorização para utilização de artigo científico em dissertação de Mestrado

Eu, Giuliana Bertozi, autorizo que a aluna Fabiana Maria das Graças Corsi Zuelli, número USP 6809211, regularmente matriculada no curso de Mestrado, no programa Medicina (Neurologia), área de concentração Neurociências, sob a orientação da Professora Dra. Cristina Marta Del-Ben, utilize em sua dissertação de mestrado o artigo científico intitulado "**Prolonged periods of social isolation from weaning reduce the anti-inflammatory cytokine IL-10 in blood and brain"** submetido ao jornal científico *Frontiers in Neuroscience* do qual sou co-autora. Declaro ainda que o artigo não será apresentado em outros trabalhos de conclusão de curso.

Giuliana Bertozi

Giuliana Bertozi
email: gbertozi@hotmail.com