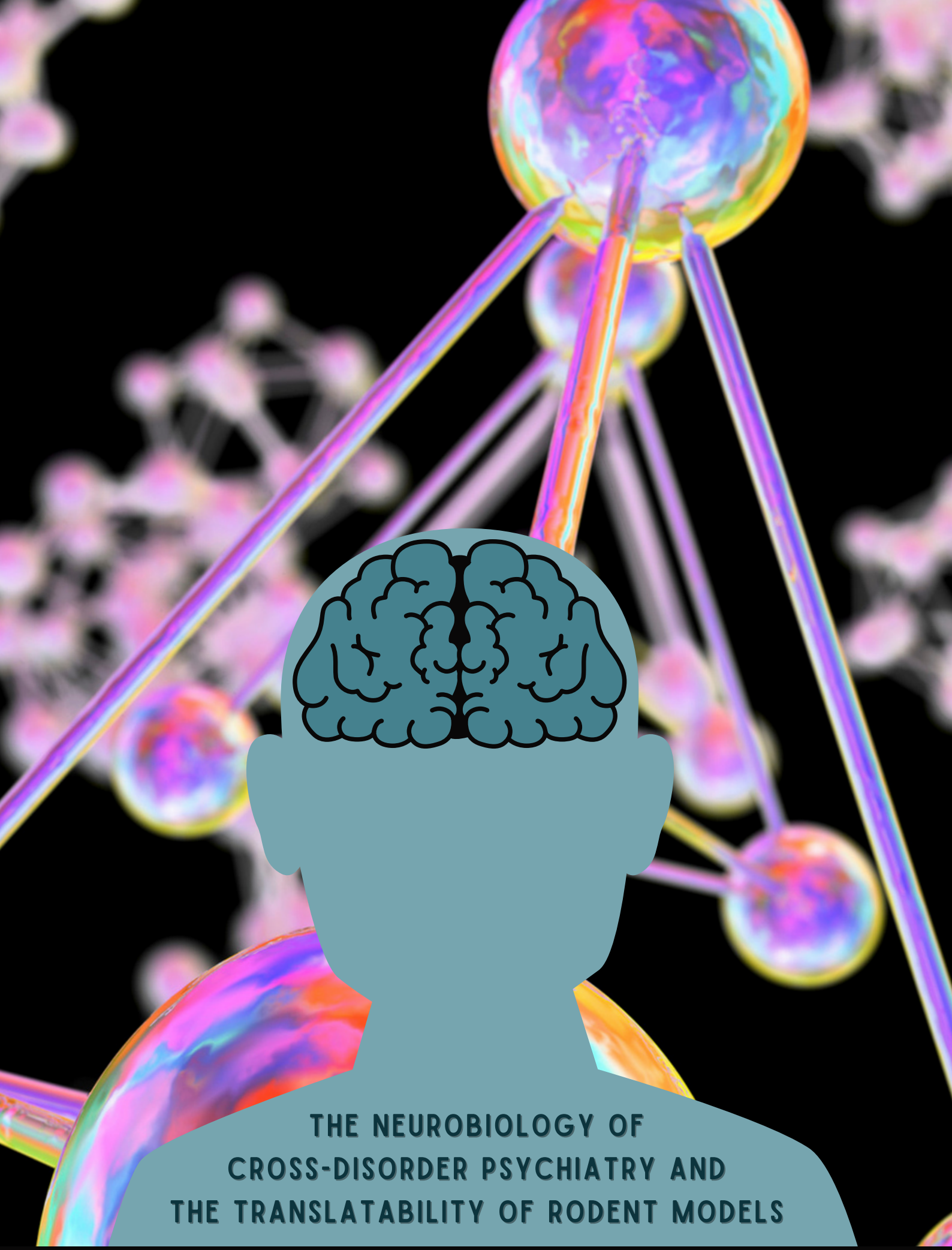

THE NEUROBIOLOGY OF CROSS-DISORDER PSYCHIATRY AND THE TRANSLATABILITY OF RODENT MODELS

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Ph.D. Thesis

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Thesis presented to Programa de Pós-Graduação em Ciências Biológicas: Bioquímica from Instituto de Ciências Básicas da Saúde from Universidade Federal do Rio Grande do Sul as partial requirement for gaining the doctor of biochemistry (Ph.D.) title.

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We inhabit a universe where atoms are made in the centers of stars; where each second a thousand suns are born; where life is sparked by sunlight and lightning in the airs and waters of youthful planets; where the raw material for biological evolution is sometimes made by the explosion of a star halfway across the Milky Way; where a thing as beautiful as a galaxy is formed a hundred billion times - a Cosmos of quasars and quarks, snowflakes and fireflies, where there may be black holes and other universe and extraterrestrial civilizations whose radio messages are at this moment reaching the Earth. How pallid by comparison are the pretensions of superstition and pseudoscience; how important it is for us to pursue and understand science, that characteristically human endeavor.

– Carl Sagan, Cosmos

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Preamble

This thesis (written in English) is organized in three parts, in accordance with the rules of the Graduate Program in Biological Sciences: Biochemistry (UFRGS):

Part I: Introduction and objectives (up to 20 pages).

Part II: Results presented as scientific articles. Each article corresponds to one chapter. Five (5) articles were published in peer-reviewed journals. Two (2) articles are under review in peer-reviewed journals. Other two (2) articles are submitted to peer-reviewed journals. Other three (3) are published as preprints in repositories.And other two (2) articles are in preparation for publication.

Part III: Discussion and references (up to 20 pages). References correspond to citation in Parts I and III. References in Part II are within each chapter.

The work presented in this thesis was performed in both the Department of Biochemistry at UFRGS (Porto Alegre, Brazil) and the Department of Translational Psychiatry at the Max Planck Institute of Psychiatry and the Technical University of Munich (Munich, Germany). This was enabled by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the German Academic Exchange Service (DAAD - german for Deutscher Akademischer Austauschdienst) through the following funding programme: Bi-nationally Supervised Doctoral Degrees/Cotutelle, 2020/21 (57507869).

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Part I

“If I have seen further than others, it is by standing on the shoulders of giants”

– Sir Isaac Newton

1 Abstract

1.1 Resumo

Transtornos psiquiátricos, como transtorno depressivo maior (TDM), transtorno bipolar (TB), esquizofrenia (ESQ) e transtorno do déficit de atenção e hiperatividade (TDAH), não apenas compartilham características clínicas e moleculares, mas também impõem um ônus socioeconômico. Esses transtornos afetam o funcionamento diário das pessoas, resultando em produtividade reduzida e aumento dos custos com saúde. Do ponto de vista clínico, a co-ocorrência de transtornos psiquiátricos é maior do que o esperado ao acaso, e certos fatores de risco, como genética, trauma na infância e inflamação, aumentam a probabilidade de desenvolver múltiplos transtornos. Essa compreensão tem impacto no diagnóstico e tratamento, destacando a necessidade de uma abordagem abrangente considerando características transdiagnósticas. Além disso, modelos animais em psiquiatria são cruciais para entender mecanismos moleculares e para a descoberta de medicamentos. E a validade desses modelos pode ser avaliada com base na validade de construto, validade de face e validade preditiva. Técnicas de neuroimagem também avançaram nossa compreensão sobre a estrutura e a função cerebral em transtornos psiquiátricos. No entanto, desafios persistem, incluindo baixa reprodutibilidade e limitações de análise. Ainda, o comportamento suicida é apresentado como um fenótipo extremo e transdiagnóstico, destacando sua natureza complexa e multifatorial. Todas essas dimensões da psiquiatria podem ser abordadas levando em consideração suas características transdiagnósticas. O primeiro estudo apresentado utiliza as vendas de medicamentos psiquiátricos como um indicador de diagnóstico no Brasil, destacando impactos socioeconômicos e a urgência da compreensão dos transtornos. O segundo e o terceiro estudo introduzem uma metodologia para identificar genes consistentemente diferencialmente expressos em transtornos psiquiátricos e neurológicos. Eles exploram sobreposições, revelando genes e vias compartilhadas, especialmente relevantes na esquizofrenia e Alzheimer (ALZ). Do quarto ao oitavo estudo, a validade de modelos animais em psiquiatria é examinada. São destacadas as limitações do modelo do rato espontaneamente hipertenso (SHR) em representar o TDAH, levando a uma investigação mais ampla sobre modelos para TDM e transtorno do espectro autista (TEA). A abordagem fornece uma ferramenta valiosa para os pesquisadores escolherem modelos relevantes para comportamentos e tratamentos específicos. O 9º e 10º estudo abordam a variabilidade na psiquiatria por meio de análises de neuroimagem. Uma meta-análise sobre espectroscopia em TDAH revelou associações com desequilíbrio de glutamato-glutamina. Um algoritmo de biclusterização foi aplicado a conjuntos de dados de neuroimagem estrutural em TDM, oferecendo entendimento sobre características transdiagnósticas e condições específicas. Do 11º ao 14º estudo, são explorados marcadores biocomportamentais do suicídio. Características de personalidade, impulsividade e trauma na infância são examinadas por meio de meta-análises, revelando associações diferenciais com tentativas de suicídio, dependendo de condições psiquiátricas comórbidas. Marcadores inflamatórios e a proteína CD33 foram identificados como potenciais contribuintes para o comportamento suicida, abrindo caminho para pesquisas futuras. Coletivamente, esta tese contribui para a compreensão das bases biológicas dos transtornos psiquiátricos, destacando a importância de abordagens interdisciplinares, métodos analíticos avançados e conjuntos de dados diversos para estratégias de medicina de precisão.

Keywords: Psychiatric Disorders, Cross-disorder Characteristics, Neuroimaging Techniques, Animal Models in Psychiatry, Suicide Behavior

1.2 Abstract

Psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), schizophrenia (SCZ), and attention-deficit/hyperactivity disorder (ADHD), not only share clinical and molecular features but also impose a significant socioeconomic burden. These disorders affect individuals' daily functioning, leading to reduced productivity and increased healthcare costs. From a clinical perspective, the co-occurrence of psychiatric disorders is higher than expected by chance, and certain risk factors like genetics, childhood trauma and inflammation, increase the likelihood of developing multiple disorders. This understanding impacts diagnosis and treatment, highlighting the need for a comprehensive approach considering cross-disorder features. Moreover, animal models in psychiatry are crucial for understanding molecular mechanisms and drug discovery. And the validity of these models can be evaluated based on construct, face, and predictive validity. Neuroimaging techniques have also advanced our understanding of brain structure and function in psychiatric disorders. However, challenges remain, including low reproducibility and analytical drawbacks. We also present suicide behavior as an extreme cross-disorder phenotype, highlighting its complex and multifactorial nature. All these dimensions of psychiatry can be dealt with taking into account their cross-disorder characteristics. The first study presented in this thesis utilizes psychiatric drug sales as a proxy for diagnosis in Brazil, shedding light on socioeconomic impacts and the urgency for improved disorder comprehension. The second and third studies introduce a methodology for identifying consistent differentially expressed genes in psychiatric and neurological disorders. It explores overlaps, revealing shared genes and pathways, particularly noteworthy in schizophrenia and Alzheimer's (ALZ). From the fourth to the eighth studies, the validity of animal models in psychiatry are scrutinized. The spontaneously hypertensive rat (SHR) model's limitations in representing ADHD are highlighted, prompting a broader investigation into models for MDD and autism spectrum disorder (ASD). The approach provides a valuable tool for researchers to choose models relevant to specific behaviors and treatments. The ninth and tenth studies tackle variability in psychiatry through neuroimaging analyses. A systematic review and meta-analysis on ADHD spectroscopy revealed associations with glutamate-glutamine imbalance. A biclustering algorithm is applied to structural neuroimaging datasets in MDD, offering insights into cross-disorder-related features and specific conditions. The eleventh to fourteenth studies explore biobehavioral markers of suicide across psychiatric disorders. Personality traits, impulsivity, and early-life trauma are scrutinized through meta-analyses, revealing differential associations with suicide attempts depending on comorbid psychiatric conditions. Inflammatory markers and the CD33 protein are identified as potential contributors to suicide behavior, providing avenues for future research. Collectively, this thesis contributes to the understanding of psychiatric disorders' biological underpinnings, emphasizing the importance of interdisciplinary approaches, advanced analytical methods, and diverse datasets for precision medicine strategies.

Palavras-chave: Transtornos Psiquiátricos, Características Transdiagnósticas, Técnicas de Neuroimagem, Modelos Animais em Psiquiatria, Comportamento Suicida

2 List of abbreviations

1H-MRS: Proton Magnetic Resonance Spectroscopy

ADHD: Attention Deficit Hyperactivity Disorder

ADHD: Attention-Deficit Hyperactivity Disorder

ALZ: Alzheimer's

APA: American Psychiatric Association

BD: Bipolar Disorder

BMI: Body Mass Index

CD33: Sialic Acid Binding Ig-like Lectin 3

CNS: Central Nervous System

DTI: Diffusion Tensor Imaging

ENIGMA: Enhancing NeuroImaging Genetics through Meta-Analysis

fMRI: Functional Magnetic Resonance Imaging

GDP: Gross Domestic Product

GWAS: Genome-Wide Association Studies

IL1B: Interleukin 1 Beta

MDD: Major Depressive Disorder

MPH: Methylphenidate

NLGN1: Neuroligin 1

OCD: Obsessive-Compulsive Disorder

PET: Positron Emission Tomography

Psychiatric Disorders and Medications:

PWAS: Proteome-Wide Association Studies

SCZ: Schizophrenia

SHR: Spontaneously Hypertensive Rat

TNF-α: Tumor Necrosis Factor Alpha

TWAS: Transcriptome-Wide Association Studies

3 Background

3.1. The health impact and socioeconomic burden of psychiatric disorders

Psychiatric disorders affect millions of people worldwide and have a profound impact on the health of individuals and societies; they can be debilitating and even life-threatening (Tripathi et al., 2018). Major depressive disorder (MDD), bipolar disorder (BD), schizophrenia (SCZ), and attention-deficit hyperactivity disorder (ADHD) are some of the most common psychiatric disorders (Cheng et al., 2018). MDD, for instance, can lead to feelings of sadness, hopelessness, and helplessness, while bipolar disorder is characterized by episodes of mania and depression. Schizophrenia, on the other hand, can cause hallucinations, delusions, and disordered thinking. And ADHD includes symptoms as innatention, hiperactivity, and impulsivity(American Psychiatric Association, 2013). But they all have something in common, and that is precisely what I dive into in this thesis.

While they have their own well-defined diagnoses, all psychiatric disorders share many features, from clinical symptoms to their molecular biology (Pelin et al., 2021). And this is no different when we take into account their impact in society, which goes far beyond the symptoms themselves. Psychiatric disorders also have a significant socioeconomic burden (Findling & Stepanova, 2018). These conditions can affect a person's ability to work and function in their daily lives, leading to decreased productivity and increased healthcare-related costs for companies. They can also contribute to poverty and homelessness, as people with severe psychiatric disorders may struggle to maintain employment and housing. Not to mention the cost of treatment itself, both for governments and for individuals (Dykxhoorn & Kirkbride, 2018).

Efforts to address the health impact and socioeconomic burden of psychiatric disorders include increasing access to mental health services and reducing stigma surrounding mental illness (Dykxhoorn & Kirkbride, 2018). However, research into the biology of psychiatry is just as crucial. I understand that investigating cross-disorder features through a variety of methods and prisms may help to elucidate both the boundaries or uniqueness of disorders as well as underlying common aspects. And those will hopefully ease the development of better mitigation strategies and to more accurately predict both disorder onset and treatment response.

3.2. An overview of the cross-disorder concept

The field of psychiatry has long been challenged by the complexity and heterogeneity of mental disorders, making diagnosis and treatment of these conditions difficult (Marquand et al., 2019). The cross-disorder concept in psychiatry has significant implications for our understanding and treatment of mental illnesses. One of the key insights from this perspective is that many psychiatric disorders share common features in terms of their clinical presentation, genetic risk factors, and neurobiological underpinnings (Pelin et al., 2021). By identifying these shared features, we can develop more comprehensive and effective interventions and also help to explain the overlap in symptoms and comorbidity between disorders (Schijven et al., 2020).

3.2.1. Clinical perspective

Research has shown that many psychiatric disorders co-occur at rates higher than would be expected by chance (Hagerty et al., 2019), and that certain risk factors, such as childhood trauma and inflammation, may increase the likelihood of developing multiple disorders .

From a clinical perspective, the cross-disorder concept has important implications for diagnosis and treatment. Clinicians need to consider comorbidity when diagnosing and treating patients, as treating one disorder may have implications for the course of another. Additionally, the identification of common underlying mechanisms may lead to the development of more targeted and effective treatments that address multiple disorders simultaneously (Womersley et al., 2022).

3.2.2. Neuroanatomical perspective

One of the key neuroanatomical perspectives that has been implicated in the cross-disorder concept is the prefrontal cortex (Guirado et al., 2020). This region of the brain is involved in a wide range of cognitive and emotional processes, including decision-making, planning, and regulation of emotion (Anastasiades & Carter, 2021). Dysfunction in the prefrontal cortex has been linked to a variety of mental illnesses, including depression, anxiety, and schizophrenia (Guirado et al., 2020).

In addition to the prefrontal cortex, other brain regions that have been implicated in the cross-disorder concept include the amygdala and hippocampus (Vila-Merkle et

al., 2021). The amygdala is a key region involved in emotional processing, while the hippocampus is involved in memory and spatial navigation.

Notwithstanding, there is a pressing need to further understand the brain characteristics associated with each disorder. As evidence is far from consolidated and the associations found are rarely specific, we should focus on applying different methods to further dissect the associations and hopefully these could be used as an adjuvant to guide clinical practice. Neuroimaging seems to be a promising and scalable approach that we touch on further in this thesis.

3.2.3. Molecular perspective

Common human diseases are generally multifactorial, being influenced by both environmental and genetic factors that may interact on producing a certain phenotype (Furlong, 2013; Quintana-Murci, 2016). Those diseases, related to the central nervous system (CNS) or otherwise, are usually associated with inflammation as well, which raises the possibility of systemic causal or resulting effects (Rocha et al., 2018; Sampson et al., 2016). In neurodegenerative diseases as Parkinson's or Alzheimer's (ALZ) this association has even inspired the coining of the term "inflammaging" (Franceschi et al., 2017; McGeer et al., 2016).

Diseases as diabetes, hypertension, psychiatric disorders or even continuous phenotypes, as body mass index (BMI) and height, are usually classified under the umbrella of complex traits. Those traits are influenced by an ensemble of different genetic variants and are therefore polygenic and "complex" (Boyle et al., 2017). This

theory had already been partially developed by Fisher in 1919 (Fisher, 1919). Fisher's "infinitesimal model" takes into account that the contribution of each variant and gene becomes smaller as the number of genes associated with a trait grows larger, resulting in a normally distributed phenotype (Barton et al., 2017).

More recently, researchers have proposed that complex traits are not only polygenic, but also "omnigenic". The hypothesis of the omnigenic model states that all genes expressed in disease-relevant cells can actually affect core disease-related genes because of highly interconnected regulatory networks. This idea would suggest that a great amount of the heritability of those traits can also be explained by the influence of genes outside core pathways (Boyle et al., 2017). While this theory is nonetheless important to understand the etiology of complex traits, when referring ourselves to common diseases this should be translated into something that can be also applied therapeutically. In this sense, the continuing research on which pathways are mainly responsible for common diseases susceptibility remains crucial.

3.2.3.1. Central Nervous System

At the molecular level, the CNS plays a critical role in the development and expression of psychiatric disorders (Trojan et al., 2019). The CNS is responsible for processing information, regulating emotions, and coordinating behavior and disruptions in its function have been linked to the onset of several psychiatric disorders (Ebneabbasi et al., 2021; Zheng et al., 2018). For example, research has shown that abnormalities in neurotransmitter systems, including dopamine, serotonin, and glutamate, are involved in various aspects of mood regulation, cognition, and

behavior, and have been implicated in the pathophysiology of many psychiatric disorders (Moore & Barnett, 2015).

3.2.3.2. Peripheral markers

While the CNS is generally where most changes occur in psychiatric disorders, it is hard to dive deep into the causes and consequences of disorders at this level due to the difficulties to study it further. However, there is compelling evidence that the peripheral systems exert a great deal of influence as well, be it in gut-brain interactions, systemic inflammation or others. Most importantly, the periphery can serve as proxy to the CNS as well as source of markers for the disorders (regardless if cause or consequence). The use of peripheral biomarkers provides a non-invasive and cost-effective way to obtain valuable biological data.

Genome-Wide Association Studies (GWAS) have also demonstrated that common genetic variations can considerably contribute to the heritability of brain disorders, in which many variants with small effect are responsible for a great deal of the phenotype. Those genetic variants overlap with high correlation, as is the case with schizophrenia, MDD and BD (Saito et al., 2023). Biochemical studies have also demonstrated strong associations between phenotypes related to the immune system and neurological disorders, as is the case with Alzheimer's and Parkinson's diseases (Wingo et al., 2022).

Multi-omics data has been vastly produced in the last few years from different types of human tissue and in several biological levels, such as in the genome,

transcriptome, proteome, and even the microbiome. All these layers interact with each other and assessing a full perspective of the omics data has been one of the greatest challenges of today's bioinformatics and biostatistics research (Hawe et al., 2019; Huang et al., 2017; Vasaikar et al., 2018). However, the evidence is not even consolidated or provenly consistent across different studies within each omic. One of the most robust and widely applied statistical method to solve similar problems in epidemiology is meta-analyzing data subsequently to a well-conducted systematic review of the literature in order to estimate overall effect sizes (Page et al., 2021). This is one of the reasons why we have chosen to investigate the transcriptome in such a manner in this thesis.

3.3. Animal models in psychiatry and their validity

It is highly difficult to study the molecular characteristics of psychiatric disorders since the brain tissue in which we understand most of the mechanisms happen is not easily accessible and is rare the occasion where these samples can be explored (Christian et al., 2020). For this reason, animal models are widely used in psychiatry to try and understand the underlying mechanisms and pathways associated with the disorders . Another scenario in which they are extremely useful is drug discovery. We can test several treatments in rodents, for example, and infer on their effects in humans by the responses and changes we can observe in the model (Mallien et al., 2020). But they are models, nonetheless, and have their limitations as to what information we can extract and how we interpret it (Costello et al., 2018).

Animal models in psychiatry can be evaluated regarding their validity. The most accepted proposed method divides the validity in three main domains: construct validity, face validity, and predictive validity (which can all be further broken down, but we will restrain ourselves to these three major categories) (Willner, 1986). *Construct validity* refers to the molecular mechanisms and aetiology of the disorder, or in other words, if the animal model presents the phenotype through the same underlying reason as do humans. This is a challenging validity to test as one can imagine. We are still driving major discoveries in the field of psychiatry in humans, trying to understand the aetiology and mechanisms – *how could we validate them in animal models?* The same animal models we are utilizing in order to better understand these underlying mechanisms. It is an ouroboros of some sort. We will partly delve into construct validity (if I dare to say so) when comparing the transcripts found to be differentially expressed in the animal models and in individuals with the disorders. When we set out to understand how was the comparison of transcripts between model and disorder, we soon realized there was the question of whether the models themselves were valid or by which extend. Therefore, we proceeded to evaluate the face and predictive validities of representative animal models in psychiatry. They are somewhat more straightforward to evaluate than construct validity. *Face validity* is the ability of the animal model to show the same phenotype that we see in humans, in this case, symptoms of the psychiatric disorders that are measured through a set of behavioural tests. While *predictive validity* is how well an animal model can respond to well-established clinical treatments, in which they should present the same response seen in humans with the disorder. To our knowledge, this is the first endeavour to measure the validity of animal models in psychiatry.

3.4. State-of-the-art neuroimaging in psychiatry

Neuroimaging in psychiatry has undergone significant advancements, providing profound insights into complex neural mechanisms. State-of-the-art techniques have propelled the field forward, shedding light on the intricate interplay between brain structure, function, and psychiatric conditions (Murray et al., 2018). *Functional Magnetic Resonance Imaging* (fMRI) continues to be a cornerstone, allowing real-time observation of brain activity and enhancing our understanding of disorders like schizophrenia, depression, and anxiety (John & Parekh, 2018).

Structural MRI has evolved to identify subtle anatomical changes associated with psychiatric disorders, providing crucial insights into conditions such as bipolar disorder and obsessive-compulsive disorder (OCD) (McCutcheon et al., 2021). *Diffusion Tensor Imaging* (DTI) has enabled the exploration of white matter tracts, contributing to our understanding of connectivity disruptions in disorders like autism, attention-deficit/hyperactivity disorder (ADHD), and schizophrenia (Meoded & Huisman, 2019). Moreover, *Positron Emission Tomography* (PET) has been instrumental in studying neurochemical processes, offering valuable information about the role of neurotransmitters in conditions like depression and schizophrenia (Herfert et al., 2020).

As these advancements continue, practical applications in clinical settings start to emerge from the new neuroimaging findings. Neural markers identified through these techniques hold promise for early diagnosis, treatment selection, and monitoring treatment response. The collaborative efforts of researchers and clinicians through large consortia such as the Enhancing neuroimaging genetics through meta-analysis

(ENIGMA) are crucial for translating these discoveries into improved strategies for the diagnosis and treatment of individuals (Medland et al., 2022).

That being said, there are still several pitfalls to be addressed and there is a lot of work to be done in order to identify differences that can be confidently used in clinical practice. Some methods are behind others when regarding successes in finding significant associations. That is also true for different psychiatric disorders, where we can find many successful characterizations of schizophrenia, for instance, but not of depressive disorder. This is a trend that can be seen in several areas, such as in the clinic and genetics, where disorders with higher heritability and more well-defined diagnostic criteria tend to be easier to study in biological psychiatry (Burmeister et al., 2008; Gallinat et al., 2008).

Proton magnetic resonance spectroscopy (1H-MRS) is a specialized neuroimaging technique that allows us to investigate the chemical composition of the brain by measuring the concentrations of specific neurochemicals. The focus often lies in examining key neurochemicals, including N-acetylaspartate (NAA), creatine (Cr), choline-containing compounds (Cho), and myo-Inositol (mI), among others (Bustillo, 2013; Dong et al., 2022). However, results from MRS have been showing low reproducibility in psychiatry, which hinders the usability of the method and confidence in this type of study. In an effort to understand which are the main pitfalls we have conducted a meta-analysis with all evidence of MRS in ADHD, which has demonstrated large variability regardless of its high heritability component (Faraone & Larsson, 2019; Perlov et al., 2009).

Although, in psychiatry, structural neuroimaging has shed light on some associations between cortical thickness and surface area of brain regions, especially for schizophrenia and bipolar disorder (Padmanabhan et al., 2015), the evidence for depressive disorder still falls short of the closely-related phenotypes' successes. One of the possible reasons for this is small sample sizes. Since we expect higher variability in MDD presentations, coupled with low heritability, it makes sense that bigger sample sizes are needed to identify the same number of associations as in schizophrenia, for instance. This has been partially mitigated by international collaborations. However, this disparity can also be due to the way in which we process and analyse the data. In this thesis we present a new biclustering method trying to deal with the high dimensionality of depressive disorder in structural neuroimaging data and that will be hopefully extrapolated to other neuroimaging methods.

3.5. Suicide behaviour as an extreme cross-disorder phenotype

Suicide behaviour is a complex and multifactorial phenotype that has become a major public health concern worldwide. Despite numerous interventions and prevention strategies, the incidence of suicide has remained high, with over 700,000 deaths by suicide occurring each year (Wasserman & Cyranka, 2019). This highlights the need for a better understanding of the underlying causes of suicide behaviour.

While it is known that suicide behaviour is associated with several psychiatric disorders such as depression, bipolar disorder, schizophrenia, substance use disorders, and anxiety disorders, it could also represent an extreme cross-disorder

phenotype. This means that there are shared genetic risk factors that contribute to the development of suicide behaviour (and are specific to it) across a range of different mental health conditions.

The identification of shared genetic risk factors across different disorders could help to explain why individuals with various psychiatric disorders are at an increased risk of suicide, and could lead to the development of more effective interventions to prevent it (Docherty et al., 2023; Mullins, Bigdeli, Power, et al., 2019). Furthermore, research into the shared genetic risk factors between suicide and other disorders could lead to the development of new therapies that target these underlying biological mechanisms (Wingo et al., 2022).

3.5.1. The heritability and genetics

Studies have shown that genetic factors account for up to 50% of the variance in suicide behavior, suggesting that these factors play a significant role in its development (O'Reilly et al., 2020). A number of genetic variants has been identified that may be associated with an increased risk of suicide. Notably, the most recent GWAS meta-analysis of suicide attempt identified 12 significant loci (Li et al., 2022), in which associations with nonpsychiatric traits such as insomnia and risk-taking behaviour remained largely unchanged after conditioning for psychiatric disorders.

By and large, genetic studies on suicide behaviour overall have found a trend of positive genetic correlations with depression, schizophrenia, pain, smoking, risk

taking behaviour, and negative genetic correlation with educational attainment and socioeconomic status (Campos et al., 2023; Li et al., 2022; Mullins et al., n.d.).

3.5.2. The environment

It is important to note that suicidal behavior is not solely determined by genetics. Suicidal behavior is the result of a complex interplay of biological, psychological, and environmental factors that interact over time. Environmental factors can interact with genetic traits to increase the risk of suicidal behavior further. For example, individuals with a genetic predisposition to depression may be more susceptible to the negative effects of stressors such as relationship breakdown or financial hardship (Uchida, 2021).

3.5.2.1. Distal factors

One of the most significant environmental factors that can influence an individual's risk of suicidal behavior is early-life events. Research has shown that individuals who experience childhood abuse, neglect, or other adverse childhood experiences are at an increased risk of suicidal behavior later in life. Childhood abuse can take many forms, including physical, sexual, or emotional abuse, and the trauma associated with such experiences can lead to a range of mental health conditions, such as depression, anxiety, and post-traumatic stress disorder (Brezo et al., 2007; Turecki et al., 2019).

Notwithstanding, individuals with a family history of suicide are also at an increased risk of suicidal behavior. This risk may be not only due to genetics but also environmental factors that run in families, including mental illness, substance abuse, and social isolation. Bad experiences and exposure to certain behaviours can trigger feelings of hopelessness, helplessness, and despair, which can contribute to suicidal thoughts and actions. Similarly, chronic health conditions, such as chronic pain, cancer, or HIV/AIDS, can also increase the risk of suicidal behavior by causing emotional distress and exacerbating psychiatric disorders (Turecki et al., 2019; Wasserman & Cyranka, 2019).

3.5.2.2. Proximal factors

Recent life stressors, such as job loss, relationship problems, financial difficulties, and legal issues, can increase the risk of suicidal behavior by triggering feelings of hopelessness, depression, anxiety, or other psychiatric conditions. Traumatic events in general, such as exposure to violence or disasters, can also significantly impact an individual's risk (Turecki et al., 2019).

Substance abuse is another significant risk factor, as individuals who abuse drugs or alcohol are more likely to experience depression, anxiety, and other psychiatric conditions that can contribute to suicidal thoughts and actions. On the other hand, the substance abuse itself can have arisen from a coping mechanism over life despair (Turecki et al., 2019).

Finally, social isolation and perceived loneliness can also contribute to an individual's risk of suicide behavior. Social isolation can lead to feelings of despair and hopelessness, which can contribute to suicidal thoughts and actions. This was particularly discussed after the COVID-19 pandemic since long-term psychiatric issues have been common, either as a result of contracting the disease or from the environmental and social changes that were imposed. In general, suicide rates go down in the beginning of a pandemic outbreak, but tend to significantly increase as time goes on and once feelings of hopelessness and other triggers (such as the loss of loved ones) start to take place (Antonello et al., 2020; Carvalho et al., 2020; Mazza et al., 2020).

3.5.3. Key-differences between suicidal ideation, suicide attempt, and death by suicide

Suicidal ideation can be described as thoughts or fantasies of suicide that an individual may experience at any point in their life (Turecki et al., 2019). Suicide attempt is a deliberate act of self-harm with the intention of dying. Suicide attempts can range from non-lethal acts such as overdosing on medications to more lethal methods such as jumping from a high place or using a firearm. Suicide attempts can be impulsive or planned and are often a sign of significant emotional distress (Kim et al., 2015). Death by suicide is the most severe outcome of suicide behavior, representing the intentional taking of one's own life. Suicide is a leading cause of death worldwide and has devastating impacts on families, communities, and society as a whole (Fortgang & Nock, 2021). It is important to recognize the key differences

between suicidal ideation, suicide attempt, and death by suicide to provide appropriate support and interventions.

Although sometimes suicide behaviour is seen as a set of progressive stages and suicidal ideation can in part predict attempt and death (Galfalvy et al., 2023), there is also evidence of differences between these conditions. Approximately 80% of deaths by suicide occur without report of suicidal ideation (Gosnell et al., 2019). We can also identify differences as to the characteristics of samples with suicidal ideation, suicide attempt or death. While the majority of suicidal ideation cases are females (Begum et al., 2017), males consistently attempt more and contribute to more than two thirds of deaths (McKean et al., 2018). Based on this evidence, several hypotheses have been proposed, such as impulsivity as a main driver of attempt (Rizk et al., 2021) or aggressiveness and extroverted personality as associated with the implication of more lethal methods (Perry et al., 2022).

However, there is also evidence of diverse genetic background, not only behavioural differences. Studies suggest a significant genetic contribution with heritability ranging from 30 to 55% to suicidal ideation (Mullins, Bigdeli, Børglum, et al., 2019), while suicide attempts present heritability estimates of 17 to 45%, even after controlling for psychiatric disorders (Levey et al., 2019). Moreover, a recent study found an association of one locus in the neuroligin 1 (NLGN1) gene with death by suicide that is independent from suicide attempt (Li et al., 2022).

4 Objectives

4.1. Main objectives

- To investigate the gene expression patterns reported for different psychiatric and neurological disorders, comparing the overlap between conditions;
- To evaluate which are the best animal models and their translatability regarding face, predictive, and construct validities across psychiatric disorders;
- To identify potential behavioural and biological markers (such as personality, inflammatory markers, or structural neuroimaging) of disorder subtypes and suicidality.

4.2. Specific objectives

- To contextualize the research regarding the importance of better understanding psychiatric conditions for future treatment development, based on Brazilian records (*Chapter 1*)
- To investigate the gene expression patterns in peripheral blood samples (reported in open access repositories) for different psychiatric and neurological disorders comparing the overlap between conditions (*Chapter 2 and Annex*)
- To evaluate which are the best animal models and their translatability regarding face and predictive validity for most rodent models of psychiatry though meta-analyses (*Chapters 3 to 5 and Annex*)

- To investigate the accuracy and reliability of spectroscopy findings as biomarkers for attention deficit/hyperactivity disorder through a systematic review and meta-analysis (*Chapter 6*)
- To understand the body of literature regarding personality, early-life trauma, and inflammation as markers for suicide behaviour, an important and extreme cross-disorder phenotype (*Chapters 7 to 10*).

Part II

“Have no fear of perfection; you’ll never reach it”

– Marie Curie

The importance of biological psychiatry research

“There is no standard normal. Normal is subjective. There are seven billion versions of normal on this planet.”

– Matt Haig

The translatability of cross-disorder endophenotypes

“Show me a sane man and I will cure him for you”

– Carl Gustav Jung

2

Gene expression overlap between neuropsychiatric disorders

Research article published as a pre-print on ***medRxiv***.

Gene expression overlap between neuropsychiatric disorders

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Abstract

Common diseases result from a mix of genetic and environmental factors, often involving inflammation. Complex traits like diabetes and psychiatric disorders are polygenic, influenced by many genetic variants. The omnigenic model suggests all expressed genes can impact disease-related genes. This study examines blood transcriptomic variations in psychiatric and neurological disorders to understand mRNA expression profiles and address field discrepancies. Animal models are explored for similar gene expressions. This study extensively searched GEO DataSets and ArrayExpress databases, identifying gene expression profiles associated with neuropsychiatric disorders. From GEO, 10,359 samples were found, with 30 series (1,897 samples) in the qualitative synthesis, revealing 1,364 differentially expressed genes in Schizophrenia, 134 in Bipolar Disorder, 11 in Autism Spectrum Disorder, and 2,784 in Alzheimer's Disorder. Comparisons with GWAS studies unveiled overlaps, with 81 genes for SCZ, two for BD, and 135 for ALZ. Notably, 441 genes were shared between ALZ and SCZ. Enrichment analyses indicated associations with signalling pathways. In animal models, 2,360 series were identified, with 175 in the qualitative synthesis, resulting in a meta-analysis focusing on ALZ with hippocampus tissue, revealing 14 consistently differentially expressed genes. Four overlapped with human data (ALOX5AP, P2RY13, RGS10, SH3GL1). These findings contribute to understanding shared and unique molecular signatures across neuropsychiatric disorders, bridging insights between human and animal models. The study efficiently identifies and tests consistent differentially expressed genes in psychiatric and neurological disorders, focusing on blood transcriptomes. Compared to transcriptome-wide or proteome-wide association studies, this approach analyses transcripts directly from individuals with disorders, offering real-world predictive capability. Shared genes between disorders suggest common molecular pathways, emphasizing the need for interdisciplinary approaches in understanding and treating psychiatric disorders. Limitations include sample characterization and the peripheral marker focus. Further investigations, including functional assays, are crucial for validation and extending these findings.

Introduction

Many common human diseases result from a complex interplay between genetic and environmental factors, which can jointly contribute to the development of specific traits (1,2). These diseases, whether related to the central nervous system (CNS) or not, often involve inflammation, suggesting potential systemic causal or consequential effects (3–5). In the case of neurodegenerative conditions like Parkinson's and Alzheimer's, this association has led to the coining of the term "inflammaging" (6–8).

Conditions like diabetes, hypertension, psychiatric disorders, and even continuous traits such as body mass index (BMI) and height fall under the umbrella of complex traits. These traits are influenced by multiple genetic variants, making them polygenic and inherently intricate (1–9). This concept was partially formulated by Fisher in 1919 through his "infinitesimal model," which posits that the contribution of each genetic variant diminishes as the number of associated genes increases, resulting in a normally distributed phenotype (10,11).

More recently, researchers have proposed that complex traits are not just polygenic but also "omnigenic." The omnigenic model posits that all genes expressed in disease-relevant cells can affect core disease-related genes due to highly interconnected regulatory networks. This theory suggests that a substantial portion of the heritability of these traits can be attributed to genes outside of core pathways(9). While this theory is important for understanding the origins of complex traits, it must be translated into therapeutic applications when addressing common diseases. Consequently, ongoing research to identify the primary pathways responsible for susceptibility to common diseases remains vital.

In recent years, multi-omics data has been extensively generated from various human tissues and biological levels, encompassing the genome, transcriptome, proteome, and even the microbiome. These layers of data interact intricately, posing a significant challenge to contemporary bioinformatics and biostatistics research (12–14). However, the evidence derived from different studies within each omic category remains inconclusive and lacks consistency. In epidemiology, one of the most robust and commonly used statistical methods to address similar challenges is meta-analysis, which involves the comprehensive evaluation of data following a well-conducted systematic literature review to estimate overall effect sizes (15). Given that the protein networks involved in disease regulation are primarily linked to gene expression, our study focuses on the transcriptome level. Additionally, because representative tissue samples for common diseases are scarce due to high heterogeneity and complexity, the investigation of peripheral factors becomes imperative. This is particularly crucial for brain disorders, and considering potential systemic effects can provide valuable insights. Therefore, we have chosen to investigate transcriptomic variations in the most readily available peripheral tissue: blood, aiming to reconcile inconsistencies across the field.

This study aims to consolidate existing evidence on transcriptomic gene expression levels in blood samples, comparing individuals with psychiatric and neurological disorders. Through this approach, we seek to gain a comprehensive understanding of the mRNA expression profiles associated with complex traits, with the goal of resolving major discrepancies in the field. We also sought for the animal models of these disorders in order to understand if they present any similar differentially expressed genes.

Methods and Analysis

For this study report, we have adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (15).

Eligibility Criteria

We incorporated studies that compare gene expression levels in blood samples from individuals with psychiatric and neurological disorders to those from healthy control groups. Included in our selection are experimental studies that have quantified gene expression using mRNA measurement techniques such as microarray or RNA-sequencing. We exclusively considered studies that feature a well-defined control or resilience group. Our criteria encompass randomized clinical trials (RCTs), cohort studies, and case-control studies, with the stipulation that they include a control group not subjected to specific, unique treatments. The studies should have collected blood samples (including leukocytes, lymphocytes, peripheral blood mononuclear cells (PBMCs)) from patients diagnosed with the respective disorder, as well as from healthy (or resilient) control individuals. We included all diagnostic approaches, regardless of the diagnostic manual or tool used. There were no restrictions on publication date, language, methodological quality, age, sex, or ethnicity of study participants.

We applied the following exclusion criteria: i) studies comprising only genetically related individuals (e.g., family-based studies); ii) studies lacking original data; iii) studies with cases displaying unique comorbidities; iv) studies devoid of healthy (or resilient) control groups; v) studies labeling remitters as healthy controls; vi) studies where blood samples underwent any form of ex vivo treatment before microarray analysis or RNA-sequencing; and vii) studies in which all case samples received a specific type of drug not categorized as "treatment as usual," such as the predominant prescription drug or class of drugs.

For the animal models, the criteria were as follows:

Inclusion criteria: study involving rats (rat, rats, Rattus norvegicus) or mice (mouse, mice, Mus musculus); animals must serve as models (or be used as models in other studies) for the specific disorder/disease (behavioral model, knockout, lineage, etc.); presence of controls without the disorder/disease (no behavioral induction, wildtype, control lineage, etc.) or resilient animals (in this case, note in the observation!); gene expression / transcriptomic method for coding mRNA (array, microarray, RNA-Seq, RNA sequencing...) in any tissue/cell type.

Exclusion criteria: studies where all animal groups were treated with any drug or treatment technique (e.g., deep brain stimulation), preventing comparison between the treated and untreated model and control; studies exclusively focused on non-coding RNA, miRNA, siRNA, methylation, genome variation, genome binding...; studies conducted solely with cell cultures.

Search and Study Identification

To identify relevant studies, we conducted an online search using two distinct electronic databases: Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/gds) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). We did not employ search filters.

Our search strategies encompassed subject headings for each specific disorder/disease, research involving human subjects, and blood-related research:

1. Schizophrenia: (("schizophrenia"[mesh] OR "SCZ") AND ("humans"[mesh] OR "Homo sapiens"[Organism]) AND ("blood"[mesh])).

2. Major depressive disorder: (("depressive disorder"[mesh] OR "depression"[mesh] OR "depressive disorder, major"[mesh] OR "MDD" OR depress*) AND ("humans"[mesh] OR "Homo sapiens"[Organism]) AND ("blood"[mesh])).

3. ADHD: (("attention deficit disorder with hyperactivity"[mesh] OR attention-deficit/hyperactivity disorder OR "ADHD" OR inattent* OR hyperact* OR impulsiv* OR "attention deficit") AND ("blood"[mesh]) AND ("humans"[mesh] OR "Homo sapiens"[Organism])).

4. ALZ: (("alzheimer disease"[mesh] OR alzheimer*) AND ("humans"[mesh] OR "Homo sapiens"[Organism]) AND ("blood"[mesh])).

5. BD: (("bipolar disorder"[mesh] OR bipolar) AND (humans[mesh] OR "Homo sapiens"[Organism]) AND ("blood"[mesh])).

6. ASD: (("autistic disorder"[mesh] OR "ASD" OR "autism spectrum disorder"[mesh]) AND (humans[mesh] OR "Homo sapiens"[Organism]) AND ("blood"[mesh])).

For the animal models the strategies were the following:

(("schizophrenia"[mesh] OR "SCZ") AND ("Models, Animal"[mesh] OR "Rats"[mesh] OR "Mice"[mesh]))

(("depressive disorder"[mesh] OR "depression"[mesh] OR "depressive disorder, major"[mesh] OR "MDD" OR depress*) AND ("Models, Animal"[mesh] OR "Rats"[mesh] OR "Mice"[mesh]))

(("attention deficit disorder with hyperactivity"[mesh] OR attention-deficit/hyperactivity disorder OR "ADHD" OR inattent* OR hyperact* OR impulsiv* OR "attention deficit") AND ("Models, Animal"[mesh] OR "Rats"[mesh] OR "Mice"[mesh]))

(("autistic disorder"[mesh] OR "ASD" OR "autism spectrum disorder"[mesh]) AND ("Models, Animal"[mesh] OR "Rats"[mesh] OR "Mice"[mesh]))

(("alzheimer disease"[mesh] OR alzheimer*) AND ("Models, Animal"[mesh] OR "Rats"[mesh] OR "Mice"[mesh]))

Study Selection

The study selection process was conducted in two stages. Initially, we evaluated titles for inclusion, followed by a thorough review of detailed information from GEO and ArrayExpress. Both stages involved a minimum of two independent reviewers (ACP, ATT, MSW, CSG, LS, or AKT). Any disagreements were resolved through consultation with a third reviewer (ACP or JCFM).

Data Collection

Descriptive data from each study were extracted independently by at least two reviewers (ACP, ATT, MSW, CSG, LS, or AKT), with discrepancies resolved by a third reviewer (ACP or JCFM). Data encompassed study descriptions (sample size, mean age, standard deviation, gender distribution, ethnicity, country/region of origin, data collection date, transcriptome analysis platform used, diagnostic manual/tool, and medication or psychotherapy details). Transcriptomic outcome data, including raw and/or normalized gene expression data were retrieved through the GEOquery R package [21] or direct downloads from GEO and/or ArrayExpress websites.

Outcome Measures

Our primary outcome measure was mRNA levels, assessed through microarray or RNA-sequencing methods. These measures are typically available in full in online repositories, allowing access to raw data.

Data Synthesis

The dataset distributions and sample quality evaluations were performed as indicated by GEO. Since not all microarray platforms had the same exact coverage, we excluded the genes that were not present in at least 85% of the studies. We imputed the remaining values with the mean from the pooled sample. All meta-analyses comprised at least 20,000 mapped genes. Gene expression data was aggregated into a single variable for each disorder using the ImaGEO shiny app, using a random effects approach and 10% missing variables allowed. Differentially expressed genes were computed collectively. We have also explored gene enrichment analyses using the FUMA tool, with at least 10 genes belonging to each set.

Results

The search for studies in the GEO DataSets (https://www.ncbi.nlm.nih.gov/gds/) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) databases was conducted without language or date restrictions (including studies from the inception of the database up to June 30, 2019) in accordance with the search strategies described.

In the GEO database, a total of 10,359 samples from 324 series (gene expression series - datasets, typically corresponding to a single study, except for rare duplicates) were found, of which 30 series, comprising 1,897 samples, were included in the qualitative synthesis. The inclusion process is illustrated in Figure 1. The search for studies in the ArrayExpress database did not yield any studies different from those already found through GEO.

We found 1,364 consistently differentially expressed genes in Schizophrenia, 134 in Bipolar Disorder, 11 in Autism Spectrum Disorder, and 2,784 in Alzheimer's Disorder. Figure 2A depicts the overall methodology of ImaGEO, while the first 1,000 genes associated with each disorder are represented in heatmaps (Figure 2B-E). Not all series were possible to meta-analyse mainly because of different built for microarrays, making it difficult to join the data.

When comparing the differentially expressed genes found here with those significantly associated with outcomes in GWAS studies (data from the GWAS Catalog - https://www.ebi.ac.uk/gwas/), we found 81 overlapping for SCZ, two for BD, and 135 for ALZ (Supplementary file 1).

We also found that the differentially expressed genes overlap to some extend in between disorders (Figure 3A), with the biggest overlap being between ALZ and SCZ (441 genes). We have investigated these genes further through enrichment analyses. SCZ genes seem to be associated with signalling pathways, proteolysis, endocytosis, and cell cycle (Figure 3B). ALZ genes also seem to be associated with signalling pathways and the cell cycle, but mainly with the ribosome, oxidative phosporilation, Huntington`s disease, and cancer (Figure 3C). When we look at the intersection between ALZ and SCZ genes we see they are mainly associated with Toll-like receptor signalling, proteolysis, endocytosis and cancer pathways (Figure 3D).

Regarding the animal models, we identified 2,360 series and included 175 in our qualitative synthesis, comprising 2,040 samples (Figure 4). However, we were only able to conduct a meta-analysis with the ALZ group with hippocampus tissue due to a lack of data or comparable tissues. We found 14 genes consistently differentially expressed (Figure 5). Four of those were also found in our meta-analysis with human data (ALOX5AP, P2RY13, RGS10, SH3GL1).

All genes described here and the qualitative synthesis of studies can be found in Supplementary file 1.

Discussion

The methodology described demonstrates an efficient technique for identifying and testing the consistency of differentially expressed genes across multiple studies with a similar design. The differentially expressed genes that were found still need to be individually further investigated for us to better interpret these results. However, the focus of the present study is on the overlap of markers between disorders and the similarities found in relation to GWAS results.

One of the main differences from our study to the ones that perform transcriptome-wide association (TWA) or proteome-wide association (PWA) is that we are meta-analysing the transcripts found directly in individuals with the disorders. The disadvantage of this approach is that it is highly improbable that we would be able to identify the whole set of causal genes or variants, since it is a cross-sectional freeze of the current characteristics, after years with the disorder. On the other hand, the advantage is that it carries more predictive capability in real-world samples, as we can identify the consequences of the disorder on the transcriptome. Future studies should focus on analyses that are carried out at different stages of disorder development, for example near the onset, or after different treatments. This could potentially lead us to better biomarkers that could be used in the clinic. Another advantage of this approach is that we can now compare what is found at the individual level with the predicted changes in TWAS and PWAS studies to better dissect what has been causal or consequential to the condition.

In a bid to contextualize our results, we compared the differentially expressed genes with those significantly associated with outcomes in GWAS studies, drawing data from the GWAS Catalog (16). Notably, 81 genes were overlapped in SCZ, two in BD, and 135 in ALZ. It shows that some of the genes associated in the transcriptome were also associated in GWA studies, and we would argue, most likely have to do with the development of the disorder.

A recent study by Wingo and colleagues, from 2022 compared the shared mechanisms across major psychiatric and neurodegenerative diseases by mapping TWAS and PWAS predictions from summary statistics of GWAS (17). They tried to infer the most likely causal genes for the overlap between disorders. They have found 13 different genes that were in the intersection between psychiatric and neurodegenerative diseases, from which we see two were also in our SCZ-ALZ overlap (HSDL1 and STXBP3). We could interpret as if those genes are part of a shared molecular pathophysiology that should be taken into account in early identification and treatment.

A particularly intriguing aspect of our investigation lies in the exploration of shared genes among the different psychiatric disorders. Subsequent enrichment analyses shed light on the functional implications of these shared genes. SCZ-associated genes were found to be linked with signaling pathways, proteolysis, endocytosis, and the cell cycle. On the other hand, ALZ-associated genes exhibited associations with signalling pathways, the cell cycle, the ribosome, oxidative phosphorylation, Huntington's disease, and cancer. The intersection of ALZ and SCZ genes was particularly noteworthy, with associations identified in Toll-like receptor signalling, proteolysis, endocytosis, and cancer pathways. The overlap between SCZ and ALZ has not been often noted in GWA studies (18,19) and it seems to be more prevalent when we investigate consequences and markers of the disorders in contrast to its genetic causes. In neuroimaging, for example, that is also true, in which several structural and functional changes are similar in between SCZ and ALZ (20–22). The environmental interactions might be at play here, in which similar environmental factors or

endophenotypes, such as cognitive decline, depressive behaviour, and others could be responsible by the shared biology that we encounter in these neuropsychiatric disorders. It is also possible that this relationship between SCZ and ALZ may still become evident in GWAS as larger samples are collected. Nevertheless, another possibility is that GWAS studies may not be sufficient to investigate the heritability of these disorders, emphasizing the importance of interdisciplinary approaches, as presented here. When we look at the overlap between ALZ and SCZ in terms of where these genes are expressed, we found that, apart from some constitutive expressions in the pancreas, heart, and liver, they were also found highly expressed in brain amygdala, putamen, hippocampus, the substantia nigra, and the caudate; suggesting key changes in CNS functions.

Some limitations should be taken into account when interpreting our results, especially: i) that a meta-analysis is just as good as its original studies, and we were limited as to characterization of samples and sample sizes; ii) that using peripheral markers, although non-invasive and good for predictions, hinders our ability to derive interpretations about the CNS and what are the changes that occur in the brain.

Our findings not only deepen our understanding of the intricate molecular landscape of psychiatric disorders but also hint at potential shared pathways and mechanisms across seemingly distinct conditions. Further investigations, including functional assays and validation studies, will be crucial to validate and extend these initial observations. The integration of diverse datasets and advanced analytical approaches continues to unveil the complex nature of psychiatric disorders, offering new avenues for targeted therapeutic interventions and precision medicine strategies.

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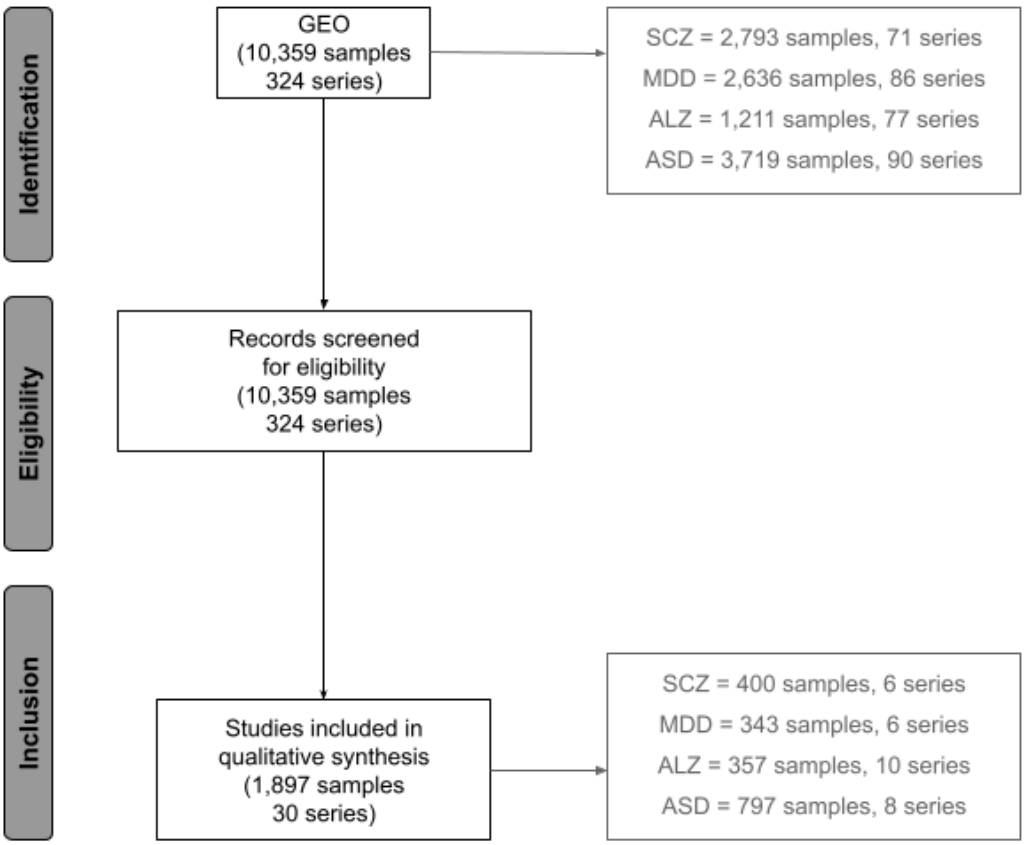


Figure 1. Flowchart of the study inclusion process from the GEO DataSets database for clinical studies. SCZ = Schizophrenia, MDD = Major depressive disorder, ALZ = Alzheimer's disease, ASD = Autism spectrum disorder.

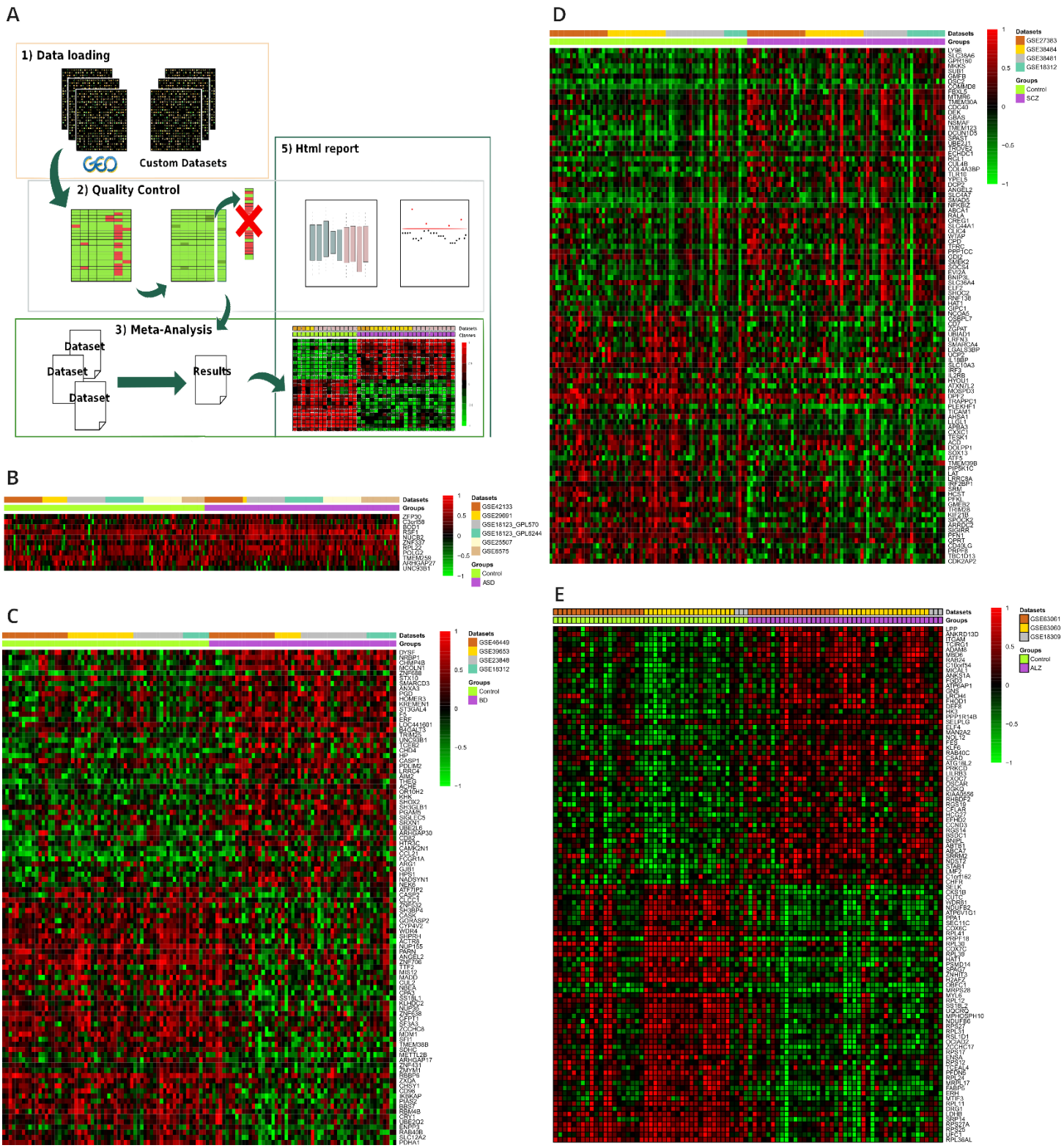
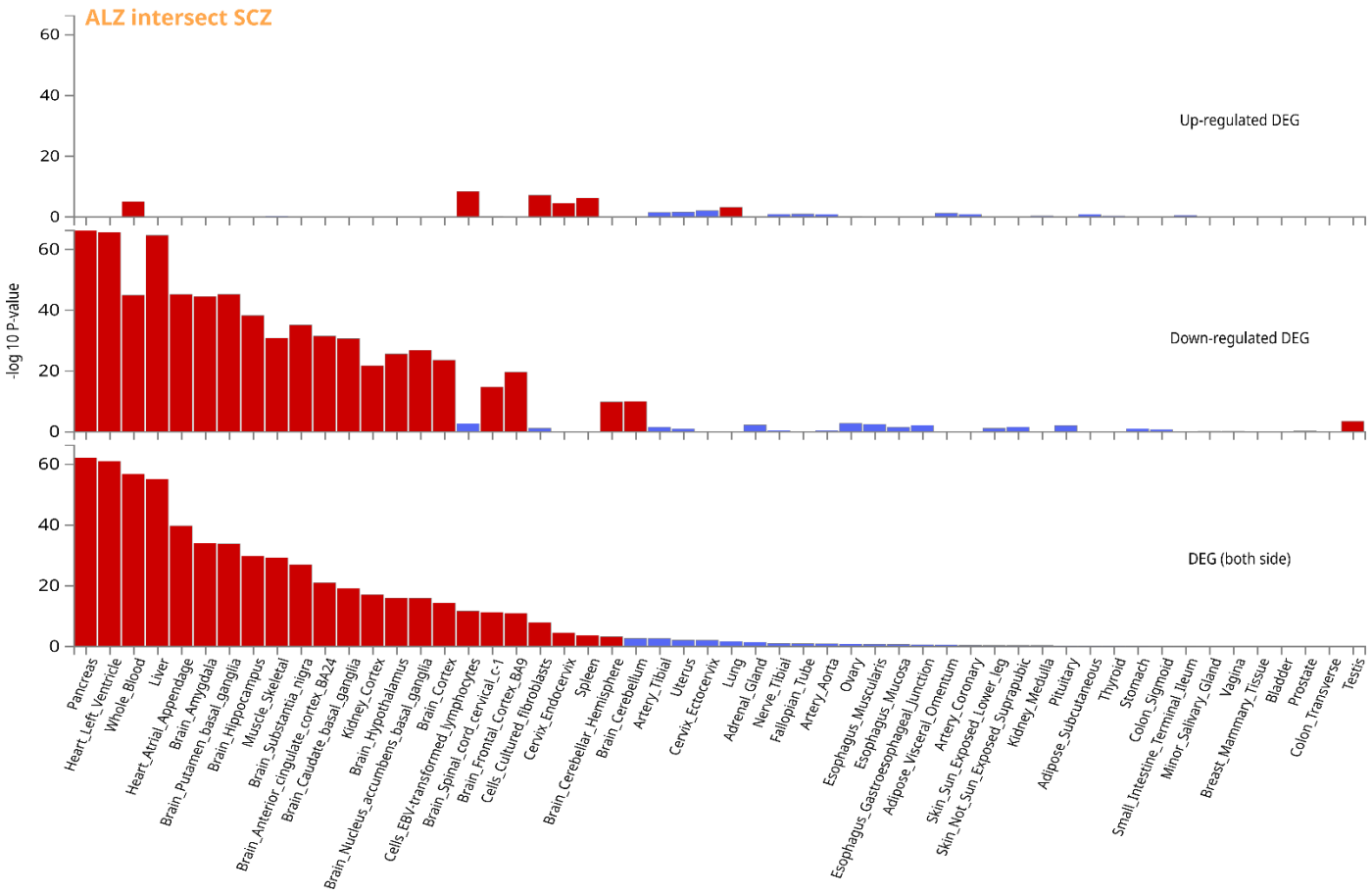


Figure 2. Gene expression meta-analysis results for the first 1000 genes. (A) The overall methodology of ImaGEO shinny app. Gene expression heatmaps representing the associations found in Autism Spectrum Disorder (B), Bipolar Disorder (C), Schizophrenia (D), and Alzheimer's disease (E).

Figure 3. Enrichment analysis of Schizophrenia and Alzheimer's associated genes. All enrichment analyses are corrected by FDR and only genets with more than 10 genes were considered. A) The overlap between all disorders tested in an UpSet style plot. B) Enrichment analysis of Schizophrenia-associated genes in the Kyoto Encyclopaedia of Genes and Genomes (KEGG). C) Enrichment analysis of Alzheimer's-associated genes in KEGG. D) Enrichment analysis of genes overlapping between Alzheimer's and Schizophrenia. E) Tissue-specific expression of genes in the overlap

between Alzheimer's and Schizophrenia; red bars represent significant associations < 0.05 after FDR correction. ALZ = Alzheimer's disease, ASD = Autism Spectrum Disorder, BD = Bipolar Disorder, SCZ = Schizophrenia.

E



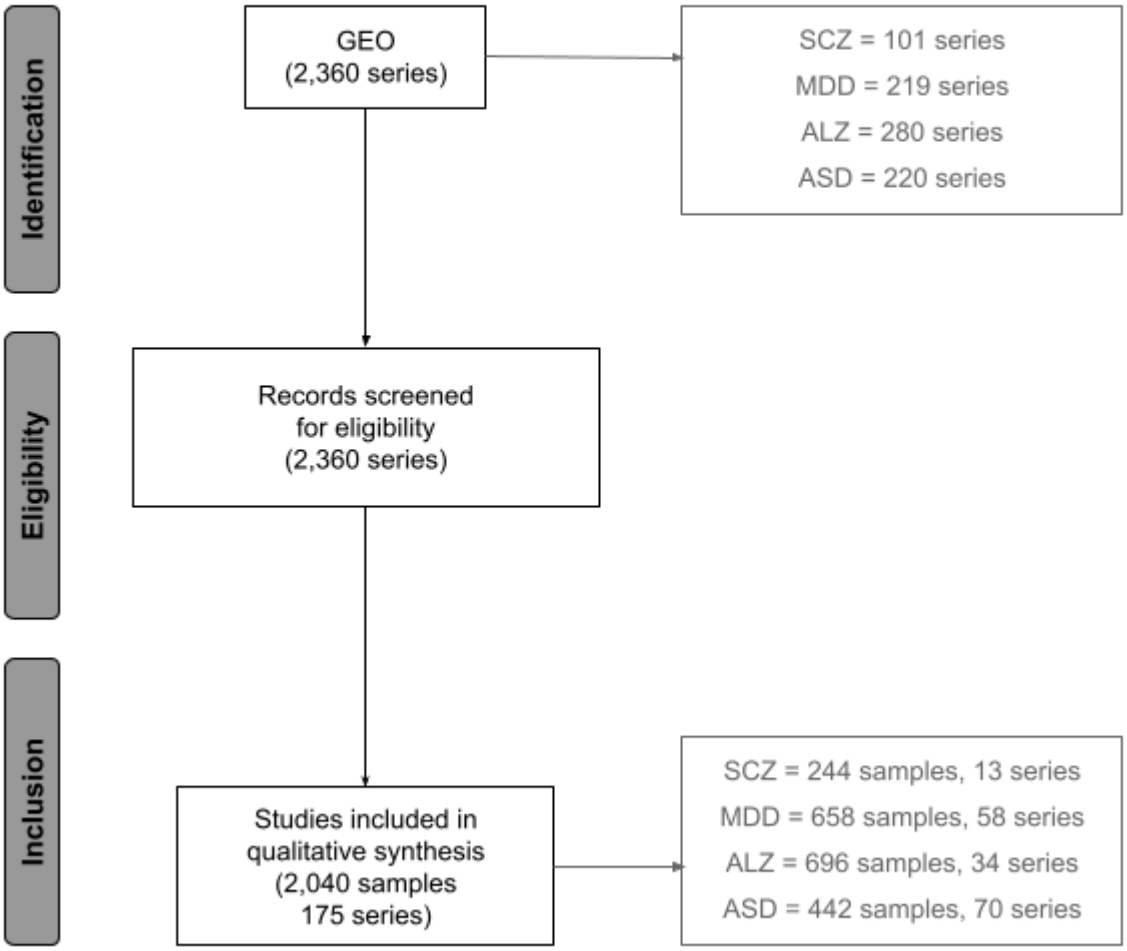


Figure 4. Flowchart of the study inclusion process from the GEO DataSets database for animal model studies. SCZ = Schizophrenia, MDD = Major depressive disorder, ALZ = Alzheimer's disease, ASD = Autism spectrum disorder.

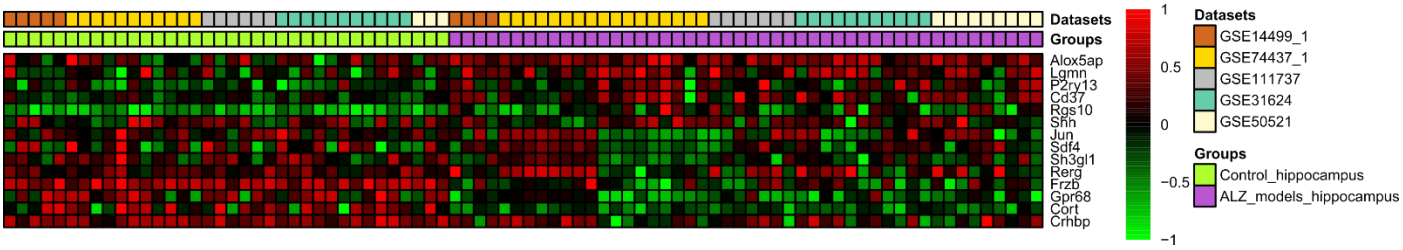


Figure 5. Gene expression meta-analysis results in heatmaps representing the associations found in Alzheimer's disease animal models hippocampal tissue.

The validity of animal models in psychiatry

“A model is a lie that helps you see the truth”

– Howard Skipper

3

Systematic review and meta-analysis of the behavioral effects of methylphenidate in the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder

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Review article

Systematic review and meta-analysis of the behavioral effects of methylphenidate in the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder

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ABSTRACT

The spontaneously hypertensive rats (SHR) are the most widely used model for ADHD. While face and construct validity are consolidated, questions remain about the predictive validity of the SHR model. We aim at summarizing the evidence for the predictive validity of SHR by evaluating its ability to respond to methylphenidate (MPH), the most well documented treatment for ADHD. A systematic review was carried out to identify studies evaluating MPH effects on SHR behavior. Studies (n = 36) were grouped into locomotion, attention, impulsivity or memory, and a meta-analysis was performed. Meta-regression, sensitivity, heterogeneity, and publication bias analyses were also conducted. MPH increased attentional and mnemonic performances in the SHR model and decreased impulsivity in a dose-dependent manner. However, MPH did not reduce hyperactivity in low and medium doses, while increased locomotor activity in high doses. Thus, since the paradoxical effect of stimulant in reducing hyperactivity was not observed in the SHR model, our study does not fully support the predictive validity of SHR, questioning their validity as an animal model for ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by impairing levels of hyperactivity, impulsivity, and inattention (Faraone et al., 2015). Although ADHD is characterized by a strong heritability of about 70–80% across the lifespan (Larsson et al., 2014), and some risk genes have been identified (Demontis et al., 2019), its pathophysiology is still not entirely known. In this sense, animal studies are considered a fundamental tool to unravel the neurobiological underpinning of the disorder (Nestler and Hyman, 2010). The spontaneously hypertensive rats (SHR) are the most widely used animal model (Sagvolden et al., 2005) of ADHD. However, questions are still open regarding the validity of using this strain as an ADHD model (Aparicio et al., 2017; Niigaki et al., 2019; Peres et al., 2018; van den Bergh et al., 2006).

In order to be considered a proper animal model, three main criteria need to be fulfilled. The first is face validity, which may be assessed by the similarity between symptoms expressed by the animal model and individuals with the disorder. The second is construct validity, which can be evaluated by the similarity of pathophysiological mechanisms.

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Also, the third is predictive validity, which may be measured by the ability of the animal model to respond to well-documented treatments for the disorder (van der Staay et al., 2009).

Several studies have provided support for the face validity of SHR as an animal model of ADHD. Behavioral similarities between SHR and patients with ADHD were observed concerning attentional deficits, impulsivity, hyperactivity, as well as motor and cognitive impulsiveness (Bayless et al., 2015; Pardey et al., 2009; Sagvolden, 2000; Sagvolden et al., 2005). Construct validity has been extensively investigated, although we should consider the limited well-known pathophysiological mechanisms of ADHD. SHR present variations in the DAT-1 gene, sex differences, decreased brain volume, dopamine hypofunction, as well as glutamate and norepinephrine dysfunctions (Meneses et al., 2011; Pardey et al., 2009; Sagvolden, 2000; Sagvolden et al., 2005). However, predictive validity has been little discussed so far (Sagvolden, 2000; Sagvolden et al., 2005); likely due to the scarcity and heterogeneity of the studies investigating SHR drug treatment. Therefore, the compilation and analysis of this data are of paramount importance.

Stimulant and non-stimulant medications with dopaminergic and noradrenergic action are usually recommended for ADHD treatment (Subcommittee on Attention-Deficit/Hyperactivity Disorder et al., 2011), being stimulants the most effective drugs and the first-line choice (Cortese et al., 2017; Faraone et al., 2006). Among stimulants, methylphenidate (MPH), a dopamine transporter inhibitor, is the most commonly used (Shier et al., 2013). Despite some heterogeneity in response to MPH treatment (Wilens et al., 2011), there is a huge response rate to the drug, which is around 80% for both children and adults (Pliszka, 2007). Therefore, MPH can be considered the best tool to test the predictive validity.

Thus, in this study, we aim at summarizing the evidence for the predictive validity of SHR as an animal model of ADHD. We evaluate and summarize all studies reporting behavioral effects of MPH in SHR following a systematic review and meta-analysis.

2. Methods and materials

The methods are described according to the guidelines for meta-analysis of animal studies (Peters et al., 2006; Vries et al., 2015). A protocol for this study has been previously published (Leffa et al., 2018).

2.1. Search strategy

Studies were identified using three different databases: Medline, Embase, and Web of Science. The complete search strategy can be found in Supplementary Material. The search was independently conducted in February 2017 by two authors (D.L. and A.S). There were no language or date of publication restrictions. The reference list of included studies was searched in order to locate additional references. Authors of the original studies were contacted in case of missing data or questions regarding data extraction. If key information was not explicitly described or calculable based on other information, and there was no reply from the author, the study was excluded.

2.2. Inclusion and exclusion steps

The study selection and inclusion were performed based on title and abstract, followed by full-text analysis. Both were conducted by two independent authors (D.L. and A.S.), and any disagreement was discussed with a third author (E.G.). We included all studies that administered MPH to SHR (SHR-MPH) and had a control group (SHR-vehicle) evaluating locomotion (hyperactivity), attention, impulsivity or memory. The following exclusion criteria were applied: use of SHR substrains (e.g., stroke-prone SHR), MPH administered only in brain slices, MPH administered only together with another drug, and MPH self-administration. Studies using a crossover approach, meaning that the same rats were used as active and vehicle, were excluded in order to avoid a carryover effect (Curtin et al., 2002).

2.3. Data extraction

Data extraction from included studies was conducted independently by two authors (D.L and A.P.). When not reported in enough detail, extraction was done by graph estimation using a digital ruler, as previously described (Pires et al., 2016a, 2016b). If both methods were not viable, the authors were contacted. After two unsuccessful attempts, the article was excluded. For each experiment, the following data were extracted: sample size; gender and age of animals; the route of drug administration; MPH dosage in mg/kg; the number of administrations per day; total days of treatment; behavioral test used; and outcome of interest. Whenever the articles reported a sample range instead of the exact value of animals per group, the lowest value was used, avoiding overestimation of effects. Also, when two different experimental groups shared the same control, the sample size of the control group was divided by the number of comparisons, as suggested by Vesterinen et al. (2014), and rounded down (minimum of 2 rats per group).

When there were multiple outcomes reported from the same behavioral test, the choice was made according to a rank of relevance organized by one review author (D.L.). Variables from each behavioral test were ranked subjectively according to their importance, and the one ranked highest was extracted. The rank was organized prior to data extraction and can be found in Table S1. If the same animals were evaluated more than once in the same behavioral test, the last one was selected for extraction. If the manuscript separated the results by time, the first time point was selected.

2.4. Bias assessment

The risk of bias assessment consists of a manual evaluation of included studies, concerning methodological quality. Risk of bias assessment was conducted by one author (A.P.), based on the SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014). This tool provides a list of general topics that an animal study should address for it to be considered unbiased. Questions related to the assessment were discussed with a second reviewer (D.L.). Ten items were evaluated in the quality assessment. Three items were related to selection bias. The first item domain was sequence generation, in which a study presents low risk of bias when the investigators describe a random process of allocating animals in enough detail to assess whether comparable groups are created. The second was baseline characteristics, which for the sake of this study comprised information on sex and age of the animals. The third item domain was allocation concealment, which concerns whether the investigator allocating the animals to intervention or control group could not foresee assignments before or during enrollment. Two items were related to performance bias. The first domain was random housing, in which a study should describe the methods to randomly housing the animals within the animal room. The second item evaluated whether caregivers were blinded to which intervention the animals received. Two items were related to detection bias. The first evaluated if the investigators selected the animals at random for outcome assessment, and the second evaluated blinding of outcome assessors. One item was related to attrition bias (incomplete outcome data); other related to reporting bias (whether there was no selective outcome reporting, i.e., the study presented all the expected outcomes); and the last one related to other sources of bias. The tenth item addressed sources of bias beyond the ones covered by other domains. Each study was evaluated considering the ten domains and, for each item, it was classified as presenting a low, unclear or high risk of bias. In order to access publication bias, funnel plots were generated, and the Egger´s regression test was performed.

2.5. Statistical analysis

Studies were grouped according to the behavioral outcomes (locomotion, attention, impulsivity or memory) and a meta-analysis was performed for each group. In order to conduct a meta-analysis, a minimum of three experiments was required. Pooled effect sizes were determined with standardized mean differences (SMD) using the Hedge´s G method with random-effects, allowing the comparison among distinct behavioral tests. The significance of pooled effect sizes was determined using the Z-test, and because we performed four statistical tests, a Bonferroni correction was applied, and a p-value ≤ 0.0125 was considered statistically significant. Individual study weights were obtained using the inverse of the variance. Data were transformed in order to obtain positive values for decreased impulsive behavior and increased attentional or memory performances.

Any variability causes heterogeneity among studies, such as methodological or population/strain differences. The heterogeneity between studies was estimated using both the Chi^2 and the I^2 tests, in which $I^2 = \left(\frac{Q-df}{Q}\right)x\ 100\%$, where Q = Chi^2 test results. A p-value ≤ 0.1 was considered significant for the Chi^2, and I^2 values of 25%, 50%, and 75% were considered as representing low, moderate, and high heterogeneity, respectively (Higgins and Thompson, 2002). SMD and heterogeneity values were obtained using the Review Manager (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). For sensitivity analyses, a p-value ≤ 0.05 was considered statistically significant.

2.6. Meta-regression

In order to evaluate the source of variability among studies, potential covariates were selected based on biological plausibility and added to a random-effects meta-regression model. The following covariates were chosen: age of animals, route of drug administration, and MPH total dosage. Covariates associated with the outcome with a p ≤ 0.1 in univariate analysis were included in a final multivariate meta-regression model. Age was categorized in "adolescent" and "adult" before inclusion in the model because some studies did not report the age of animals in days or weeks. Animals with 60 or more days of age were categorized as "adults," while animals with 28 to 60 days of age were categorized as "adolescents," as previously proposed (Spear, 2000). MPH total dosage was calculated by multiplying the dosage received in mg/kg by the number of administrations per day and by the total number of days of treatment. Studies with missing values were excluded from the meta-regression analysis. Categorical variables were included in the model using dummy variables. Meta-regression analyses were conducted using Stata 13.0 (College Station, TX: StataCorp LP), as previously described (Harbord and Higgins, 2008).

2.7. Sensitivity analyses

Sensitivity analysis consists of a series of methods used to evaluate whether any particular study or group of studies, as well as any main methodological decision, may have significantly skewed the analyses. For that, the following tests were performed: (1) the jackknife method (Miller, 1974); (2) inclusion of effect sizes extracted from the same behavioral test; (3) including studies with crossover designs; (4) including only one MPH dosage at a time; (5) excluding studies presenting a concerning risk of bias, defined as either a high risk of bias in one category or an unclear risk of bias in 7 categories or more. All sensitivity analyses were performed with a minimum of three experiments. An additional sensitivity analysis was performed including only studies with experiments in female rats.

2.8. Secondary analyses

In the secondary analyses, we compared SHR submitted to MPH to a normotensive control strain submitted to vehicle stimulation. From these analyses, one can evaluate whether MPH is effective in normalizing SHR behavior to the level of control strains. For that, we selected studies reporting behavioral effects of vehicle stimulation in Wistar Kyoto Rats (WKY) or Wistar rats, when compared to SHR. The selection was performed from the final list of included studies. The sample size and the outcome of interest were extracted as previously mentioned for the primary analysis. Whenever there were multiple control strains, the WKY, followed by the Wistar rats, were selected for extraction. For control strain analyses, a p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Study characteristics

The initial search identified 218 articles after excluding duplicates. A total of 82 studies were excluded after the title and abstract review, and 100 after the first full-text review (Fig. 1). Eleven authors were contacted, from which seven were unresponsive. In the end, one study reporting all outcomes, one study reporting attention outcomes, and five crossover studies were excluded. A total of 36 articles were included in the primary analysis, among which 22 reported locomotion (Chelaru et al., 2012; Cheng et al., 2017; Dela Peña et al., 2013; Fox et al., 2002; Hong et al., 2009; Kim et al., 2011, 2016; Pardey et al., 2012; Pires et al., 2010; Robinson et al., 2012; Robinson and Bucci, 2014; Somkuwar et al., 2016; Tamburella et al., 2012; Umehara et al., 2013a, 2013b; van den Bergh et al., 2006; Vendruscolo et al., 2008; Warton et al., 2009; Yang et al., 2015, 2011, 2006; Yoon et al., 2008), 14 attention (Aspide et al., 2000; Cao et al., 2012; Cheng and Li, 2013; Dela Peña et al., 2013; Fox et al., 2002; Harvey et al., 2013, 2011; Hong et al., 2009; Kantak et al., 2008; Kawaura et al., 2014; Kim et al., 2016; Robinson et al., 2012; Robinson and Bucci, 2014; Yoon et al., 2013), 6 impulsivity (Adriani et al., 2004; Dela Peña et al., 2013; Kim et al., 2012; Somkuwar et al., 2016; Yoon et al., 2013, 2008), and 8 memory outcomes (Cheng et al., 2017; Guo et al., 2012; Hong et al., 2011; Kim et al., 2011; Pires et al., 2010, 2009; Tamburella et al., 2012; Tian et al., 2009) (some studies reported more than one behavioral category). Descriptions of included studies can be found in Table 1. From the 36 studies included in the primary analysis, 28 also reported behavioral effects of vehicle stimulation in control strains. Among those, 17 reported locomotion (Chelaru et al., 2012; Cheng et al., 2017; Dela Peña et al., 2013; Kim et al., 2011, 2016; Pardey et al., 2012; Pires et al., 2010; Robinson et al., 2012; Robinson and Bucci, 2014; Somkuwar et al., 2016; Tamburella et al., 2012; Umehara et al., 2013a; van den Bergh et al., 2006; Warton et al., 2009; Yang et al., 2011, 2006; Yoon et al., 2008), 12 attention (Aspide et al., 2000; Cao et al., 2012; Cheng and Li, 2013; Dela Peña et al., 2013; Harvey et al., 2013, 2011; Kantak et al., 2008; Kawaura et al., 2014; Kim et al., 2016; Robinson et al., 2012; Robinson and Bucci, 2014; Yoon et al., 2013), 5 impulsivity (Dela Peña et al., 2013; Kim et al., 2012; Somkuwar et al., 2016; Yoon et al., 2013, 2008), and 6 memory outcomes (Cheng et al., 2017; Kim et al., 2011; Pires et al., 2010, 2009; Tamburella et al., 2012; Tian et al., 2009). The digital ruler was used for the retrieving of data from 33 studies.

3.2. Meta-analyses

A total of 22 studies evaluating hyperactivity were meta-analyzed, comprising 46 experiments with 418 rats in the MPH group and 239 rats in the vehicle group. There was no statistically significant effect of MPH (Z = 0.99, p = 0.32) and the overall effect size was -0.18 (95% CI = -0.54, 0.18; Fig. 2). In the attention analysis, 14 studies were

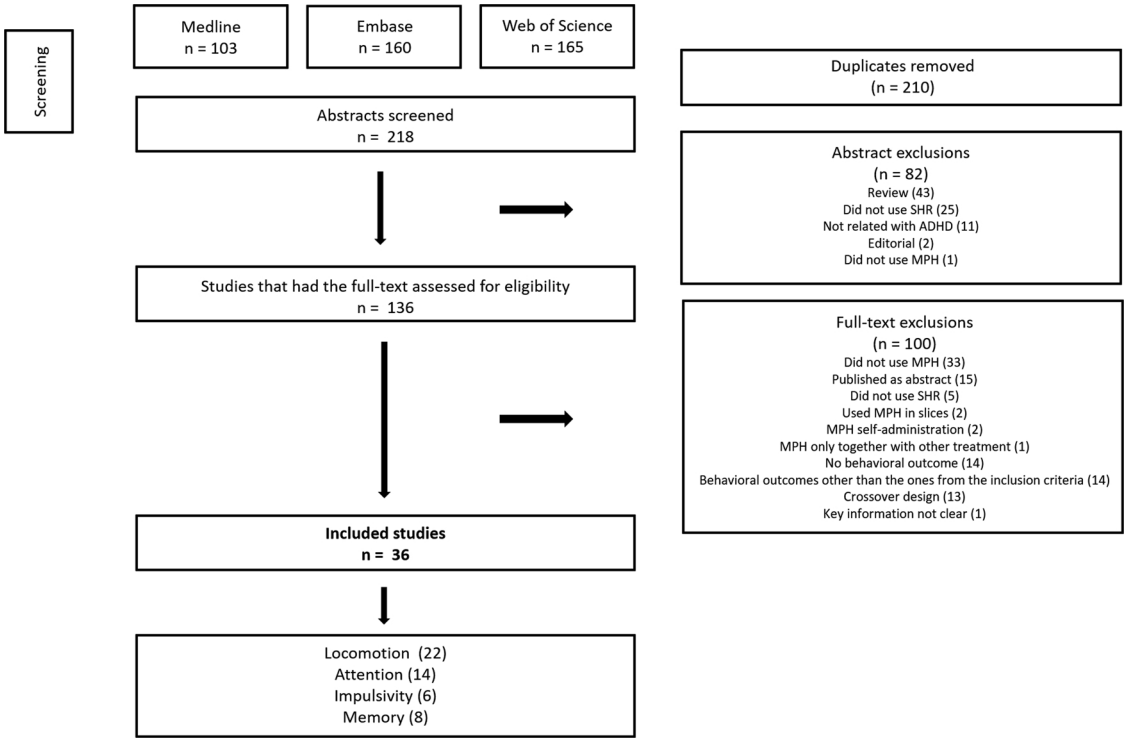

Fig. 1. Flowchart of included studies. MPH: methylphenidate; SHR: Spontaneously Hypertensive Rats.

included comprising 24 experiments with 224 rats in the MPH group and 126 rats in the vehicle group. A statistically significant positive effect of MPH was detected ($Z = 3.57$, $p < 0.001$) with a pooled effect size of 0.86 (95% CI = 0.39, 1.33; Fig. 3), showing that MPH increased attentional performance. Six studies evaluating impulsive behavior were included, comprising nine experiments with 82 rats in the MPH group and 62 rats in the control group. A statistically significant positive effect of MPH was shown ($Z = 4.20$, $p < 0.001$), demonstrating that MPH decreased SHR impulsive behavior. The overall effect size was 0.81 (95% CI = 0.43, 1.18; Fig. 4). A total of 8 studies evaluating mnemonic performance were meta-analyzed, comprising 14 experiments with 133 rats in the MPH group and 93 rats in the vehicle group. A statistically significant positive effect of MPH was detected ($Z = 4.71$, $p < 0.001$) with a summary effect size of 1.01 (95% CI = 0.59, 1.43; Fig. S1), showing an increase in memory performance with MPH treatment. All meta-analysis results are summarized in Table 2.

3.3. Bias assessment

Overall, studies failed to report aspects that could lead to selection bias (allocation concealment), as well as performance and detection bias. The quality assessment of locomotion studies did not reveal any high risk of bias, although the unclear risk remains considerably high (Figs. S2 and S3). The bias assessment of attention studies revealed that 14.3% of studies presented high risk of bias considering baseline characteristics (Figs. S4 and S5). Among impulsivity studies, 16.6% presented high risk of bias in the allocation sequence generation (Figs. S6 and S7). Quality assessment of memory studies revealed a similar pattern to the locomotion studies (Figs. S8 and S9). Finally, the Egger´s regression test indicates no publication bias for all categories (Fig. S10). Visual inspection of funnel plots also demonstrates a relatively symmetrical distribution (Fig. S10).

3.4. Heterogeneity

High heterogeneity was detected in the hyperactivity analysis with an $I^2 = 70\%$ and a $Chi^2 = 151.56$ ($df = 45$, $p < 0.001$; Fig. 2). In the attention analysis, high heterogeneity was also found, with an $I^2 = 68\%$ and a $Chi^2 = 72.68$ ($df = 23$, $p < 0.001$; Fig. 3). For impulsivity analysis, there was no heterogeneity. The analysis showed an $I^2 = 9\%$ and a $Chi^2 = 8.8$ ($df = 8$, $p = 0.36$; Fig. 4). In the memory analysis, a moderate heterogeneity was found, with an $I^2 = 43\%$ and a $Chi^2 = 22.97$ ($df = 13$, $p = 0.04$; Fig. S1).

3.5. Meta-regression

A univariate regression model was conducted in order to test the association between the pre-defined covariates and the effect sizes for locomotion, attention, and memory. We did not perform a regression for the impulsivity results since no heterogeneity was detected. Results are presented in Table S2. Since there was no covariate associated with the effect size for a p-value less than 0.1, the multivariate regression model was not performed.

3.6. Sensitivity analyses

Sensitivity analyses were conducted as previously described. In the first sensitivity analysis, no study was skewing the overall result. In the second sensitivity analysis, the effects of MPH were analyzed for each behavioral test. A total of 36 experiments evaluated locomotion using the open field, and there was no effect of MPH; 3 experiments used the automated activity monitor, with increased locomotion after MPH treatment; and four experiments were conducted using the locomotor activity during the social interaction test, with no effect of MPH (Fig. S11). For attention, 4 experiments used the Y-maze, with a positive effect of MPH in increasing attentional performance; 5 used the attentional set-shifting, with a positive effect of MPH; 5 performed the orienting behavior, with a positive effect of MPH; 4 used the lat maze, with no effect of MPH; and 3 experiments conducted the five-trial inhibitory avoidance test, with a positive effect of MPH (Fig. S12). For impulsivity, five experiments performed the electro-foot shock aversive water drinking test, with a positive effect of MPH in decreasing

Table 1
Description of included studies.

Study	Gender	Age (days)	Route of drug administration	MPH dosage (mg/kg)	Administrations per day	Days of treatment	Behavioral test	Outcome extracted
Locomotion								
Chelaru et al. (2012)-1	Female	40	IP	0.6	1	7	Open field	Distance moved
Chelaru et al. (2012)-2	Female	40	IP	2.5	1	7	Open field	Distance moved
Chelaru et al. (2012)-3	Female	40	IP	10	1	7	Open field	Distance moved
Chelaru et al. (2012)-4	Male	40	IP	0.6	1	7	Open field	Distance moved
Chelaru et al. (2012)-5	Male	40	IP	2.5	1	7	Open field	Distance moved
Chelaru et al. (2012)-6	Male	40	IP	10	1	7	Open field	Distance moved
Cheng et al. (2017)	Male	28-35	IP	0.5	1	1	Midline crossing task	Number of midline crossings
dela Pena et al. (2013)-1	Male	28	IP	2	1	1	Open field	Distance moved
dela Pena et al. (2013)-2	Male	28	IP	3	1	1	Open field	Distance moved
Fox et al. (2002)-1	Male	20-24	Subcutaneous	1	1	1	Automated activity monitor	Mean distance traveled
Fox et al. (2002)-2	Male	20-24	Subcutaneous	3	1	1	Automated activity monitor	Mean distance traveled
Fox et al. (2002)-3	Male	20-24	Subcutaneous	10	1	1	Automated activity monitor	Mean distance traveled
Hong et al. (2009)	Not clear	Not clear	Oral	2	2	14	Lat maze	Horizontal activity
Kim et al. (2011)	Male	Adult	Oral	1	1	28	Open field	Distance moved
Kim et al. (2016)	Male	49	Oral	1	2	15	Open field	Distance moved
Pardey et al. (2012)	Male	25	Oral	2	2	20	Operant conditioning chambers	Mean locomotor activity
Pires et al. (2010)	Female	25	IP	2	1	14	Open field	Distance moved
Robinson et al. (2012)	Female	49-56	IP	0.125	1	1	Locomotor activity during the social interaction test	Number of line crossings
Robinson and Bucci (2014)-1	Female	49-56	IP	0.03125	1	1	Locomotor activity during the social interaction test	Number of line crossings
Robinson and Bucci (2014)-2	Female	49-56	IP	0.0625	1	1	Locomotor activity during the social interaction test	Number of line crossings
Robinson and Bucci (2014)-3	Female	49-56	IP	0.125	1	1	Locomotor activity during the social interaction test	Number of line crossings
Somkuwar et al. (2016)	Male	77	Oral	1.5	1	28	Open field	Distance moved
Tamburella et al. (2012)-1	Male	60	IP	1	1	1	Open field	Distance moved
Tamburella et al. (2012)-2	Male	60	IP	3	1	1	Open field	Distance moved
Tamburella et al. (2012)-3	Male	60	IP	6	1	1	Open field	Distance moved
Umehara et al. (2013a)-1	Male	28-35	IP	0.1	1	1	Open field	Distance moved
Umehara et al. (2013a)-2	Male	28-35	IP	0.3	1	1	Open field	Distance moved
Umehara et al. (2013a)-3	Male	28-35	IP	1	1	1	Open field	Distance moved
Umehara et al. (2013b)	Male	28-49	IP	0.3	1	1	Open field	Distance moved
van den Bergh et al. (2006)	Male	30-44	Oral	1	1	1	Open field	Distance moved
Vendruscolo et al. (2008)-1	Female	63-70	IP	2	2	16	Open field	Peripheral locomotion
Vendruscolo et al. (2008)-2	Male	63-70	IP	2	2	16	Open field	Peripheral locomotion
Warton et al. (2009)-1	Male	29-36	Oral	0.5	1	1	Open field	Distance moved
Warton et al. (2009)-2	Male	29-36	Oral	1	1	1	Open field	Distance moved
Warton et al. (2009)-3	Male	29-36	Oral	2	1	1	Open field	Distance moved
Yang et al. (2006)-1	Male	34-41	IP	0.6	1	7	Open field	Distance moved
Yang et al. (2006)-2	Male	34-41	IP	2.5	1	7	Open field	Distance moved
Yang et al. (2006)-3	Male	34-41	IP	10	1	7	Open field	Distance moved
Yang et al. (2011)-1	Male	40	IP	0.6	1	7	Open field	Distance moved
Yang et al. (2011)-2	Male	40	IP	2.5	1	7	Open field	Distance moved
Yang et al. (2011)-3	Male	40	IP	10	1	7	Open field	Distance moved
Yang et al. (2011)-4	Male	62	IP	0.6	1	7	Open field	Distance moved
Yang et al. (2011)-5	Male	62	IP	2.5	1	7	Open field	Distance moved
Yang et al. (2011)-6	Male	62	IP	10	1	7	Open field	Distance moved
Yang et al. (2015)	Male	42-56	IP	0.1	1	1	Open field	Distance moved

(*continued on next page*)

Table 1 (*continued*)

Study	Gender	Age (days)	Route of drug administration	MPH dosage (mg/kg)	Administrations per day	Days of treatment	Behavioral test	Outcome extracted
Yoon et al. (2008)	Male	28	IP	3	1	1	Open field	Distance moved
Attention								
Aspide et al. (2000)-1[a]	Male	Juveline	IP	3	1	14	Lat maze	Duration of hearing episodes in a novel situation
Aspide et al. (2000)-2	Male	Juveline	IP	3	1	1	Lat maze	Duration of hearing episodes in a novel situation
Aspide et al. (2000)-3[b]	Male	Juveline	IP	3	1	14	Lat maze	Duration of hearing episodes in a novel situation
Cao et al. (2012)-1	Male	35	IP	2.5	1	14	Attentional set-shifting	Percentage or number of regressive errors
Cao et al. (2012)-2	Male	35	IP	5	1	14	Attentional set-shifting	Percentage or number of regressive errors
Cheng and Li (2013)	Male	35	IP	0.6	1	1	Attentional set-shifting	Trials to criterion Rev1
dela Pena et al. (2013)-1	Male	28	IP	2	1	1	Y-maze	Percentage or number of spontaneous alternations
dela Pena et al. (2013)-2	Male	28	IP	3	1	1	Y-maze	Percentage or number of spontaneous alternations
Fox et al. (2002)-1	Male	20-24	Subcutaneous	1	1	1	Repeated acquisition version of an inhibitory avoidance task	Transfer latency (trials 2-5 summarized)
Fox et al. (2002)-2	Male	20-24	Subcutaneous	3	1	1	Repeated acquisition version of an inhibitory avoidance task	Transfer latency (trials 2-5 summarized)
Harvey et al. (2011)	Male	28	Oral	1.5	1	28	Visual discrimination in a T-maze	Trials to criterion
Harvey et al. (2013)	Male	28	Oral	1.5	1	28	Attentional set-shifting	Percentage or number of regressive errors
Hong et al. (2009)	Not clear	Not clear	Oral	2	2	14	Lat maze	Frequencies of leaning
Kantak et al. (2008)	Male	63	Oral	1.5	1	14	Attentional set-shifting	Percentage or number of regressive errors
Kawaura et al. (2014)-1	Male	28	Subcutaneous	0.3	1	1	Five-trial inhibitory avoidance task	Total transfer latency on trials 2–5
Kawaura et al. (2014)-2	Male	28	Subcutaneous	1	1	1	Five-trial inhibitory avoidance task	Total transfer latency on trials 2–5
Kawaura et al. (2014)-3	Male	28	Subcutaneous	3	1	1	Five-trial inhibitory avoidance task	Total transfer latency on trials 2–5
Kim et al. (2016)	Male	49	Oral	1	2	15	Y-maze	Percentage or number of spontaneous alternations
Robinson et al. (2012)	Female	49-56	IP	0.125	1	1	Orienting behavior	Beam breaks in block 3
Robinson and Bucci (2014)-1	Female	49-56	IP	0.015625	1	1	Orienting behavior	Beam breaks in block 3
Robinson and Bucci (2014)-2	Female	49-56	IP	0.03125	1	1	Orienting behavior	Beam breaks in block 3
Robinson and Bucci (2014)-3	Female	49-56	IP	0.0625	1	1	Orienting behavior	Beam breaks in block 3
Robinson and Bucci (2014)-4	Female	49-56	IP	0.125	1	1	Orienting behavior	Beam breaks in block 3
Yoon et al. (2013)	Male	28	IP	2	1	1	Y-maze	Percentage or number of spontaneous alternations
Impulsivity								
Adriani et al. (2004)-1[c]	Male	30	IP	3	2	15	Nose poking test	Percentage of self-control in delay 80
Adriani et al. (2004)-2[d]	Male	30	IP	3	2	15	Nose poking test	Percentage of self-control in delay 80
dela Pena et al. (2013)-1	Male	28	IP	2	1	1	Electro-foot shock aversive water drinking test	Drinking attempts
dela Pena et al. (2013)-2	Male	28	IP	3	1	1	Electro-foot shock aversive water drinking test	Drinking attempts
Kim et al. (2012)-1	Male	28	IP	2	1	1	Electro-foot shock aversive water drinking test	Water area frequency
Kim et al. (2012)-2	Male	28	IP	5	1	1	Electro-foot shock aversive water drinking test	Water area frequency

(*continued on next page*)

Table 1 (*continued*)

Study	Gender	Age (days)	Route of drug administration	MPH dosage (mg/kg)	Administrations per day	Days of treatment	Behavioral test	Outcome extracted
Somkuwar et al. (2016)	Male	77	Oral	1.5	1	28	Differential reinforcement of low-rate schedules	Response efficiency under DRL30
Yoon et al. (2008)	Male	28	IP	3	1	1	Elevated plus maze	Percentage of staying time in closed area
Yoon et al. (2013)	Male	28	IP	2	1	1	Electro-foot shock aversive water drinking test	Water area frequency
Memory								
Cheng et al. (2017)	Male	28-35	IP	0.5	1	1	Temporal order recognition memory test	Discrimination ratio
Guo et al. (2012)	Male	28-35	IP	10	1	14	Morris water maze	Percentage of time in target quadrant
Hong et al. (2011)	Not clear	35	Oral	2	2	14	Morris water maze	Mean latency to find the platform in day 3
Kim et al. (2011)	Male	Adult	Oral	1	1	28	8-arm maze test	Number of correct choices before the first error
Pires et al. (2009)-1	Female	90	IP	2	1	1	Object recognition test	Discrimination index
Pires et al. (2009)-2	Male	90	IP	2	1	1	Object recognition test	Discrimination index
Pires et al. (2010)	Female	25	IP	2	1	14	Object recognition test	Discrimination index
Tamburella et al. (2012)-1	Male	60	IP	1	1	1	Active avoidance test	Latency to re-enter the dark box in the first retention
Tamburella et al. (2012)-2	Male	60	IP	3	1	1	Active avoidance test	Latency to re-enter the dark box in the first retention
Tamburella et al. (2012)-3	Male	60	IP	6	1	1	Active avoidance test	Latency to re-enter the dark box in the first retention
Tamburella et al. (2012)-4	Male	60	IP	1	1	1	Passive avoidance test	Latency to re-enter the dark box in the first retention
Tamburella et al. (2012)-5	Male	60	IP	3	1	1	Passive avoidance test	Latency to re-enter the dark box in the first retention
Tamburella et al. (2012)-6	Male	60	IP	6	1	1	Passive avoidance test	Latency to re-enter the dark box in the first retention
Tian et al. (2009)	Male	49	IP	10	1	6	Morris water maze	Crossings to the target area

[a] 0.5 h after the last drug administration. [b] 24 h after the last drug administration. [c] Non-sensitive to delay rats. [d] Impulsive rats.

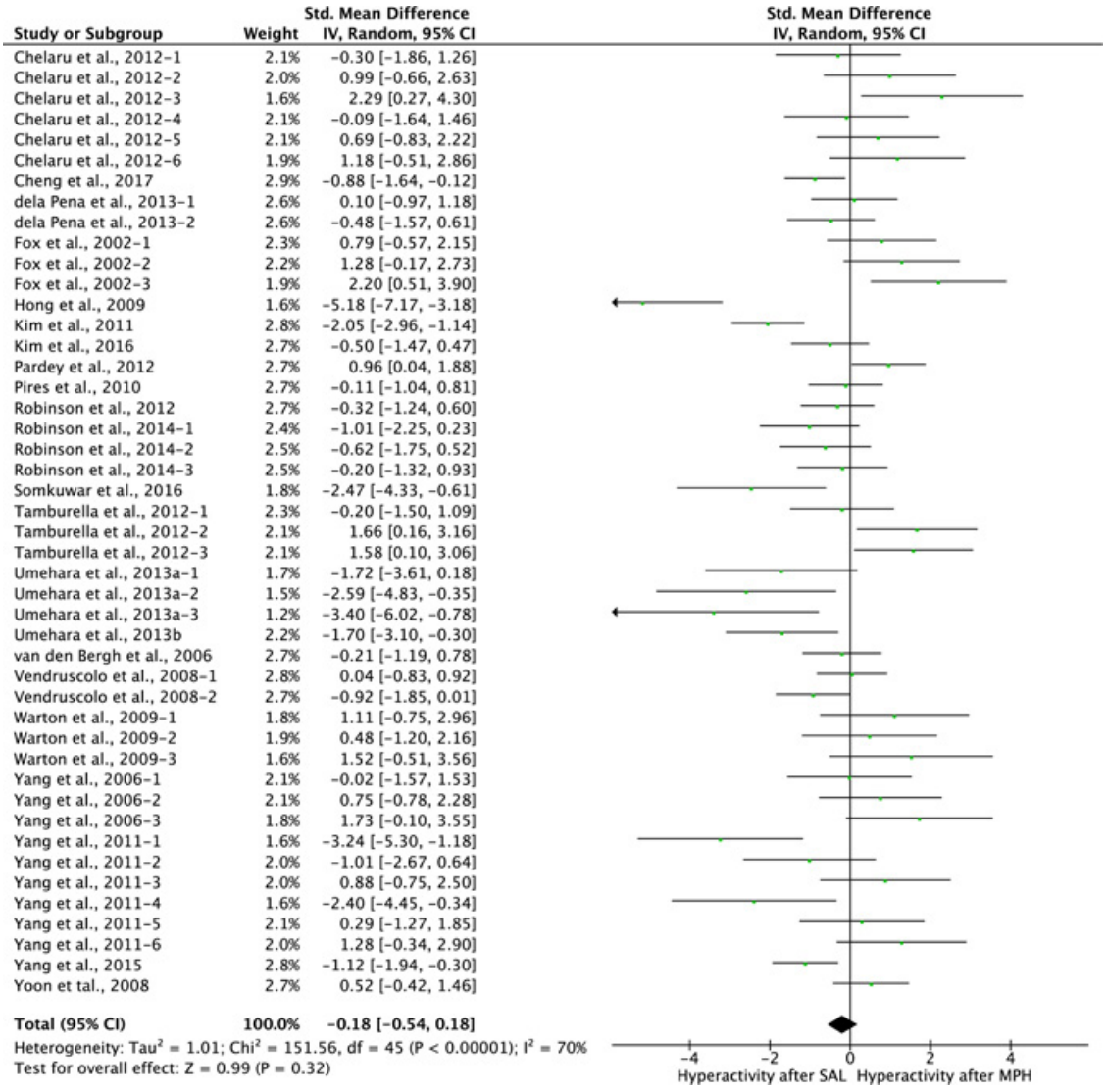

Fig. 2. Forest plot of included studies evaluating locomotion outcomes. Horizontal lines represent the effect size ± the confidence interval (95%). Summary effect size is represented by the diamond. CI = confidence interval. SAL = saline treatment. MPH = methylphenidate treatment.

impulsivity (Fig. S13). For memory, 3 experiments used the morris water maze, with no effect of MPH; 3 used the object recognition test, with a positive effect of MPH in improving memory performance; 3 used the passive avoidance test, with no effect of MPH; and 3 experiments conducted the active avoidance test, with a positive effect of MPH (Fig. S14).

In the third analysis, the inclusion of crossover studies did not change the significance of the results. Three new studies were included in the locomotion analysis (Guo et al., 2012; Hong et al., 2011; Pires et al., 2009; Fig. S15), two in the attention analysis (Tian et al., 2009; van den Bergh et al., 2006; Fig. S16) and five in impulsivity analysis (Dommett, 2014; Ferguson and Cada, 2003; Natsheh and Shiflett, 2015; van den Bergh et al., 2006; Yang et al., 2003; Fig. S17).

In the fourth analysis, we included only one MPH dosage at a time. For hyperactivity, there was an increase in locomotion only after treatment with an MPH dosage of 10 mg/kg (Fig. S18). For attention, there was a positive effect of MPH with dosages of 1 mg/kg and 2 mg/kg (Figure S19). For impulsivity, there was a positive effect of MPH with dosages of 2 mg/kg and 3 mg/kg (Fig. S20). For memory, there was a positive effect of MPH with a dosage of 2 mg/kg (Fig. S21).

In the fifth analysis, three studies (Hong et al., 2009; Pardey et al., 2012; Vendruscolo et al., 2008) were excluded from the locomotion analysis for having 7 or more categories with an unclear risk of bias. In the attention analysis, two studies (Aspide et al., 2000; Hong et al., 2009) were excluded for presenting a high risk of bias on the baseline characteristics. In impulsivity analysis, one study (Adriani et al., 2004) was excluded for presenting a high risk of bias on the sequence generation. No significant differences were observed concerning the primary analysis (effect sizes can be found in Figs. S22, S23, and S24).

Finally, 6 of the 36 studies reported experiments in female SHR rats. Among these, 5 reported locomotion outcomes (Chelaru et al., 2012; Pires et al., 2010; Robinson et al., 2012; Robinson and Bucci, 2014; Vendruscolo et al., 2008); 2 reported attentional outcomes (Robinson et al., 2012; Robinson and Bucci, 2014); and 2 reported memory outcomes (Pires et al., 2010, 2009). None reported impulsivity outcomes for female SHR. The evaluation of those experiments revealed the same effects of the primary analyses (Figs. S25, S26, S27).

3.7. Secondary analyses

For the control strain comparisons, 17 studies evaluating locomotion were analyzed, comprising 38 experiments. A statistically significant difference was found ($Z = 4.84$, $p < 0.001$; Fig. S28), demonstrating increased locomotion of SHR after MPH in relation to the

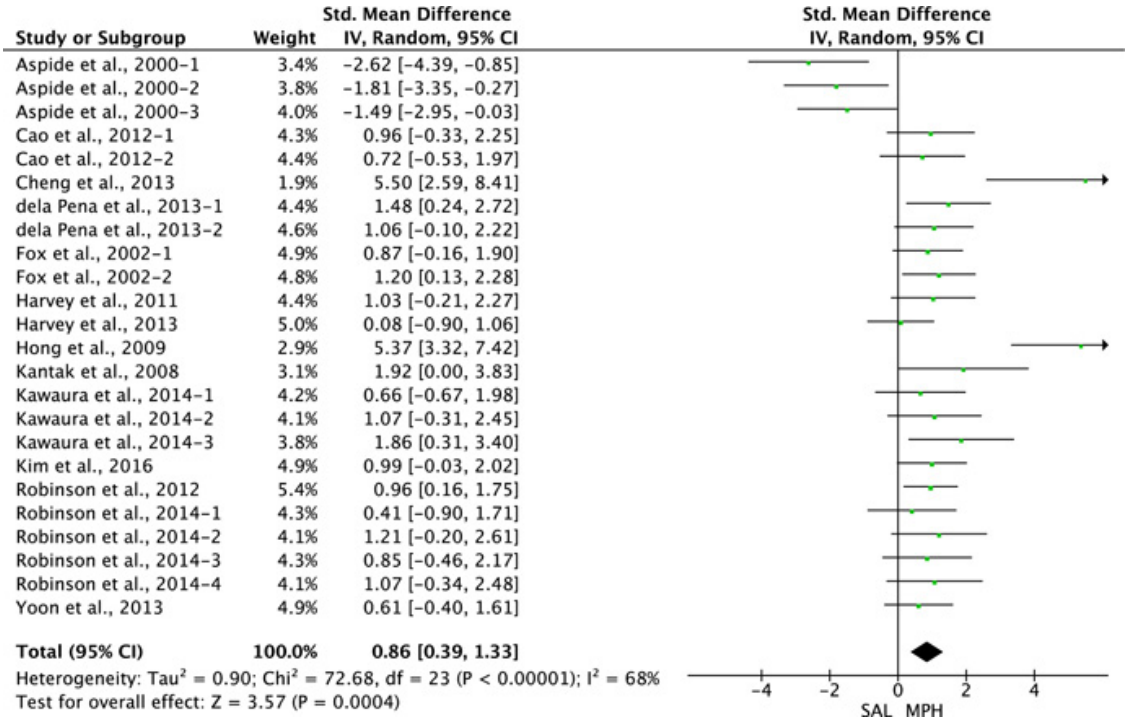

Fig. 3. Forest plot of included studies evaluating attentional outcomes. Horizontal lines represent the effect size ± the confidence interval (95%). Summary effect size is represented by the diamond. CI = confidence interval. SAL = saline treatment. MPH = methylphenidate treatment.

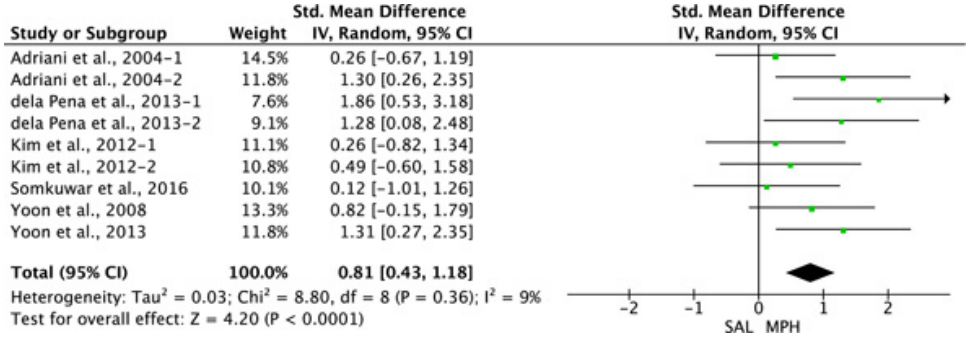

Fig. 4. Forest plot of included studies evaluating impulsivity outcomes. Horizontal lines represent the effect size ± the confidence interval (95%). Summary effect size is represented by the diamond. CI = confidence interval. SAL = saline treatment. MPH = methylphenidate treatment.

Table 2
Summary of main meta-analyses results by behavioral outcome.

Outcome	SMD	[95% CI]	Z test	Z test p-value	Chi² p-value	I² %	SMD	[95% CI]	Z test	Z test p-value	Chi² p-value	I² %
	SHR + MPH vs. SHR + SAL						*SHR + MPH vs. Control SAL*					
Locomotion	−0.18	[-0.54, 0.18]	0.99	0.32000	< 0.00001	70	1.07	[0.63, 1.50]	4.84	< 0.00001	< 0.00001	67
Attention	0.86	[0.39, 1.33]	3.57	0.00040	< 0.00001	68	−0.21	[-0.52, 0.11]	1.29	0.20000	0.15000	24
Impulsivity	0.81	[0.43, 1.18]	4.20	< 0.00010	0.36000	09	−0.41	[-0.95, 0.12]	1.52	0.13000	0.14000	38
Memory	1.01	[0.59, 1.43]	4.71	< 0.00001	0.04000	43	−0.40	[-0.78, -0.02]	2.04	0.04000	0.15000	30

SHR = spontaneously hypertensive rats. MPH = methylphenidate. Control = Wistar-Kyoto rats or Wistar rats. SMD = Standard Mean Difference. CI = confidence interval.

control group. For attention, 12 studies were analyzed, comprising 21 experiments. There was no statistically significant difference (Z = 1.29, p = 0.20; Fig. S29). For impulsivity, five studies with seven experiments were analyzed, and there was also no statistically significant difference between groups (Z = 1.52, p = 0.13; Fig. S30). For memory, six studies comprising 12 experiments were included. A statistically significant effect was found (Z = 2.04, p = 0.04; Fig. S31), showing a better mnemonic performance in the control strain when compared to SHR submitted to MPH. Results are summarized in Table 2.

4. Discussion

We performed a systematic review and meta-analyses of studies evaluating behavioral effects of MPH on the SHR model. As far as we know, we are the first to perform a systematic review and meta-analyses of studies on an animal model of ADHD. Although they have been

already used in the last decades in order to guide clinical practice, systematic reviews and meta-analyses are relatively new in preclinical research (Sena et al., 2014) and are an essential tool to allow objective conclusions from the huge amount of evidence available. Our main findings show that MPH increased attentional and mnemonic performances in the SHR and decreased their impulsive behavior. No effect on locomotion (hyperactivity) was observed.

MPH improved the performance of SHR in tests requiring attention, which corroborates results from patients with ADHD. A meta-analysis published by Tamminga and colleagues (2016) showed that MPH improves sustained attention with an effect size of 0.42 (95% CI = 0.26,0.59), while the effect size found in our study was of 0.86 (95% CI = 0.39, 1.33). The coordination and computation of relevant information necessary to appropriate problem-solving reactions is a function conducted primarily by the prefrontal cortex (PFC) (Miller and Cohen, 2001). The computational properties of the PFC are densely modulated by brainstem nuclei and have a preeminent role among dopaminergic circuits (D'Ardenne et al., 2012). Thus, MPH appears to improve attention by modulating PFC activity, which is found to be deficient in patients with ADHD (Hart et al., 2013). Our sensitivity analyses indicate that there was no effect of MPH in attention when it was evaluated with the lat maze. This suggest that the lat maze test may be less sensible for detecting a difference in attentional performance. Moreover, there was a statistically significant effect when using higher doses of MPH (1 mg/kg and 2 mg/kg).

Treatment with MPH decreased impulsive behavior of the SHR, similar to what is observed in patients with ADHD (Tamminga et al., 2016). A meta-analysis and meta-regression on the effects of MPH in improving impulsivity in patients with ADHD (Tamminga et al., 2016) found an effect size of 0.40 (95% CI = 0.22, 0.58), agreeing with our results (effect size of 0.81 (95% CI = 0.43, 1.18)). Impulsivity is a core symptom of ADHD (Lijffijt et al., 2005) and the dopaminergic system has been pointed as an essential player in response inhibition (for a review see Pattij and Vanderschuren (2008)). During tests requiring the ability to inhibit inappropriate behaviors, patients with ADHD seem to have reduced activation in the right inferior frontal gyrus, supplementary motor area, anterior cingulate cortex and striato-thalamic areas (Hart et al., 2013).

MPH treatment improved memory performance in the SHR model, with a pooled effects size of 1.01 (95% CI = 0.59, 1.43). The storage and retrieval of declarative memory (information regarding events, facts, and places, for example) is a function attributed mainly to the hippocampal formation and adjacent medial temporal lobe structures (Kandel et al., 2014). Memory impairment is not considered a primary symptom of ADHD, yet memory tests were conducted in 8 of the selected studies. Furthermore, declarative memory impairments have been reported in this population and appear to be present mainly in the encoding of information (Skodzik et al., 2017). A mega-analysis of MRI data (Hoogman et al., 2017) has shown that children with ADHD have decreased hippocampal size when compared to a control population, thus giving support to this hypothesis. Besides, hippocampal ability to use internal and external cues in order to guide both the encoding and the retrieval of memories seems to be strongly influenced by the PFC (Preston and Eichenbaum, 2013), a structure knowingly involved in ADHD etiology. The storage of short and long-term memories also appears to be dependent on a regulated dopaminergic transmission (Rossato et al., 2009). Moreover, in patients with ADHD, MPH has been shown to improve memory (Fuermaier et al., 2017). In our sensitivity analyses, a positive effect of MPH was observed in the object recognition and active avoidance tests. Besides, only the dosage of 2 mg/kg showed a statistically significant effect.

Different from the response pattern observed for the treatment of attention, impulsivity, and memory, our results on hyperactivity demonstrated that MPH was ineffective when in low and medium dosages. Moreover, an increase in hyperactivity was observed under high dose administration (10 mg/kg). This suggests a distinct response to MPH when SHR are compared to patients with ADHD. Our results regarding hyperactivity may be attributed to differences in the pathophysiology intrinsic to the SHR model. SHR present large genetic and gene expression differences when compared to Wistar-Kyoto rats (established from the same parental Wistar stock). These differences likely comprise genetic variants beyond those associated with hypertension and ADHD (Williamson and Tai, 2017; Zhang-james et al., 2018). Furthermore, the increased locomotion in the SHR model could represent the clinical syndrome observed in patients following MPH induced neurotoxicity (Klein-Schwartz, 2002; Spiller et al., 2013). In this sense, it might be hypothesized that the increased locomotion observed in the SHR following administration of a high dosage of MPH may be the result of sympathetic overstimulation. Our results also raise the possibility of this pattern of response being more related to the effect observed in non-ADHD individuals using MPH as a cognitive enhancer (Tomasi et al., 2011).

In the secondary analyses, SHR treated with MPH were compared to a control normotensive strain. MPH had a positive effect on attention and impulsivity, reverting the symptoms to the WKY control level. The effect sizes found in different dosage protocols are similar to those found in humans. Although memory impairment is improved after MPH administration in the SHR, treated animals did not revert to WKY control levels. Moreover, MPH did not revert symptoms of hyperactivity, which was expected based on our primary analysis. The fact that SHR presented memory impairments not fully recovered after MPH administration should be better investigated in the future.

The behavioral effects of MPH reported in this study are likely linked to a regulation of the dopaminergic, glutamatergic and noradrenergic systems. As discussed above, patients with ADHD present several neurobiological alterations, some of which are shared with the SHR. The dopaminergic system is the most studied, and evidence indicates that this system is less active in the animal model of ADHD. It has been shown, for instance, that SHR present decreased dopamine release after neuronal depolarization when compared to WKY (Miller et al., 2012; Russell et al., 1998), possibly due to reduced vesicular storage (Russell et al., 1998). In addition, several studies suggest that SHR exhibit an increased density of dopamine transporter in the brain (mostly in frontocortical and striatal terminals). Increased dopamine transporter activity enhances dopamine uptake from the synaptic cleft (Chen et al., 2017; Miller et al., 2012; Pandolfo et al., 2013; Roessner et al., 2010). Synaptic abnormalities are also seen in glutamatergic and noradrenergic systems. Glutamatergic neurotransmission in pyramidal neurons of the PFC was shown to be reduced in SHR (Cheng et al., 2017). In the noradrenergic system, there was an increased noradrenaline uptake in the orbitofrontal cortex (Somkuwar et al., 2015).

Our results should be viewed in light of several limitations. The accuracy of the meta-analyses depends on the quality of the individual studies. Small sample sizes, behavioral tests evaluated, or dosages tested may slightly influence the precision of the analyses. SHR substrains, like the stroke-prone SHR, have been proposed as a better animal model for ADHD (Hiraide et al., 2013; K. Ueno et al., 2002a). However, we decided to include only studies performed in the SHR model, since they are most frequently used (Hiraide et al., 2013; Jesmin et al., 2004; K.-I. Ueno et al., 2002b; K. Ueno et al., 2002b; Yabuki et al., 2014). In this sense, the validity of other strains as a model of ADHD should be investigated, especially in face of the lack of response concerning hyperactivity. It is also important to stress that there has been consistent evidence showing genetic and behavioral differences between SHR and WKY rats obtained from distinct breeding facilities (Sagvolden et al., 2009). These data suggest they may even represent different ADHD subtypes, thus including another confounder in our analyses.

MPH treatment has been shown to improve ADHD symptoms consistently at different doses (Faraone et al., 2004). Nevertheless, the doses used in the included studies ranged from 0.03125 to 10 mg/kg, while in patients with ADHD it usually ranges from 0.2 mg/kg to 2 mg/

kg (Huss et al., 2017). Besides, more than half of the included experiments administered MPH to the SHR only once. Since ADHD treatment usually requires a long-term pharmacological intervention, the translational impact of ADHD preclinical studies may be compromised. The route of administration is also essential when evaluating preclinical studies and, ideally, it should reflect how the drug is administered in patients. Most studies administered MPH using intraperitoneal injections, with few using oral administrations. With intraperitoneal injections, the drug undergoes hepatic metabolism in a similar way to orally administered drugs. However, there are considerable differences in the pharmacokinetic and pharmacodynamic parameters when compared to an oral administration (Kuczenski and Segal, 2005). Finally, animal studies evaluating behavioral outcomes often use only male rats, since the behavior of females may change according to the estrous cycle. This phenomenon introduces new variables to the analyses and hampers the interpretation of results (Llaneza and Frye, 2009; Pompili et al., 2010; Sell et al., 2005). We have also extracted data regarding gender and observed that six studies from the 36 included used females. However, analyzing female rat experiments separately did not change any meta-analysis effect.

Clinical studies have been able to demonstrate positive effects of MPH on the symptomatological dimensions of ADHD (Catala-Lopez et al., 2017; Faraone and Buitelaar, 2010; Faraone and Glatt, 2010; Storebo et al., 2015), diminishing inattention, hyperactivity/impulsivity symptoms (Huss et al., 2014; Willcutt et al., 2012). The paradoxical effect of stimulants in decreasing hyperactivity is the hallmark of the ADHD treatment since Charles Bradley's serendipity discovery of the calming effect of Benzedrine (Bradley et al., 1937). Therefore, a valid animal model of ADHD should ideally replicate this effect. This was not the case in our study.

In summary, although our analyses show improvement in three of the behavioral outcomes evaluated, there is no effect of MPH regarding hyperactivity, a core symptom of ADHD. Therefore, our study does not fully support the predictive validity of SHR. Further studies should investigate different animal model strains and substrains, as well as variations in protocol, in order to simulate human treatment more accurately, such as including doses that are analogous to those used in humans.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neubiorev.2019.02.019.

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An animal model of what? The case of spontaneously hypertensive rats

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Letter to the Editor

An animal model of what? The case of spontaneously hypertensive rats

Dear Editor,

We have read with interest the research paper recently published in PNPBP entitled "Young spontaneously hypertensive rats (SHRs) display prodromal schizophrenia-like behavioral abnormalities" by Niigaki et al. (2019). The authors demonstrated that the SHR, a strain commonly used as an animal model of attention deficit hyperactivity disorder (ADHD), may (also) be an animal model of schizophrenia. This data corroborates another recent research published in PNPBP showing a continuous schizophrenia-like trait in rats, where SHRs presented higher values when compared to the Wistar group (Peres et al., 2018). It is important to mention that the authors of both papers highlighted that some preclinical studies have shown that SHRs do not respond to stimulants. Those facts call into question SHR use as a model of ADHD.

We recently developed a protocol of a systematic review and meta-analyses of animal studies to summarize all the evidence for the predictive validity of the SHR as a model of ADHD, focusing on the behavioral effects of methylphenidate (MPH) (Leffa et al., 2018). Several meta-analyses have supported the use of MPH to treat ADHD; thus, it could be considered the best tool to test the predictive validity of this strain as a model of ADHD. However, our results were not enough to support a robust predictive validity of SHR as a model of the disorder. Despite observed significant effects for inattention and impulsivity in our study, we did not demonstrate significant effects of MPH on hyperactivity in those animals (Leffa et al., 2019). The effect of stimulants in decreasing the core symptom of hyperactivity is a hallmark of ADHD treatment; therefore, an animal model of ADHD should present this characteristic.

Based on all the pieces of evidence presented (Niigaki and Peres studies and our meta-analysis findings), our position is at least of concern, since SHR appears to be highly heterogeneous regarding pathology, presenting hypertension, schizophrenia and ADHD-like traits, such as hyperactivity, inattention, impulsivity, and cognitive deficits. So, what is the meaning of studying an animal model that presents this broad range of abnormalities occurring at the same time or in its lifespan? Is the SHR a reliable and validated animal model of what?

Disorders with relatively low heritability estimates, such as major depressive disorder, post-traumatic stress disorder, and anxiety disorder present well-documented animal models that are induced mainly through environmental stressors (Czéh et al., 2016; Harro, 2018; Schöner et al., 2017). Conversely, the successful breeding of animals that accurately represent complex disorders with high heritability estimates is especially challenging to achieve, which is the case for both ADHD and schizophrenia. Indeed, SHRs present large genetic and gene expression differences when compared to control strains, which comprise genetic variants beyond those associated with hypertension, ADHD, and probably schizophrenia (Williamson et al., 2017; Zhang-James et al., 2013). Moreover, according to the evidence mentioned, distinct behavioral outcomes seem to represent distinct disorders better. Perhaps SHR is a good model for an underlying generalized behavior spectrum, *i.e.* endophenotypes, that are part of the characterization of psychiatric disorders.

A considerable complicator seen in neurobiology studies in psychiatry is the phenotypic heterogeneity found in humans. For instance, around 70% of patients with ADHD present at least one psychiatric comorbidity; and genomic analyses of the Brainstorm and Psychiatric Genomics Consortia (Anttila et al., 2018; Demontis et al., 2019) have shown that psychiatric disorders do not have clear biological boundaries. Therefore, animal models are of paramount importance to the study of Neuropsychopharmacology in a way that helps to disentangle the specificities of each psychiatric disorder. Nevertheless, study findings from a broadly altered animal model, presenting traits that fulfill criteria for 'psychopathology' in general and not exclusively for a specific disease, may be difficult to translate. Therefore, more rigorous evaluations regarding face, construct, and predictive validities should be taken in order to consider SHR an animal model proper to give clues about the biological underpinnings of ADHD or schizophrenia.

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Expanding the discussion on experimental models of attention deficit hyperactivity disorder

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Commentary

Expanding the discussion on experimental models of attention deficit hyperactivity disorder

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A recent paper reviewed models of attention deficit hyperactivity disorder (ADHD) (Regan et al., 2022). ADHD presents a complex etiology and is highly heterogeneous in presentation and comorbidity profile, making it challenging to validate an animal model for the disorder. However, animal experimentation is of enormous importance to understanding neuropharmacological aspects of ADHD (Panzenhagen et al., 2019). This relevance became even more substantial after new genes were implicated in ADHD etiology from genome-wide association studies (GWAS) (Demontis et al., 2022). Although Regan et al. deeply reviewed the rodent models of ADHD, there are some important points we would like to add to the discussion: *First,* ADHD is not a monogenic or oligogenic disorder. Models based on gene knock-out or knockdown (e.g., DAT-KO/KD) can hardly represent the complex nature of ADHD and its endophenotypes. Therefore, we suggest that we recognize these experimental models as relevant tools to study disturbance effects in specific physiological pathways but do not label them as particular models for any given disorder (in fact, dopamine transporters are also relevant for other psychiatric phenotypes).

Second, genes such as *DAT1* and *DRD4* are far from being associated with ADHD or biologically correlated psychiatric disorders in the latest GWASs (https://atlas.ctglab.nl/PheWAS). Nonetheless, it is possible that small effects will be detected with the rise in GWAS sample sizes. Although environmental factors play a role, for most of the exposures investigated in meta-analyses, the quality of evidence is weak to moderate, the level of heterogeneity is high (e.g., for lead exposure), effect sizes tend to be small (e.g., acetaminophen), and the findings are susceptible to potential confounders (e.g., high genetic correlations between ADHD and smoking). Therefore, establishing exposure-based models falls into the same perspective as KO/KD approaches.

Third, construct validity is hard to achieve; however, face and predictive validity can be better tested. In this regard, it is time to scrutinize the evidence from animal models as we do for clinical and epidemiological studies in humans. For instance, we conducted a systematic review and meta-analysis to evaluate the predictive validity of the Spontaneously Hypertensive Rat (SHR) as a model of ADHD (Leffa et al., 2019). The SHR is the most widely used animal model of ADHD, and the number of published studies was sufficiently robust to carry out a meta-analytic approach following the required standards for human studies. However, we could not adequately support the SHR as an ADHD model since methylphenidate (MPH), a first-line treatment for ADHD, was ineffective on hyperactivity. Despite improving other ADHD dimensions, the lack of significant effects of MPH in decreasing hyperactivity (a core symptom of the disorder) - additionally to other model concerns, such as hypertension - jeopardizes the use of SHR in ADHD studies. This does not mean, however, that SHRs cannot be successfully used for studying other behavioral dimensions (e.g., inattention,

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impulsivity, and memory deficits).

Fourth, we should also consider the new animal models being developed. For instance, Zebrafish use is becoming popular in ADHD research (Fontana et al., 2019). This model is promising since it presents the behavior in the adult and at the larval stage and the phenotype can be studied across the Zebrafish lifespan, which may be crucial due to the neurodevelopmental nature of most ADHD cases (Breda et al., 2020). Furthermore, they seem to respond to atomoxetine, a consolidated ADHD medication. There are several differences between rodent (e.g., SHR) and Zebrafish models, and each has its inherent advantages and limitations. For example, rats are closer to humans in the evolution tree and present behaviors more comparable to humans. Conversely, while this can make the SHR an interesting ADHD model, its organism complexity introduces variables that often make it challenging to translate to human biology. The questionable predictive validity of the model is one of them. On the other hand, Zebrafish is a simpler organism, which facilitates the investigation of baseline variables, but hampers the inference on more complex and intertwined systems. In the case of SHR, researchers hopefully achieved a disorder-like phenotype from the inbreeding of strains to change their genetic background. This approach is entirely different from Zebrafish models, where the animal is genetically altered by targeting single genes or sets of genes involved with the disorder. While the first is unpredictable, the second is possibly too strict when investigating polygenic disorders (as we mentioned for rodent KO/KD models). We do not yet have information on the entirety of genes involved in ADHD etiology to produce a comparable model based solely on genetics. Another concern is that the genes commonly manipulated in Zebrafish ADHD models are not associated with the disorder in GWASs.

Fifth, we acknowledge the fact that we are now in the early journey of understanding the biological basis of ADHD, which highlights the need for the pre-clinical research endeavor. However, as new studies arise, ADHD animal models should be carefully investigated regarding their validities. The current and modest literature on new ADHD models is not enough to confirm their face, construct, or predictive validities. However, we argue that at this point, researchers should be stricter when evaluating such requirements. Increasingly available methodologies, such as meta-analyses or systems biology approaches, serve us well when making these assumptions. Face validity is usually considered appropriate when the animal model presents human-like phenotypes. However, this hardly assures its validity if the phenomenon is inconsistent across studies or if the intervention (when there is one) has a low phenotype conversion rate in animals.

Sixth, it may be time for researchers to step back and re-evaluate ADHD models. The current models are based mainly on rodents, fish, and flies; however, the successful breeding/manipulation of an animal model that represents disorders with high heritability is challenging. In this sense, other models are possible, as the dedifferentiation of somatic cells from probands into pluripotent stem cells (hiPSCs), which has become an encouraging tool since it allows other cell derivation types from a genetic background that somehow knowingly contributes to the investigated phenotype. From hiPSCs a new study model named “brain organoid” has gained traction. Organoids are 3D cell cultures that mimic the central nervous system’s anatomical and functional properties. This model is a promising way to assess the influence of specific genetic backgrounds in the neurodevelopmental trajectory. Additionally, crossing data of cerebral organoids from patients and tissue data from the animal models available can be a feasible strategy to assess construct validity in the future. One of the major disadvantages of these models, of course, is the lack of network connection capability, cognition, or indicators that could be associated with behavior. These are not models that could achieve face or predictive validities but could help in the understanding of specific molecular mechanisms impacting neurodevelopment. Another downside for hiPSCs and 3D cultures is the challenge of having isogenic comparisons, or to ‘repair’ causative variations in these lines (which proves to be a consistently growing issue with the increasing number of associated *loci* in complex phenotypes). However, there are approaches being developed, such as CRISPR editing in combination with biochemical and physiological neuronal assays or “village-in-a-dish” methods, in which many individuals are grown in a single experiment. These methods have proven to be promising alternatives for identifying neuronal phenotypes and interactions with expression quantitative trait *loci* known to be associated with psychiatric disorders (Kelley and Paşca, 2022). Therefore, we expect to have further progress in this area so that better (if not completely isogenic) controls are generated.

Finally, there are many other rodent models that were not discussed here due to their limited use so far compared to the SHR. However, some have shown promising predictive validity for core behaviors associated with ADHD, as do the Atxn7-overexpressing mice when treated with atomoxetine for impulsivity, for example (Dela Peña et al., 2019). We would like to stress that we are not by any means advocating for or against any of the models available. Indeed, it is an arduous task to model such a heterogeneous and complex disorder. It is unlikely that all core symptoms/behaviors of ADHD (impulsivity, inattention, and hyperactivity) will be modeled in any one single model. That would be a naive assumption to say the least. We intend to raise the questions regarding the available models and provide ideas on a few directions researchers could take in going forward. As the renowned oncologist and researcher Howard Skipper once said, "a model is a lie that helps you see the truth.” Hence, we need a representative "lie" while keeping it as transparent and realistic as possible.

So, what do we propose we do? *First*, solve inconsistencies on the face and predictive validities throughout meta-analyses and hopefully investigate construct validity (e.g., through brain organoids and *ex vivo* samples from animal models). *Second*, there is a huge space to develop new models, both within rodents and other groups, as we exemplified with Zebrafish. In this sense, it would be of great value testing manipulation on GWAS-oriented sites as an exploratory tool to unravel molecular mechanisms. *Third*, be stricter in acknowledging that we are far from modeling a whole ADHD symptomatology and consider different models for specific endophenotypes, which ultimately means that we might have to combine them in our quest to understand psychiatric disorders. Moreover, as in the comorbid nature of psychiatry itself, we will see models with similar backgrounds leading to different phenotypes and vice-versa (Panzenhagen et al., 2019). We will most likely not find the perfect separate model for each disorder, as many are looking for, but ironically that is precisely the most translational aspect we can get when talking about behavior.

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Ways to tackle variability in psychiatry through neuroimaging analyses

“There is no scientific study more vital to man than the study of his own brain. Our entire view of the universe depends on it.”

– Francis Crick

6

Emerging findings of glutamate-glutamine imbalance in the medial prefrontal cortex in attention deficit/hyperactivity disorder: systematic review and meta-analysis of spectroscopy studies

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ORIGINAL PAPER

Emerging findings of glutamate–glutamine imbalance in the medial prefrontal cortex in attention deficit/hyperactivity disorder: systematic review and meta-analysis of spectroscopy studies

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Abstract

One of the main challenges in investigating the neurobiology of ADHD is our limited capacity to study its neurochemistry in vivo. Magnetic resonance spectroscopy (MRS) estimates metabolite concentrations within the brain, but approaches and findings have been heterogeneous. To assess differences in brain metabolites between patients with ADHD and healthy controls, we searched 12 databases screening for MRS studies. Studies were divided into 'children and adolescents' and 'adults' and meta-analyses were performed for each brain region with more than five studies. The quality of studies was assessed by the Newcastle–Ottawa Scale. Thirty-three studies met our eligibility criteria, including 874 patients with ADHD. Primary analyses revealed that the right medial frontal area of children with ADHD presented higher concentrations of a composite of glutamate and glutamine ($p = 0.02$, SMD = 0.53). Glutamate might be implicated in pruning and neurodegenerative processes as an excitotoxin, while glutamine excess might signal a glutamate depletion that could hinder neurotrophic activity. Both neuro metabolites could be implicated in the differential cortical thinning observed in patients with ADHD across all ages. Notably, more homogeneous designs and reporting guidelines are the key factors to determine how suitable MRS is for research and, perhaps, for clinical psychiatry. Results of this meta-analysis provided an overall map of the brain regions evaluated so far, addressed the role of glutamatergic metabolites in the pathophysiology of ADHD, and pointed to new perspectives for consistent use of the tool in the field.

Keywords MRS · Spectroscopy · ADHD · Attention deficit hyperactivity disorder · Systematic review · Meta-analysis

Marcos Vinícius Vidor and Alana Castro Panzenhagen have contributed equally to this work.

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Introduction

Attention deficit/hyperactivity disorder (ADHD) is a neurobehavioral disorder characterized by age-inappropriate patterns of inattention, hyperactivity, and impulsivity [1, 2], being a prevalent psychiatric diagnosis among children [3–5] and often persisting into adulthood [6–9]. ADHD is a major cause of impairment in quality of life [10], producing an economic loss estimated at billions of dollars each year [11]. Despite its impact on both individual and collective domains, the neurobiology of ADHD remains poorly understood [12]. One of the main impediments for testing pathophysiological hypotheses of ADHD is our limited capacity for examining the neurochemistry of the central nervous system in vivo [13].

Apart from post mortem analyses [13], which are currently lacking for ADHD [14], the main approaches for studying neurochemistry in humans are positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) [15]. By and large, MRS displays higher resolution, no radiation harm, and is a low-cost method compared to other non-invasive techniques [16], constituting a viable and practical tool for clinical purposes. In a hydrogen based MRS (^{1}H-MRS), the metabolites usually reported are *N*-acetyl aspartate (NAA), a compound of glutamate and glutamine (glx), creatine (cr), choline (cho), and myo-inositol (mI). These molecules are generally interpreted as surrogate markers for neuronal integrity (NAA), excitatory neurotransmission (glx), energetic metabolism (cr), membrane turnover (cho), and glial proliferation (mI) [17]. These interpretations are connected to the current clinical applications of MRS, chiefly for neurological conditions such as brain tumors, multiple sclerosis, and brain abscesses. However, other neurochemical readings may prove suitable for analyzing the functional deficits of psychiatric disorders. For instance, NAA is thought to act as an excitatory neurotransmitter [18]; high choline levels might indicate chronic cholinergic deficits [19]; as well as myo-inositol, a precursor of intracellular second messengers [20], might be correlated to lithium treatment [21] and perhaps neuronal activity [22, 23].

The first study to analyze spectroscopy data in ADHD was performed more than 2 decades ago [24]. Since then, two meta-analyses have tried to make sense of the available studies on MRS and ADHD [25, 26]. In 2009, a review pooled 16 studies on the subject [25]. Due to insufficient studies for each brain area, the authors were compelled to perform meta-analyses with as few as two studies, concluding that patients with ADHD had higher choline concentrations in several brain areas [25]. Four years later, a new systematic review of spectroscopy studies was published using narrower inclusion criteria—only proton MRS studies were included, and few brain areas of interest were selected (frontal cortex, striatum, and cerebellum) [26]. Findings from the first systematic review were not replicated, with results showing a higher concentration of NAA in the medial prefrontal cortex (mPFC) of children with ADHD, highlighting a possible neurophysiological difference compared to adults [26]. Despite the methodological differences, these studies had to use a nonspecific selection of brain areas (such as disregarding brain laterality) and samples (such as disregarding age) to pool enough data for the meta-analyses.

Several publications on spectroscopy and ADHD have arisen since the reviews mentioned earlier [27–39]. Apart from the increased statistical power provided by new original data, it also allows for a narrowed methodological approach. For example, it enables to deal with the heterogeneity so far displayed in MRS studies, especially regarding voxels of choice and the lack of a standardized unit of measurement—MRS results are reported in arbitrary units that vary with specific characteristics of the study, such as voxel size and radiofrequency coil sensitivity [40]. In this review of more than two decades of studies, we aimed to take advantage of these possibilities to investigate whether there is a difference in the concentration of metabolites measured by ^{1}H-MRS between patients with ADHD and non-ADHD controls in specific brain volumes. Besides, we hope to delineate a landscape for future MRS studies in terms of regions of interest to be selected and other possible optimal approaches.

Materials and methods

Protocol and registration

This review was registered in the International Prospective Register of Systematic Reviews PROSPERO under the title "Magnetic resonance spectroscopy on attention deficit/hyperactivity disorder: systematic review and meta-analysis" (ID CRD42018112418). A more detailed protocol was also published elsewhere [41]. We followed the Preferred Reporting Items for Systematic Review and Meta-analyses PRISMA checklist as a guide to our reporting [42, 43].

Eligibility criteria

To fulfill the eligibility criteria, studies should be in accordance with the following requirements: (1) to be an original study with proton MRS data of brain metabolites; (2) to contain at least one group of patients with ADHD; (3) to contain at least one non-ADHD control group; (4) cases should be defined according to DSM-III (Attention Deficit Disorder-ADD), DSM-III-R, DSM-IV, DSM-IV-TR, or DSM 5, following professional assessment and/or screening tools and controls must be defined as not having the condition by similar means. Studies were excluded if they fulfilled

the following criteria: not containing a group for which the only psychiatric diagnosis required was ADHD—e.g., case groups that required two psychiatric conditions simultaneously (e.g., requiring both ADHD and borderline personality disorder diagnoses). There were no direct requirements for active screening for other psychiatric conditions (comorbidities). Nonetheless, this information was used as a quality criterion for evaluating studies and bias assessment later on. There were no restrictions on the sex or age of participants.

Information sources and study selection

A comprehensive search for studies was performed on the following databases: Embase, Google Scholar, PubMed, ScienceDirect, Scielo, Scopus, and Web of Science. Additionally, broadly targeting gray literature, ERIC, CINAHL, GreyGuide, OpenGrey, and WorldCat were screened. The combination of keywords was divided into two axes, one for ADHD and another for MRS. ADHD terms included "ADHD" and "attention deficit hyperactivity disorder", while MRS terms covered "mrs", "mr spectroscopy", "nuclear magnetic resonance spectroscopy", and "nmr spectroscopy" (Table S1). Databases were searched on August 18, 2020. After duplicates removal, the first set of entries was removed based on titles and abstracts by two independent authors (MVV and RBC). Finally, the remaining records were examined in full-text reading for eligibility criteria by the same two authors. A third author (EHG) decided for eventual inconsistent decision-making between evaluators. References of all studies on the final list were then screened for further works to be included in our review. As a final step, studies were compared for possible sample overlapping due to multiple reports of the same study based on authors' names, sponsors, location and setting of research, sample characteristics, and equipment [44].

Data extraction

All studies were independently assessed by two authors (MVV and CEB) who collected the following data in a worksheet: year of publication, the sample size of each group, number of male and female individuals in each group, age category of the population studied ('children and adolescents' or 'adults', aged 18 or older [6, 7]), the mean age of each group, regions of the brain studied, metabolites measured, and main results. For the meta-analysis, the means and standard deviations of all reported metabolites were extracted independently by two authors (MVV and ARM) for each brain region of each group, along with the sample size of both cases and controls. Preferable data sources were as follows: (1) tables on the study or supplementary material; (2) description of the data within the text; (3) graph estimation using a digital ruler [45–47]. When data was not reported in the aforementioned formats, communication with the correspondent author was attempted via email requesting the information. In case of no response after one month, data was deemed missing for meta-analysis purposes.

Studies diverged on how to report metabolites. When absolute concentrations and ratios with creatine were both available, the latter was preferred. Overall, creatine ratios represent a more prevalent format for reporting MRS results and are believed to correct the data for uncontrollable experimental circumstances, even though this rationale is still a source of controversy [48].

Quality assessment

The risk of bias was assessed using the Newcastle–Ottawa Scale (NOS) for nonrandomized studies in its case–control format [49]. NOS is a quality assessment tool that generates a score from 0 to 9 by evaluating nine items (officially eight, one item split in two) divided into three sections: selection, comparability, and exposure. Two authors (MVV and ACP) applied the NOS tool for each study, and inconsistencies were discussed by reviewing the methodological description of the studies. Funnel plots and Egger's regression test were expected to be used for publication bias assessment if ten or more studies were included in a single meta-analysis, which has not occurred for any specific brain region and metabolite [50].

Summary measures

To estimate a standardized mean difference between cases and controls for each metabolite of each brain area, we performed random-effects meta-analyses. As MRS data output is not uniform, we used standardized mean differences through Hedge's G method. The inverse of the variance established individual study weights. Significance was determined through a Z test and alpha = 0.05. I^2 tests assessed study heterogeneity. Levels of heterogeneity were assumed as low (25%), moderate (50%), and high (75%) [51]. The Review Manager 5.3 software was used to analyze standardized mean differences, analyze heterogeneity values, and generate forest plots for each analysis [52].

Synthesis of results

All the data was divided into two major groups based on age categories: 'children and adolescents' (< 18 yo) and 'adults' (≥ 18 yo). To address a more comprehensive review, no specific brain regions were preliminarily set as inclusion criteria. Instead, all studies meeting the eligibility criteria were included, and clusterization of brain areas was performed later for data synthesis. Our aim was to be as specific as possible, following a hierarchy of preferences applied to the

available information. The first author (MVV) and a psychiatrist with neuroimaging experience (FAP) classified the regions. In the first level, data of the same specific area in a defined side were grouped (e.g., left dorsolateral prefrontal cortex). When the available data was insufficient, we gathered data of related brain regions but still on the same side (e.g., all data from the left frontal lobe).

All brain areas with more than five studies were meta-analyzed and reported as primary analyses. Meta-analyses of two–five studies were used to conceive a map of opportunities for future studies, classifying brain regions according to the number of studies with enough data for meta-analyses and their partial results. These analyses were disclosed as a supplement to this work (Tables S2, S3). In studies with more than one ADHD group (e.g., ADHD inattentive subtype and ADHD hyperactive subtype), all ADHD groups were compared to the control group. In those cases, the sample size of the control group was divided by the total number of ADHD groups (rounded down to avoid overestimation of effects).

Multiple comparisons, sensitivity and subgroup analyses

All primary meta-analyses were tested for multiple comparisons with the Benjamini–Hochberg procedure with a false discovery rate established at 10% ($Q = 0.1$) [53, 54]. Following our initial procedures, we performed a Jackknife resampling of each analysis for detecting outlier datasets. To explore potential effects of biases, we reran all analyses in a variety of ways: excluding studies that scored less than one standard deviation from the mean NOS score; selecting studies by treatment status, separating medication free from patients currently on stimulants; and selecting by the field strength of MRS, only analyzing studies using magnetic resonance equipment stronger than 1.5 Tesla. Meta-regression analyses were not conducted since all meta-analyses presented a small number of independent studies (less than ten each), which prevents us from generating reliable statistical results for this kind of analysis.

Results

Study selection

The initial search identified 1636 entries, with 1335 remaining after removing duplicates. A total of 1234 entries were excluded based on titles and abstracts. The remaining 101 studies proceeded to the full-text appreciation for eligibility criteria evaluation. At this step, 68 studies were excluded: 22 for not containing a healthy control group (mostly, studies on pharmacodynamics), 16 for not reporting ^{1}H-MRS data, 13 studies for not reporting original data, 10 for sample overlapping or alternative reports of already included studies, and seven had as inclusion criteria the need of ADHD plus a specific other mental disorder. A total of 33 studies [24, 27–32, 34–36, 38, 39, 55–76] met the criteria of our systematic review, from those, 25 studies reported enough data to be included in the meta-analyses (Fig. 1) [27–29, 31–34, 37–39, 55, 57–59, 63–71, 73, 75].

Characteristics of studies

The 33 studies compiled in our review pooled an aggregate of 874 patients with ADHD and 775 control individuals. The mean long-lasting age of ADHD groups ranged from 7.7 to 36.1 years, while healthy controls' mean age ranged between 7.4 and 36.8 years. Twenty studies focused on children and adolescents samples [27, 29, 33, 35, 37–39, 55, 57, 59, 61–63, 65–68, 71, 73, 75, 76], 12 studies focused on adult samples [24, 28, 31, 32, 34, 36, 58, 60, 64, 69, 70], and one work studied both age categories [30]. Male individuals represented 68% of patients with ADHD. Only three studies had a majority of female individuals in the ADHD group [31, 58, 62]. In 14 studies, the patients were under no pharmacological treatment [24, 27, 32, 37, 56–58, 62–64, 69, 70, 73], whereas three studies recruited only patients treated with stimulant medication [33, 59, 71]. Ten studies with patients under treatment specified a washout period of at least 16 h. Other studies have either not mentioned treatment status or not used it as inclusion or exclusion criteria. Forty-eight percent of studies (16) presented their data in absolute quantification (arbitrary units), 52% (17) as ratios per creatine, 6% (2) as ratios per inositol, 3% (1) as ratios per H_2O, and 3% (1) as ratios per creatine plus inositol—the sum exceeds 100% since a few studies reported their findings in two formats (Table 1).

As anticipated, studies in our final list diverged on targeted volumes of interest (VOI). Based on the authors' description and illustrative pictures of VOIs, it was possible to cluster children's right medial frontal area (mFA) for meta-analyses. Brain regions with less than six studies were pooled and reported as supplementary material (Tables S2, S3). All areas were examined considering laterality; both sides were analyzed separately.

Risk of bias

Having applied the case–control model of the Newcastle–Ottawa Scale, we established the mean score among all studies was 5.4 (SD 1.58) on a scale of zero to nine. Most studies (91%) failed to correctly describe the non-response

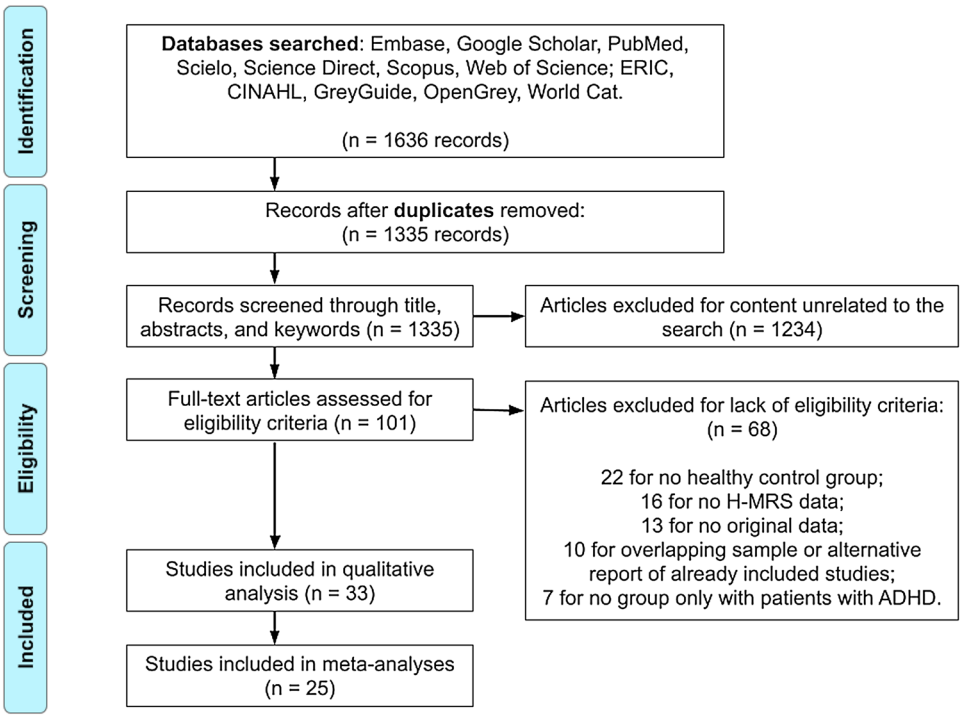

Fig. 1 Preferred reporting items for systematic reviews and meta-analyses studyflow

rate of their samples. Evaluation of case definition (39%), case representativeness (39%), and selection of controls (30%) were also accomplished only by a minority of studies. All other NOS criteria were fulfilled for at least 50% of articles. For controlling for the most important factor, ascertainment of exposure, and method of ascertainment, all studies (100%) scored positively (Figs. S1, S2).

Meta-analyses of metabolites per brain region

Regarding children and adolescents, in the right medial frontal area, pooling six studies together, there was a higher concentration of glx for patients with ADHD ($p = 0.02$, (i/m) $Q = 0.033$, $I^2 = 62\%$, SMD = 0.53). No other meta-analyses indicated significant differences between brain metabolites of cases and healthy controls. In addition, sub-analyses using only samples undergoing pharmacological treatment or drug naïve patients found no statistical differences between those groups and healthy controls.

It was possible to devise a map of opportunities for studies in both child and adult populations independently. For children and adolescents, most brain areas had few studies with data for meta-analyses. Children's right medial frontal area showed elevated glx—as referred above—while the left medial frontal area had no statistically significant meta-analyses. In adults, only the left dorsolateral prefrontal cortex (NAA) and the left striatum (cho, glx, NAA) pooled at least three studies for meta-analyses, with no statistically significant results. All other brain regions had no more than two studies with sufficient data for meta-analyses (Figs. 2, 3).

Discussion

In this review, we tried to approach MRS literature regarding ADHD in a way that would be both comprehensive on the level of search strategies and specific on the level of data analysis. Eight brain regions for children and 12 for adults had less than three studies with enough data for meta-analyses, establishing an open field for future work. Since our work identified 13 new studies compared to the previous systematic reviews [25, 26], it was possible to split the analyses aiming for child and adult samples specifically and with a higher cutoff for minimum datasets. Overall, the only meta-analysis to find a statistically significant result showed that children with ADHD had higher levels of glx in the right medial frontal area.

While the medial prefrontal cortex as a whole is often associated with decision-making processes [77, 78] and emotional regulation [79], specific roles have been attributed to the right medial prefrontal cortex [80, 81]. Worth mentioning that neuroanatomical evidence shows that the right ventromedial prefrontal cortex plays an essential role in impulsive behavior inhibition [82]. For that matter, a neurochemical imbalance within this region is potentially implicated in the pathogenesis of ADHD, especially in children, who commonly exhibit a more obvious pattern of impulsivity and hyperactivity [83].

Glx is defined as the combined concentrations of glutamate (Glu) and glutamine (Gln) [25, 84–86]. Although glutamate and glutamine are closely related through the glutamate–glutamine cycle [87, 88], it is well known that these

Table 1 Characteristics and main findings of studies

Study	ADHD group			Control group			Diagnosis	ADHD treatment status	Drug washout time	Magnet. field strength (Tesla)	Brain regions	Metabolite presentation (ratios)	Main findings for ADHD Group
	n	m/f	Mean age	*n*	m/f	Mean age							
Alvarado et al. [55]	40	31/9	11	30	14/6	12	DSM-IV	N/A	N/A	1.5	L frontal lobe R frontal lobe	Cr + Cr+Cho	↓ NAA—L frontal lobe ↓ Cho—L frontal lobe ↓ Cho—R frontal lobe
Arcos-Burgos et al. [56]	13	8/5	33.7	19	7/12	34	DSM-IV DICA-IV-P	No treatment	N/A	1.5	L striatum R striatum L ACC R ACC L MCC R MCC L PCC R PCC L lat. thalamus R lat. thalamus L med. thalamus R med. thalamus Corpus callosum Cerebellar vermis	Cr	↑ glx—R PCC
Bae et al. [27]	27	27/0	15.3	42	42/0	16.4	K-SADS-PL	No treatment	N/A	3.0	R frontal lobe	absolute quantification	↓ NAA ↑ Glx
Bauer et al. [28]	27	17/10	31.1	27	15/12	27	DSM-IV	Mixed 16 on 11 off	24 h	3.0	ACC L DLPFC	absolute quantification	↑ Glu
Benamor et al. [29]	102	32/13 43/14 75/27	8.8 9.7 9.3	38	25/13	8.2	DISC-IV DSM-IV	Drug naïve (n=45) On simulants (n=57)	N/A	1.5	L PFC R PFC L striatum R striatum L cerebellum	Cr	Regarding children on stimulants: ↑ Cho—L PFC ↓ NAA—L striatum ↓ Glx—L cerebellum

Table 1 (continued)

Study	ADHD group			Control group			Diagnosis	ADHD treatment status	Drug washout time	Magnet. field strength (Tesla)	Brain regions	Metabolite presentation (ratios)	Main findings for ADHD Group
	n	m/f	Mean age	*n*	m/f	Mean age							
Bollman et al. [30]	16 children 16 adults 32 total	9/7 8/8 17/15	10.8 38.4 19.2	19 38 57	11/7 19/19 30/26	10.8 31.6 24.7	K-SADS-PL DSM-IV	Mixed	72 h	3.0	L basal ganglia L DLPFC (adults)	H_2O	↑ GABA—L BG (adults) ↑ Gln—L BG (children)
Carrey et al. [57]	13	13/0	8.12	10	10/0	8.39	K-SADS CPRS and CTRS CGI-S	No treatment	N/A	1.5	R PFC L striatum L occipital lobe	absolute quantification	↑ Glu—L striatum ↑ Glx—L striatum ↑ Cr—L striatum
Colla et al. [58]	15	7/8	36.1	10	6/4	33.2	DSM-IV CPRS WURS	No treatment	N/A	1.5	L ACC R ACC	absolute quantification	↑ Cho—R ACC
Courvoisie et al. [59]	8	N/A	N/A	8	N/A	N/A	K-SADS	On stimulants	24 h	1.5	L frontal lobe R frontal lobe	Cr	↑ Glx—L frontal lobe ↑ Glx—R frontal lobe ↑ NAA—R frontal lobe ↑ Cho—R frontal lobe
Dramsdahl et al. [11]	29	15/14	32.9	38	15/23	29.8	ICD-10 or DSM-IV K-SADS	Mixed 16 on 13 off	48 h	3.0	L midfrontal region R midfrontal region	Cr	↓ Glu—L midfrontal region
Edden et al. [61]	13	11/2	10.2	19	12/7	10.6	DICA-IV CPTRS-R ADHD-RS-IV	Mixed 7 on 6 off	~48 h	3.0	Primary somatosensory cortex (hand knob)	Absolute quantification	↓ GABA
Ende et al. [31]	22	0/22	30.05	30	0/30	27.53	WURS Connor Adult ADHD RS Wender-Reimherr ADHD Scale	N/A	N/A	3.0	ACC	Absolute quantification + Cr	↓ GABA
Endres et al. [32]	113	57/56	33.9	82	40/42	36.8	DSM-IV WURS	No treatment	N/A	3.0	ACC L cerebellum	Absolute quantification	–

Table 1 (continued)

Study	ADHD group			Control group			Diagnosis	ADHD treatment status	Drug washout time	Magnet. field strength (Tesla)	Brain regions	Metabolite presentation (ratios)	Main findings for ADHD Group
	n	m/f	Mean age	*n*	m/f	Mean age							
Fayed and Modrego [62]	8	3/5	9.1	12	5/7	7.7	DSM-IV	No treatment	N/A	1.5	L centrum semiovale	Cr	↑ NAA
Fayed et al. [63]	22	18/4	9	8	4/4	7.5	DSM-IV-TR	No treatment	N/A	1.5	L centrum semiovale R PFC	Cr	↑ NAA—L centrum semiovale ↑ NAA—R PFC
Ferreira et al. [64]	19	N/A	20.84	12	N/A	N/A	DSM-IV K-SADS	No treatment	N/A	1.5	L VMPFC R VMPFC L putamen R putamen L thalamus R thalamus	Cr	Regarding ADHD-C (10): ↓ mI—R VMPFC ↑ Glx—L thalamus
Hai et al. [33]	21	17/4	10.41	15	6/9	9.9	DSM 5	On stimulants	N/A	3.0	R PFC L striatum	Absolute quantification	↓ Glu—R PFC ↓ NAA—R PFC ↓ Cho—R PFC
Hammermes et al. [65]	10	N/A	14.2	12	N/A	12.8	DSM-IV-TR K-SADS-E	No treatment	N/A	4.0	ACC	mI	–
Hesslinger et al. [24]	5 inatt 5 hyper	5/0 5/0	27.2 27.8	5	5/0	27.8	DSM-IV	No treatment	N/A	2.0	L DLPFC L striatum	Absolute quantification	↓ NAA—DLPFC (ADHD)
MacMaster et al. [67]	9	6/3	9.6	9	N/A	9.36	K-SADS-PL	Mixed 8 on 1 off	At least 48 h	1.5	R PFC L striatum	Cr	↑ Glx—R PFC
Maltezos et al. [34]	40	31/9	30.6	20	15/5	33	Conners Adult ADHD DSM-IV	Mixed 16 on 24 off	N/A	1.5	L DLPFC L striatum L parietal lobe	Absolute quantification	↓ Cr—L DLPFC ↓ Glx—L striatum ↓ Cr—L striatum ↓ NAA—L striatum
Moore et al. [68]	15	N/A	N/A	7	N/A	N/A	DSM-IV	Mixed 3 on 12 off	N/A	1.5	ACC	Cr + mI	↑ Glx/mI

Table 1 (continued)

Study	ADHD group			Control group			Diagnosis	ADHD treatment status	Drug washout time	Magnet. field strength (Tesla)	Brain regions	Metabolite presentation (ratios)	Main findings for ADHD Group
	n	m/f	Mean age	*n*	m/f	Mean age							
Naaijen et al. [35]	39	21/18	10.7	53	38/15	10	DSM 5 K-SADS	Mixed 24 on 15 off	48 h	3.0	ACC L striatum	Absolute quantification	–
Naaijen et al. [36]	28	21/7	19.7	27	17/10	21.22	K-SADS	Mixed	48 h	3.0	ACC L striatum	Cr	↓ Glu—ACC
O'Neill et al. [39]	10 PAE⊕ 13 PAE⊖ 4 PAE ⧄ 27 total	9/1 11/2 2/2 22/5	12.4 11.9 12.6 12.19	7 PAE⊕ 8 PAE⊖ 2 PAE ⧄ 17 total	3/4 2/6 0/2 5/12	12.4 12.9 12.0 12.59	DSM-IV NIMH DISC-IV SNAP-IV	Mixed 5 on 22 off	N/A	1.5	Corona radiata ACC	Absolute quantification	–
Perlov et al. [69]	28	17/11	32.4	28	15/13	30.5	DSM-IV WURS	No treatment	N/A	1.5	L ACC R ACC	Cr	↓ Glx—R ACC
Perlov et al. [70]	30	18/2	30.2	30	15/15	29.9	DSM-IV WURS ADHD-CL	No treatment	N/A	1.5	L cerebellum R cerebellum Vermis of cerebellum	Cr	↑ Glx—L cerebellum
Puts et al. [37]	26	N/A	7.7	24	N/A	7.38	DICA-IV CPRS-R CTRS-R	No treatment	N/A	7.0	L DLPFC L ACC L striatum L premotor cortex	Cr	↓ GABA—L striatum
Soliva et al. [71]	17	15/2	10.41	17	15/2	10.76	DSM-IV-TR CPTRS-R CB-CL	On stimulants	24 h	1.5	R DLPFC L cerebellum	Absolute quantification	↓ NAA—L cerebellum ↓ mI—L cerebellum
Sun et al. [73]	10 ADHD-I 10 ADHD-C 20 total	10/0 10/0 20/0	12.64 12.43 12.49	10	10/0	12.67	CDIS DSM-IV	No treatment	N/A	1.9	L striatum R striatum	Cr	↓ NAA—L striatum (ADHD-C) ↓ NAA—R striatum (ADHD-C)
Tafazoli et al. [38]	13	8/5	12.3	13	8/5	12.2	DSM-IV	Mixed 3 on 10 off	N/A	1.5	L DLPFC R DLPFC	Absolute quantification	↓ NAA—R DLPFC ↓ Cho—R DLPFC ↓ Cr—R DLPFC

Table 1 (continued)

Study	ADHD group			Control group			Diagnosis	ADHD treatment status	Drug washout time	Magnet. field strength (Tesla)	Brain regions	Metabolite presentation (ratios)	Main findings for ADHD Group
	n	m/f	Mean age	*n*	m/f	Mean age							
Yang et al. [75]	15	13/2	13.88	22	14/8	14.85	DSM-IV K-SADS-E	Mixed 12 on 3 off	N/A	1.5	L PFC R PFC	Absolute quantification + Cr	↑ NAA—R PFC ↓ Cr—R PFC
Yeo et al. [76]	23	17/6	9.47	24	16/8	9.4	DSM-IV CTPRS	Mixed 17 on 6 off	16 h	1.5	L DLPF volume R DLPF volume	Absolute quantification	–

n sample size, *m/f* number of male and female individuals in the sample respectively, on stimul. individuals being treated with stimulants, *ADHD* attention deficit/hyperactivity disorder, *ADD* attention deficit disorder, *ADHD-I* ADHD subtype inattentive, *ADHD-C* ADHD subtype combined, *PAE* prenatal alcohol exposure, *DSM* Diagnostic and Statistical Manual of Mental Disorders, *DICA-IV-P* Diagnostic Interview for Children and Adolescents Revised Parents Version, *K-SADS-PL* Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, *DISC-IV* Diagnostic Interview Schedule for Children, *K-SADS* Kiddie Schedule for Affective Disorders and Schizophrenia, *CPRS* Conners Rating Scale for Parents, *CTRS* Conners Rating Scale for Teachers, *CGI-S* Clinical Global Impression Severity Scale, *WURS* Wender Utah Rating Scale, *ICD10* International Classification of Diseases, *CPTRS-R* Conners Parent and Teacher Rating Scales Revised Long Form, *ADHD-RS-IV* ADHD Rating Scale, *DSM-IV-TR* DSM-IV Text Revision, *K-SADS-E* Kiddie Schedule for Affective Disorders And Schizophrenia—Epidemiologic Version, *NIMH* NIMH Diagnostic Interview Schedule for Children, *SNAP-IV* parent rated and/or teacher-rated Inattentive and Hyperactive–Impulsive subscales of the Swanson Nolan and Pelham Scale, *ADHD-CL* ADHD Checklist, *CPRS-R/CTRS-R* Conners Rating Scale for Parents—Revised/Conners Rating Scale for Teachers—Revised, *CB-CL* Child Behavior Checklist, *CDIS* Clinical Diagnostic Interviewing Scales, *Conners* Teacher and Parent Rating Scales, *L* left, *R* right, *ACC* anterior cingulate cortex, *MCC* midcingulate cortex, *PCC* posterior cingulate cortex, *lat.* thalamus lateral thalamus, *med.* thalamus medial thalamus, *DLPFC* dorsolateral prefrontal cortex, *PFC* prefrontal cortex, *VMPFC* ventromedial prefrontal cortex, *DLPF* volume dorsolateral prefrontal volume, *NAA* *n*-acetylaspartate, *Cho* choline, *Glx* glutamate and glutamine, *Glu* glutamate, *Gln* glutamine, *GABA* gamma aminobutyric acid, *Cr* creatine, *mI* myo-inositol. ~ around, *N/A* not available or not applicable, *PAE⊕* with prenatal alcohol exposure, *PAEϴ* without prenatal alcohol exposure, *PAE*[?] uncertain prenatal alcohol exposure, ↑ higher concentrations in comparison to controls, ↓ lower concentrations in comparison to controls

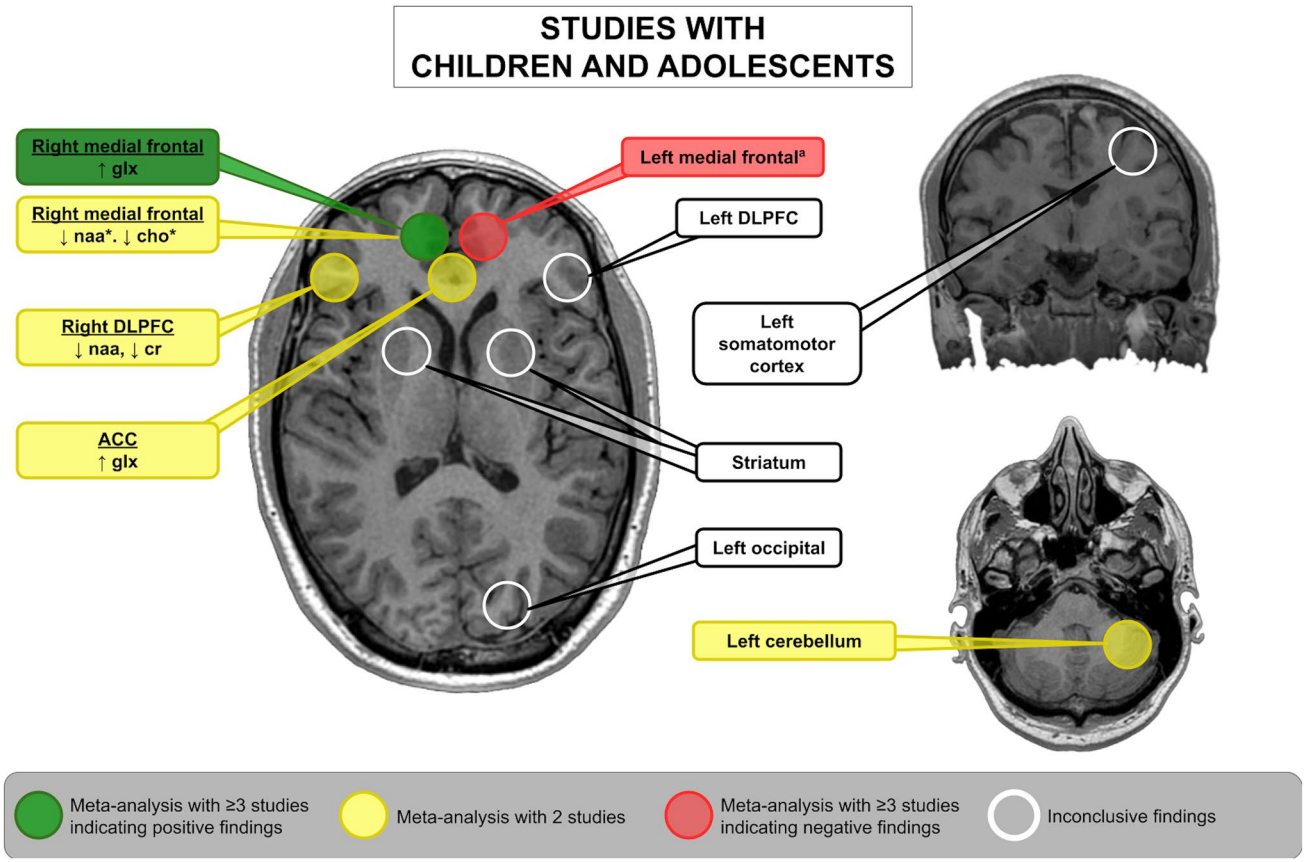

Fig. 2 Mapping the window of opportunities for MRS studies with children with ADHD. The circles are not placed over the exact volumes of interest of the studies, but rather indicate the areas in a schematic fashion. Meta-analyses with three or more studies with a statistically significant result ("positive finding") are displayed as a green box. Meta-analyses with only two studies are displayed on the respective yellow boxes. Meta-analyses with three or more studies without a statistically significant result ("negative finding") are displayed as red boxes. Inconclusive findings account for regions for which there was only one study or not enough data to be extracted for meta-analysis. *Meta-analysis only with studies using field strengths higher than 1.5 T. [a]Meta-analyses with ≥ 3 studies for naa, glx, and cho. *naa* n-acetylaspartate, *cr* creatine, *glx* glutamate–glutamine, *DLPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex. Background brain images were courtesy of PRODAH-A library of neuroimages

two substances might vary their concentrations in opposite directions [89–91], a subtlety for which the generic identification of glx is not sensible for [85]. This uncertainty of what is being measured allows for opposing hypotheses to be viable neurobiological rationales to our findings. For instance, excessive glutamate is known to act as an excitotoxin implicated in several neurodegenerative processes [92]. At the same time, higher glutamine levels could represent the end line of glutamate depletion [88, 90, 91]. In both scenarios, the neurochemical imbalance could lead to cortical thinning, a known finding for children with ADHD [93].

Also noteworthy is the fact that the results of this review did not match the findings of the two previous meta-analyses of MRS for ADHD [25, 26]. Remarkably, they also had not matched their results between themselves. Two concurrent motives could explain this: the technique's sensitivity and the lack of specific guidelines. First, it might be relevant to discuss how suitable MRS is for the subtle functional changes of psychiatric disorders. Although this is a question for which we have no definitive answer, it is imperative to continuously consider that MRS has eminent limitations as conflicting results consistently emerge. For example, a metabolite is required to exist in high concentrations (on the millimolar range) to be assessed by the technique, and there is uncertainty on how much overlap between molecules exists when we finally see it on the chart [94]; this limitation precludes MRS from detecting key neurotransmitters, such as acetylcholine, norepinephrine, dopamine, and serotonin [95].

Nevertheless, an alternative explanation is that the MRS still lacks consensual specialized protocols to investigate most psychiatric disorders. As the clinical use of the method is predominantly directed to conditions in the fields of neurology and neurosurgery, most MRS guidelines are not committed to the nuances that might be crucial for research in psychiatry [96–100]. Notably, to our knowledge, there is no

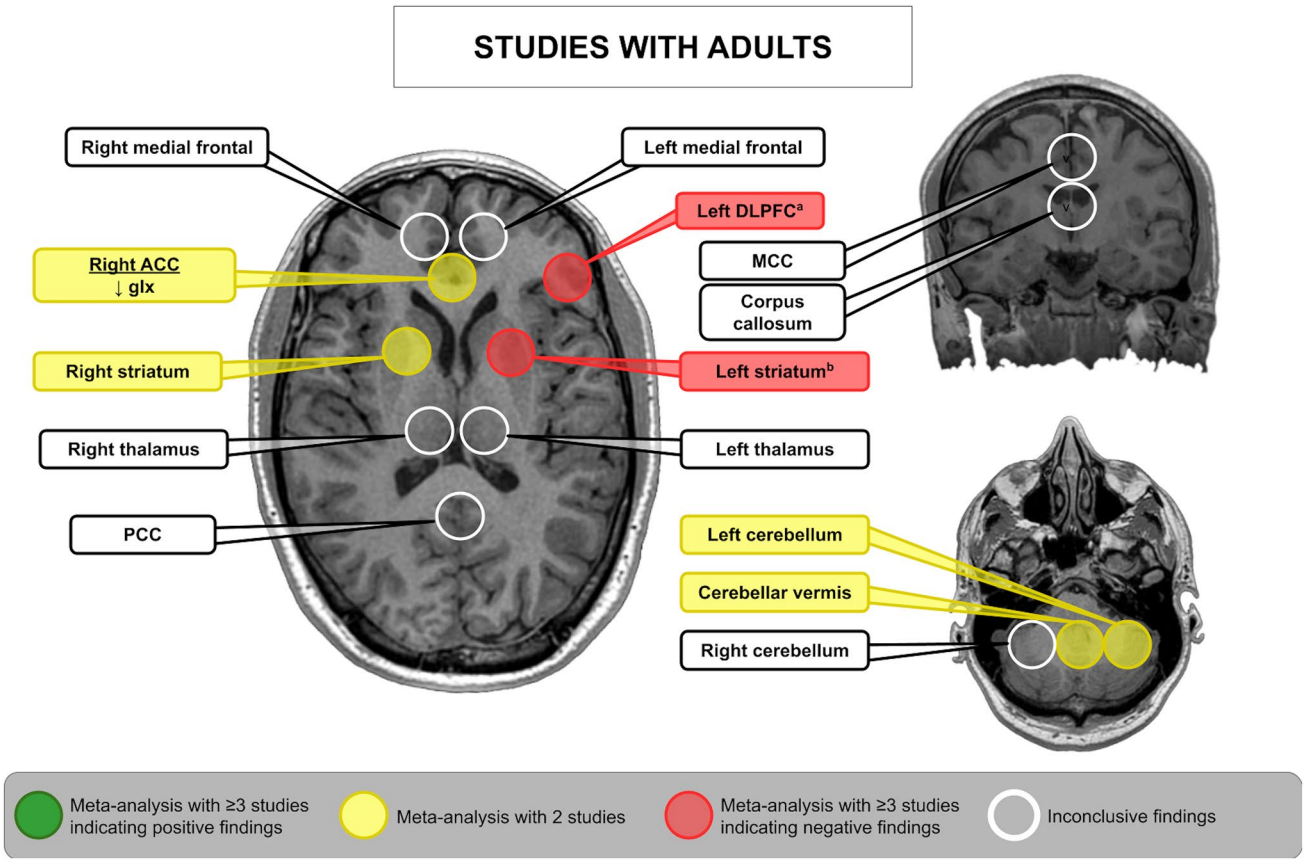

Fig. 3 Mapping the window of opportunities for MRS studies with adults with ADHD. Mapping the window of opportunities for MRS studies with adults with ADHD. The circles are not placed over the exact volumes of interest of the studies, but rather indicate the areas in a schematic fashion. Meta-analyses with only two studies are displayed on the respective yellow boxes. Meta-analysis with three or more studies without a statistically significant result ("negative finding") are displayed as red boxes. Inconclusive findings account for regions for which there was only one study or not enough data to be extracted for meta-analysis. [a]Meta-analysis with ≥ 3 studies for naa (meta-analyses with two studies for cr, glx, and cho also had no significant results). [b]Meta-analyses with ≥ 3 studies for naa, glx, and cho. *glx* glutamate–glutamine, *DLPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex, *MCC* midcingulate cortex. Background brain images were courtesy of PRODAH-A library of neuroimages

general guide on how to sample key regions of the brain, which might result in considerable differences in terms of outcomes. This is a particular problem for achieving consistent and replicable findings and, therefore, also challenging for clustering data in meta-analyses. Besides, there is no consensus on how to report results, as some studies find it appropriate to use creatine ratios, whereas other studies use either absolute quantifications or different ratios [28, 29, 65].

Apart from that, any meta-analysis relies on the volume of data available and the homogeneity of such data. There was enough data for assessing one key region in children in this review. Nonetheless, the studies displayed some degree of heterogeneity, as described earlier.

Altogether, the employment of MRS for ADHD—and likely to psychiatric disorders as a whole—lacks uniformity and consistency as a research field. One of the main aspects that might be addressed in future studies is a refined description of the volumes analyzed, including information on the anatomical limits used as reference points to establish the volume of interest. Ideally, brain regions would be spatially pre-defined, similar to what is seen in the stereotaxy of animal models such as rats. However, the latter might be less feasible than the former in practice [101–103]. Likewise, another issue worth mentioning is the reporting on the treatment status of patients. As MRS sheds light on neurochemical features of neurobiology, any external influence on these grounds should be closely monitored. In the case of ADHD, several studies have demonstrated how stimulant medication can change metabolites levels in the brain [29, 104–106]. It is reasonable to speculate that every psychotropic drug would perceptively affect MRS studies [107–110]. Besides that, treatment status is sometimes poorly reported or unregarded in studies. It would be of great importance that all medications potentially impacting the neurochemistry of patients are controlled in some way, at least with washout periods reasonably greater than the half-lives of the drugs.

Finally, we consider that providing the data in absolute quantification would be the best approach for all studies. Even if the authors' analysis is ultimately performed using ratios, presenting absolute quantification values would allow for independent appreciation of the data and converge to more transparent and comparable results. Within the studies included in our review, data on metabolites were reported on absolute values, creatine ratios, choline ratios, and H_2O ratios, which further burdens the process of putting those findings together.

Regarding the limitations of our review, it is crucial to consider some bottlenecks in the quality of the present work. First, despite our efforts to search for gray literature, unpublished data is a ubiquitous problem for the scientific appraisal of any subject. In the case of our work, this issue is further complicated by the limited number of studies for the specific areas analyzed, which precluded any publication bias assessment, such as Egger's tests. Furthermore, some studies do not provide their results in a usable format for meta-analysis, and most of those with usable data are of rather small sample sizes. The shortage of information was also the cause that hindered the use of some methodologies previously thought to be applied. For example, meta-regression was not employed for the risk of relying on very few studies determining statistical imprecisions. Similarly, some sub-analyses were also not accomplishable, such as dividing patients according to DSM types (inattentive, hyperactive-impulsive, and combined) [1]. Lastly, although the quality control instrument used (Newcastle–Ottawa Scale) [49] was the most suitable tool identified to be employed in our review, it does not accurately reflect key quality points to be addressed in an MRS study. Characterization of how each item of the scale was evaluated and a description of the scores are present on supplementary material. Despite these limitations, the relevance of the present review is on raising the awareness that after more than two decades of studies, there is still a lack of consistent data to be analyzed. There is value per se in highlighting how so few studies examined the same metabolites in the same brain regions. In this sense, our meta-analyses have the potential role of identifying the most consistent results and providing a map of the field for future studies.

In this study, we found that children with ADHD may present higher concentrations of glx in the right medial prefrontal area and speculated on how it could reflect underlying metabolic imbalances. Nonetheless, our considerations still do not fall under the category of solid conclusions. Moreover, as a research field for mental disorders, MRS seems to lack common grounds on methodological approaches up until now. Finally, we advocate that more homogeneous designs and reporting guidelines are the key factors determining how suitable MRS is for research and, perhaps, for clinical psychiatry.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00406-022-01397-6.

Declarations

Conflict of interest Mr. Vidor is the recipient of a PhD scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES—PBE-DPM, Bolsa Especial para Doutorado em Pesquisa Médica). Mr. Martins is affiliated to private companies related to clinical imaging services. Luis Augusto Rohde has received honoraria, has been on the speakers' bureau/advisory board, and/or has acted as a consultant for Medice, Novartis/Sandoz, and Shire/Takeda in the last two years; and receives authorship royalties from OxfordPress and ArtMed. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by him has received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Janssen-Cilag, Novartis/Sandoz, and Shire/Takeda. Eugênio Horácio Grevet has served as a speakers' bureau/advisory board for Shire Pharmaceuticals in the past 3 years; and has received travel awards from Shire for taking part in psychiatric meetings. Ms. Panzenhagen, Dr. Cupertino, Ms. Bandeira, Dr. Picon, Dr. Silva, Dr. Vitola, Dr. Rovaris, and Dr. Bau reported no biomedical financial interests or potential conflicts of interest.

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Biobehavioral markers of suicide across psychiatric disorders

“Life on earth is more like a verb. It repairs, maintains, re-creates, and outdoes itself.”

– Lynn Margulis

8

Early-life trauma, impulsivity and suicide attempt: a systematic review and meta-analysis

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Early-life trauma, impulsivity and suicide attempt: a systematic review and meta-analysis

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ABSTRACT

Introduction: Suicide is a worldwide health concern and up to date there is no good predictor of it except a previous suicide attempt. Therefore, there are increasing efforts in the understanding of which factors, genetic or environmental, are associated with suicide behaviour. Objective: To review evidence of the effect of childhood trauma and impulsivity on suicidal behavior through a systematic review and meta-analysis. Methods: Searches were conducted on the 12th of June 2021 in the PubMed, Scopus, and Web of Science

databases. Two reviewers evaluated each record for eligibility and discussed upon disagreement, when no consensus was reached, a third reviewer was involved to make a decision. Results: A total of 11,530 records were identified through the searches. After duplicates were removed, 6,595 records remained to be screened. The full text was sought for 1,561 records. Our qualitative synthesis included 22 studies, from which 9 were included in the meta-analyses. We found a significant effect of sexual abuse, physical abuse, emotional abuse and physical neglect on suicide attempts in the prisoners, and Substance Use Diorder (SUD) subgroups. Moreover, there was a significant effect of Childhood Trauma Questionnaire (CTQ) total score and emotional neglect dimension for all the subgroups.

Conclusion: The present study has provided an overview of the state-of-the-art research on childhood trauma and impulsivity and their association with suicidal behavior and quantified their effects on suicide attempts. Hopefully this evidence will be considered in future research and harnessed for clinical gain in detection and treatment of suicide behaviour.

Keywords: behaviour, childhood, trauma, psychiatry, suicide.

INTRODUCTION

Suicide is a World Health Organization (WHO) priority health concern, affecting mainly low- and middle-income countries (77%). Every 40 seconds one person dies by suicide, this amounts to more than 700,000 people each year [1]. Suicide is a leading cause of death worldwide, ahead of malaria, AIDS, breast cancer, war, and homicide [1,2]**.** In 2019, suicide accounted for 1.3% of all deaths. Moreover, for each completed suicide there were more than 20 other attempts. The behavior is present throughout life, being the fourth leading cause of death among 15-29 year-old [2,3].

Advances in our understanding of suicidal behaviour have shown that it is indeed a complex phenotype, comprising environmental, social, clinical, genetic, and other biological factors [4]. Proximal and distal components might affect suicide risk, such as genetic susceptibility [5,6] or traumatic and stressful events [7–9]. The multifactorial nature of suicidal behaviour makes it challenging to investigate its aetiology or predict individual hazards. The best predictor of suicide risk to date is a previous suicide attempt [10], which is far from an ideal indicator for suicide prevention.

Furthermore, suicidal behaviour is a shared comorbid phenotype in different psychiatric disorders, although more prevalent in mood and affect-related disorders, such as major depressive disorder (MDD), bipolar disorder (BD), schizoaffective disorder (SZA), and

schizophrenia (SCZ) [4]. Due to its cross-disorder characteristic, one would also expect to find shared features between disorders that might influence suicide risk, and maybe even the same associated predictors. This has been demonstrated by the first genome-wide association studies (GWAS) investigating suicide attempts in different disorder samples, revealing that indeed there seems to be a common genetic, and hence biological, component to the pathophysiology of suicide [5,11].

There is also evidence in the literature about shared behavioral traits, such as self-criticism, hopelessness, guilt, anxiety, and personality, including impulsivity. The latter, in fact, does have common domain profiles across many disorders and is worthy of further investigation since it is usually formed early in life and changes little across the lifespan, which makes personality traits good candidate predictors. Among these, impulsivity seems to be a key factor for suicide attempt and death by suicide, contributing for trigger to the act itself [4,12]. Moreover traumatic events seem to play a role as distal factors that can be present early in life, and influence the coping capabilities in adulthood [5,13]. Therefore, we aim at i) providing a systematically-gathered overview of the literature on childhood trauma, personality traits and suicidal behaviour; ii) quantifying one of the main behaviours, suicide attempt, through a meta-analysis, which could guide future research and hint at potential predictors.

METHODS

This systematic review and meta-analysis was pre-registered on the International prospective register of systematic reviews (PROSPERO) under registration CRD42022345915. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for reporting this work [14].

Eligibility criteria

The inclusion criteria for this systematic review comprised any study: with adult individuals (Population) presenting suicidal behaviour, namely suicide risk, suicidal ideation, suicide attempt, or suicide completion (Exposure), with or without a control group for suicidal behaviour (Comparison), presenting an evaluation of personality and childhood trauma through documented questionnaire scale instruments (Outcome), and designed as a cross-sectional, cohort, case-control or clinical trial study.

The exclusion criteria were: non-human studies, such as animal models, *in vitro*, or *in silico*; studies with adolescents or children in the sample; studies with individuals diagnosed with any personality or neurological disorder, such as borderline personality disorder or Alzheimer's disease; studies presenting only subjective records of personality and childhood trauma assessment (not based on a scored questionnaire scale); studies with no original data, such as reviews or commentaries; and studies published in other languages than English.

Information sources and search strategies

Searches were conducted on the 12th of June 2021 in the PubMed, Scopus, and Web of Science databases. No limitations were imposed on the date of publication. The following strategies were used: *PubMed* - (("personality"[tiab] OR "personality traits"[tiab] OR "BIG-5"[tiab] OR "Big Five personality"[tiab] OR "PID-5"[tiab] OR "PID-V"[tiab] OR "Personality Inventory for DSM-5"[tiab] OR "Tridimensional Personality Questionnaire"[tiab] OR (("five-factor"[tiab] OR "five factor"[tiab] OR "five-factors"[tiab] OR "five factors"[tiab]) AND "personality"[tiab])) AND ("suicidal"[tiab] OR "suicide attempts"[tiab]OR "suicide"[tiab] OR "Suicide"[mesh])) NOT (review[title] OR "personality disorder"[title] OR "borderline disorder"[title] OR "antisocial disorder"[title] OR "Schizoid Personality Disorder"[title] OR "Narcissistic Personality Disorder"[title]); *Scopus* - TITLE-ABS ("personality" OR "personality traits" OR "BIG-5" OR "Big Five personality" OR "PID-5" OR "PID-V" OR "Personality Inventory for DSM-5" OR "Tridimensional Personality Questionnaire" OR (("five-factor" OR "five factor" OR "five-factors" OR "five factors") AND "personality")) AND TITLE-ABS ("suicidal" OR "suicide attempts" OR "suicide" OR "suicides" OR "parasuicide" OR "parasuicides" OR "fatal Attempt" OR "fatal attempts") AND NOT TITLE (review OR "personality disorder" OR "borderline disorder" OR "antisocial disorder" OR "Schizoid Personality Disorder" OR "Narcissistic Personality Disorder"); *Web of Science* - TOPIC("personality" OR "personality traits" OR "BIG-5" OR "Big Five personality" OR "PID-5" OR "PID-V" OR "Personality Inventory for DSM-5" OR "Tridimensional Personality Questionnaire" OR (("five-factor" OR "five factor" OR "five-factors" OR "five factors") AND ("personality"))) AND ("suicidal" OR "suicide attempts" OR "suicide" OR "suicides" OR "parasuicide" OR "parasuicides" OR "fatal Attempt" OR "fatal attempts") NOT TITLE:(review OR "personality disorder" OR "borderline disorder" OR "antisocial disorder" OR "Schizoid Personality Disorder" OR "Narcissistic Personality Disorder").

Selection of studies and data collection

Records were uploaded to the rayyan.ai online tool, where duplicates were removed by human screening after detecting possible duplicate records using Rayyan's algorithm. Two independent reviewers evaluated all studies for eligibility, first through title and abstract screening and second by full-text evaluation. Disagreements were resolved by discussion between reviewers or by a third reviewer when consensus could not be reached. The hierarchy of exclusion criteria was the language of the record, design of the study, non-human samples, studies without suicidal behaviour, studies without an objective personality evaluation, studies including individuals with personality disorders or neurological disorders, and studies without an objective childhood trauma evaluation.

Data collection was conducted manually by one reviewer, and information was inserted into a spreadsheet. The process was divided into two main steps: 1) screening of studies and summary data collection; and 2) data collection for meta-analysis. The following data was extracted in the first step: first author last name, year of publication, the sample size of cases, the sex ratio of individuals, mean age or age range reported, if the study included a control group and its sample size, which was the population the sample was based on, which personality and childhood trauma evaluation scales the study used, the type of suicidal behaviour, and the scale used to evaluate suicidal behaviour, when available. In the second step, the specific sample size for groups of interest, mean, and standard deviation or standard error of the mean were collected.

Synthesis methods

The study results are summarised in a characteristics table with all main demographic information, and instruments used. A meta-analysis was conducted for the main groups of suicide attempters versus non-attempters, in prisoners, major depressive disorder (MDD) patients, and substance use disorder (SUD) patients. The meta-analyses were performed by calculating random-effects estimates using inverse variance weighting for pooling. The method used for estimating the standardised mean difference (SMD) was the Hedges' g method. Heterogeneity was also estimated through I^2 and τ^2 methods. Subgroup analyses were conducted by the base population group. Meta-regressions were performed, including age and sex ratio as independent variables through the restricted maximum likelihood mixed model. Publication bias was assessed by funnel plotting and the Egger's regression test. All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021). The meta-analyses, meta-regressions, Egger's regression, forest plots, and

funnel plots were generated using the R packages *meta* (v5.2-0; Balduzzi 2019) and *metafor* (v3.4-0; Viechtbauer 2010).

RESULTS

Study selection

A total of 11,530 records were identified through the following searches: 3,024 in PubMed, 4,679 in Web of Science, and 3,827 in Scopus. After duplicates were removed, 6,595 records remained to be screened. With the title and abstract exclusions, 5,034 records were eliminated. The full text was sought for 1,561 records, which were assessed for eligibility. From those, 1,539 were excluded, the majority because they did not present any objective personality evaluation, did not include original data, or did not present any objective childhood trauma evaluation. Our qualitative synthesis included, therefore, 22 studies, from which 9 were included in the meta-analyses. The reasons for exclusion and the whole selection process are depicted in Figure 1. All the 22 included report references and the personality assessments conducted in them are cited in the Additional File 1.

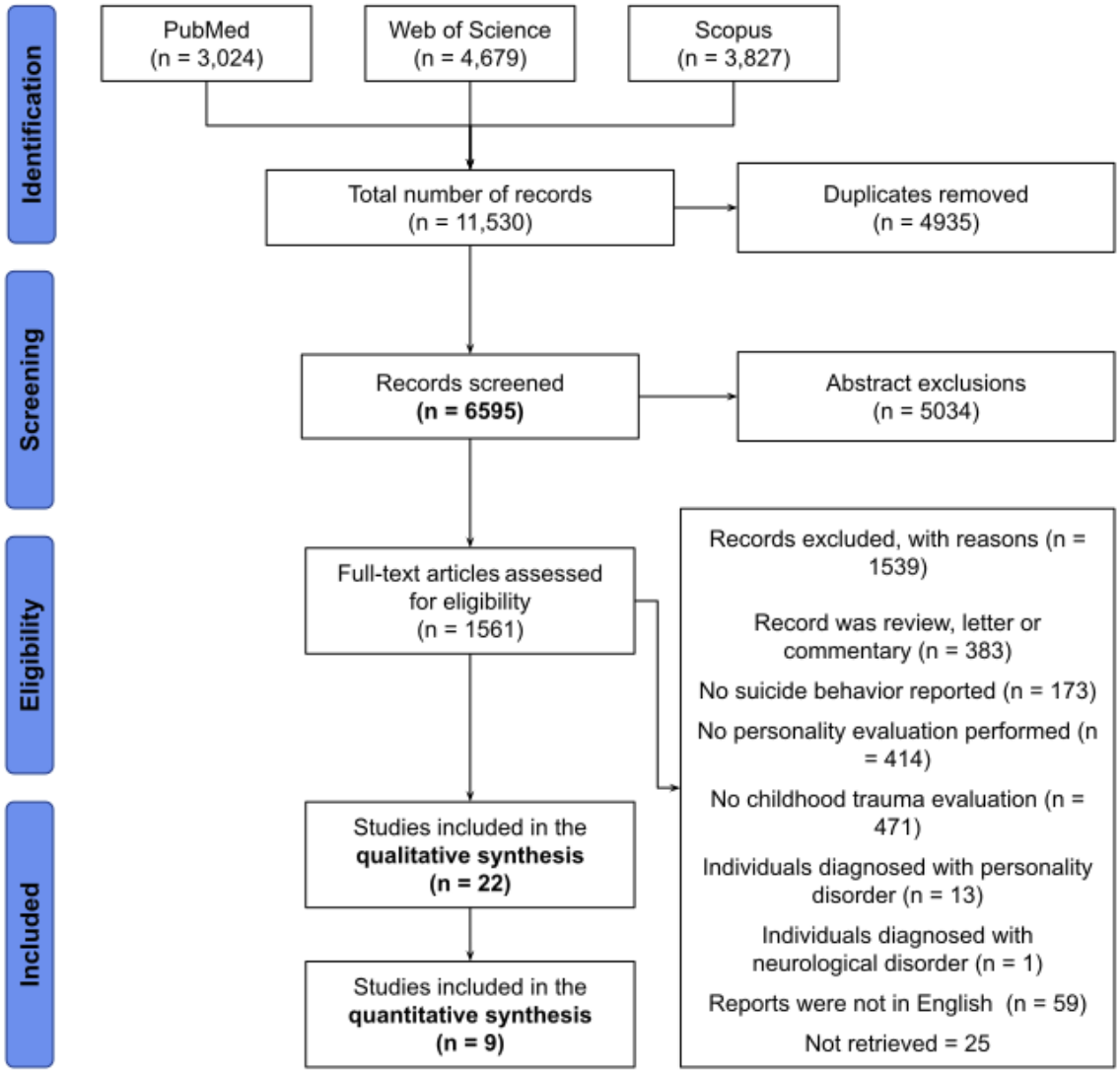

Figure 1. Flowchart of the screening procedure.

Study characteristics

The description of studies included in the qualitative synthesis is presented in Table 1. The studies comprised mostly suicide attempt samples. The base population (healthy individuals, patients with psychiatric disorders, etc), sex, and age information is provided. Studies could include or not a control group, which is also described with the number of individuals in the group.

Table 1. Description of the studies included in the qualitative synthesis.

Paper	Childhood Trauma Scale	Sex	Age (years)	Population	Suicide behavior
Bach 2017	CTQ	78,2% women, 21,8% men	mean 29, 18-56	psychiatric outpatients	suicidal ideation, suicide attempt
Blasco-Fontecilla 2013	CTQ	72% women, 28% men	18-83	hospitalized suicide attempters	suicide attempt
Blasco-Fontecilla 2014	CTQ	71% women, 29% men	18-83	suicide attempters	suicide attempt
Carli 2010	CTQ	100% men	mean 39	incarcerated individuals	suicidal ideation, suicide attempt
Carli 2011	CTQ-34	100% men	18-77	prisioners	suicide attempt
Carli 2013	CTQ-34	100% men	mean 39	prisioners	suicide attempt
Carlier 2016	CTQ	60,4% female, 39,6% men	mean 38, 18-79	mood, anxiety and somatoform outpatients	suicidal ideation, suicide attempt
Dalsanto 2020	CTQ-28	66% female, 34% men	mean 54	MDD outpatients	suicide attempt
Gorodetsky 2016	CTQ-34	100%	mean 41	prisioners	suicide attempt
Kamali 2018	CTQ	65,5% women, 34,5% men	mean 40	bipolar patients with follow-up	suicide attemp, suicide ideation
Lopez-Castroman 2012	CTQ-28	72% women, 28%	18-75	survivors of a current suicide attempt	suicide attempt
Marzano 2011	CTQ	100%	mean 25	prisioners	suicide attempt
Pompili 2009	CTQ	77,4% women, 22,6%	mean 42	physically or sexually abused psychiatric inpatients	suicide ideation
Rivlin 2013	CTQ	100% men	>18	prisioners	suicide attempt
Roy 2003	CTQ	31,4% women, 68,6% men	mean 43	alcoholics	suicide attempt
Roy 2003	CTQ-34	22,2% women, 77,8% men	mean 43	drug addicts	suicide attempt
Roy 2003b	CTQ	21,7% women, 21,7% men	mean 43	substance dependent suicide attempters	suicide attempt
Roy 2014	CTQ-34	100% men	mean 40	prisioners	suicide attempt

Sarchiapone 2009	CTQ	100% men	mean 39, 18-81	prisioners	suicidal ideation, suicide attempt
Sarchiapone 2009	CTQ-34	100% men	mean 40	prisioners	suicide attempt
Stewart 2015	CTQ	76,6% women, 23,4% men	13-18	MDD and/or dysthymia patients	suicide attempt
Velasco 2019	CTQ	58,2% women, 41,8%	mean 50	major depressive disorder patients	suicide attempt, suicide ideation

Suicide attempts and the Childhood Trauma Questionnaire (CTQ)

A total of 22 studies used the Childhood Trauma Questionnaire (CTQ) [15] to assess childhood trauma, from which nine (9) presented the data necessary for a meta-analysis. The studies included in the quantitative synthesis amounted to 1,063 suicide attempters and 1,854 controls. Five (5) studies had the prisoners as base population for the sample, two (2) has MDD patients, and another two (2) had SUD patients.

The total score of CTQ was higher in suicide attempts for all the subgroups (prisoners, MDD patients, and SUD patients). The overall effect was significant, in which childhood trauma seems higher in suicide attempters with a moderate standardised mean difference of 0.64 (0.51; 0.77). The test for the overall effect presented a z = 9.94 and P-value < 0.01. The heterogeneity was low overall (I^2 = 33%; τ^2 = 0.0118, p = 0.16). The subgroup and overall meta-analysis results are depicted in Figure 2. The funnel plot and Egger's regression test indicate there is no evidence of publication bias (t = -1.86, P-value = 0.11; Figure 9A).

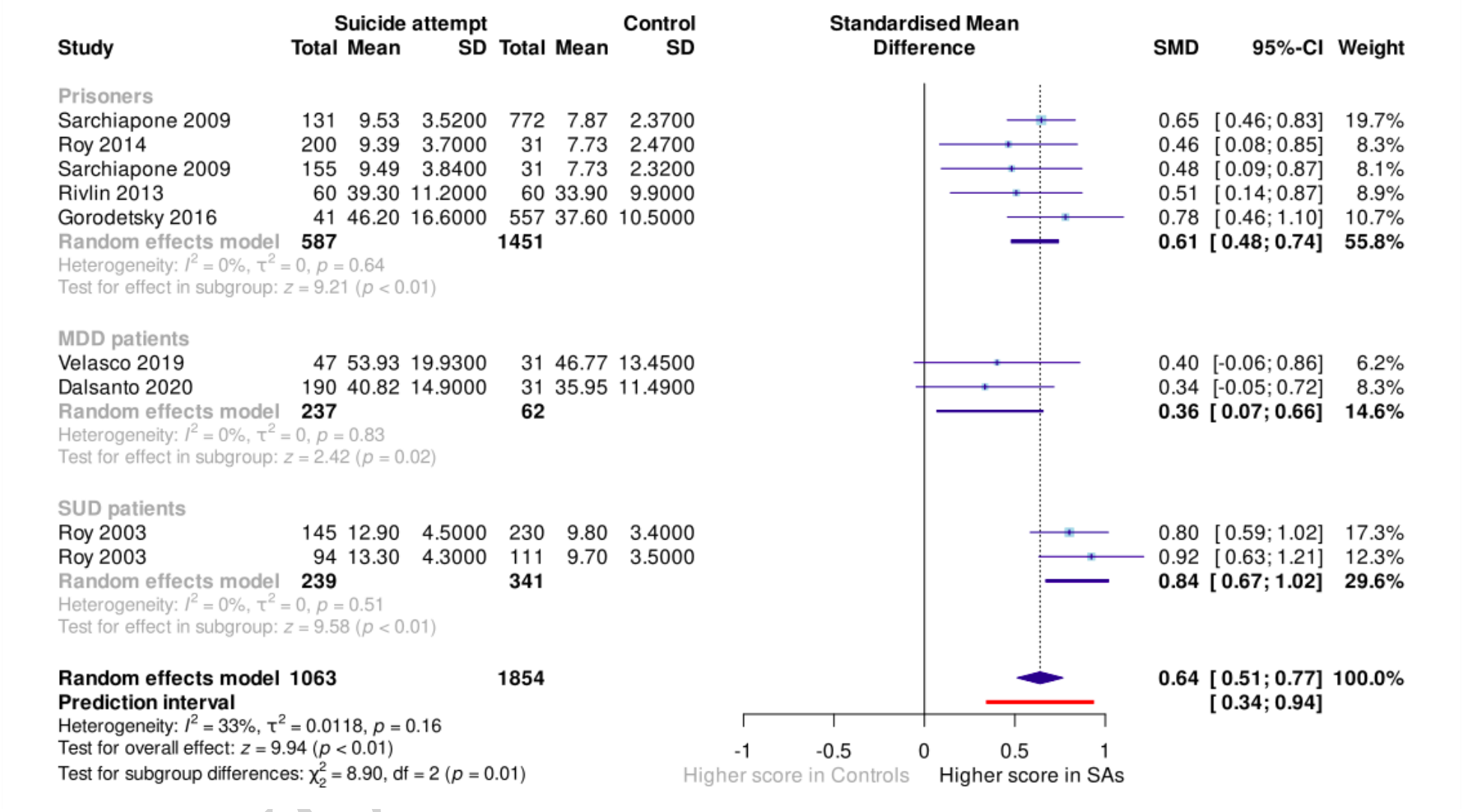

Figure 2. Forest plot of the meta-analysis of CTQ's total score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

For the sexual abuse dimension of CTQ, only the prisoners (SMD = 0.21 (0.06; 0.37); z = 2.75, p < 0.01) and the SUD (SMD = 0.68 (0.51; 0.85); z = 7.80, p < 0.01) subgroups were associated. However, the overall effect was also significant, with a mild mean difference (0.38 (0.19; 0.57)), showing moderate heterogeneity overall (I^2 = 66%; τ^2 = 0.0371, p < 0.01), but very low for the subgroups (I^2 = 0%). The subgroup and overall meta-analysis results are depicted in Figure 3. The funnel plot and Egger's regression test indicate there is no evidence of publication bias (t = -0.42, P-value = 0.69; Figure 9C).

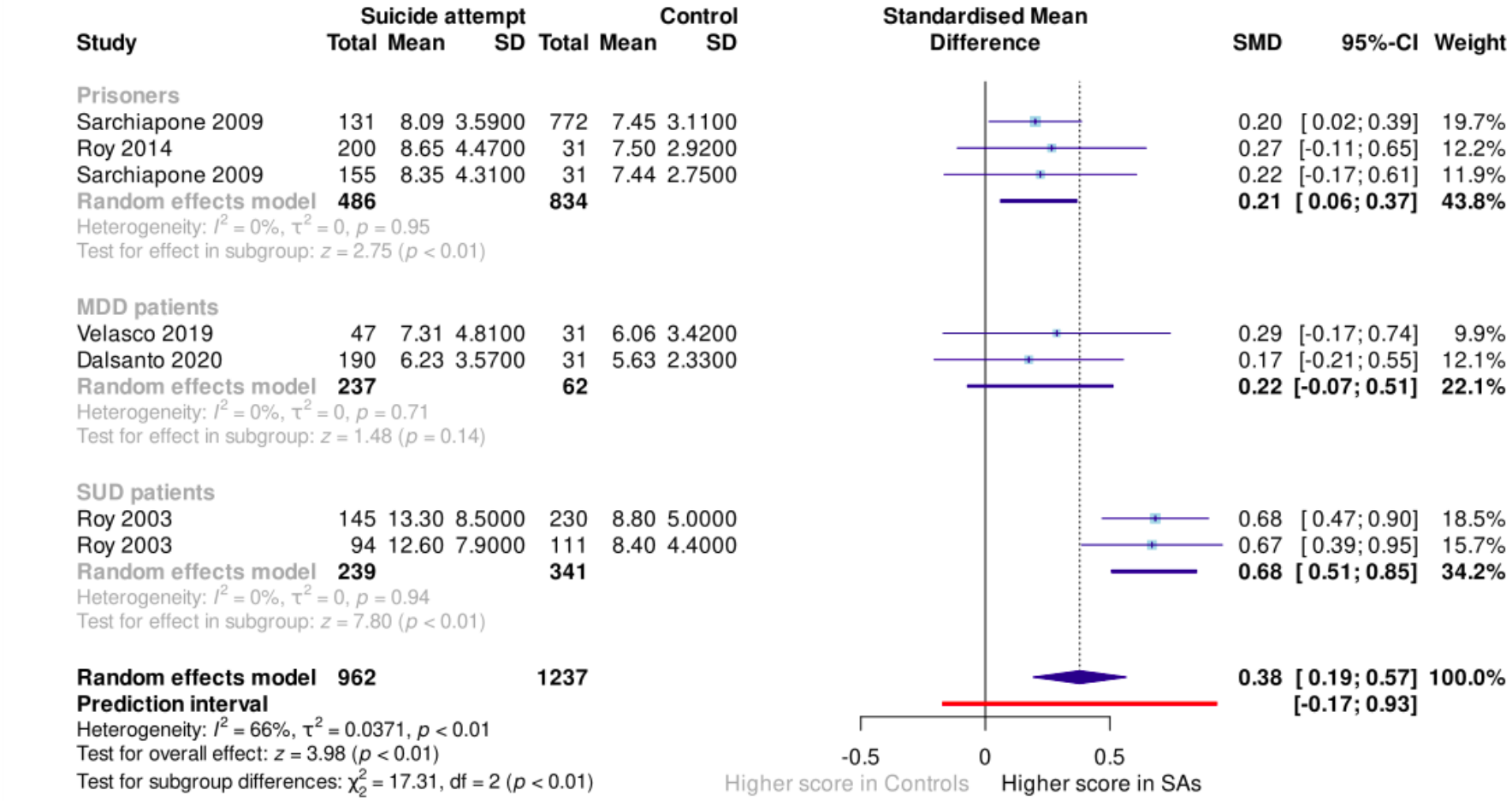

Figure 3. Forest plot of the meta-analysis of CTQ - Sexual Abuse dimension score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

For the physical abuse dimension of CTQ, similar to sexual abuse, only the prisoners (SMD = 0.48 (0.32; 0.63); z = 6.08, p < 0.01) and the SUD (SMD = 0.62 (0.45; 0.79); z = 7.16, p < 0.01) subgroups were associated. Additionally, the overall effect was significant, with a mild mean difference (0.48 (0.35; 0.60)), showing low heterogeneity overall (I^2 = 26%; τ^2 = 0.0057, p = 0.23), and very low for the subgroups (I^2 = 0%). The subgroup and overall meta-analysis results are depicted in Figure 4. However, the funnel plot and Egger's regression test indicate there is some evidence of publication bias (t = -3.09, P-value = 0.03; Figure 9E).

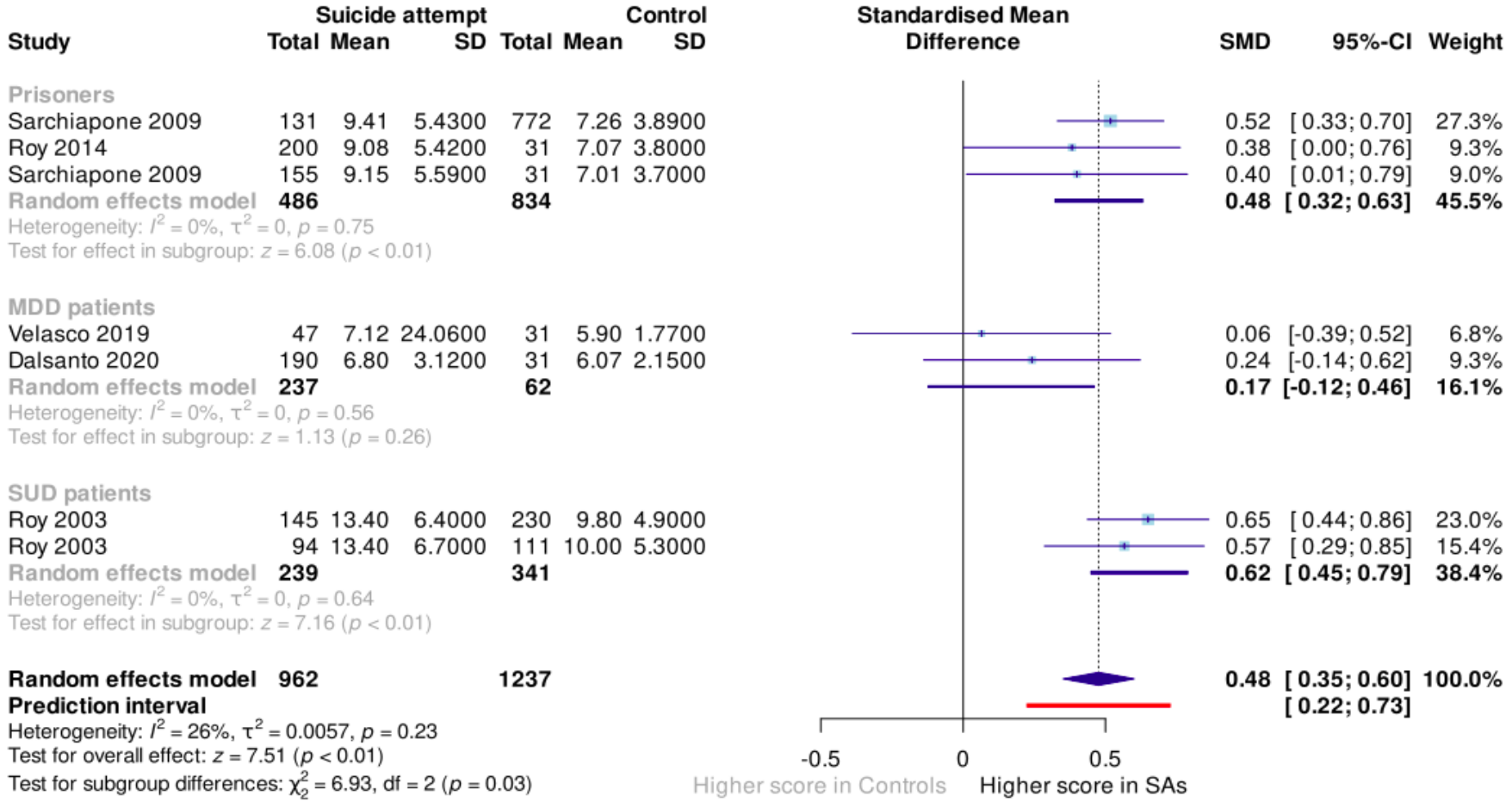

Figure 4. Forest plot of the meta-analysis of CTQ - Physical Abuse dimension score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

For the emotional abuse dimension of CTQ, only the prisoners (SMD = 0.52 (0.37; 0.68); z = 6.67, p < 0.01) and the SUD (SMD = 0.76 (0.58; 0.93); z = 8.65, p < 0.01) subgroups were associated once again. The overall effect was also significant, with a moderate mean difference (0.56 (0.42; 0.70)), showing low heterogeneity overall (I^2 = 35%; τ^2 = 0.0128, p = 0.16), and very low for the subgroups (I^2 = 0%). The subgroup and overall meta-analysis results are depicted in Figure 5. The funnel plot and Egger's regression test indicate there is no evidence of publication bias (t = -1.96, P-value = 0.11; Figure 9B).

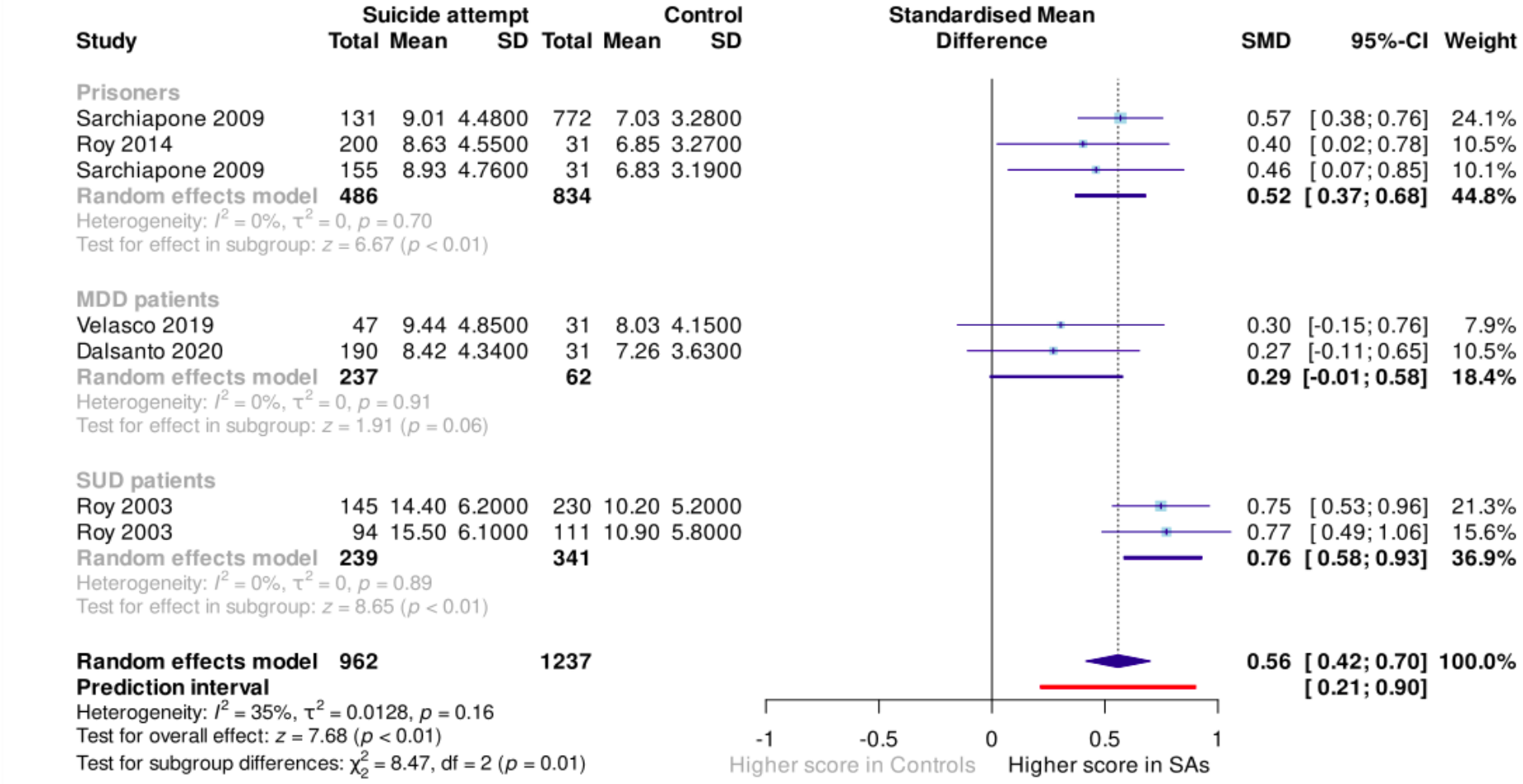

Figure 5. Forest plot of the meta-analysis of CTQ - Emotional Abuse dimension score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

For the physical neglect dimension of CTQ, only the prisoners (SMD = 0.45 (0.30; 0.60); z = 5.75, p < 0.01) and the SUD (SMD = 0.49 (0.17; 0.81); z = 3.04, p < 0.01) subgroups were associated. Additionally, the overall effect was significant, with a moderate mean difference (0.42 (0.32; 0.53)), showing very low heterogeneity overall (I^2 = 0%; τ^2 = 0.0004, p = 0.44). Moreover, a very low heterogeneity was identified for the prisoners and MDD subgroups (I^2 = 0%), but not for the SUD subgroup (I^2 = 69%). The subgroup and overall meta-analysis results are depicted in Figure 6. The funnel plot and Egger's regression test indicate there is no evidence of publication bias (t = -0.81, P-value = 0.46; Figure 9D).

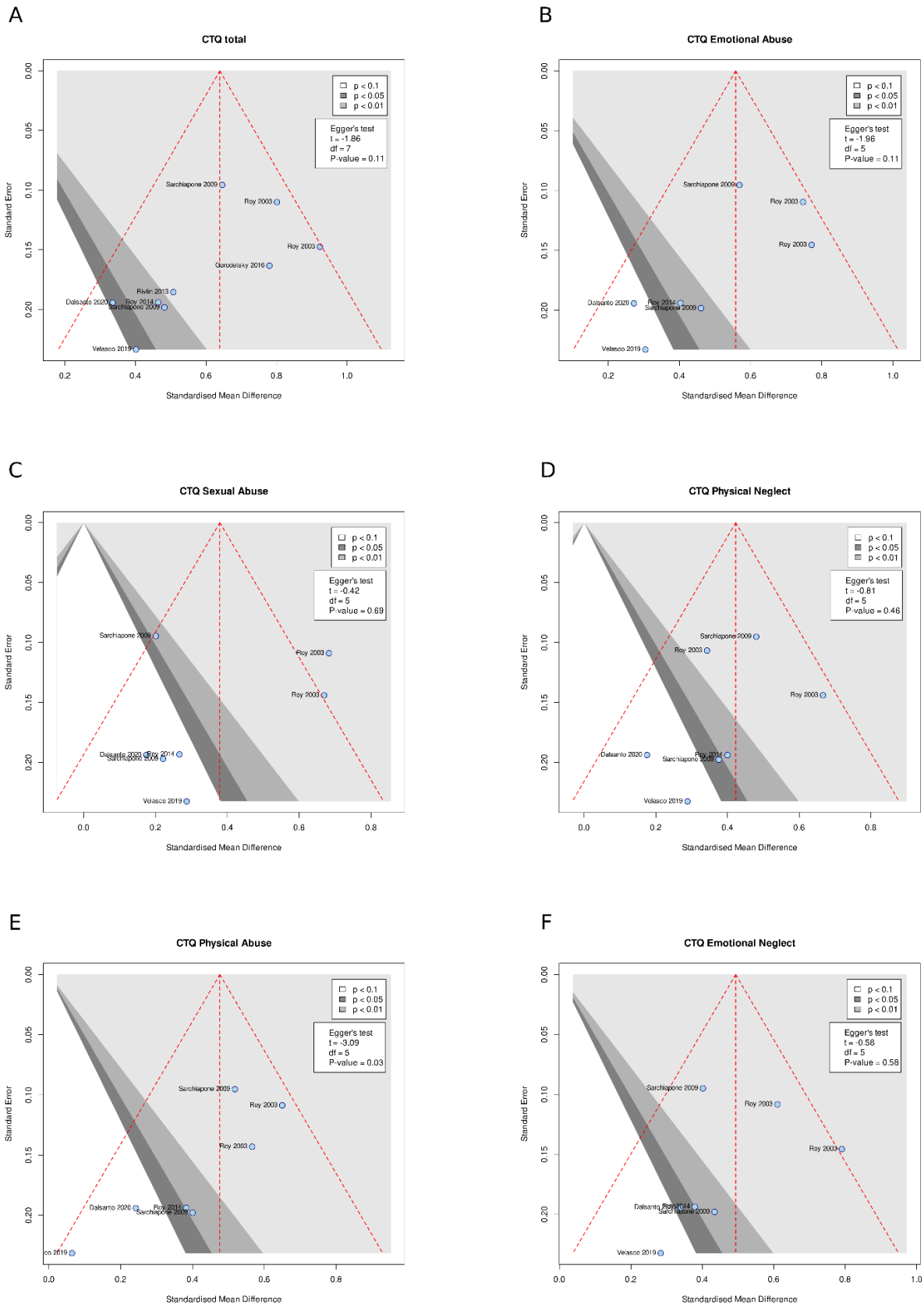

Figure 9. Funnel plots indicating the publication bias of studies included in the meta-analysis of impulsivity (A), harm avoidance (B), novelty seeking (C), reward dependence (D), neuroticism (E), extroversion (F), and psychoticism (G).

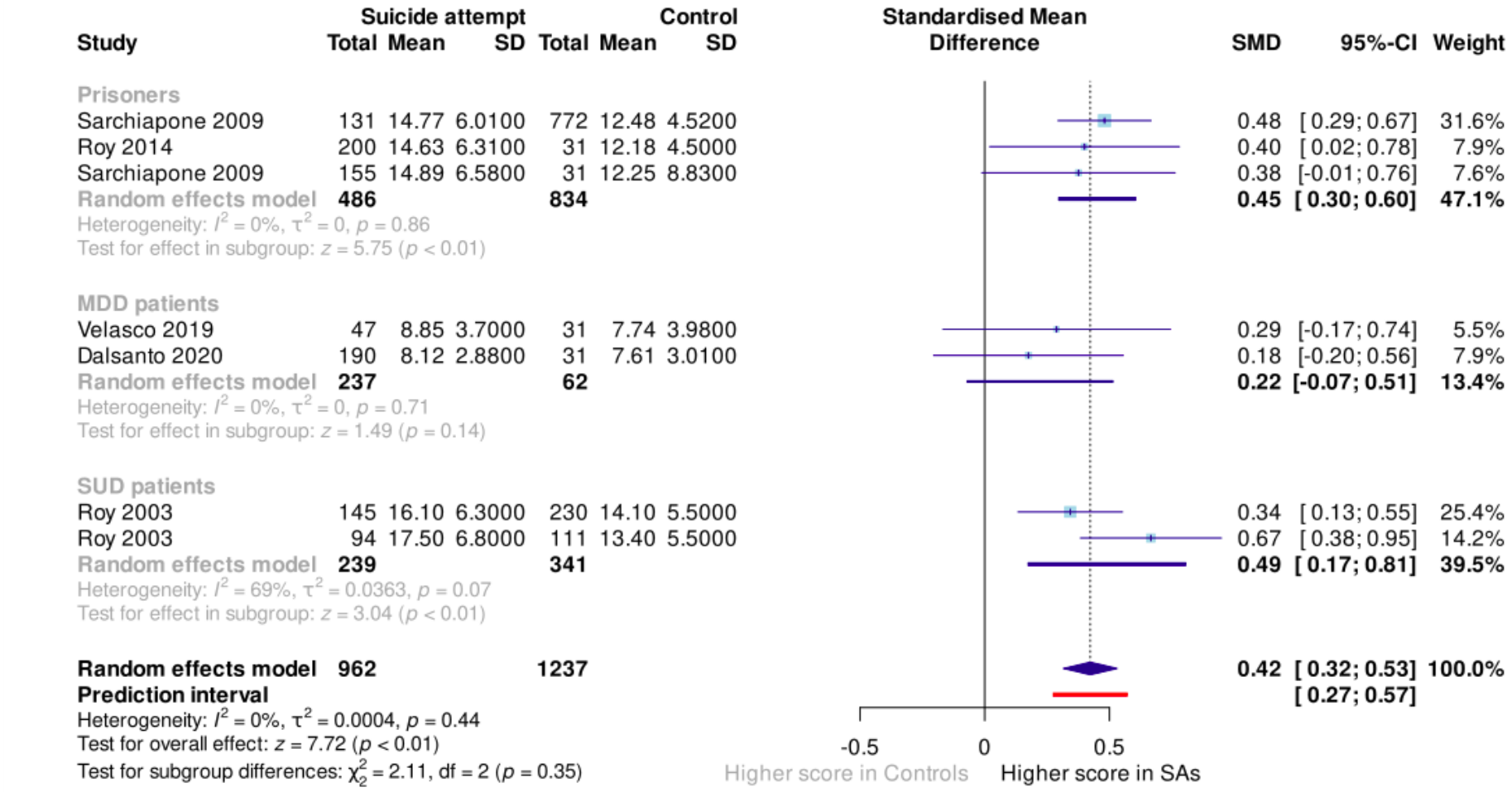

Figure 6. Forest plot of the meta-analysis of CTQ - Physical Neglect dimension score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

For the emotional neglect dimension of CTQ, all subgroups were associated with the outcome: prisoners (SMD = 0.40 (0.25; 0.56); z = 5.15, p < 0.01), MDD patients (SMD = 0.32 (0.02; 0.61); z = 2.12, p = 0.03) and SUD patients (SMD = 0.49 (0.36; 0.80); z = 7.76, p < 0.01). Additionally, the overall effect was significant, with a moderate mean difference (0.49 (0.36; 0.63)), showing low heterogeneity overall (I^2 = 26%; τ^2 = 0.0098, p = 0.23). Moreover, a very low heterogeneity was identified in all subgroups (I^2 = 0%). The subgroup and overall meta-analysis results are depicted in Figure 7. The funnel plot and Egger's regression test indicate there is no evidence of publication bias (t = -0.58, P-value = 0.58; Figure 9F).

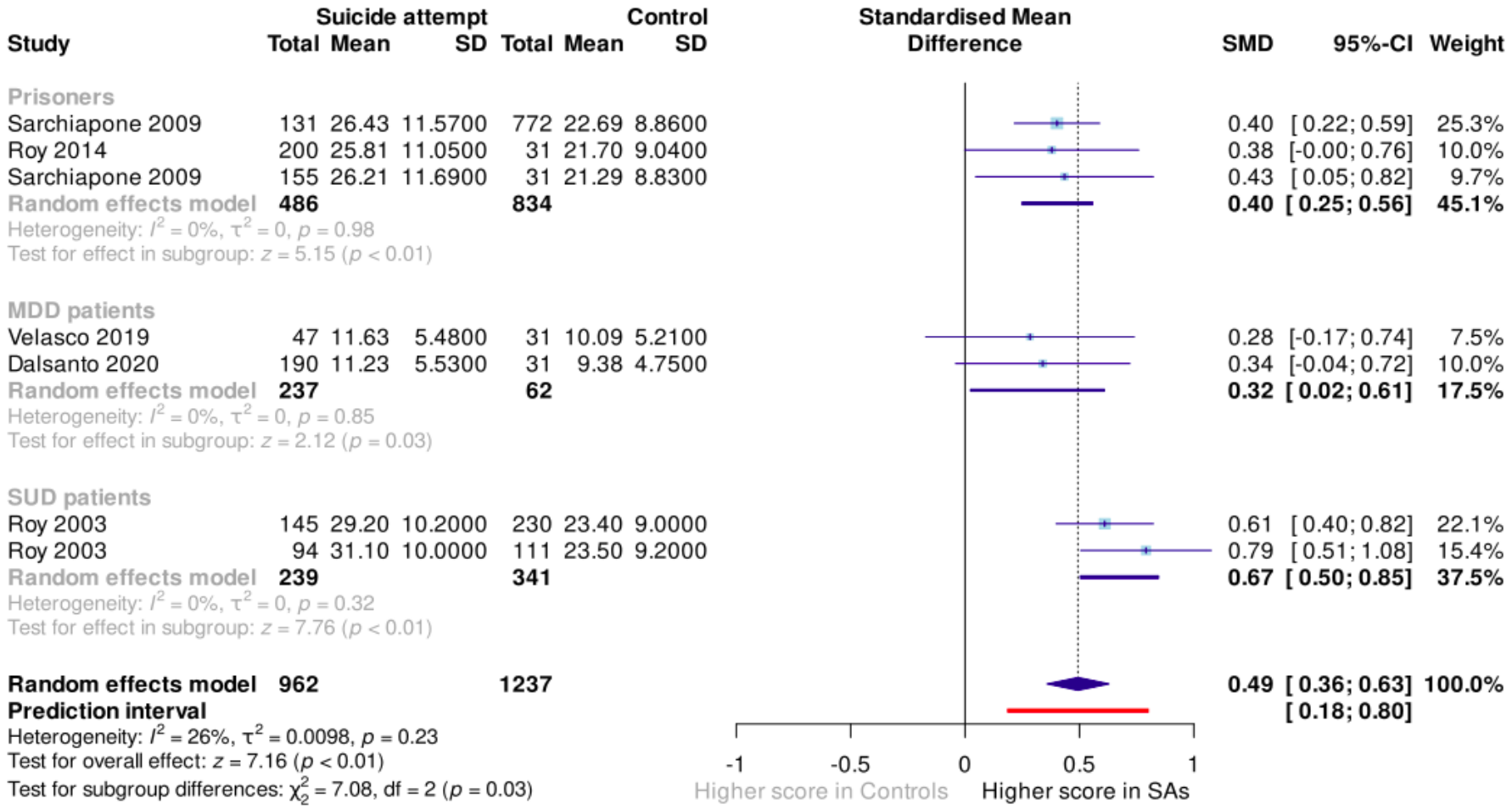

Figure 7. Forest plot of the meta-analysis of CTQ - Emotional Neglect dimension score in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

Barratt Impulsiveness Scale

Regarding the personality assessments, only two studies [16,17] from the ones included in the quantitative synthesis also presented personality data, namely impulsivity evaluated using the Barratt Impulsiveness Scale [18]. Results show that the suicide attempt group present higher impulsivity scores (Figure 8), while also presenting more emotional neglect in CTQ compared to controls (Figure 7). These reports are coincidently the ones in the MDD patient subgroup [16,17].

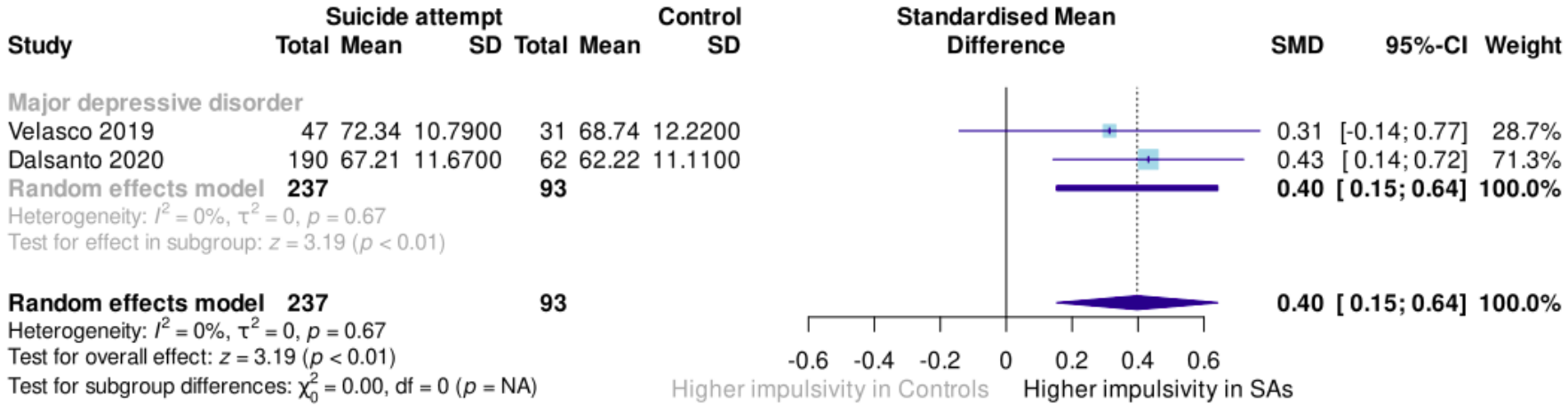

Figure 8. Forest plot of the meta-analysis of impulsivity in suicide attempt. SD = standard deviation, SMD = standardised mean difference, SA = suicide attempt.

Meta-regression analysis

The meta-regression analysis showed that sex or age accounted for 100% of the variability for emotional abuse, physical neglect, and emotional neglect; 68.47% for sexual abuse, and 47.06 for physical abuse. Unfortunately sex ratio and age did account for the heterogeneity found in the total score meta-analysis. However, there seems to be little residual heterogeneity left from the analysis. We would like to stress that both sex ratio and age are particularly related to sexual abuse, emotional abuse, and emotional neglect. The results for the main estimators are summarized in Table 2 and for the mixed-effects model in Table 3.

Table 2. Residual heterogeneity and moderators after meta-regression using sex ratio and age as covariates

							Residual Heterogeneity			Moderators		
Analysis	**tau²**	**SE**	**tau**	**I² (%)**	**H²**	**R² (%)**	**QE**	**df**	**P-value**	**QM**	**df**	**P-value**
Total score	0.017	0.025	0.13	43.25	1.76	0.00	8.524	5	0.130	2.010	2	0.367
Sexual abuse	0.012	0.025	0.108	33.69	1.51	68.47	5.413	4	0.248	6.628	2	**0.037**
Physical Abuse	0.003	0.017	0.055	11.56	1.13	47.06	4.475	4	0.346	3.261	2	0.196
Emotional Abuse	0.00	0.015	0.00	0.00	1.00	100.00	3.337	4	0.503	5.869	2	0.053
Physical Neglect	0.00	0.014	0.00	0.00	1.00	100.00	2.436	4	0.656	3.382	2	0.184
Emotional Neglect	0.00	0.015	0.00	0.00	1.00	100.00	1.387	4	0.847	6.754	2	**0.034**

tau² = estimated amount of residual heterogeneity; tau = square root of estimated tau^2 value; I² = residual heterogeneity / unaccounted variability; H² = unaccounted variability / sampling variability; R² = amount of heterogeneity accounted for; QE = test for residual heterogeneity QM = test of moderators (coefficients 2:3).

Table 3. Meta-regression estimates using sex ratio and age as covariates

	Estimate	SE	Z-value	P-value	CI
CTQ total score					
Intercept	1.9117	0.8968	2.1317	**0.0330**	0.1541; 3.6693
Sex ratio	-0.0020	0.0025	-0.8218	0.4112	-0.0069; 0.0028
Age	-0.0261	0.0186	-1.4070	0.1594	-0.0625; 0.0103
Sexual Abuse					
Intercept	9.2809	3.4831	2.6645	**0.0077**	2.4541; 16.1077
Sex ratio	-0.0307	0.0120	-2.5691	**0.0102**	-0.0542; -0.0073
Age	-0.1492	0.0592	-2.5225	**0.0117**	-0.2651; -0.0333
Physical Abuse					
Intercept	4.1692	2.9642	1.4066	0.1596	-1.6404; 9.9789
Sex ratio	-0.0096	0.0101	-0.9552	0.3395	-0.0293; 0.0101
Age	-0.0678	0.0506	-1.3391	0.1805	-0.1670; 0.0314
Emotional Abuse					
Intercept	6.9356	2.7846	2.4907	**0.0127**	1.4779; 12.3933
Sex ratio	-0.0194	0.0094	-2.0696	**0.0385**	-0.0378; -0.0010
Age	-0.1114	0.0477	-2.3376	**0.0194**	-0.2049; -0.0180
Physical Neglect					
Intercept	4.5685	2.7652	1.6521	0.0985	-0.8512; 9.9883
Sex ratio	-0.0116	0.0093	-1.2505	0.2111	-0.0299; 0.0066
Age	-0.0747	0.0474	-1.5767	0.1149	-0.1675; 0.0182
Emotional Neglect					
Intercept	7.7142	2.7847	2.7702	**0.0056**	2.2563; 12.1721
Sex ratio	-0.0241	0.0094	-2.5760	**0.0100**	-0.0425; -0.0058
Age	-0.1226	0.0477	-2.5702	**0.0102**	-0.2160; -0.0291

DISCUSSION

We found a significant effect of sexual abuse, physical abuse, emotional abuse and physical neglect on suicide attempts in the prisoners, and SUD subgroups. Moreover, there was a significant effect of CTQ total score and emotional neglect dimention for all the subgroups. These results show that suicide attempt in MDD may be closely related to

emotional neglect, since this was the only subgroup with a positive meta-analysis result for it.

We show that suicide attempt is associated with early-life trauma assessed by CTQ, and in all its dimentions, across most of the populations included. Childhood trauma is a distal factor that is associated with several, if not all, disorders across the psychiatry spectrum [19,20]. Negative influences during the neurodevelopment, a fragile time window, have exacerbated effects on emotional memories. Baseline susceptibility to mental disorders added to stressors in everyday-life and traumatic events might be triggering enough to predict suicide attempt [21]. Moreover, there are also special components that might differentiate ideation and attempt [22]. We hypothesize that this differentiation may be related to the interaction of traumatic events with personality traits, more specifically, impulsivity.

The action of suicide attempt seems to require a level of action-taking behaviour. Bipolar disorder (BD) and MDD, for exemple, appear in different frequencies when we compare suicidal ideation and suicide attempt. Some argue that this is due to the more externalizing, and impulsive component of BD, more specifically, mania [18,23]. Another estimate that seems to corroborate this idea is that more women present ideation, but mostly men attempt and complete suicide [24]. The distribution of psychiatric disorders has a different presentation between men and women, in which men are more likely to be diagnosed with externalizing disorders, and women with internalizing disorders. For instance, a study that examined the differential effects of childhood maltreatment and impulsivity on interpersonal violence, suicide attempts and self-injury, with a sample of 34,653 US adults, showed that childhood impulsivity and maltreatment independently increased the risk of attempts of suicide, self-mutilation and interpersonal violence. Childhood maltreatment was a stronger predictor of self-directed violence in both sexes, while impulsivity had a greater effect on self-injury than suicide attempt or interpersonal violence only in men [25]. These sex differences should be explored further, since suicide attempt and completion in women might require a specific set of commorbidities to take place, due to the relative low impulsive component.

Furthermore, regarding the lack of association between most CTQ dimensions and the MDD subgroup, we hypothesize that trauma as a whole might be already too associated with MDD, so that the signal is lost in the noise. Impulsivity was unfortunately only evaluated in the MDD subgroup, so no conjectures about how it could be in other subgroups can be drawn, but it is also elevated in suicide attempters. Many have hypothesized that it would be one of the triggers necessary to the attempt/completion act, especially in women [26].

This work should be viewed in light of some limitations: 1) although publication bias does not seem to be of concern, there was low to moderate heterogeneity in some analyses. Fortunately, this could be mostly explained by the meta-regression analyses including sex ratio and age mean; 2) impulsivity or trauma will hardly be a determinant factors, but when combined with polygenic risk scores [27] and with other predicting variables it might add important value; 3) the evidence summarized here is not useful to clinical practice yet, though any additional care in this sense should be certainly exploited in identifying individuals at risk; 4) the review process might have overlooked a few studies on the subject, although unlikely due to the standardized procedures.

The present study has provided an overview of the state-of-the-art research on childhood trauma and impulsivity and their association with suicidal behaviour and quantified their effects on suicide attempts. We hope these results can guide future research since the evidence regarding the influence of trauma on suicidal behaviour might be important for phenotype aetiology and as a candidate predictor.

Conflicts of interest? No

Acknowledgements

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Part III

“The Universe is under no obligation to make sense to you”

– Neil deGrasse Tyson

1 Discussion

1.1. The importance of biological psychiatry research

In Chapter 1 we showed how psychiatric drug sales have increased in Brazil in the last few years and estimated how it impacts the country economically. Needless to point out it will most likely also result in other associated country-wide issues, such as lack of productivity, and worsening of the already unequal socioeconomic scenario as higher income provides more access to quality healthcare.

In the first study in this thesis, we used drug sales as a proxy for psychiatric diagnosis. Of course, it is a sub-optimal measure since other factors could influence the sales of psychiatric disorders, such as differences in purchasing power. However, we have reasons to believe it is an underestimation of the actual scenario since we assessed only private records from pharmacy consumers. It is most likely that a bigger impact would be observed in the public sector.

We are also aware of several differences between the public and private sectors in Brazil. We found that escitalopram is one of the most sold antidepressants in the private sector. Conversely, it is rarely a first-option antidepressant to be offered free of cost in public facilities, where fluoxetine is more common due to its lower cost (de Sousa et al., 2018; Marinho et al., 2019). Cipriani and colleagues established Escitalopram as the most effective antidepressant (Cipriani et al., 2018), and many countries have switched their ranking for the availability of antidepressants based on it (G Patel et al., 2018; Jyotiranjan et al., 2021). Regardless of the response seen from authorities worldwide, there is a pressing need for further understanding of psychiatric disorders at the biological level in order to better predict, prevent, and treat these conditions.

1.2. The translatability of cross-disorder endophenotypes

In our study we introduce an effective methodology for identifying and testing consistent differentially expressed genes in psychiatric and neurological disorders. It focused on the overlap of markers between disorders and their connection to GWAS results. The approach involved meta-analyzing transcripts directly from individuals with disorders, offering real-world predictive insights. While this method may not identify the entire set of causal genes, it provides a freeze-frame of current characteristics, allowing for the identification of disorder consequences on the transcriptome. Future studies should explore different disorder development stages for improved biomarker identification, and the approach allows comparison with predicted changes in other studies to distinguish causal from consequential factors.

Contextualizing results involved comparing differentially expressed genes with those associated with GWAS outcomes, revealing overlaps in genes related to schizophrenia (SCZ), bipolar disorder (BD), and Alzheimer's (ALZ). A recent study on shared mechanisms across psychiatric and neurodegenerative diseases (Wingo et al., 2022) identified genes that intersect with our SCZ-ALZ overlap, suggesting shared molecular pathophysiology.

Our investigation explored shared genes among psychiatric disorders, revealing functional associations. SCZ-associated genes link with signaling pathways, proteolysis, endocytosis, and the cell cycle, while ALZ-associated genes associate with various pathways including the ribosome, oxidative phosphorylation, and cancer. Notably, the overlap between SCZ and ALZ, often overlooked in GWAS, becomes evident when exploring consequences and markers, such as neuroimaging

(Demirhan, 2018; Hassanzadeh et al., 2022; Noor et al., 2020), suggesting shared biology influenced by environmental factors or endophenotypes.

Examining gene expression locations in the overlap between ALZ and SCZ, besides constitutive expressions in peripheral tissues, reveals high expression in brain regions like the amygdala, putamen, hippocampus, substantia nigra, and caudate, indicating key changes in central nervous system (CNS) functions.

Some limitations of our study include reliance on original studies' quality and sample sizes, as well as the challenge of deriving CNS interpretations from peripheral markers.

Our findings deepen understanding of psychiatric disorders' molecular landscape, suggesting shared pathways across seemingly distinct conditions. Further investigations, including functional assays and validation studies, are crucial for extending these observations. The integration of diverse datasets and advanced analytical approaches continues to unveil the complex nature of psychiatric disorders, providing new avenues for targeted therapeutic interventions and precision medicine strategies.

1.3. The validity of animal models in psychiatry

In our first study, investigating the predictive validity of the spontaneously hypertensive rat (SHR) model of ADHD, we found that it is unable to represent the disorder as a whole, especially regarding the hyperactivity response to MPH. MPH is classically effective for hyperactivity in humans (Leffa et al., 2019) and it raises

questions as to the SHR model's usability to investigate other drugs for this phenotype. However, the SHR seem to mimic impulsivity and inattention well in terms of predictive validity. On the other hand, it is important to mention that the main issue regarding hyperactivity might be the way in which it is measured in animals, which is far from how we characterize it in humans. Nonetheless, one of the key points of discussion from this study (and hence the follow-up letters bringing up the issue) is that we should refrain from looking at animal models in psychiatry as true proxies for the disorder as a whole. It seems to be more advantageous and accurate to work with animal models for specific behaviours and symptoms and not complex presentations. They are important tools for drug discovery and molecular investigation since there are many restraints to conducting such experiments in patients. However, we should be careful as researchers when interpreting the data not to raise false analogies between species.

With this in mind, we proceeded to investigate other models, with the idea to bring better estimates and help guide researchers on their choice of models to be used for different behaviours and treatments. Major depressive disorder (MDD) is an interesting condition for this investigation since there are many animal models that include mainly environmental interventions (Czéh et al., 2018; Rana et al., 2022). This is possible because of MDD's relatively low heritability. We have included the majority of animal models for MDD that are induced by stress or olfactory bulbectomy and we are now mapping the behaviours and treatments with the most common antidepressants to each model. Therefore, one will be able to search for specific behaviours or drugs and see an estimate of which would be the best animal model to choose for their research question.

Another disorder for which there are many proposed animal models is autism spectrum disorder (ASD) (Das et al., 2019). We proceeded with the same methodology as for MDD models, however, separating the models into two groups: exposure-induced animal models and genetic models. Here we established a more descriptive protocol that can be followed in future endeavors (Panzenhagen et al., 2022). This research is still ongoing and hopefully will be finalized soon after this thesis.

1.4. Ways to tackle variability in psychiatry through neuroimaging analyses

As I hope it has become clear with the questions we have raised in this thesis, understanding how different psychiatric disorders are associated and overlap is key to understanding their biological underpinnings. This can be done at different levels as already described in the transcriptomic analyses that were compared to GWAS, TWAS, and PWAS studies. Another dimension that is certainly closer to the clinic is neuroimaging. Neuroimaging data is already extensively applied for diagnosing neurological disorders, such as Alzheimer's (Lei et al., 2019; Mandal & Perry, 2022). In psychiatry, however, results still fall short of being translatable to clinical settings or useful for diagnosis and treatment. Nonetheless, it is a promising piece to be included when understanding the puzzle of cross-disorder psychiatry. Since the neuroimaging field of research in psychiatry is still in its infancy, with relatively low sample sizes overall (Schmaal et al., 2020), we have focused on different approaches to try and tackle the variability in psychiatry that could also be applied to neuroimaging as it is.

In an endeavour to understand the state of the art of spectroscopy research on ADHD, we have conducted a systematic review and meta-analysis of the published findings. We showed that glutamate-glutamine imbalance in the medial prefrontal cortex seems to be associated with ADHD. Moreover, we discuss how the field would benefit from more comparable approaches, unifying voxels and measurements so that the different studies become more comparable and can be meta-analyzed to increase the chances of finding relevant associations.

In the second study investigating methods for neuroimaging, we have applied a biclustering algorithm adapted from transcriptomics in hopes of taking advantage of the high dimensional characteristic of these datasets. The adaptation of the method has been successful, resulting in four robust biclusters that seem consistent across the cortical thickness dataset. We also incorporated data from what we called "positive controls", which consisted of three independent samples of other neuropsychiatric disorders, namely schizophrenia, Huntington's disease, and Alzheimer's disease. There are two aspects here that I would like to highlight: 1) the positive controls served as a validation of the biclustering algorithm since they should be different enough from the MDD and healthy control samples to be at least roughly separated into some of the biclusters. This seemed to happen, strengthening the validity of the algorithm for structural neuroimaging; 2) from the results we gathered, it seems that the positive controls could prove useful in separating what is cross-disorder-related and what is specific from each condition. This happened once brain areas more strongly associated with Alzheimer's and SCZ drove the separation of some biclusters. This is very interesting in our opinion, and using positive controls in machine learning and clustering approaches can be useful when trying to dissect the

common and specific features belonging to each condition. It will be obviously dependent on how refined the characterization of samples is and on sample size, but it could prove to be a valuable practice going forward.

1.5. Biobehavioural markers of suicide across psychiatric disorders

Suicide behaviour encompasses a set of conditions widespread across psychiatric disorders and highly comorbid to several of them (Lönnqvist, 2021; Wasserman et al., 2021). I see the task of disentangling the differences between suicidal ideation, suicide attempt and death by suicide as partly cross-disorder in nature since there is a need to identify true predictors of it while accounting for confounding features that are most likely also associated with a set of symptomatologies in psychiatry. Thus, we have investigated environmental (personality and early-life trauma) and biological (inflammatory and candidate marker) factors contributing to suicide behaviour.

Since 2006 there has been no systematic review on personality traits and how they might be associated with suicide behaviour (Brezo et al., 2006). Additionally, since then, the body of literature has expressively increased, making it now possible to expand the methodological rigour and better report the results, including a meta-analysis. Impulsivity is widely regarded to be important for suicide attempts (Reich et al., 2019; Smaoui et al., 2021) and is most likely the key to the fact that most attempts are in samples of individuals with bipolar disorder and not depression (Herrera, 2018), as one would think from the high correlation between suicidal ideation and MDD (Chiang et al., 2022). However, ours is the first study to quantify this through a meta-analysis regardless of diagnosis, finally estimating the overall

effects. Moreover, neuroticism, psychoticism, and extroversion are highly associated with suicide attempts overall, but might not be informative within MDD because of their likely correlation with the disorder itself. We show that apart from impulsivity, personality does not seem to be a good predictor specifically within MDD. Our meta-analysis suggested that there are specific features of personality that are differentially associated with suicide attempts depending on the comorbid psychiatric condition. This should be taken into account in future studies to further dissect disorder-specific and disorder-independent associations in suicide behaviour as a whole.

When we evaluated the effects of early-life trauma on suicide attempts we found a significant effect of sexual abuse, physical abuse, emotional abuse and physical neglect on suicide attempts in the prisoners, and substance use disorder subgroups. Furthermore, regarding the lack of association between most early-life trauma dimensions within the MDD subgroup, we hypothesized that trauma as a whole might be already strongly associated with MDD, making the signal lost in the noise. This is the second time that we found behavioural features associated with suicide attempts are not informative within MDD individuals. We should be aware of that trend going forward to better design experiments that try to associate environmental factors with suicide behaviour.

When investigating inflammatory markers of suicide, we found that the protein expression of TNF-α was increased in the brain of people who died by suicide, but not suicide attempts. This is an interesting finding since TNF-α is an important pro-inflammatory marker, both an adipokine and cytokine (Chumakova et al., 2021). Moreover, suicide attempters showed lower levels of peripheral IL1B. IL1B is a pro-

inflammatory cytokine, which goes against the general idea of systemic inflammation being associated with suicidal behaviour (Lengvenyte et al., 2021). Nevertheless, IL1B has been linked to differential response to pain by mediating COX-2 expression, causing hyperalgesia when IL1B is overexpressed (Bergqvist et al., 2019; Nagura et al., 2019; Ohnishi et al., 2019). At the same time, there is evidence that chronic pain and its genetic associations are particularly associated with suicide attempts (Docherty et al., 2023; Fanelli et al., 2022). Although inflammatory markers are usually associated with other conditions, aetiologies and diseases, when combined with other predicting variables they might add important value; especially concerning identification and prognosis to avoid attempts and deaths.

The sialic acid binding Ig-like lectin 3 (CD33) protein is a transmembrane receptor that seems to enable protein phosphatase binding activity and sialic acid binding activity. It is located in several cellular components, including the Golgi apparatus; the external side of the plasma membrane; and the peroxisome (Rendina et al., 2020). CD33 has been widely studied in the context of cognition and Alzheimer's disease (Miles et al., 2019; Tortora et al., 2022; Wißfeld et al., 2021). Although depression is one of the most prevalent comorbidities in Alzheimer's disease (Chiba-Falek et al., 2020), there appears to be no study in the literature investigating the relationship between CD33 and depressive states or suicidal ideation. In our investigation, we showed that CD33 protein levels are higher in individuals with suicide attempts, independently from cognitive decline or depressive symptoms. We will continue to investigate this association by increasing sample size and trying to understand if there is also a connection between mRNA levels and genomic variants within CD33.

1.6. Conclusion

Exploring the significance of advancing research in biological psychiatry, this thesis provides valuable insights into the molecular landscapes of psychiatric disorders and potential therapeutic interventions. In the first chapter, we highlighted the increasing sales of psychiatric drugs in Brazil, which not only have economic implications but also draw attention to broader societal issues, emphasizing the urgent need for a deeper understanding of psychiatric disorders at the biological level. We also examined the translatability of cross-disorder endophenotypes, introducing an effective methodology for identifying differentially expressed genes across studies and revealing shared molecular pathophysiology across psychiatric and neurodegenerative diseases. By exploring gene expression in the overlap between schizophrenia and Alzheimer's, for instance, we introduce crucial insights into the role of the central nervous system in these disorders.

Additionally, we carefully examined the effectiveness of animal models in psychiatry and found that using spontaneously hypertensive rats as a comprehensive model for ADHD has its limitations. The research into animal models for major depressive disorder and autism spectrum disorder continues with the goal of improving researchers' choices based on specific animal behaviours and responses to treatments. It is important to exercise caution when using animal models as proxies for complex human disorders and to interpret the results carefully.

Moreover, we expand the role of neuroimaging analysis in understanding the biological basis of psychiatric disorders and how it can address the variability in psychiatry. We introduced novel approaches to tackle the complexity and variability in

psychiatry, including a systematic review and meta-analysis of spectroscopy research on ADHD and the application of a biclustering algorithm to neuroimaging data.

Furthermore, the analysis of biobehavioral indicators of suicide behaviour across mental disorders underscores the complex interplay between environmental and biological factors that contribute to suicide. The combination of a meta-analysis on personality traits and their correlation with suicide attempts, and research on the effects of early-life trauma and inflammatory markers, provides a nuanced comprehension of the multifaceted nature of suicidal behavior. The discovery of a potential link between CD33 protein levels and suicide attempts adds a new dimension to the exploration of biological markers in psychiatric research.

In essence, this thesis provides a comprehensive and multidimensional approach to unravelling the complexities of psychiatric disorders. The integration of biological, neuroimaging, and behavioural perspectives contributes to a holistic understanding, paving the way for future research in biological psychiatry.

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1. Protocols pertaining to the research in this thesis

1.1 Peripheral blood as tool to determine gene expression patterns in patients with psychiatric, neurological and other common disorders: a systematic review and meta-analysis protocol

1.2 Protocols for a systematic review and network meta-analysis comparing animal models of depression

1.3 Behavioral manifestations in rodent models of autism spectrum disorder: protocol for a systematic review and network meta-analysis

Animal review

1. * Review title.

Give the working title of the review. This must be in English. The title should have the interventions or exposures being reviewed and the associated health or social problems.

A systematic review and network meta-analysis of the predictive validity of the animal models of depression

2. Original language title.

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3. * Anticipated or actual start date.

Give the date when the systematic review commenced, or is expected to commence.

01/01/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

30/06/2021

5. * Stage of review at time of this submission.

Indicate the stage of progress of the review by ticking the relevant Started and Completed boxes. Additional information may be added in the free text box provided.
Please note: Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. Should evidence of incorrect status and/or completion date being supplied at the time of submission come to light, the content of the PROSPERO record will be removed leaving only the title and named contact details and a statement that inaccuracies in the stage of the review date had been identified.
This field should be updated when any amendments are made to a published record and on completion and publication of the review.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	Yes	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here (e.g. Funded proposal, protocol not yet finalised).

6. * Named contact.

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

Alana Castro Panzenhagen

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7. * Named contact email.

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9. Named contact phone number

Enter the telephone number for the named contact, including international dialling code.

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10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as ‘none’ if the review is not affiliated to any organisation.

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11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country are now mandatory fields for each person.**

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12. * Funding sources/sponsors.

Give details of the individuals, organisations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

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Taquari.

Grant number(s)

13. * Conflicts of interest.

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

None

14. Collaborators.

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

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Miss Gabriela Esper Kallás Lopes. Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto

15. * Review question.

Give details of the question to be addressed by the review, clearly and precisely.

What are the behavioral and memory effects of pharmacological treatments in rat and mouse animal models of depression?

Context and rationale

Provide a brief description of the context and rationale of the review, including information on the relevance of your review for human health (max 250 words).

Major depressive disorder (MDD) is a highly impairing condition that affects up to 364 million people worldwide. Appropriate treatment for MDD therefore poses a great health and also economic concern. In the US alone, it is estimated that the costs due to MDD impairing effects are as high as $210.5 billion per year. Moreover, approximately 50% of patients do not respond to first and second line pharmacological treatments, leading to overall low response rates. These factors raise major concerns in the field, pointing to the desperate need for antidepressant drug discovery. Animal models are key to understanding the pathophysiology of psychiatric disorders, but even more crucial to the investigation of new treatment approaches. Notwithstanding, models are not always ideal and should follow a set of rules and validities for them to be accepted as reliable tools. Although some authors have stated that there are several validities to be fulfilled, they are commonly still divided in three main dominia suggested primarily by Willner in 1984; these are construct, face, and predictive validity. In the herein focused predictive validity, animals should respond to well-documented treatments in clinical practice in a similar way as patients do. In this case, it means the reduction of depressive-like behaviors. Taking all this into account, in this systematic review and network meta-analysis, the consistency of the predictive validity of animal models of MDD will be tested through the evaluation of the behavioral responses of those models to pharmacological treatments.

16. * Searches.

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

The databases MEDLINE via PubMed, Web of Science, and Scopus will be searched. No restrictions will be imposed on language or publication period.

17. URL to search strategy.

Give a link to the search strategy or an example of a search strategy for a specific database if available (including the keywords that will be used in the search strategies).

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Human disease modelled.

Give a short description of the disease, condition or healthcare domain being modelled.

Major depressive disorder (MDD) is a highly impairing condition that affects up to 364 million people worldwide. Appropriate treatment for MDD, therefore, poses great health and also an economic concern. In the US alone, it is estimated that the costs due to MDD impairing effects are as high as $210.5 billion per year. Moreover, approximately 50% of patients do not respond to first and second-line pharmacological treatments, leading to overall low response rates.

19. * Animals/population.

Give summary criteria for the animals being studied by the review, e.g. species, sex, details of disease model. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

The following rat or mouse models of depression: olfactory bulbectomy, chronic unpredictable mild stress, chronic mild stress, mild stress, acute stress, social defeat, learned helplessness, and restrain stress. All age and gender groups will be included.

Exclusion criteria:

Studies that do not include at least of the animal models cited above.

20. * Intervention(s), exposure(s).

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed (e.g. dosage, timing, frequency). Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Any pharmacological intervention added or not by a second pharmacological intervention.

Exclusion criteria:

Studies that do not include pharmacological interventions or only have groups in which the pharmacological intervention is added by a non-pharmacological intervention.

21. * Comparator(s)/control.

Where relevant, give details of the type(s) of control interventions against which the experimental condition(s) will be compared (e.g. another intervention or a non-exposed control group). Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

A control/vehicle or any other pharmacological intervention.

Exclusion criteria:

Other non-pharmacological interventions.

22. * Study designs to be included.

Give details of the study designs eligible for inclusion in the review. If there are no restrictions on the types of study design eligible for inclusion, or certain study types are excluded, this should be stated. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

All designs will be included

Exclusion criteria:

None

23. Other selection criteria or limitations applied.

Give details of any other inclusion and exclusion criteria, e.g. publication types (reviews, conference abstracts), publication date, or language restrictions.

24. * Outcome measure(s).

Give detail of the outcome measures to be considered for inclusion in the review. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Behavioral or memory test outcomes.

Exclusion criteria:

Studies that do not perform behavioral ou memory tests in the model/intervention groups included.

25. N/A.

This question does not apply to systematic reviews of animal studies for human health submissions.

26. * Study selection and data extraction.

Procedure for study selection

Give the procedure for selecting studies for the review, including the screening phases (title and/or title-abstract and/or full-text), the number of researchers involved, and how discrepancies will be resolved.

1) pre-screening based on title and abstract

2) full-text screening of the eligible articles

Prioritise the exclusion criteria

Multiple exclusion criteria may apply to an abstract/paper, which can cause discrepancies between reviewers in the reason for exclusion recorded. To avoid this, it is helpful to prioritize the exclusion criteria (e.g. 1) not an animal study; 2) not a myocardial infarction model, etc.) and record the highest ranking applicable criterion as the reason for exclusion. Please sort the exclusion criteria defined in questions 19 to 24. If

applicable, do so for each screening phase.

Selection phase 1:

1. The study is a review, letter, or commentary
2. The study does not include rats or mice
3. The study does not include one of the chosen models
4. The study does not include any pharmacological intervention
5. The study does not include a second group with no treatment, vehicle, or intervention with another drug.
6. The study does not perform any type of behavioral or memory test with all included experimental groups

Selection phase 2:

1. The study is a review, letter, or commentary
2. The study does not include rats or mice
3. The study does not include one of the chosen models
4. The study does not include any pharmacological intervention
5. The study does not include a second group with no treatment, vehicle, or intervention with another drug.
6. The study does not perform any type of behavioral or memory test with all included experimental groups

Methods for data extraction

Describe methods for data extraction, including the number of reviewers performing data extraction, extraction of data from text and/or graphs, whether and how authors of eligible studies will be contacted to provide missing or additional data, etc.

First extraction from text and tables, then from graphs and figures using WebPlotDigitizer v 4.3. When the information required is not available, the authors of the original studies will be contacted. If no answer is received in two months the records will be excluded from the analysis. All data will be extracted independently by two reviewers. Discrepancies will be resolved by a third reviewer data extraction.

Data to be extracted: study design

Specify the data to be extracted related to characteristics of the study design, e.g. controlled versus cross-over, number of experimental groups, etc.

Controlled trial or cross-over, number of experimental groups, sample sizes.

Data to be extracted: animal model

Specify the data to be extracted related to characteristics of the animal model, e.g. species, sex of the animals, etc.

Species, gender, type of disease induction, age of the animal upon induction and outcome assessment.

Data to be extracted: intervention of interest

Specify the data to be extracted related to characteristics of the intervention of interest, e.g. dose, timing, etc.

Type of intervention, dosage, timing, duration, route of administration.

Data to be extracted: primary outcome(s)

Define the primary outcome measure(s). For each outcome measure, specify in which format data will be extracted, including the eligible units of measurement, and data type (continuous/dichotomous). A description of any other manipulation or transformation of the extracted data that is planned may be included.

For behavioral or memory tests outcome measures: mean, standard deviation (SD) by group, and sample size. Or other effect measures and p-values when the mean and SD are not available.

Data to be extracted: secondary outcome(s)

Define the secondary outcome measure(s). For each outcome measure, specify in which format data will be extracted, including the eligible units of measurement, and data type (continuous/dichotomous). A description of any other manipulation or transformation of the extracted data that is planned may be included.

None

Data to be extracted: other

Specify any other data or study characteristics to be extracted, e.g. bibliographical details, such as author, year and language.

First author, year of publication, journal name, DOI.

27. * Risk of bias and/or quality assessment.

State whether and how risk of bias and/or study quality will be assessed. Assessment tools specific for pre-clinical animal studies include SYRCLE's risk of bias tool and the CAMARADES checklist for study quality

No risk of bias and/or quality assessment planned

No

By use of SYRCLE's risk of bias tool

Yes

By use of SYRCLE's risk of bias tool adapted as follows:

No

By use of the CAMARADES checklist for study quality

No

By use of the CAMARADES checklist for study quality, adapted as follows:

No

Other criteria, namely

No

Method for risk of bias and/or quality assessment

Give the procedure for the risk of bias and/or quality assessment, including the number of reviewers

involved, their contribution, and how discrepancies will be resolved.

Two independent trained reviewers will assess the risk of bias assessment of each study. Discrepancies will be resolved by discussion between reviewers or assessment by a third reviewer when necessary.

28. * Strategy for data synthesis.

Planned approach

For each outcome measure, specify whether a quantitative or narrative synthesis is planned and how this decision will be made.

For all outcome measures the descriptive summary and effect sizes will be compared qualitatively. A meta-analysis will be conducted whenever there are at least three studies with the same design reporting data for the same animal model, drug, comparison group, and type of behavioral or memory test.

If a meta-analysis is planned, please specify the following:

Effect measure

For each outcome measure, specify the effect measure to be used (e.g. mean difference, odds ratio etc.).

Mean or median and standard deviation or interquartile interval when continuous and incidence/percentage in each group when dichotomous outcomes of all behavioral and memory tests applied in the original records.

Effect models

For each outcome measure, specify the statistical model of analysis (e.g. random-effects or fixed-effect model).

Standardised mean difference will be used and odds or risk ratios when the first is not a possibility. A random effects model will be used in order to account for heterogeneity.

Heterogeneity

Specify the statistical methods to assess heterogeneity (e.g. I^2, Q). For further guidance please refer to the introduction and practical guide to pre-clinical meta-analysis.

I^2 and Cochran Q statistics

Other

Specify other details of the meta-analysis methodology (e.g. correction for multiple testing, correction for multiple use of control group).

Bonferroni post hoc analysis will be conducted according to the number of tests performed. Whenever one control is used multiple times the number will be adjusted by dividing the total sample size by the number of times it is used in the analysis. Additionally, study and research group (same last author) will be included as an additional random variable to account for neglected heterogeneity. A network meta-analysis will also be performed including all drugs in

each model.

29. * Analysis of subgroups or subsets.

Subgroup analyses

Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.

Subgroup meta-analyses (meta-regression or stratified regression) according to the following potential heterogeneity introducing variables: species, strain, sex, drug delivery route, drug dosage, age and/or weight of animals.

Sensitivity

For each outcome measure, specify any sensitivity analyses you propose to perform.

Sensitivity analyses will be performed as follows: a) following the Jackknife method for all main meta-analysis groups. b) according to risk of bias quality score of original studies (poorly classified studies in the 25% bottom quartile will be excluded). c) in case of doubts regarding the assumptions and interpretation of previous analyses.

Publication bias

Specify whether an assessment of publication bias is planned. If applicable, specify the method for assessment of publication bias.

Funnel plotting and Egger's regression test.

30. * Review type.

Type of review

Animal model review
No

Experimental animal exposure review
No

Pre-clinical animal intervention review
Yes

31. Language.

Select each country individually to add it to the list below, use the bin icon to remove any added in error.
English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved.

Brazil

33. Other registration details.

List other places where the systematic review protocol is registered. The name of the organisation and any unique identification number assigned to the review by that organisation should be included.

34. Reference and/or URL for published protocol.

Give the citation and link for the published protocol, if there is one.

Give the link to the published protocol.

Alternatively, upload your published protocol to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

The results of this study will be published in leading peer-reviewed journals of the area and also presented at relevant national and international conferences.

Do you intend to publish the review on completion?

No

36. * Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line.

depression; animal models; antidepressants; behavior effects; memory

37. Details of any existing review of the same topic by the same authors.

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38. * Current review status.

Review status should be updated when the review is completed and when it is published.
Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any further information the review team consider relevant to the registration of the review.

40. Details of final report/publication(s) or preprints if available.

This field should be left empty until details of the completed review are available OR you have a link to a preprint. Give the full citation for the preprint or final report or publication of the systematic review.

Give the link to the published review.

PERIPHERAL BLOOD AS TOOL TO DETERMINE GENE EXPRESSION PATTERNS IN PATIENTS WITH PSYCHIATRIC, NEUROLOGICAL AND OTHER COMMON DISORDERS: A SYSTEMATIC REVIEW AND META-ANALYSIS PROTOCOL

Alana Castro Panzenhagen[1], Alexsander Alves-Teixeira[1], Martina Schroeder Wissmann[2], Carolina Saibro Girardi[1], Lucas Santos[1], Alexandre Kleber Silveira[1], Daniel Pens Gelain[1], José Cláudio Fonseca Moreira[1,3,4]

ABSTRACT

Introduction Common diseases are influenced by a variety of factors that can enhance one person's susceptibility to developing a specific condition. Complex traits have been investigated in several biological levels. One that reflects the high interconnectivity and interaction of genes, proteins and transcription factors is the transcriptome. In this study, we disclose the protocol for a systematic review and meta-analysis aiming at summarizing the available evidence regarding transcriptomic gene expression levels of peripheral blood samples comparing subjects with psychiatric, neurological and other common disorders to healthy controls.

Methods and analysis The investigation of the transcriptomic levels in the peripheral blood enables the unique opportunity to unravel the etiology of common diseases in patients *ex-vivo*. However, the experimental results should be minimally consistent across studies for them to be considered as the best approximation of the true effect. In order to test this, we will systematically identify all transcriptome studies that compared subjects with common disorders to their respective control samples. We will apply meta-analyses to assess the overall differentially expressed genes throughout the studies of each condition.

Ethics and dissemination The data that will be used to conduct this study are available online and have already been published following their own ethical laws. Therefore this study requires no further ethical approval. The results of this study will be published in

leading peer-reviewed journals of the area and also presented at relevant national and international conferences.

Strengths and limitations of this study

- ➢ We present a new and systematically centered method to assess the overall effect of transcriptomic levels in the blood of subjects with common conditions.
- ➢ Meta-analyses are a robust statistical method to assess effect sizes across studies.
- ➢ The analysis is limited by the availability of studies, as well as their quality and comprehensiveness.
- ➢ Subgroup and meta-regression analyses will be also limited by the amount and quality of sample characterization variables made available by original studies.

INTRODUCTION

Common human diseases are generally multifactorial, being influenced by both environmental and genetic factors that may interact on producing a certain phenotype [1,2]. Those diseases, related to the central nervous system (CNS) or otherwise, are usually associated with inflammation as well, which raises the possibility of systemic causal or resulting effects [3–9]. In neurodegenerative diseases as Parkinson's or Alzheimer's this association has even inspired the coining of the term "inflammaging" [10–12].

Diseases as diabetes, hypertension, psychiatric disorders or even continuous phenotypes, as body mass index (BMI) and height, are usually classified under the umbrella of complex traits. Those traits are influenced by an ensemble of different genetic variants and are therefore polygenic and "complex" [1,13]. This theory had already been partially developed by Fisher in 1919 [14]. Fisher's "infinitesimal model" takes into account that the contribution of each variant and gene becomes smaller as the number of genes associated with a trait grows larger, resulting in a normally distributed phenotype [15].

More recently, researchers have proposed that complex traits are not only polygenic, but also "omnigenic". The hypothesis of the omnigenic model states that all genes expressed in disease-relevant cells can actually affect core disease-related genes because of highly interconnected regulatory networks. This idea would suggest that a great amount of the heritability of those traits can also be explained by the influence of genes outside core pathways [13]. While this theory is nonetheless important to understand the etiology of complex traits, when referring ourselves to common diseases this should be translated into something that can be also applied therapeutically. In this sense, the continuing research on which pathways are mainly responsible for common diseases susceptibility remains crucial.

Multi-omics data has been vastly produced in the last few years from different types of human tissue and in several biological levels, such as in the genome, transcriptome, proteome, and even the microbiome. All these layers interact with each other and assessing a full perspective of the omics data has been one of the greatest challenges of today's

bioinformatics and biostatistics research [16–18]. However, the evidence is not even consolidated or provenly consistent across different studies within each omic. One of the most robust and widely applied statistical methods to solve similar problems in epidemiology is meta-analyzing data subsequently to a well-conducted systematic review of the literature in order to estimate overall effect sizes [19]. Having in mind that the interacting protein networks that influence diseases are mainly related to the regulation of gene expression, we have chosen to focus our study on the transcriptome level. Moreover, as tissue sample availability that is representative of common diseases is scarce, due to high heterogeneity and complexity, the investigation of peripheral factors becomes imperative. This is especially true for brain disorders and, as stated before, systemic effects might bring valuable insight to the matter. Hence, we have chosen to investigate transcriptomic differences in the most abundantly collected peripheral tissue: blood; which will hopefully solve inconsistencies across the field.

This study, therefore, aims at summarizing the available evidence regarding transcriptomic gene expression levels of blood samples comparing subjects with psychiatric, neurological and other common disorders to healthy controls. With this approach, we intend to identify the overall scenario regarding mRNA expression levels profile of complex traits, hopefully clarifying major inconsistencies in the field. Here we disclose the protocols for a systematic review and meta-analysis with the purpose of standardizing our methodology in order to increase quality and comprehensiveness, making it possible to be meticulously replicated hereafter.

METHODS AND ANALYSIS

For the present study report, we have followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) [20].

Eligibility criteria

We will include studies that compared the gene expression levels in blood of patients with psychiatric, neurological, and other common disorders with healthy controls. We will include experimental studies that assessed gene expression levels by coding mRNA quantification through both microarray and RNA-sequencing methodology. Only studies with a specified control or resilience group will be considered. We will include randomized clinical trials (RCTs), cohort, and / or case-control studies, provided that they present a control group without specific and unique treatment administration. Studies should have collected blood samples (leukocytes, lymphocytes, peripheral blood mononuclear cells (PBMCs)) from patients affected with the disorder and healthy (or resilient) controls. All types of diagnosis will be included regardless of diagnosis manual or tool. Nevertheless, the diagnosis manual/tool will be included as variables in later sensitivity analyses. We have not imposed any restrictions regarding publication date, language or methodological quality, as well as age, sex or ethnicity of participants.

The following exclusion criteria will be applied: i) studies with a sample that comprises only genetically related individuals (*e.g.* family-based studies); ii) studies without original data; iii) studies in which every case has a unique type of comorbidity; iv) studies without healthy (or resilient) controls; v) studies in which remitters are referred to as healthy controls; vi) studies in which the blood samples received any kind of treatment *ex vivo* before microarray analysis or RNA-sequencing; and vii) studies in which all the case sample was receiving a specific type of drug that is not listed as "treatment as usual", such as the mainly prescripted drug or class of drugs.

Search and study identification

Studies will be identified through an online search using two different electronic databases: Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/gds) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). No search filters will be used and the reference list of published studies that were included will be searched for additional independent records. A further database search will be conducted for studies published since the last search date in GEO.

Search strategies will include subject headings for each disorder/disease (**Table 1**), indication for studies with human subjects, and blood. Full search strategies will be provided upon the final publication of the completed study.

Study selection

Study selection and inclusion will be performed in two steps. Firstly through title screening evaluation and secondly, by full GEO / ArrayExpress description details perusal. Both will be conducted by at least two independent reviewers (ACP, ATT, MSW, CSG, LS or AKT). Disagreements will be resolved in consultation with a third reviewer (ACP or JCFM).

Data collection

The descriptive data of each study will be extracted by at least two independent reviewers (ACP, ATT, MSW, CSG, LS or AKT). Discrepancies will be resolved by a third reviewer (ACP or JCFM). Data will be extracted as follows: description of studies (sample size, age mean and standard deviation, gender frequencies in the sample, ethnicity and country/region of origin, date of data collection, type of platform used for transcriptome analyses, diagnostic manual / tool, and data on medication or psychotherapy being used). The transcriptomic outcome data (raw and/or normalized gene expression data) will be assessed through the *GEOquery* R package [21] or by downloading directly from the GEO

and/or ArrayExpress websites. Whenever there is missing data, the authors of the original studies will be contacted. If no response is received in two months, the study will be excluded from the analyses.

Outcome measures

Our primary outcome measure is mRNA levels assessed through microarray (or any other kind of specific array) or by RNA-sequencing methods. Those measures are available in full in the online repositories, and we will, therefore, be able to have access to raw data most of the time. Normalized data will not be a problem in the analyses, as we can convert raw data into normalized if the information on which method was used is provided. Whenever only the mean and standard deviation/error values are provided, the meta-analysis will still remain possible after appropriate measurement conversions.

Bias assessment

Risk of bias will be assessed by two independent raters (ACP and MSW) through the NIH Quality Assessment of Case-Control Studies tool [22]. Any discrepancies in the assessment will be resolved through discussion and, where necessary, by a third rater (JCFM). Studies are rated as presenting high, unclear or low risk of bias. The NIH Quality Assessment tool comprises 12 questions about methodology quality of original case-control studies. However, we chose not to evaluate items 9 to 11, since they are not an issue in transcriptomic studies. This is because the exposure assessment, in this case, is performed by the automatic measurement of microarray or RNA-sequencing of samples. Furthermore, publication bias will be assessed using funnel plots and Egger's regression test [23].

Data synthesis

Gene expression data will be collapsed into one variable for each disorder through the *MetaMA* R package [24] and the overall differentially expressed genes computed. Subgroup analyses will be conducted for every study descriptive characteristics (e.g. gender, age, diagnostic tool, etc.) in which groups of at least three studies are formed.

Heterogeneity and subgroup analyses

Heterogeneity between studies will be assessed using both the χ^2 and the I^2 tests, in which a p-value ≤ 0.1 will be considered statistically significant in the first, and I^2 values of 25%, 50% and 75% will be considered as low, moderate and high heterogeneity, respectively. We will conduct separate analyses of studies in groups by gender, age, and ethnicity of subjects when the data is available. We will also separate studies by disorder/disease subtypes or symptom grouping when pertinent, and by diagnostic tool when the data is available. Whether studies present high heterogeneity, we will also perform meta-regression analyses when enough data is available.

Sensitivity analyses

Sensitivity analyses will be also performed in order to evaluate result differences related to the effects of individual or specific groups of studies. The following sensitivity analysis are planed: i) the jackknife method, a common procedure used to test the stability of the outcome after excluding one result at a time [25], ii) excluding studies without formal diagnostic criteria from the analyses, and iii) excluding studies presenting a concerning risk of bias (the 25% worst ranked studies). The exploratory objectives of our sensitivity analyses will be to i) observe if any study skews the overall result, ii) examining the impact of studies without formal diagnostic criteria, and iii) evaluate the impact of studies with a high risk of bias.

Pilot study

Each step of the analyses described above will be primarily tested in a pilot study, with a selected set of conditions, which are indicated by an asterisk in **Table 1**. We have chosen this set ambitioning to have more data on a broad variety of common conditions. This pilot study was thought of with the purpose of detecting possible misdesigns in the methodology and spot eventual issues that may emerge during the execution of the systematic review and/or meta-analysis.

ETHICS AND DISSEMINATION

The data that will be used to conduct this study are available online and have already been published following their own ethical laws. Therefore this study requires no further ethical approval. The results of this study will be published in leading peer-reviewed journals of the area and also presented at relevant national and international conferences.

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Authors' contributions

ACP and JCFM conceived the study. ACP designed and drafted the protocol and ATT, MSW, CSG, LS, AKT, DPG, and JCFM critically revised it. ACP, ATT, MSW, CSG, LS, and AKT will conduct the paired study search and paired inclusion of studies. Data extraction will be performed by ACP, AAT, and MSW. ACP will perform the analyses and draft the systematic review and meta-analysis manuscript and all authors will revise it. All authors have contributed to and approved the final protocol paper, as well as agreed on submission to BMJ Open.

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Competing interests statement

The authors declare no competing interests.

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Table 1 Common conditions and phenotypes that will be included in the study

Psychiatric disorders	**Neurological disorders**	**Immune-related diseases**	**Common diseases and phenotypes**
Attention-deficit/hyperactivity disorder	Alzheimer's disease*	Allergy	Body mass index*
Anorexia	Epilepsy	Asthma	Coronary artery disease*
Anxiety disorders	Stroke	Celiac disease	Diabetes*
Autism Spectrum Disorder*	Migraine disorders	Crohn's disease	Height*
Bipolar disorder	Multiple sclerosis	Lupus	Intelligence quotient
Major depressive disorder*	Parkinson's disease*	Psoriasis	Myocardial infarction
Obsessive compulsive disorder		Rheumatoid arthritis	Schooling years
Post-traumatic stress disorder			
Schizophrenia*			
Tourette syndrome			

*Phenotypes that will be used in the pilot study

Systematic Reviews

PROTOCOL

Open Access

Behavioral manifestations in rodent models of autism spectrum disorder: protocol for a systematic review and network meta-analysis

Alana Castro Panzenhagen[1,2], Amanda Cavalcanti[1,3], Dirson João Stein[1,4], Ligia Lins de Castro[1,5], Mailton Vasconcelos[1,6], Mariana Boechat Abreu[1,5], Roberto Farina Almeida[1,7], Leandro José Bertoglio[1,8] and Ana Paula Herrmann[1,9*]

Abstract

Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition associated with severe social communication, interaction, and sensory processing impairments. Efforts to understand its etiology and pathophysiology are crucial for improving treatment and prevention measures. Preclinical models of ASD are essential for investigating the biological mechanisms and should present translatability potential. We aim to evaluate the consistency of the most commonly used rodent models of ASD in displaying autistic-like behavior through a systematic review and meta-analysis.

Methods: This review will focus on the most frequently used autism models, surveying studies of six genetic (*Ube3a*, *Pten*, *Nlgn3*, *Shank3*, *Mecp2*, and *Fmr1*), three chemically induced (valproic acid (VPA), lipopolysaccharide (LPS), and polyinosinic:polycytidylic acid (poly(I:C))), and one inbred model (BTBR T+ Itpr3tf/J mouse strain). Two independent reviewers will screen the records. Data extraction of behavioral outcomes and risk of bias evaluation will be performed. We will conduct a meta-analysis whenever at least five studies investigate the same model and behavioral outcome. We will also explore the heterogeneity and publication bias. Network meta-analyses are planned to compare different models.

Discussion: By shortening the gap between animal behavior and human endophenotypes or specific clinical symptoms, we expect to help researchers on which rodent models are adequate for research of specific behavioral manifestations of autism, which potentially require a combination of them depending on the research interest.

Systematic review registration: PROSPERO CRD42021226299.

Keywords: Animal model, Autism, Autism spectrum disorder, Rodent model, Systematic review, Network meta-analysis, Protocol

Background

Autism spectrum disorder (ASD), or simply autism, is a neurodevelopmental condition characterized by severe impairments in social communication, interaction, and sensory processing, often accompanied by repetitive behaviors and restricted interests. At the most severe

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level, patients with ASD may also present varying levels of intellectual disability. Moreover, attention deficit/hyperactivity, anxiety, major depressive disorders, and epilepsy are relatively frequent comorbidities in people with autism, making their therapeutic management further challenging [1, 2].

The worldwide prevalence of autism is below 1% [3], yet its diagnosis and identification have dramatically increased in the last decades. Epidemiological studies indicate significant variability in prevalence globally, although it is remarkably higher in high-income countries. ASD is highly heritable, occurs in all ethnic and socioeconomic groups, and is over four times more common among males than among females [4].

Despite extensive clinical and preclinical studies, the etiology and pathogenesis of ASD remain unclear. It has gradually led scientists to use in vitro and in vivo animal models to uncover the causes of ASD and improve treatment. This endeavor provided advances in understanding ASD pathophysiology, shedding light on new targets for therapy. Overall, animal models rely on a single gene dysfunction, epigenetic manipulations, or environmental interventions that ultimately influence the expression of risk genes. Although the behavioral assays mimicking specific symptoms are excellent translational research tools to investigate and identify the biological mechanisms underlying the core features of ASD [5], there has been no systematic investigation on whether they are interchangeable or complement each other. Considering the heterogeneity and complexity of ASD, it is hypothesized that a combination of various animal models is necessary to recapitulate its main behavioral manifestations. As a result, compiling information by reviewing and comparing existing data may more effectively guide future researchers' efforts.

Three criteria have been considered for assessing the validity of a given animal model, namely, face validity (i.e., does the model exhibit the salient features of the condition in humans?), construct validity (i.e., is the condition arising from the same biological background?), and predictive validity (i.e., will the model respond to well-established treatments?) [6]. Das et al. [7] present a manually curated annotation tool that used to be updated quarterly and gathered information on ASD research for circa 10 years. AutDB (https://gene.sfari.org/database/animal-models/genetic-animal-models/) is a database platform. In 2019, as reported by Das et al., there were 787 articles identified and 18 behavioral phenotypes most frequently evaluated in genetic, induced, and inbred ASD rodent models. These behaviors were classified as core and auxiliary. Core behaviors are represented by social interaction, ultrasonic vocalization, and repetitive behavior. This classification was proposed by Basu et al. [8] when creating AutDB based on their similarity to the human phenotype (impairment related to social interaction, communication, and repetitive behavior). Das et al. [7] (from the same research group) provide an update regarding the annotated data so far. In order to summarize the evidence from the original studies annotated in AutDB, the researchers separated the results into qualitative terms to indicate the direction of change compared to control animals. Regarding the core categories, "no change" represented at least 36% of the annotations in the autism models used in the analyzed articles. In the auxiliary categories, the lack of changes was even more expressive, reaching 70%.

AutDB is a manually curated tool; however, its methodology for search strategy and selection of studies is far from a systematic review. Moreover, the platform started with a focus on genes for ASD. The search consisted of the terms "gene" AND ("autism" OR "autistic") restricted to the titles and abstracts of the publications for retrieval, only on PubMed. Therefore, we understand that the literature on ASD-like animal models is expressively larger than the one reported in AutDB. Furthermore, there is no quality control or assessment of the risk of bias in the studies annotated, nor is there any data extraction process for conducting meta-analyses. In view of this gap, and considering that Basu et al. [8] and Das et al. [7] are the only reports on attempts to gather the literature on ASD-like animal models, we intend to conduct a thorough systematic review to evaluate the validity and compare different rodent models of ASD available to date. Nonetheless, we based our inclusion criteria on Das et al. [7] report to choose the likely most frequently used models and evaluated behaviors.

This review aims to test model face validity and assess whether the most commonly used rodent ASD models reproduce behavioral phenotypes related to the core symptoms of the condition in humans. Moreover, we mean to understand what secondary phenotypes are also altered in such models. Finally, we will hopefully show which rodent models are more suitable for research in this area, enabling the assertion of models or compilation of models that present specific behavioral manifestations of ASD.

Methods

The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement [9] was followed to elaborate this protocol, which was registered in PROSPERO under registration number CRD42021226299. We intend to answer the following question through this study: what are the differences and similarities between the behavioral manifestations

commonly assessed in the most widely used induced, inbred, or genetic rodent ASD models?

Model selection

This review will focus on the most frequently used ASD rodent models according to the estimate by Das et al. [7], which was based on data retrieved from AutDB. This comprehensive and integrated database collates in-depth annotation of genetic and non-genetic ASD models [8]. A frequency cutoff of at least ten references of either mouse or rat studies was set, leading to the selection of six genetic model groups based on the manipulated genes (*Ube3a*, *Pten*, *Nlgn3*, *Shank3*, *Mecp2*, and *Fmr1*), three chemically induced models (valproic acid (VPA), lipopolysaccharide (LPS), and polyinosinic:polycytidylic acid (poly(I:C))), and one inbred model (BTBR T+ Itpr3tf/J mouse strain). The number of references refers directly to what is reported in Das et al. [7], not necessarily corresponding to the number of studies or experiments. This corresponds to the "number of reports" in AutDB (https://gene.sfari.org/database/animal-models/genetic-animal-models/). This evidence has been used to choose the models to be included in the present systematic review. However, none of the data will be retrieved from the annotation tool. A full systematic review will be performed since the methodology used in AutDB does not compare to it in terms of criteria, accuracy, or inclusion of the whole literature on the matter.

Eligibility criteria

We will include preclinical studies evaluating behavioral outcomes in selected ASD models in mice and rats (*P-participants/individuals*). The *E-exposure* will include alterations of the whole organism at the DNA level for the genetic model groups based on the manipulated genes, without restriction for temporally controlled conditional models. We will include genetic alterations, such as knock-down, knock-in, or heterozygous models. In this sense, we will group the models as to the risk gene function (increased or decreased gene expression). Although we are confident there will be enough studies by model (we expect to find more studies through a systematic review than AutDB did), we also would like to see the results by groupings, which would make sense due to their biological nature. However, if heterogeneity is too high because of the variability in model development, we will refrain from making any conclusions from these analyses. Only interventions (*E-exposure*) administered prenatally or postnatally until weaning day (PND21) will be included for the induced models. Only studies with a comparison group (*C-comparison*) will be included; these will include control (without any intervention), sham (same staged intervention with a vehicle application or no actual induction), and wild-type (background genetic architecture). Any behavioral (*O-outcome*) related to the following broad categories will be considered: social or repetitive behavior, communication, emotion, learning and memory, and sensory and motor function (seizure-related behavior will not be included). There will be no date or language restrictions.

Any publication that does not include original data will be excluded (e.g., review, letter, editorial, comment). We will also exclude studies (1) conducted in species other than rats or mice or purely ex vivo, in vitro, or in silico; (2) without a comparison group (model control); (3) using cell- or tissue-specific genetic models; (4) in which VPA, LPS, or poly(I:C) was not administered prenatally or postnatally until the weaning day (or PND21); (5) in which the groups of interest were subjected to an additional experimental intervention (e.g., stress, rescue treatment); and (6) lacking the report of a behavioral outcome in the categories of interest.

Search strategy

Studies will be identified through a literature search using three electronic databases: MEDLINE, Web of Science Core Collection, and Scopus. The basic combination of search concepts consists of (((Genetics or strain ASD model terms) OR ((Neonatal developmental terms) AND (Induced ASD model terms))) AND (Animal model identification terms). The detailed combination of search terms constructed for each database is shown in Additional file 1. No search filters are going to be used.

Report identification and selection

The identification and selection of reports (both for abstract and full-text screening) will be performed by nine reviewers using a web-based/smartphone application systematic research tool: Rayyan (Rayyan Systems Inc.). After gathering the search results from the three selected databases, duplicates/triplicates will be removed manually with Rayyan's automatic suggestions. Next, the list of unique hits will be randomly split into sets. Each set will be evaluated by a pair of independent reviewers, with a third reviewer as a tiebreaker for conflict cases, ensuring that at least two reviewers evaluate each identified report. The teams of reviewers will be formed at random, and each one will be combined with at least four other reviewers in four sets of unique studies to guarantee diversity in the decision-making style of the report selection. As reviewers will also serve as tiebreakers in other groups, meetings will be held regularly throughout the selection process to discuss identified error biases or address specific cases; this approach will unify decision rules across all reviewers.

The report selection and inclusion will be performed with a two-step screening process. Initially, the decision will be based on the title and abstract, and, if necessary, a full-text screening will be performed to include a report. The full-text review step will also be performed if an exclusion criterion is not observed in the first step.

Data collection

Five reviewers will extract all data, and a training procedure will be implemented to assure standardization of the process. The quality control procedures will be implemented in the early extraction stages. During the pilot phase of data collection, we expect to determine common variables for the more frequent phenotypic analysis, reducing data amount and facilitating the training for homogeneous and reliable data extraction. When the information required is not available, the corresponding author or the first and last authors of the original studies will be e-mailed. If no answer is received in 2 months, the records will be excluded from the analysis. Data and tables with relevant information will be accessed manually. Information of graphs and figures will be extracted using the WebPlotDigitizer version 4.3 software.

The following data will be extracted: study design (controlled trial or cross-over, number of experimental groups, and sample sizes) and animal model (species, strain, sex, type of disease induction, age of the animal upon induction, age at measurements, number of control groups, type of housing after weaning, and outcome assessment). In the case of induced models, intensity, dose, and administration route will be assessed. All reported measures will be extracted for the tests described in the "Outcomes" section.

The summary statistics to be extracted are mean, standard deviation (SD) by group, sample size when data are continuous, or percentage and sample size when data are dichotomous. Other effect measures will be extracted when the mean and SD or percentage are not available. We will recheck a random sample of 10% of studies and, based on the results, cross-check all the remaining data only for the variables where the highest proportion of mistakes are observed ("tricky variables").

Outcomes

A complete profile of behavioral outcomes in rodent ASD models adapted from AutDB will be used in this study. We will include tests of social memory, social interaction, social approach, self-grooming, repetitive digging, ultrasonic vocalization, exploratory activity, anxiety, spatial reference memory, spatial learning, object recognition memory, cued or contextual fear conditioning, motor coordination and balance, general locomotor activity, startle response, sensorimotor gating, and pain or nociception. A complete description of each behavioral category is listed in Additional file 2, where the phenoterms and tests, as described by Das et al. [7], are classified into seven categories (social behavior, repetitive behavior, communications, emotions, learning and memory, sensory, motor). In ASD models with face validity, we would expect the following differences (as compared to control animals) in the core behaviors: reduced social behavior (e.g., three-chamber sociability test: time in the social chamber), reduced communication (e.g., ultrasonic vocalizations: number of calls), and increased repetitive behavior (e.g., home cage behavior: repetitive rearing and climbing). The evaluation of outcomes from the auxiliary categories of phenoterms (emotion, learning and memory, sensory, motor) will be exploratory for the different studied models.

Risk of bias

Assessment of risk of bias in included studies will be evaluated by the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE's) risk of bias tool (RoB) [10], with suitable modifications adjusted by aspects that play relevant roles in ASD-associated rodent models. Each report criterion of the SYRCLE RoB tool to detect the risk of bias will be judged by experienced investigators according to the following items: (1) reporting of random allocation, (2) reporting of baseline characteristics, (3) reporting regarding if the animals were randomly housed during the experiment, (4) reporting the blinding methods used by caregivers and investigators, (5) reporting animal random outcome assessment, (6) reporting of blinded assessment of outcome, (7) reporting of animal exclusions, (8) selective outcome reporting, (9) reporting the correct unit-of-analysis, and (10) reporting of sample size calculation. Classification of low, high, or unclear risk of bias will be assigned for each item evaluating every included report, except the first item (*reporting of random allocation*) that will be characterized as low risk of bias (authors describe the method used to randomize), unclear (authors only say "random" without any specification), uncertain (authors did not describe the method used to randomize the sample), and high risk of bias (it is not random). After the risk of bias assessment, we will recheck a random sample of 10% of reports. Tricky items will be considered and discussed when interpreting the results.

Data synthesis

A meta-analysis will be conducted whenever at least five studies have the same design, reporting data for the same animal model, comparison group, and behavioral test type. The effect measures used to perform the meta-analysis will be standardized mean difference and odds or risk

ratios when the first is impossible. The analysis will follow a random-effects model to account for heterogeneity. We will use "report" as a random factor. Notwithstanding, I^2 and Cochran *Q* statistics will be employed to quantify the statistical heterogeneity among studies. We will investigate any possible source of heterogeneity after conducting a subgroup analysis and consider adding them to the random-effects model.

Whenever one control is used multiple times, the final sample (of that control group) will be adjusted by dividing the total sample size by the number of times that group is included in the analysis.

A network meta-analysis will also be performed, comparing the models for each outcome, provided that each model has at least five different studies investigating the same behavior. Subgroup meta-analyses (meta-regression or stratified regression) will also be conducted according to the following potential heterogeneity introducing variables: species, strain, sex, intensity and duration of model behavior induction, age and weight of animals, lab/study group, and analyses by specific behavioral tests. All these will be performed when the subgroup is composed of at least ten different original studies.

When there are multiple and comparable outcomes reported for the same behavioral test (e.g., for elevated plus-maze: time spent in open arms, distance traveled in open arms, number of entries in open arms), we will choose the most frequently reported metric across studies; if a report does not report the chosen metric, then we will use the second most frequently reported metric, and so forth. The sign of the effect size will be reversed (multiplying it by minus one) when needed so that the direction of the effect can be interpreted consistently if the metrics have opposite meanings for the behavioral trait (e.g., exploration in the open versus closed arms in the elevated plus-maze; correct choices versus the number of errors in learning tests). Behavioral variables that are not the most common across reports for the same behavioral test will not be used in the meta-analysis. Moreover, we will refrain from mixing different behavioral tests that are not based on the same apparatus (e.g., grooming in the open field test and grooming in the elevated plus-maze test).

Sensitivity analyses will be performed as follows: (a) following the Jackknife method for all main meta-analysis groups, (b) according to the risk of bias quality score of original studies (poorly classified studies with two or more items rated as high risk of bias will be excluded—this represents around a quarter of the studies based on our pilot data), and (c) in case of doubts regarding the assumptions and interpretation of previous analyses. Publication bias will be investigated through funnel plotting and Egger's regression test [11]. These will only be conducted whenever at least five (for Funnel plots) or ten (for Egger's regression) studies evaluating the same outcome are available.

All analyses will be conducted using the R Project for Statistical Computing (https://www.r-project.org/) [12] packages *metafor* (https://cran.r-project.org/package=metafor) [13] and *ggplot2* [14].

Whenever a meta-analysis is not possible to be conducted, the descriptive summary and effect sizes from the original studies will be compared qualitatively, also following the five (5) studies' rule. We will summarize the effect estimates, discussing the range and distribution of observed effects when a comparable estimate of effect is provided (or can be arrived upon through conversion). A narrative summary will be done carefully considering the study quality (including the risk of bias) and sample sizes. Figures (including scatterplots, barplots, or radar plots) will also be constructed to visualize the differences in effect, *P*-value, and direction of findings over the years.

Piloting

Every step of the methods for this protocol either has been piloted or is planned to be piloted in the next steps of the systematic review. The search described in Additional file 1 and performed on November 5, 2020, identified 18,336 reports in Scopus, 14,202 in Web of Science, and 17,648 in PubMed. The duplicates were removed manually with the help from the Rayyan AI for identification. After this step, the remaining reports (24,983) were randomized, and a sample of 378 reports was used for report selection piloting. Agreement between all nine reviewers reached 95%. Moreover, 6% of the reports were included, leading us to estimate around 1500 to be included in the systematic review.

We have decided to include a variety of behavioral tests, as described in Additional file 2. However, only studies within the same animal model and that have data on the same variables for the same behavioral tests will be compared in the traditional meta-analysis. This means that we have to identify all common tests before going further. With this in mind, the first step in our data extraction procedure will be a screening of all included studies to identify tests and variables used in the original reports. This will guide us as to which studies can be compared. From the screening, we will have data on which animals were used for which behavioral assessment and if the same variables were reported within and between different reports. This will allow us to identify and control the analyses (if applicable) for duplicated samples, repeated measures, and which outcomes are more widely reported for each comparable behavioral assay.

The entire protocol has been based on small pilot studies. A large part of the group has been trained to conduct systematic reviews with meta-analyses of preclinical studies; pilots are the form of training we have adopted. We had pilot phases that often consisted of familiarization, followed by training, and finally, a comparison of reliability or other metrics among researchers. Researchers met regularly to discuss the issues and misunderstandings. That happened for the use of Rayyan and initial selection of the studies and will happen for the data extraction. For the screening pilot, four rounds of judgment were performed with 50–60 hits of random abstracts per round; agreement between all nine reviewers reached 95%.

For the risk of bias analysis pilot, the SYRCLE's tool for risk of bias assessment was adapted for the two groups of studies based on the induced or genetically altered models of ASD. After three rounds of judgment with 20 full-text studies per round, we obtained an agreement of 85%, on average, for studies of induced models. Training for risk of bias in models based on genetic alterations, data extraction, and conducting meta-analyses has not yet been completed.

Discussion

We established the current protocol to synthesize and compare the behavioral outcomes of studies using common genetic- (*Pten*, *Fmr1*, *Ube3a*, *Nlgn3*, *Shank3*, or *Mecp2*) and chemically induced (VPA, LPS, or poly(I:C)) rodent models of ASD, besides the BTBR mice, an inbred strain naturally expressing manifestations similar to the core human phenotypes [15]. It is anticipated that researchers interested in this field, especially those aiming at combining complementary models to advance the neurobiology and therapeutic interventions for autism spectrum disorders in humans, will benefit from having comprehensive information on this subject to plan new study designs. Another expected contribution of this review is improving research reproducibility and translatability, minimizing research costs and waste. Finally, by intending to bridge the gap between animal behavior and human endophenotypes or specific clinical symptoms, we expect to foster clinical ASD research indirectly.

Abbreviations

ASD: Autism spectrum disorder; PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols; Ube3a: Ubiquitin-protein ligase E3A; Pten: Phosphatase and tensin homolog; Nlgn3: Neuroligin-3; Shank3: SH3 and multiple ankyrin repeat domains 3; Mecp2: Methyl CpG binding protein 2; Fmr1: Fragile X mental retardation 1; LPS: Lipopolysaccharide; VPA: Valproic acid; Poly(I:C): Polyinosinic:polycytidylic acid; BTBR: BTBR $T^{+}Itpr3t^{f/J}$ mouse strain; PND: Postnatal day; SYRCLE: SYstematic Review Centre for Laboratory animal Experimentation; RoB: Risk of bias; SD: Standard deviation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13643-022-02028-w.

Additional file 1. Search strategy. The complete search strategy syntax for PubMed, Web of Science, and Scopus databases.

Additional file 2. Behavioral outcomes. List of behavioral categories and examples of experimental paradigms.

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Authors' contributions

ACP (guarantor) devised and designed the research and contributed to writing the manuscript. All authors contributed to the writing and deciding critical points of the methodology, critically evaluated methodological planning, and read and approved the final manuscript. In addition, APH provided invaluable clinical translatability inputs and supervised the protocol.

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Availability of data and materials

All raw data, processed and meta-data, analysis codes used, lists of included and excluded full-text studies, and the datasets generated and analyzed during the current study will be made available in the Open Science Framework repository (https://doi.org/10.17605/OSF.IO/DXMW7) [15].

Declarations

Ethics approval and consent to participate

Since this protocol is for a systematic review and meta-analysis, ethical approval is not required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

2. Co-authored research articles published during the PhD

On the following pages there are presented research articles published in peer-reviewed journals as co-author.

In summary:

1. Systematic review and meta-analysis of 10 years of unpredictable chronic stress in zebrafish. Lab Anim (IF = 6.9). Oct 2023.

2. Site effects how-to and when: An overview of retrospective techniques to accommodate site effects in multi-site neuroimaging analyses. Front Neurol (IF = 4.086). Oct 2022.

3. Neurological impairment caused by Schistosoma mansoni systemic infection exhibits early features of idiopathic neurodegenerative disease. JBC. (IF = 5.486) Jul 2021.

4. Incidence of lung cancer and mortality among civil construction industry workers: A protocol for a systematic review and meta-analysis. PLOS ONE (IF = 3.582). Apr 2021.

5. Attention-deficit/hyperactivity disorder and brain metabolites from proton magnetic resonance spectroscopy: a systematic review and meta-analysis protocol. Trends Psychiatry Psychother (Impact Score: 2.30). Mar 2021.

6. Effects of foetal and breastfeeding exposure to methylmercury (MeHg) and retinol palmitate (Vitamin A) in rats: Redox parameters and susceptibility to DNA damage in liver. Mutat Res Genet (IF = 3.189). Dec 2020

7. Virtual meetings and social isolation in COVID-19 times: transposable barriers. Trends Psychiatry Psychother (Impact Score: 2.30). Sep 2020.

8. Hepatic and neurobiological effects of foetal and breastfeeding and adulthood exposure to methylmercury in Wistar rats. Chemosphere (IF = 8.943). Apr 2020.

3. Attendance in conferences

3.1. Work presented as poster

“Inflammatory markers and suicide behaviour: a systematic review and meta-analysis,” World Congress of Psychiatric Genetics, Montreal, Canada, 2023.

"Biclustering Algorithm Applied to Structural Magnetic Resonance Imaging in Major Depressive Disorder," Society of Biological Psychiatry Meeting, San Diego, USA, 2023.

"Biclustering Algorithm Applied to Structural Magnetic Resonance Imaging in Major Depressive Disorder," World Congress of Psychiatric Genetics, Florence, Italy, 2022.

"Hippocampal histone trimethylation and neurogenesis are associated with resilience in a rat model with depressive-like behavior," ISN-APSN Meeting, Honolulu, USA, 2022.

"Behavioral Manifestations in Genetic Rodent Models of Autism Spectrum Disorder: A Systematic Review and Meta-Analyses," World Congress of Psychiatric Genetics, Virtual, 2021.

"Peripheral Blood as a Tool to Determine Gene Expression Patterns in Patients With Psychiatric and Neurological Disorders: A Systematic Review and Meta-Analysis," World Congress of Psychiatric Genetics, Virtual, 2020.

"The evolutionary interplay of human apoptosis, senescence, autophagy and genome- stability gene pathways," Natal Bioinformatics Forum, Natal, Brazil, 2019.

3.2. Speaker

"A investigação de marcadores inflamatórios através das ômicas em transtornos de humor," XV Congresso Gaúcho de Psiquiatria, Virtual, 2022

Chair in the Systematic review in psychotherapy session, XIV Congresso Gaúcho de Psiquiatria, Bento Gonçalves, 2019

4. Awards and positions within scientific initiatives

4.1. Awards

PGC Trainers Program

October 2023

Society of Biological Psychiatry Travel Fellowship Award

April 2023

International Society of Neurochemistry Travel Award

August 2022

WCPG Early Career Investigator Program Award

October 2020

4.2. Positions in scientific initiatives

Latin American Genomics Consortium - Suicide Working Group

Member since 2022

Trends in Psychiatry and Psychotherapy

Junior Editor since 2020

Brazilian Reproducibility Initiative in preclinical Systematic review and meta-Analysis

Founding member since 2020 and co-coordinator (2020-2022)

5. Co-supervised BSc and MSc theses

M.Sc.

• Present. Student: Lorena Pigoso. University: Universidade Federal de Santa Catarina (UFSC)

M.D.

• 2021. Student: Isabel de Carvalho Cyrne. University: Universidade do Vale do Taquari (UNIVATES)

• 2020. Student: Luciano Gouvea De Moraes Silva. University: Universidade do Vale do Taquari (UNIVATES)

• 2019. Student: Lucas Pires Freitas. University: Universidade do Vale do Taquari (UNIVATES)

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