Universidade do Minho
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PREFRONTAL CORTEX: DEFINING THE NEUROANATOMICAL SUBSTRATES OF INHIBITORY CONTROL IN BINGE DRINKING

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Sónia da Silva Sousa
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Tese de Doutoramento em Psicologia Básica

Trabalho efetuado sob a orientação da
Doutora Adriana da Conceição Soares Sampaio
e do
Doutor Manuel Alberto Crego Barreiro

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DECLARAÇÃO DE INTEGRIDADE

Declaro ter atuado com integridade na elaboração da presente dissertação. Confirmo que em todo o trabalho conducente à sua elaboração não recorri à prática de plágio ou a qualquer forma de falsificação de resultados.

Mais declaro que tomei conhecimento integral do Código de Conduta Ética da Universidade do Minho.

Universidade do Minho, 29 de Junho de 2017

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"Believe in Better"

I see myself as an athlete, although amateur. Everyday I step into the gym I take a glance at the Nike wallpaper illustrating a runner with the citation "Believe in Better". I definitely identify myself with this quote! Work hard! Give all you have in every single thing you do in your life. I try to do my best.

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PREFRONTAL CORTEX: DEFINING THE NEUROANATOMICAL SUBSTRATES OF INHIBITORY CONTROL IN BINGE DRINKING

Abstract

Adolescence is a period characterized by improvements in complex cognitive functions such as inhibitory control and self-regulation, in parallel with a set of undergoing developmental processes (e.g., gray matter thinning and white matter growth) in the prefrontal regions that underlies the maturation of such cognitive abilities. The immature self-control has been associated to the adolescent vulnerability to risky environmental factors (e.g., drugs or alcohol misuse) and despite the high prevalence of heavy alcohol consumption among youth, research on the neural signatures of this particular behavior, is lacking.

The pattern of heavy alcohol consumption in a brief period of time followed by intervals of abstinence is known as Binge Drinking (BD). This highly risky behavior has gathered several followers in the last decades among young teenagers and college students.

Structural, functional and electrophysiological studies have shown alterations in the binge drinkers brains in widespread areas including the frontal cortex, in addition to several cognitive deficits, including the inhibitory control, although not very explanatory about the underlying processes that might prompt the BD behavior. As such, the present report aim to search for neurodevelopmental markers contextualized in the addiction models that could signal alcohol misuse among young consumers.

Likewise, the first study describes the healthy development of the prefrontal white matter, since this is a major event that strongly contributes to improving cognitive skills, mainly in adolescence. Data gathered in this study reveals a protracted course of prefrontal white matter maturation (e.g., increased myelination, axonal density, and fibers' organization) until the third decade of life. Significant white matter alterations were observed between adolescence and early adulthood, suggesting a continuous

reorganization of the prefrontal cortex in response to environmental demands, and associated with advanced cognitive abilities.

Taking into account the vulnerability of the adolescent brain, the aim of our second study was to search for abnormalities in the prefrontal network associated to self-regulation and inhibitory control in binge drinkers. The findings revealed gray matter abnormalities in the binge drinkers' prefrontal regions, compared to their alcohol-abstinent peers, associated to diminish self-control.

Considering these results, in the third study white matter analysis were conducted in the same prefrontal regions in order to complete the anatomic assessment associated with inhibitory control. The results revealed white matter alterations in the binge drinkers' prefrontal cortex, in comparison with alcohol-abstinent controls, which were associated to the intensity and frequency of BD.

Overall, these findings revealed abnormalities in the prefrontal gray and white matter in the binge drinkers, compared to alcohol-abstinent controls, associated to abnormal self-regulatory processes. The neurodevelopmental specificities found in binge drinkers might be premorbid, existing even before their first contact with alcohol, eventually prompting to alcohol initiation. Further investigation should deeper explore these findings.

CORTEX PREFRONTAL: DEFINIÇÃO DOS SUBSTRATOS NEUROANATÓMICOS DO CONTROLO INIBITÓRIO NO BINGE DRINKING

Resumo

A adolescência é um período caracterizado pelo aperfeiçoamento de funções cognitivas complexas, nomeadamente o controlo inibitório e a capacidade de autorregulação, em paralelo com um conjunto de processos desenvolvimentais (por exemplo, diminuição da substância cinzenta e aumento da substância branca) em regiões do córtex pré-frontal, subjacentes à maturação destes processos cognitivos. Um autocontrolo subdesenvolvido tem sido associado à vulnerabilidade dos adolescentes face a factores ambientais adversos (por exemplo, drogas ou abuso de álcool) e, apesar da alta prevalência do consumo intensivo de álcool entre os jovens, falta investigação sobre as assinaturas neurais associadas a este comportamento.

O padrão de consumo intensivo de álcool durante um breve período de tempo, seguido de intervalos de abstinência, é conhecido com Binge Drinking (BD). Este comportamento de risco reuniu vários seguidores nas últimas décadas entre os jovens adolescentes e os estudantes universitários.

Estudos estruturais, funcionais e eletrofisiológicos têm revelado alterações em diversas áreas do cérebro dos jovens consumidores, incluindo o córtex pré-frontal, para além de vários défices cognitivos, que incluem o controlo inibitório, embora os resultados encontrados até ao momento não sejam suficientemente esclarecedores sobre os processos subjacentes ao BD, e que possam conduzir a este comportamento.

Neste sentido, no presente trabalho pretende-se investigar marcadores neurodesenvolvimentais, ancorados nos modelos de abuso e dependência de substâncias, que poderiam contribuir para sinalizar o uso indevido do álcool entre os jovens consumidores.

Assim, o primeiro estudo descreve o desenvolvimento normativo da substância branca do córtex pré frontal, uma vez que este é um evento de grande relevância no aperfeiçoamento das capacidades cognitivas, principalmente durante a adolescência. Os resultados revelaram um trajeto prolongado de maturação da substância branca pré-frontal (por exemplo, aumento da mielinização, densidade axonal e organização das fibras) até à terceira década de vida. Alterações significativas da substância branca foram observadas entre a adolescência e início da idade adulta, sugerindo uma reorganização contínua do córtex pré-frontal em resposta a estímulos ambientais e associada a capacidades cognitivas complexas.

Tendo em consideração a vulnerabilidade do cérebro adolescente, o objetivo do nosso segundo estudo foi procurar anomalias na rede pré-frontal associada à autorregulação e ao controlo inibitório nos jovens binge drinkers. Os resultados revelaram alterações da substância cinzenta nas regiões pré-frontais dos binge drinkers, em comparação com seus pares não consumidores, associadas a uma diminuição do autocontrolo.

Considerando estes resultados, no terceiro estudo, foi conduzida uma análise da substância branca nas mesmas regiões pré-frontais, com a finalidade de completar a avaliação anatómica associada ao controlo inibitório. Os resultados revelaram alterações da substância branca no córtex pré-frontal dos binge drinkers, em comparação com os controlos, associadas à intensidade e frequência do BD.

No geral, estes resultados revelaram alterações da substância cinzenta e branca pré-frontal nos binge drinkers, em comparação com os controlos não consumidores, associadas a processos de autorregulação deficitários. As especificidades neurodesenvolvimentais encontradas nos binge drinkers podem eventualmente ser pré-mórbidas, existindo mesmo antes do primeiro contato com o álcool, sendo percussivas na iniciação ao consumo de álcool. Futura investigação deverá explorar mais aprofundadamente estas hipóteses.

Abbreviations

AAC	Alcohol-abstinent Control
AACs	Alcohol-abstinent Controls
AAL	Anatomical Automatic Labeling
AD	Axial diffusivity
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AUD	Alcohol Use Disorders
AUDIT	Alcohol Use Disorder Identification Test
AUQ	Alcohol Use Questionnaire
BD	Binge Drinking
BDs	Binge Drinkers
BIS	Barratt Impulsiveness Scale
CNS	Central Nervous System
DARTEL	Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra
DLPFC	Dorsolateral Prefrontal Cortex
DSI	Diffusion Spectrum Imaging
DSM-V	Diagnostic and Statistical Manual of Mental Disorders (5^{th} edition)
DTI	Diffusion Tensor Imaging
DTT	Diffusion Tensor Tractography
DWI	Diffusion-weighted Imaging
EEG	Electroencephalography
ERP	Event-related Potential
FA	Fractional Anisotropy
FHAM	Family History Assessment Module
fMRI	Functional Magnetic Resonance Imaging
FoV	Field of View
FSL	fMRI Software Library
FWHM	Full Width at Half Maximum
FWM	Frontal White Matter
GLM	General Linear Model
GM	Gray Matter

GSI	Global Score Index
HARDI	High Angular Resolution Diffusion Imaging
IAM	Individual Assessment Module
ICBM	International Consortium for Brain Mapping
IFG	Inferior Frontal Gyrus
iFOF	Inferior Fronto-occipital Fasciculus
iRISA	Impaired Response Inhibition and Salience Attribution
MD	Mean Diffusivity
MFG	Middle Frontal Gyrus
MNI	Montreal Neurological Institute
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NIAAA	National Institute on Alcohol Abuse and Alcoholism
OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RD	Radial Diffusivity
ROI	Region of Interest
SCL-90-R	Symptom Checklist-90-Revised
SD	Standard Deviation
SFG	Superior Frontal Gyrus
sFOF	Superior Fronto-occipital Fasciculus
sLF	Superior Longitudinal Fasciculus
SPM	Statistical Parametric Mapping
SSAGA	Semi-Structured Assessment for the Genetics of Alcoholism
TBSS	Tract-based Spatial Statistics
TE	Echo Time
TR	Repetition Time
UF	Uncinate Fasciculus
US	United States
VBM	Voxel-based Morphometry

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Preamble

The pattern of high alcohol intake in a short time followed by periods of abstinence is known as Binge drinking (BD). This pattern of consumption is very common in adolescence, a stage of life characterized by advancements in complex cognitive functions such as inhibitory control and self-regulation that allow avoiding impulsive and risky behaviors like alcohol and/or drug abuse. The neuroanatomical substrates underlying the refinement of these self-control abilities during adolescence involve the prefrontal–striatal network, brain regions with a critical role in cognitive control.

Between adolescence and early adulthood a set of maturational processes (e.g., gray matter thinning and white matter growth) is observed in the prefrontal regions, associated to improved cognitive skills. The ability to regulate behavior and suppress inappropriate emotions or actions seems to be underdeveloped in this developmental stage, fact that has been associated to structural immaturity of cortical and subcortical regions, enhancing adolescent vulnerability to risky environmental factors (e.g., drugs or alcohol misuse) and predisposed to act impulsively.

Despite the growing prevalence of BD among young people, research on the putative neural abnormalities related to this particular pattern of high alcohol ingestion, either as a precursor and/or as an effect of alcohol abuse, is still scarce. Of particular importance is to understand how this behavior might be enhanced as a result of disruptions in the normative brain development or how this BD pattern affects the optimal brain maturation and integrity. Therefore, the main purpose of this dissertation is to contribute to the binge-drinking research field in the pursuit of evidencing neurodevelopmental markers that could be predictive of alcohol misuse among young consumers, and/or understand how this disruptive behavior might affect the brain structure.

Therefore, the current dissertation is divided in 5 chapters. Chapter I addresses the neurobiological aspects underlying the teenage brain development and associated cognitive skills, especially the inhibitory control ability. Additionally, the vulnerability factors that may impact the immature adolescent brain development are explored, namely the exposure to harmful environmental

factors (e.g., binge drinking). Then for a better characterization of the BD phenomena, aspects such as prevalence, diagnosis, and neurocognitive, neurostructural, and neurofunctional phenotypes are included. The main objectives and hypothesis of the present work are presented in the end of this chapter. For a comprehensive view of these issues, Chapter II addresses in a systematic review the developmental pathway of the prefrontal cortex, in terms of its microstructural connectivity, and its association with cognitive improvement. The relation between brain structural data and inhibitory control will be discussed in the context of the addiction models in the chapter III and IV. Specifically, chapter II presents the empirical study conducted first, in which morphological alterations (e.g., gray matter) within core brain regions associated with self-regulatory processes of binge drinkers (BDs) in comparison to its alcohol-free counterparts, were explored. Chapter III will present the second empirical study where we address the white matter abnormalities of the dorsolateral prefrontal cortex in BDs, compared to its alcohol-free peers, and its association with the intensity and frequency of alcohol consumption. Finally, a general discussion integrating all the findings and future directions will be present in chapter V.

Chapter I

General Introduction

1. The Adolescent Brain and Vulnerability to Adverse Environmental Factors

Brain development, is a dynamic process that occurs during half of the human lifecycle (Bartzokis et al., 2012; Giedd & Rapoport, 2010; Lenroot & Giedd, 2006). From birth to adulthood, a set of progressive (e.g., myelination) and regressive (e.g., neural pruning) neurobiological events is responsible for regional brain modifications of specific neural circuits. Fluctuations in the gray and white matter volumes, reorganization and refinement of the synaptic connectivity and fine tuning of the neural connections apparently follow a particular temporo-spatial sequence within the process of brain development (Durston et al., 2001; Giedd & Rapoport, 2010; Giedd et al., 1999; Giedd, 2008; Huttenlocher, 1979; Shaw et al., 2008), resulting in an inferior-to-superior and posterior-to-anterior and central-to-peripheral direction of maturation (Colby, Van Horn, & Sowell, 2011; Dubois et al., 2013; Snaidero & Simons, 2014).

Two major brain cellular events have been associated with improved neural efficiency, namely, the synaptic and neural pruning (i.e., gray matter reduction) and the increased myelination (i.e., white matter growth and organization) (Giedd, 2004; Lenroot & Giedd, 2006; Petanjek et al., 2011). With development, while a major increase of gray matter is observed between neonatal ages and childhood, during adolescence the gray matter volumes diminish (Durston et al., 2001; Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Lenroot & Giedd, 2006; Petanjek et al., 2011). With a different trend, the white matter mostly increases throughout life (Bennett, Diamond, Krech, & Rosenzweig, 1964; Dubois et al., 2013; Durston et al., 2001; Giedd et al., 1999; Giedd, 2004; Lenroot & Giedd, 2006; Snaidero & Simons, 2014) with both events contributing to sustain the individuals' increasing behavioral and cognitive complexity. As we can observe, age-related structural and functional specificities are present in different developmental stages and seem to be associated with the emergence of particular cognitive

milestones (Bennett et al., 1964; Casey, Giedd, & Thomas, 2000; Paus et al., 1999; Schmithorst, Wilke, Dardzinski, & Holland, 2005). In particular, a development period characterized by highly complex demanding tasks in several domains - cognitive, emotional and social – is the adolescence.

Adolescence is characterized by important changes either in terms of the organism in itself as in its interaction with the environment. In fact, it is likely that the newish, complex and highly demanding experiences, such as advanced academic formation, engagement in a job, new social relations, and further familiar responsibilities, arising in this phase of the individuals' life may ultimately influence and be influenced by the architecture of the brain, as brain development is highly experience-dependent (Scholz, Klein, Behrens, & Johansen-Berg, 2009). In particular, between adolescence and young adulthood, structural alterations in the frontal lobes are largely observed (e.g., pruning, myelination, synaptic remodeling) and these changes have been associated to improvements in higher-level cognitive functions including inhibitory control abilities (Gogtay et al., 2004, Madsen et al., 2010; Nagy, Westerberg & Klingberg, 2004; Urger et al., 2015).

Adolescents are also particularly susceptible to environmental factors that might interfere with the optimal brain development; such is the case of drugs or alcohol misuse. Adolescence is a phase to embrace new experiences leaving individuals willing to seek new sensations, novelty, and social interactions, thus adolescents seem to be highly sensitive towards external stimuli, either positive or negative, which in turn might modulate their actions (Crews, He, & Hodge, 2007; De Lorme, Bell, & Sisk, 2013; Peper, & Dahl, 2013).

In fact, there is a tendency to refer to adolescence as a particularly vulnerable period of life since young teens are seen as more risk-prone individual than adults. This risky behavior has been related with two main cognitive processes: reward sensitivity and self-control (Casey & Caudle, 2013; Galván, 2013). The reward system seems to be especially reactive during adolescence, since reward promotes the approach behavior towards new social external stimuli (e.g., alcohol) and facilitate new

learning. In fact, greater neuronal activity in subcortical regions (e.g., ventral striatum), in response to appetitive reward cues has been observed in adolescents, compared to children and adults (Galvan et al., 2006), which was additionally associated to a higher tendency to engage in risky behaviors (Galvan, Hare, Voss, Glover, & Casey, 2007). Moreover, adolescent self-control, the cognitive process that allows the individuals to suppress inadequate actions, emotions or thoughts, seems to additionally contribute to poorly chosen, risk-taking options. It is likely that adolescents are less efficient in correctly inhibit actions/emotions or thoughts that might have negative outcomes, as their self-regulation abilities are still immature (Casey & Caudle, 2013). However, apparently adolescent self-control is not always defective. Actually, it seems to be less effective when the situation involves stimuli with emotional content (e.g., appetitive social/external stimuli), but efficient towards neutral stimuli, paralleling adults' performance (Tottenham, Hare, & Casey, 2011).

In sum, adolescents' decisions towards external stimuli (e.g., drugs, alcohol) seem to be reward-driven; however, although this may play an adaptive role in facing the new incoming social exigencies, it may also adversely affect the individuals' well being, when risky choices are taken under social pressure (Albert, Chein, & Steinberg, 2013). Thus, a reward-based decision, associated to a weak self-control that is not able to counteract the over-reactive reward system, increases the probability of risky situations as the case of alcohol initiation and binge drinking (BD) that will be comprehensively analyzed below.

2. Binge Drinking

Binge drinking is a sub cluster of substance abuse that has received much attention over the last decade due to its high prevalence among youth, and is defined as a pattern of high alcohol intake, which brings blood alcohol concentration levels to 0.08 g/dL, in a brief period of time (±2h), followed by periods of abstinence (NIAAA, 2015).

2.1. Binge Drinking - Prevalence

The age onset of substance abuse, including alcohol consumption, is before age 18 (https://www.centeronaddiction.org), (Courtney & Polich, 2009) and according to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), nearly 60% of US college students (age range 18–22 years) report alcohol consumption and 40% exhibit a BD pattern. This highly risky behavior is seriously harmful and carries disastrous consequences such as, the death of approximately 1,825 US college students each year, 696,000 assaults perpetrated by another drinker student, 97,000 suffered a alcohol-related sexual assault or rape, and 1 out of 4 students reported negative academic results (NIAAA, 2015; SAMHSA, 2015).

In Europe, BD is likewise considered a major concern. The greatest percentage of frequent BD involves young consumers; i.e., 33% of the individuals ranging in age between 15 and 24 years have reported a BD pattern (Eurobarometer, 2010).

Few information is available regarding the BD phenomena among Portuguese college students (age range: 18-23), however some data was collected in a 2015 survey among Portuguese students aged 13-18 years (ESPAD, 2015). According to the data, alcohol is the substance with the highest amount of consumers. The college students that reported a BD pattern in the past 30 days were 36% overall, 43% male and 31% female. At age of 13, 4% of the students reported BD, but the higher number of binge drinkers (BDs) was observed among the 18 years-old students. The current European alcohol consumption (as measured in the 2015 survey) compared to previous surveys (e.g., 2011) has diminished among the youngest consumers but remained rather stable among the older students (17-18 years), (ESPAD, 2015).

2.2. Binge Drinking - Diagnosis

In order to establish a BD diagnosis, some characteristics of the behavior have to be taken into account, namely the quantity, the time frame, the frequency, and its consequences (Parada et al., 2011a).

The quantity of alcohol intake considered as a binge episode is five or more units or standard drinks for a male and four or more drinks for a female. A unit or standard drink represents the amount of alcohol (g/L) an adult is able to process within an hour but the alcohol metabolization may vary according to the individual (e.g., weight) and may worsen due to the strength of the alcoholic beverage (Alcohol Guidelines, 2012). Therefore, an alcohol intake episode in order to be considered as BD needs to happen in a single occasion, during a two-hour interval, because in this time frame it is not possible for the organism to process the high amount of alcohol consumed. Indeed, the time frame or speed of drinking defined for a binge episode is approximately 2 hours (NIAAA, 2015). However due to the individual differences and the amount of alcohol variation contained in a standard drink among countries, the NIAAA established the level of blood alcohol concentration as the cut point for a binge episode. Therefore, in order for high alcohol ingestion to be considered as a binge episode, it has to increase blood alcohol concentration to 0,08 g/L, at least (NIAAA, 2015).

The frequency of alcohol consumption is likewise an important factor to take into account, when defining BD (Parada et al., 2011a). Intensive alcohol ingestion side effects will not be the same as regular alcohol consumption effects. Additionally if the individuals report a lifetime binge episode, it will not account as a BD diagnosis. On the other side, individuals with highly intensive alcohol consumption might present the DSM-V criteria for an alcohol use/abuse disorder diagnosis. Therefore, it is imperative to define how often individuals drink alcohol in order to establish whether or not it is considered BD. Therefore, BD is likewise defined when the binge episodes occurs at least once per

month in the past year, with periods of no alcohol consumption (NIAAA 2015; Parada et al., 2011; SAMHSA 2015).

The mostly used instruments to establish a BD diagnosis are the Alcohol Use Disorders Identification Test (AUDIT) (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001), the Alcohol Use Questionnaire (AUQ) (Townshend & Duka, 2002) and a diary of alcohol ingestion. The AUDIT was validated to assess risky alcohol consumption and personal troubles, especially among college students providing an accurate measure of harmful drinking across gender, age and culture (Allen, Litten, Fertig, & Babor, 1997; Conigrave, Saunders, & Whitfield, 1995; Fleming, Crombie, & Cross, 1991). The AUDIT is a brief self-report questionnaire, which comprises 10 questions regarding the actual alcohol consumption, alcohol dependence symptoms and alcohol related problems, promptly allowing the screening of individuals with alcohol-related problems. Total AUDIT score reflects the individual's level of risk due to harmful alcohol intake: scores in the range of 8-19 reveal hazardous drinking, while scores of 20 or above warrant further diagnostic evaluation for alcohol dependence (Babor et al., 2001). Specifically, the item 3 of the AUDIT is used to determine if the individuals present a binge pattern of alcohol consumption (e.g., how frequently do you consume 6 or more drinks in a single occasion?). Additionally, the items 10, 11 and 12 of the Alcohol Use Questionnaire (AUQ) (Townshend & Duka, 2002), i.e., speed of drinking - average number of drinks consumed per hour - number of times getting drunk in the past 6 months, and percentage - average - of times getting drunk during drinking episodes), as well as a diary of alcohol ingestion, gather other relevant information on risky drinking and allows knowing the individuals' consumption pattern, the frequency of consumption and speed of drinking (e.g., grams/h during BD episodes).

Finally it is important to note that several individual and social risk factors were observed as contributing to the binge-drinking phenomena among college students. Specifically, at the individual level, impulsivity and sensation seeking, self-regulation and impaired control, were the mostly reported.

At the social level, peer conformity, social acceptance, fun and pleasure, violence at school, permissive parental style, family history of alcoholism, and university settings with easy access to alcohol, were among the most referred factors (Donath et al., 2012; SAMHSA, 2015). Differently, low sensation seeking, importance of the religion, prosocial activities such as volunteering, friends not consuming substances, high levels of parental monitoring, not being positive about alcohol consumption, school commitment, social integration and low economic status seem to be the mostly protective factors observed among college students non-BDs (Donath et al., 2012; SAMHSA, 2015).

2.3. Binge Drinking - Neurocognitive Phenotype

Neuropsychological studies have found deficits during the execution of tasks involving several higher order cognitive skills such as decision-making and planning ahead, verbal, episodic and visuospatial memory, working memory, cognitive flexibility, and inhibitory control (see Hermens et al., 2013 and Lopez-Caneda et al., 2014 for a review).

Specifically, BDs showed an increased number of perseverations and a lower capacity to retain verbal information when compared to controls (Parada et al., 2011b; Sanhueza, Garcia-Moreno, & Exposito, 2011). Spatial working memory and spatial orientation processing appears to be also impaired, with BDs showing more difficulty than their counterparts' non-binge, in manipulating, integrating and recalling spatial information (Blankenship, Blackwell, Ebrahimi, Benson, & Wallace, 2016; García-Moreno, Expósito, Sanhueza, & Angulo, 2008; García-Moreno, Expósito, Sanhueza, & Gil, 2009).

BDs also show deficits in the capacity to plan ahead, performing worse than non-binge in the Tower of Hanoi test (Hartley, Elsabagh, & File, 2004; Sanhueza, Garcia-Moreno, & Exposito, 2011), and poor decision-making taking less advantageous choices, as measured by the Iowa Gambling task, (Goudriaan, Grekin, & Sher, 2007).

Attentional skills seem to be additionally compromised. Thus, BDs present difficulties in the attentional networks related to alerting and executive control, and in sustaining attention (Hartley, et al., 2004; Lannoy et al., 2017; Sanhueza et al., 2011).

Finally, BDs present deficits in inhibitory control related tasks (Poulton, Mackenzie, Harrington, Borg, & Hester, 2016; Sanhueza et al., 2011). In fact, when compared to controls, BDs needed more time to complete the Stroop task, probably associated to a decreased resistance capacity to stimuli interference (Sanhueza et al., 2011) and showed by poor inhibitory control in the presence of immediate reward (Poulton et al., 2016). On the other hand, when performing the reaction time Matching to Sample visual search task, BDs were faster then controls in making their choice, which may be associated to higher impulsivity (Townshend, & Duka, 2005).

Likewise, an inefficient inhibitory control seems to be highly associated to adolescents' tendency to engage in risky-driven behaviors, such is the case of alcohol misuse, and has been referred in addiction models as having a major role in substance use/abuse (Crews & Boettiger, 2009; Bava & Tapert, 2010; Bari & Robbins, 2013). Inhibitory control is commonly defined as the capacity to suppress or restrain automatic pre-potent responses, thoughts or actions, including resistance to the interference of irrelevant stimuli, in order to overtake a goal-directed behavior. It can be seen as a selective mask of relevant information to the ongoing task/behavior, ignoring everything else (Bari & Robbins, 2013; Dillon & Pizzagalli, 2007; Miyake et al., 2000).

Poor inhibitory processes dramatically affect one's life, because it generally causes the opposite behavior, which is impulsiveness, and is generally related to harmful outcomes for the individual. Impulsivity has been defined as an incapability to hold or stop an action or thought, even when facing negative consequences (Bari & Robbins, 2013). Impulsiveness is also related with novelty and sensation seeking; preference of an immediate reward, although less profitable, over a delayed but more advantageous reward; and an increased predisposition to engage in risky behaviors (Bari &

Robbins, 2013) and has been consistently linked to several disorders including drug addiction, alcohol dependence, and more recently with the subclinical BD (Bari & Robbins, 2013; Crews et al., 2007).

Indeed, addiction models refer to impulsivity as a core trait associated to the early stages of substance dependence. Specifically, the model proposed by Koob & Volkow (2010), approaches addiction as a disorder anchored in two extreme behaviors or traits - impulsivity and compulsion - that belong to the same "pathological" spectrum or continuum although sustained by independent neurobiological mechanisms. Conceptually, the drug-seeking behavior involves a three-phase cycle including binge, withdrawal and craving being the binge consumption, which is characterized by a loss of control and partially driven by impulsiveness, manifested in the first phase or early onset of the addiction cycle (see figure 1). As such, and based on the Impaired Response Inhibition and Salience Attribution Model (iRISA), BD might be considered as a behavior that precedes alcohol addiction.

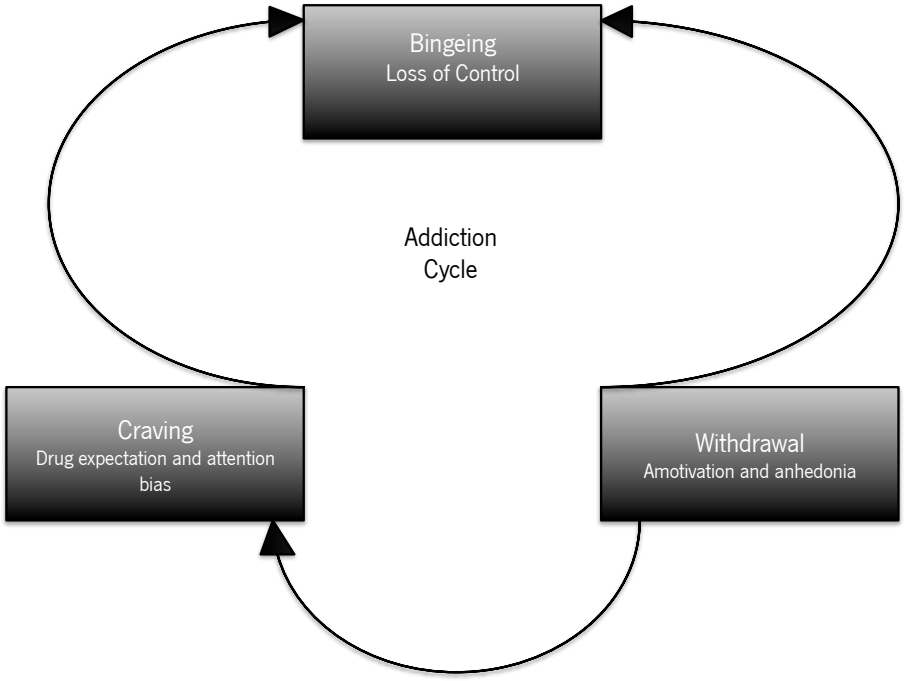

FIGURE 1 | Illustrates the Behavioural manifestations of the impaired response inhibition and salience attribution (iRISA) syndrome of drug addiction (adapted from Goldstein & Volkow, 2011)

The underlying brain structural and functional correlates of this cognitive profile will be addressed.

2.4. Binge Drinking- Neuroanatomical Phenotype: Gray matter/White matter

Structural neuroimaging studies reported gray and white matter alterations of the binge-drinker brain (Doallo et al., 2014; Heikkinen et al., 2017; Luciana et al., 2013; Squeglia et al., 2014; 2015). Increased gray matter was observed in several cortical and subcortical regions, namely in the dorsolateral prefrontal cortex (DLPFC), the middle frontal and temporal gyri, the cingulate cortex, and in the subcortical striatum of BDs, in comparison to controls (Doallo et al., 2014; Heikkinen et al., 2017; Howell et al., 2013; Wilson, Malone, Thomas, & Iacono, 2015).

On the other hand, other studies found decreased gray matter in the temporal gyri, the superior and middle frontal gyri, and in the inferior frontal gyrus of BDs, in relation to their non-consumers peers (Luciana, Collins, Muetzel, & Lim, 2013; Wilson, et al., 2015). Additionally, reduced gray matter in the cingulate, and inferior and middle frontal cortexes was found in prospective BDs, before alcohol initiation, compared to continuous non-drinkers (Cheetham et al., 2014; Squeglia et al., 2014; 2017; Wilson et al., 2015).

Regarding white matter, decreased volumes were observed in widespread areas such as the superior frontal gyrus, middle temporal and lingual gyrus, cerebellum and corpus callosum in young BDs, when compared to non-consumers (Lisdahl, Thayer, Squeglia, McQueeny, & Tapert, 2013; Luciana et al., 2013; Squeglia et al., 2014; 2015).

Finally, diffusion tensor imaging studies, which addresses microstructural white matter changes such as axons' myelination, axonal damage and integrity of fibers, revealed abnormalities (as shown by lower fractional anisotropy values) in the inferior fronto-occipital fasciculus, inferior and superior longitudinal fasciculus, uncinate, corpus callosum, corona radiata, internal and external capsule and in the caudate nucleus of BDs, compared to non-binge (Jacobus et al., 2009; Jacobus, Squeglia, Bava, &

Tapert, 2013; Luciana et al., 2013; McQueeny, 2009).

2.5. Binge Drinking - Neurofunctional Phenotype: fMRI/EEG

Functional magnetic resonance studies (fMRI) and electrophysiological studies (e.g., ERP) revealed differences in the brain activation between BDs and non-BDs, while executing specific cognitive tasks as verbal learning, spatial and verbal working memory, inhibitory control, and decision-making. Despite performance differences were not observed between BDs and non-BDs in the majority of the studies, brain functional activations were found.

Specifically, during verbal and spatial working memory encoding, BDs presented higher patterns of functional activations in fronto-parietal regions, when compared to non-binge controls (Campanella et al., 2013; Schweinsburg, McQueeny, Nagel, Eyler, & Tapert, 2010). Consistent with these results, higher N2 amplitudes in parietal regions, and larger P3b amplitudes in frontal, central and parietal regions, were observed in BDs while executing a visuospatial working memory and oddball tasks, respectively (Crego et al., 2009; 2012). These results suggest that BDs need to recruit more attentional resources to efficiently perform these cognitive tasks, when compared to non-binge controls. In the same line, BDs showed larger P3b no-go associated with a hyperactivation of the right inferior frontal cortex during response inhibition (as measured through the Go/NoGo task). Thus, this may suggest that, in order to successfully perform the task, BDs' brain require higher levels of effort of the region subjacent to response inhibition (Lopez-Caneda et al., 2012). In addition, less activity in regions related to inhibitory control (e.g., middle frontal gyrus and putamen), was also observed in prospective BDs, however; when the same individuals effectively became BDs they presented higher brain activity in the same regions, when compared to controls (Wetherill, Squeglia, Yang, & Tapert, 2013). Similarly, lower fronto-parietal activation was also observed in prospective BDs, prior to alcohol initiation (Jones, Cservenka, & Nagel, 2016).

Conversely, reduced brain activation of the dorsal striatum was observed in young BDs when performing a decision making task (The Wheel of Fortune) that was associated to the level of recent alcohol use (Jones, Cservenka, & Nagel, 2016).

Concluding, these findings suggest abnormalities in the brain activation of widespread areas underlying different cognitive tasks in young BDs, in comparison to their peers' non-binge and that premorbid factors are important, contributing to alcohol initiation.

3. Objectives and Hypotheses

Among substance abuse, one of the widely abuse substance of the moment is alcohol, which has been widely misuse in underage individuals and has a higher rate of prevalence especially among college students. Taking this into consideration, we acknowledge the relevance of the BD pattern of consumption in the context of more severe alcohol abuse or dependence. Therefore, the main purpose of this dissertation is to assess if BDs effectively display abnormalities in brain regions related with inhibitory processes and impulsiveness, as settled by the iRISA model. With this research, we expect to add a contribution to the knowledge on the neurobiological signatures of BD.

Under this assumption, the main goals of our investigation were:

1. To assess the gray and white matter morphometry associated with BD, in the prefrontal-striatal network involved in inhibitory control and impulsivity processes
2. To analyze the associations between the morphometric measures, both in gray and white matter, and two behavioral measures: the Barratt Impulsiveness Scale (BIS) and data regarding alcohol consumption (e.g., number of binge episodes per month)

The main hypotheses were defined as such:

1. BDs will display gray matter abnormalities in regions of the prefrontal-striatal network, compared to alcohol-abstinent controls (AACs), associated with impulsiveness scores. This

hypothesis was address in the first empirical study that will be presented in chapter III. In accordance with our initial hypothesis, the findings suggest frontal GM abnormalities in BDs, associated to diminish self-regulatory processes, possibly indicating that developmental disruptions may contribute to brain alterations in BDs, compared to alcohol-free controls.

2. BDs will present white matter abnormalities in regions of the prefrontal-striatal network, compared to alcohol-abstinent controls, associated with alcohol-related measures. This hypothesis was address in the second empirical study that will be presented in chapter IV. The results showed alterations in the dorsolateral prefrontal structural connectivity of BDs, eventually related with myelination processes and axonal integrity in brain regions related with self-regulation processes, associated with the intensity and frequency of alcohol consumption.

4. References

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Chapter II

Developmental Trajectory of the Prefrontal Cortex: A Systematic Review of Diffusion Tensor Imaging Studies

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Abstract

Fluctuations in gray and white matter volumes, as shown through morphometric indexes, in addition to the fibers' reorganization and refinement of synaptic connectivity, apparently happen in a particular temporo-spatial sequence during the dynamic and prolonged process of cerebral maturation throughout most of the human life cycle. These developmental events are associated to regional modifications of brain tissues and neural circuits, contributing to networks' specialization and enhancing cognitive processing.

According to several studies, improved cognitive processes are possibly myelin-dependent, thus associated to white matter maturation. Of particular interest is the developmental pattern of the prefrontal cortex (PFC), more specifically the PFC white matter, due to its role in high-level executive processes such as attention, working memory and inhibitory control. Diffusion Tensor Imaging (DTI) is a widely used non-invasive technique to assess white matter maturation, considering its sensitivity in evaluating axons' organization using the random motion of water molecules within tissues as measure.

A systematic literature review was conducted using the Web of Science, PubMed and Embase databases to analyze the development of PFC white matter using DTI data. Both the research and reporting of results were based on Cochrane's recommendations and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines. The results document an increased myelination, organization and integrity of frontal white matter with age, as revealed by DTI indexes

(fractional anisotropy [FA], mean diffusivity [MD], radial diffusivity [RD] and axial diffusivity [AD]), highlighting the extended developmental course of the frontal structural connectivity, which parallels the improvements in higher level cognitive functions observed between adolescence and early adulthood.

Keywords: Prefrontal cortex, white matter, myelination, diffusion tensor imaging, development.

1. Introduction

According to neuroimaging findings, the human brain presents a specific maturing schedule throughout life, which is related to progressive and regressive neurobiological events and reflects a dynamic and extended developmental process both in terms of structure and cognitive functioning (Giedd, 2004; Lenroot & Giedd, 2006). Fluctuations of gray and white matter (as measured through morphometric indexes: volumes, cortical thickness, etc.) and the reorganization and refinement of synaptic connectivity apparently happen in a particular temporo-spatial sequence since birth until early adulthood, and are associated to regional modifications of brain tissues and neural circuits (Durston et al., 2001; Giedd & Rapoport, 2010; Giedd et al., 1999; Giedd, 2008; Huttenlocher, 1979; Shaw et al., 2008). Accordingly, these findings suggest that particular structural and functional characteristics arise along brain development, eventually showing interdependency with specific cognitive milestones (Bennett, Diamond, Krech, & Rosenzweig, 1964; Casey, Giedd, & Thomas, 2000; Paus et al., 1999; Schmithorst, Wilke, Dardzinski, & Holland, 2005).

Namely, a major increase of global gray matter (GM) observed between birth and childhood is associated to an overproduction of synapses and is followed by a decline throughout adolescence (Durston et al., 2001; Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004) This GM thinning, associated to pruning, among other cellular maturational processes, has been associated to the networks' specialization (Giedd, 2004; Lenroot & Giedd, 2006; Petanjek et al., 2011) and to improved

cognitive skills, which seems to occur in the frontal lobes between adolescence and early adulthood (Durston et al., 2001; Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Lebel & Beaulieu, 2011; Lenroot & Giedd, 2006; Petanjek et al., 2011).

On the other hand, whole brain white matter generally increases throughout life, which has been associated with the growing need of additional and effective inter-neuronal connections to sustain the increasing central nervous system (CNS) complexity (Bennett et al., 1964; Dubois et al., 2013; Durston et al., 2001; Giedd et al., 1999; Giedd, 2004; Lenroot & Giedd, 2006; Snaidero & Simons, 2014).

Myelin, the major white matter compound, grabs around axons basically acting as an electrical insulator that speeds impulse conduction between neurons and increases brain efficiency (Fields, 2005; 2008; Snaidero & Simons, 2014). The process of axons' myelination during brain development seems to be region-specific and follows a specific temporal sequence during the CNS maturation, associated to the interaction between brain structure and environmental factors that conversely modulate brain functioning (Dubois et al., 2013; Fields, 2005; Snaidero & Simons, 2014).

Actually, it seems that the major myelin formation is an on-going process during ± 30 to 40 years of the human lifecycle, following an inferior to superior and posterior-to-anterior (Colby, Van Horn, & Sowell, 2011) and central-to-peripheral direction of maturation (Dubois et al., 2013; Snaidero & Simons, 2014). Specifically, myelination is first observed in the dorsal sensorimotor areas (temporo-occipital and parieto-frontal areas) while temporo-parietal and frontal association areas need more time to be fully myelinated, probably due to its relation with complex cognitive processes (Deoni et al., 2011; Dubois et al., 2013; Gogtay et al., 2004; Lenroot & Giedd, 2006; Paus et al., 2001). Specifically, the prefrontal cortex (PFC) maturation, including the dorsolateral PFC and orbitofrontal PFC, is not finished until the 20's (Giedd, 2004; Gogtay et al., 2004) and progresses in a posterior to anterior direction (Colby et al., 2011; Gogtay et al., 2004) A peak of maturation was firstly attained in the caudal middle

fontal gyrus (around the twenties), while in the rostral middle frontal the peak was observed in the 25 years for fractional anisotropy (FA) and 33 for mean diffusivity (MD) (Tames et al., 2010).

Additionally, it seems these maturational processes are likely to be associated with PFC functional role in supporting high-level cognitive functions such as attentional processing, working memory and inhibitory control (Kraus et al., 2007; Martino, Brogna, Robles, Vergani, & Duffau, 2010) which parallel the maturation of the major frontal tracts (superior longitudinal fasciculus; inferior fronto-occipital fasciculus; and uncinate fasciculus), suggesting that frontal white matter maturation might be the neural substrate of improved executive processes.

Finally, changes in white matter organization have been broadly observed, *in vivo,* through diffusion tensor imaging (DTI) metrics, which are believed to be quite sensitive to myelin changes, providing insight into subtle microstructural alterations (e.g. axons' myelination or demyelination; axonal injury or degeneration; axonal packing), whether these alterations happen during the healthy developmental pathway of the brain or due to clinical conditions (e.g. multiple sclerosis (MS), Wallerian degeneration) (Aung, Mar, & Benzinger, 2013; Fox et al., 2011; Le Bihan et al., 2001; Song et al., 2003; Sun et al., 2006; Xie, Wang, Wu, Song, & Sun, 2011). Once DTI provides information about axonal organization and integrity of neural pathways that ensure communication among several regions of the brain, which could not be retrieved from standard MRI measures (e.g. volumes or cortical thickness), it may provide relevant findings about PFC white matter developmental course.

Next, a brief explanation of DTI basic concepts and applications are described.

Several studies up to now have assessed white matter maturation through non-invasive imaging techniques such as DTI, due to its sensitivity in evaluating axons' features, using the random motion of water molecules (thermal Brownian motion) within tissues as measure (Hagmann et al., 2006; Le Bihan, 2003; Thomason & Thompson, 2011). The random displacement of water molecules observed in biological tissues, seems to be driven by its anatomical architecture, being more or less restricted

depending on tissues' histological properties such as cellular (e.g. cell membrane, myelin) and molecular elements (Hagmann et al., 2006; Mori & Zhang, 2006). In this sense, water diffusion within a specific milieu might be classified as isotropic; i.e., water molecules travel randomly in all directions, or anisotropic; i.e., water molecules displace into a specific direction (Hagmann et al., 2006; Thomason & Thompson, 2011). Usually the micromotion of the water molecules in GM is considered isotropic, once water molecules spread around more freely, which is associated to the less fibrillar and intricate nature of GM, compared to the white matter; whereas in the healthy white matter the movement of water molecules is essentially considered anisotropic, meaning that typically water molecules travel along a preferred direction, guided by structural barriers (e.g. glial cells, axonal membranes, myelin bundles), present in white matter. Since the white matter structure is characterized by compactly packaged and coherently aligned axons organized in bundles, the displacement of water molecules seems to be usually aligned with axons' orientation (even in non-myelinated axons), being greater in the direction parallel to axonal bundles rather than perpendicularly (Beaulieu, 2002; Hagmann et al., 2006; Pierpaoli, Jezzard, Basser, Barnett, & Di Chiro, 1996;).

Diffusion-weighted image (DWI) acquisition consists in the application of a gradient sequence, within a commonly used range of 700-1300 s/mm^2 (although these are variable values, depending on the acquisition protocol) to the entire brain covering a minimum of six directions (e.g. X, Y and Z), plus, at least, an image with no gradient (b = 0 s/mm^2). (Alexander, Lee, Lazar, & Field, 2007; Alger, 2012; Chilla, Tan, Xu, & Poh, 2015; Nishikawa et al., 2013). The b0 image (s) is used to compare the behavior of the water molecules between its initial state (acquired with no diffusion gradient) and after gradients' application. To estimate the diffusion values from each voxel of the acquired DWI, the main direction of diffusivities (eigenvectors), which result from estimating the diffusion along three orthogonal directions, and the diffusion values associated to each main direction (eigenvalues) need to be determined, thus describing the properties of the diffusion tensor. (Hagmann et al., 2006; Le Bihan et

al., 2001). The classification of the eigenvalues is as follows, $\lambda_1 \geq \lambda_2 \geq \lambda_3$, and each one matches one eigenvector. The eigenvector that corresponds to the largest eigenvalue (λ_1) represents the principal direction of the diffusion. The diffusion transversal to the main direction is represented by λ_2 and λ_3. The relation between the eigenvalues reveals the diffusion properties within a specific image, e.g. if the eigenvalues significantly change from each other, diffusion is assumed to be anisotropic, but if all the eigenvalues are similar, diffusion is presumed to be isotropic. Once the diffusion tensor is obtained, the scalar indexes (e.g. mean diffusivity and anisotropy) proposed to describe water diffusion in a specific voxel may be calculated.

Several diffusivity-based measures are referred In the DTI research field, however fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) are the most widely used indexes to characterize water diffusivity in the brain, more specifically in the white matter (Alexander et al., 2007; Alger, 2012; Hagmann et al., 2006, Le Bihan et al., 2001; Mori & Zhang, 2006; Pierpaoli et al., 1996). Specifically, if the relation between the eigenvalues assumes an elongated ellipsoid shape, than the diffusion is assumed to be anisotropic, as represented by the FA index. FA is a

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

scalar measure of the magnitude of diffusion anisotropy in a voxel, derived from a ratio of the principal diffusivities.

Basically, this measure is computed through the comparison of each eigenvalue ($\lambda_1, \lambda_2, \lambda_3$) with the diffusion coefficient (*D)*. FA represents the degree in which the diffusion of water molecules is anisotropic or unidirectional, moving into a preferred direction, varying between 0 (representing isotropic diffusion of water molecules with no directional preference) and 1 (representing a unidirectional displacement of water molecules or anisotropy). A second largely used measure is MD, which derives from the mean of the three eigenvalues, ($\lambda_1 + \lambda_2 + \lambda_3$) */3,* and indicates the overall displacement of water molecules in a voxel, although not providing information about the direction of

the movement. Two additional indexes studied on a smaller scale are AD and RD. AD is derived from the largest of the three eigenvalues (λ_1) in the Z or longitudinal axis and represents the rate of water diffusion in the direction of greatest diffusion that is usually considered to be parallel to the axonal tracts (when the tissue is mainly composed of myelin sheaths). RD derives from the average of the second λ_2 and third λ_3 eigenvalues in the X and Y-axes representing the diffusion perpendicular to the fiber direction, which is possibly constrained by cellular structure and myelination (Alexander et al., 2007; Alger, 2012; Hagmann et al., 2006; Le Bihan et al., 2001; Mori & Zhang, 2006; Pierpaoli & Basser, 1996; Thomason & Thompson, 2011). A great advantage of DTI is providing rotationally-invariant diffusivity measures, independent of the position of the fibers in space or how the subjects' head was positioned in the scan (Alexander et al., 2007; Alger, 2012).

Several methods are used to analyze DTI data. Namely, voxelwise and tract-based spatial statistics (TBSS), which are both fully automated voxel-based approaches, allowing investigating the whole brain. Voxelwise analysis runs in SPM and compares group means using a univariate approach in which each voxel is processed separately; while TBSS runs in FSL and allows investigating all fiber bundles in the brain, comparing each subject to a group mean skeleton created based on a center value found at each tract (Smith et al., 2006). Diffusion tensor tractography (DTT) allow the reconstruction of white matter bundles using the diffusion tensor of each voxel and the region of interest (ROI) method is used to assess regions defined à priori by the researcher (Mukherjee, Berman, Chung, Hess, & Henry, 2008; Froeling, Pullens, & Leemans, 2016). The retrieved mean values are compared between subjects.

Interpreting the DTI indexes in the light of brain maturation is not straightforward, however, some assumptions regarding the hypothetical associations between diffusivity measures, in special the anisotropic diffusion, and biological mechanisms have been referred in the literature. Commonly anisotropy is associated to increased myelination; however, it seems that myelin is not a key factor for

anisotropic diffusion to exist; instead it is believed that it may modulate the degree of anisotropy (Beaulieu, 2002). Specifically, it seems that changes in FA are related to the architecture of the white matter myelinated fibers (Beaulieu, 2002) and may detect rapid microstructural changes in the brain such as neuronal plasticity, axonal structure remodeling, fiber density and reorganization (Ding et al., 2013) and activity-dependent myelination mechanisms (Scholz, Klein, Behrens, & Johansen-Berg, 2009). Thus, high values of FA have been associated with enhanced neural connectivity, white matter packing, greater fiber integrity and myelination. Although myelin is not considered as a condition of the anisotropic diffusion, (Beaulieu, 2002), myelin depletion or damaged myelin significantly changes measures of anisotropy (Assaf & Pasternak, 2008). Namely, It seems that the degree of anisotropic diffusion rises during the myelination process, i.e. as myelination increases along neurodevelopment, the values of anisotropy also increase; whereas, reporting from myelin-related disorders such as MS (Preziosa et al., 2017; Roosendaal et al., 2009) or Wallerian degeneration (Xie et al., 2011), it seems that decreased anisotropic values are associated to damaged myelin (Neil, Miller, Mukherjee, & Huppi, 2002). While myelination is not a condition to find significant changes in FA, it may be important in detecting alterations in MD. In fact, it seems that MD increases largely in the absence of myelin, prompting the water displacement to be greater in the perpendicular direction to axonal bundles, than in the parallel direction (main axe) (Beaulieu, 2002). Thus, low MD values seem to be associated with white matter integrity since it reflects diminished free diffusion within tissues; whereas increased MD values have been associated with myelin lesions (Preziosa et al., 2017). Regarding AD, it is thought to reflect the integrity/damage of axons, meaning that higher AD values are associated with intact healthy axons and lower values are associated to injured axons (Beaulieu, 2002) as, for example, in Wallerian degeneration (Sun, Liang, Cross, & Song, 2008). In fact, obstructions to parallel diffusion seem to increase from the breakdown of the longitudinal axonal structure (Beaulieu, 2002). Lastly, RD appears to be associated with myelination/demyelination processes (Song et al., 2003; Sun et al., 2006) once

diffusion perpendicular to axonal tracts seems to be influenced (i.e. reduced) by interactions of the water molecules with membranes and myelin sheaths, causing diffusion to become hindered as water molecules do not move freely in the presence of obstacles (Alexander et al., 2007; Medana & Esiri, 2003; Thomason & Thompson, 2011). That is why, transverse diffusivity increases in injured axons (e.g. MS, Wallerian degeneration) (Roosendaal et al., 2009; Sun et al., 2008; Xie et al., 2011).

Finally, it seems that these water diffusion related measures effectively change during development reflecting underlying tissues' structural changes (Neil et al., 2002). Therefore, the main purpose of the present review was to systematize current knowledge, on the developmental pathway of the frontal cortex in terms of its microstructural connectivity, as shown by DTI metrics, using the PRISMA guidelines - **www.prisma-statement.org** - (Moher, Liberati, Tetzlaff, Altman, & The PRISMA Group, 2009).

2. Method

A search in peer-review publications was performed using the Web of Science, PubMed and Embase databases to identify relevant papers that evaluated the development of the frontal white matter in healthy individuals, using DTI. A total of 53 articles were identified through database searching. Records were retrieved using the key-expressions: "("Diffusion tensor" OR "DTI") AND (typical OR normal OR healthy) AND (brain development AND "white matter" AND frontal)". In detail, the search through Embase database was performed as follows: advanced search in "Journals", in the fields of Neuroscience and Medicine, using the "key-expressions" in all fields, open access articles, short communications and articles in press; search through Web of Science and PubMed was performed using the "key-expressions". No time restriction was applied. Additional articles that were referenced in other studies were also assessed. In order to pursue the database searching and the assessment for eligibility some inclusion and exclusion criteria were formerly defined. Specifically,

articles were included if: 1) were published in a peer-review journal; 2) assessed the frontal white matter development in healthy subjects employing cross-sectional or longitudinal designs; 3) used DWI acquisitions and DTI measures; 4) evaluated one or more out of the four DTI indexes: FA, MD, AD and RD, As exclusion criteria was established: 1) research using clinical samples (not typical development); 2) studies correlating DTI measures with clinical variables; 3) other imaging method that was not DTI; and 4) the frontal lobes or the frontal association tracts were not included in the analysis; 5) aging studies including only adults (>40 years old). After a careful selection of 53 studies, 12 duplicates were removed, 41 were assessed for eligibility and only 27 articles were included in this systematic review (see the diagram in figure 1). Table 1 lists all the articles revised and provides the first author, year of publication, as well as brief information concerning the participants enrolled in the study in addition to details related to the methodology, regions of interest, DTI indexes, main results and statistical analyses.

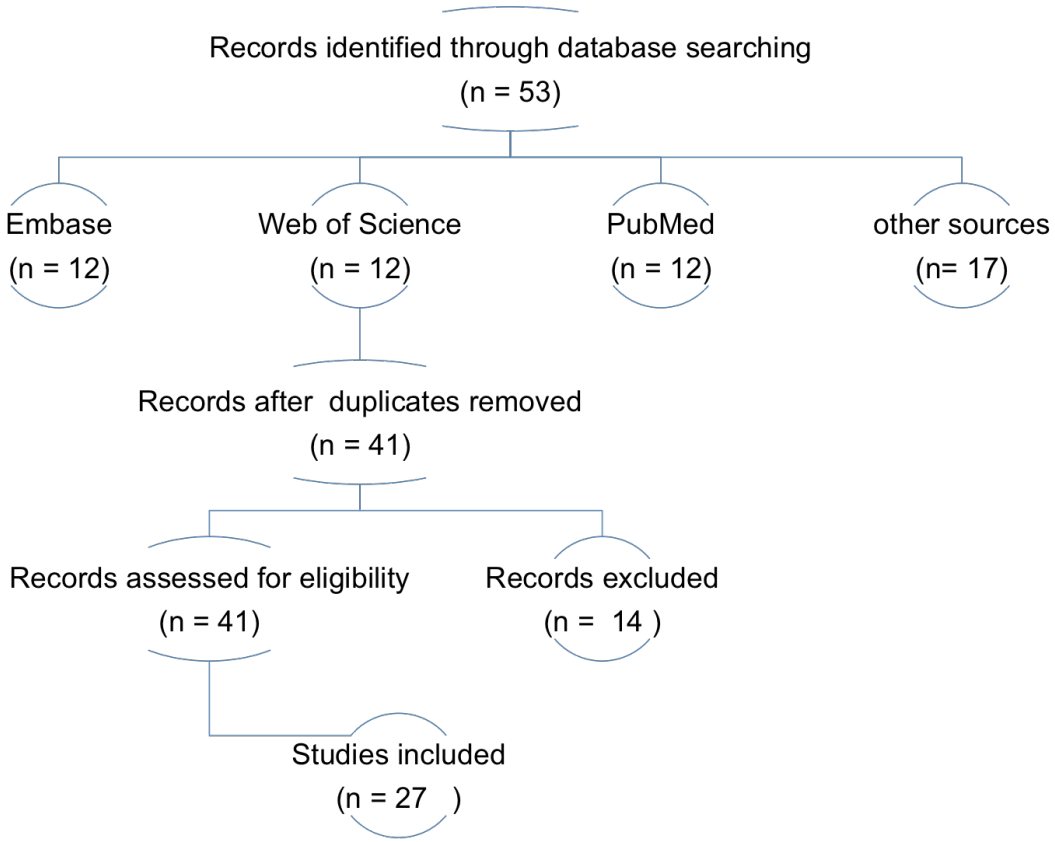

FIGURE 1 | Flow Diagram for paper selection

3. Results

Overall, the data gathered in this systematic review document a growing of FA over the distinct periods of development, concomitant with a reduction of MD and RD and to a lesser extent AD, in the frontal cortex and in its main association tracts. The findings disclosed by the analysis of the DTI indexes in healthy individuals, from neonatal ages until adulthood, suggested a late process of frontal white matter maturing, which still remains in progress throughout the third decade of life.

The majority of the studies that evaluated the FA index in the frontal white matter showed a linear anisotropic increase with age, since the neonatal period until early adulthood (range: birth-30 years), being the lowest FA values observed in children and the highest in young adults (Colby et al., 2011; Kumar, Nguyen, Macey, Woo, & Harper, 2012; Schneider, Il'yasov, Hennig, & Martin, 2004; Simmonds, Hallquist, Asato, & Luna, 2014). Comparisons between groups revealed that children (range: 8-13) showed the lowest FA values in comparison to older adolescents (range: 16-18) or young adults (range: 18-31, Klingberg, Vaidya, Gabrieli, Moseley, & Hedehus, 1999; Qiu, Tan, Zhou, & Khong, 2008; Qiu, Li, Liu, Xie, & Wang, 2010), yet no FA changes were observed between younger (range: 6-8) and older children (range: 9-11, Qiu et al., 2008). Additionally, no significant correlations were found between age and FA values in a cohort ranging in age between 5 and 19 years old (Bonekamp et al., 2007).

In terms of the diffusivity measures (MD, RD and AD) more variability was observed among studies. In relation to MD, most of the studies that assessed this index showed similar results, i.e. decreasing MD with age (range: 5-43, Bartzokis et al., 2012; Bonekamp et al., 2007; Qiu et al., 2008; 2010) and a peak was observed around 43 years old (minimum MD value), (Bartzokis et al. 2012); although, a previous study had found that infants (range: 24-36 months) displayed similar MD to adults (Schneider et al., 2004).

Regarding the RD an AD indexes, the findings revealed an overall decline with age (range: 6-54, Bartzokis et al., 2012; Kumar et al., 2012; Qiu et al., 2008; Simmonds et al., 2014), and a peak (lowest value) was noted at approximately 35 years old for RD and at age 54 for AD (Bartzokis et al. 2012); exceptionally, decreased RD but no changes in AD over age (range: 5-28) were observed in the study conducted by Colby et al., (2011).

When analyzing specific frontal regions, namely, the superior, middle and inferior frontal gyri, the dorsolateral and orbitofrontal cortex, a pattern of increased anisotropy and decreased diffusivity levels along the distinct developmental periods had emerged. A rise of FA levels with age was observed between postnatal ages and early adulthood in the superior frontal gyrus (range: 21 days-27 years, Lobel et al., 2009; Moon et al., 2011; Snook, Paulson, Roy, Phillips, & Beaulieu, 2005; Tamnes et al., 2010), middle frontal gyrus (range: 6-35, Barnea-Goraly et al., 2005; Li & Noseworthy, 2002; Tamnes et al., 2010), inferior frontal gyrus (range: 21 days-30 years, Ashtari et al., 2007; Barnea-Goraly et al., 2005; Lobel et al., 2009; Tamnes et al., 2010), dorsolateral, and orbitofrontal cortex (range: 6-30, Barnea-Goraly et al., 2005; Tamnes et al., 2010). Overall, studies documented that adult individuals displayed the highest FA levels whether compared with adolescents or children or infants.

Regarding the diffusivity indexes MD, RD and AD, a trend for decreasing values with age was observed in the superior frontal gyrus (range: 21 days-30 years, Lobel et al., 2009; Moon et al., 2011; Snook et al., 2005; Tamnes et al., 2010), middle frontal gyrus (range: 8-30, Tamnes et al., 2010), inferior frontal gyrus (21 days-30 years, Lobel et al., 2009; Tamnes et al., 2010) and in the orbitofrontal cortex (range: 8-30, Tamnes et al., 2010). Overall, all studies but one (Ashtari et al. 2007) documented that adult individuals displayed the lowest diffusivity levels (MD, RD or AD) whether compared with adolescents or children or infants. Specifically, Ashtari et al. (2007) found an augmentation of AD without significant changes in RD and MD in the inferior frontal gyrus of older adolescents (range: 17-20) compared to their younger peers (range: 10-16).

A tendency to increased FA and decreased MD and RD as a function of age was also observed in the frontal association tracts, but not so consistent results were observed regarding the AD. Specifically, increased FA with age was observed since early ages until adulthood in the inferior fronto-occipital fasciculus (range: 4-30, Eluvathingal, Hasan, Kramer, Fletcher, & Ewing-Cobbs, 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Muftuler et al., 2012; Taki et al., 2013), superior fronto-occipital fasciculus (range: 4-30, Bonekamp et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Snook, Plewes, & Beaulieu, 2007; Simmonds et al., 2014), superior longitudinal fasciculus (range: 2 months-42 years, Bava et al., 2010; Giorgio et al., 2008; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Muftuler et al., 2012; Snook et al., 2007; Simmonds et al., 2014; Taki et al., 2013; Uda et al., 2015) and uncinate fasciculus (range: 4-30, Eluvathingal et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Muftuler et al., 2012; Simmonds et al., 2014; Taki et al., 2013).

The findings observed in the association tracts were not consistent among studies, especially in relation to RD and AD rate of change with age. Specifically, decreased MD values across age in the inferior fronto-occipital fasciculus were found by nearly all the studies included in this review (range: 4-30, Eluvathingal et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Muftuler et al., 2012; Taki et al., 2013), excluding the one of Bava et al., (2010), which found inter-hemispheric variations. Likewise, decreasing MD was found in the superior fronto-occipital fasciculus (range: 4-30, Bonekamp et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Snook et al., 2007), in the superior longitudinal fasciculus (range: 2 months-30 years, Bava et al., 2010; Bonekamp et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Muftuler et al., 2012; Snook et al., 2007; Taki et al., 2013; Uda et al., 2015) and in the uncinate fasciculus (range: 4-30, Eluvathingal et al., 2007; Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008; Muftuler et al., 2012; Taki et al., 2013).

A reduction of RD with age was observed in the inferior fronto-occipital fasciculus (range: 4-30, Asato, Terwilliger, Woo, & Luna, 2010; Eluvathingal et al., 2007; Krogsrud et al., 2016), in the superior fronto-occipital fasciculus (range: 4-30, Krogsrud et al., 2016; Simmonds et al., 2014), in the superior longitudinal fasciculus (range: 2 months-30 years, Asato et al., 2010; Bava et al., 2010; Krogsrud et al., 2016; Simmonds et al., 2014; Uda et al., 2015) and in the uncinate fasciculus (range: 4-30, Asato et al., 2010; Eluvathingal et al., 2007; Krogsrud et al., 2016; Simmonds et al., 2014).

Regarding AD, reduced values across age were noted in the inferior fronto-occipital fasciculus (range: 5-17, Eluvathingal et al., 2007; Lebel & Beaulieu, 2011), in the superior fronto-occipital fasciculus (range: 8-29, Simmonds et al., 2014), in the superior longitudinal fasciculus (range: 2 months-30 years, Lebel & Beaulieu, 2011; Simmonds et al., 2014; Uda et al., 2015), and in the uncinate fasciculus (range: 4-14, Krogsrud et al., 2016; Lebel & Beaulieu, 2011); differently, an increased AD (range: 8-32) was found in the uncinate fasciculus (Lebel & Beaulieu, 2011; Simmonds et al., 2014) and in the inferior fronto-occipital fasciculus and superior longitudinal fasciculus (range: 14-32, Lebel & Beaulieu, 2011).

However, some exceptions were found. Specifically, no age-related changes in the FA values were found between childhood and late adolescence (range: 5-19) in the superior longitudinal fasciculus (Bonekamp et al., 2007) and between late-adolescence and early-adulthood (range: 16-21) in the inferior fronto-occipital fasciculus (Bava et al., 2010). Inter-hemispheric variances were observed in the inferior fronto-occipital fasciculus such as increased values of MD and AD in the right inferior fronto-occipital fasciculus but both measures were reduced in the left inferior fronto-occipital fasciculus (Bava et al., 2010).

No changes in RD values were observed between late-adolescent individuals and early-adults (range: 14-32, Bava et al., 2010; Lebel & Beaulieu, 2011) in the inferior fronto-occipital fasciculus, superior fronto-occipital fasciculus, superior longitudinal fasciculus, and uncinate fasciculus, neither in

AD between childhood (range: 4-9) and early adolescence (range: 5-11) in the inferior fronto-occipital fasciculus (Krogsrud et al., 2016).

Moreover, distinct maturation timings have been observed in some of the tracts. Non-linear trajectories (increased FA and decreased MD) were found in the bilateral inferior fronto-occipital fasciculus (Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Lebel et al., 2008) and in the left inferior fronto-occipital fasciculus (decreased RD, Krogsrud et al., 2016), showing an exponential curve that peaked by the end of the adolescence and reached a plateau throughout early adulthood (Bava et al., 2010).

Non-linear trajectories (increased FA and decreased MD) were also found in the superior fronto-occipital fasciculus, bilaterally (Krogsrud et al., 2016; Lebel & Beaulieu, 2011) and in the left superior fronto-occipital fasciculus (decreased RD, Krogsrud et al., 2016), being fully matured (as shown by increased FA and decreased RD and AD) by early adolescence (Simmonds et al., 2014).

Non-linear trajectories (increased FA and decreased MD and RD and AD) were found in the superior longitudinal fasciculus (Krogsrud et al., 2016; Lebel & Beaulieu, 2011; Simmonds et al., 2014), showing an exponential curve that peaked in adolescence and reached a plateau throughout early adulthood (Bava et al., 2010; Lebel et al., 2008; Simmonds et al., 2014); despite a previous study revealed a linear growth curve of FA in the right superior longitudinal fasciculus of young adults (range: 23 to 42, Giorgio et al., 2008)

Non-linear trajectories (increased FA and decreased MD) were found in the uncinate fasciculus (Krogsrud et al., 2016; Lebel & Beaulieu, 2011) and in the left uncinate fasciculus (decreased RD, Krogsrud et al., 2016), with its development course continuing beyond the 28 years old (as shown by increased FA and AD), but in terms of decreased RD, it was matured in early adolescence (range: 11-13, Simmonds et al., 2014); however a previous study revealed a linear developmental trajectory for increased FA until age 25 (Lebel et al., 2008). In accordance, Muftuler et al., (2012) shown that in

youngest ages (6-11) the association tracts (inferior fronto-occipital fasciculus, superior longitudinal fasciculus and uncinate fasciculus) displayed a lower rate of change (between 0.5 to 1% per year. Additionally, a recent study showed a greater increase in FA and decrease of MD, AD and RD, between birth and 6 years old in the superior longitudinal fasciculus, followed by a plateau. The percentage of growth after 6 years old was near zero and 90% of the maximum FA values were reached around 16 years old and at 9 for MD (Uda et al., 2015).

Ultimately, some gender differences were found in the frontal association tracts, pointing to some variations in the maturation timings of the frontal bundles, between females and males. Specifically, lower RD values were observed in females' inferior fronto-occipital fasciculus, superior longitudinal fasciculus and uncinate fasciculus (range: 6-17, Asato et al. 2010; Eluvathingal et al., 2007) compared to males. An exception was observed in the frontal portion of the superior longitudinal fasciculus, which showed ongoing maturation until early adulthood in the female group; while in males just the parietal portion of the superior longitudinal fasciculus was fully matured by adolescence (range: 13-17) with the other tracts maturing throughout young adulthood (Asato et al, 2010). Higher FA values were found in males' superior longitudinal fasciculus and uncinate fasciculus, compared to females and greater values of MD were found in females' superior fronto-occipital fasciculus in comparison to its males' counterparts (range: 5-32, Lebel & Beaulieu, 2011).

However, other studies found no gender differences in the developmental trajectory of frontal white matter and its association tracts (Bartzokis et al., 2012; Bava et al., 2010; Giorgio et al., 2008; Krogsrud et al., 2016; Kumar et al., 2012, Muftuler et al., 2012; Tamnes et al., 2010, Uda et al., 2015).

Table 1 | Lists the revised articles with key information about the authors, year of publication, sample size, age range, design, method, regions of interest, DTI indexes, main results and statistics

Study	N	Age ranges	Design	Method of analysis	DTI Indexes	Region	Results	Statistics
Asato et al., 2010	114	8-12 (n=36); 13-17 (n=45); 18-28 (n=33); mean age: 15.5 ± 4.49	Transversal	TBSS	RD	sLF; iFOF; UF	êRD, plateau end of adolescence in sLF + iFOF	One-way ANOVA
Ashtari et al., 2007	24 (all males)	10-16 (n=12); 17-20 (n=12); mean age: 16.6 ± 2.5	Transversal	Voxelwise	FA, MD, RD, AD	IFG	éFA, éAD No differences in RD, MD	Voxelwise ANCOVA
Barnea-Goraly et al., 2005	34 (18 male)	6 -19; mean age: 12.7 ± 3.3	Transversal	Voxelwise	FA	DLPFC; OFC; MFG; IFG	éFA	Linear regression
Bartzokis et al., 2012	171 (93 male)	14-93; mean age: 51 ± 22	Transversal	ROI	FA, MD, RD, AD	FWM	êRD, MD, AD	Pearson correlation
Bava et al., 2010	22 (15 male)	Time 1: 16-20 (mean age: 17.8 ± 1.4); Time 2: 17-21 (mean age: 19.2 ± 1.4)	Longitudinal	TBSS	FA, MD, RD, AD	iFOF; sLF	sLF: éFA, êMD, êRD; Right iFOF: éMD, éAD; Left iFOF: ê MD, êAD	t-tests
Bonekamp et al., 2007	40 (22 male)	5-19; mean age: 13.7 ± 3.5	Transversal	ROI	FA, MD	FWM; sLF; sFOF	FWM + sLF: êMD sFOF: éFA êMD	Univariate GLM
Colby et al., 2011	32 (16 male)	5-28; mean age: 14.4 ± 7.2	Transversal	TBSS	FA, AD, RD	FWM	éFA, êRD, no changes in AD	Regression
Eluvathingal et al., 2007	31 (15 male)	6-17; mean age: 11.4 ± 3.1	Transversal	DTT	FA, MD, AD, RD	iFOF; UF	éFA, êMD, êAD, êRD	Pearson correlation
Giorgio et al., 2008	62 (33 male)	13.5-21 (n=42); 23-42 (n=20)	Transversal	TBSS	FA	Right sLF	éFA in adults	Pearson correlation
Klingberg et al., 1999	12	8-12 (n=7); 20-31 (n=5)	Transversal	ROI	FA	FWM	éFA	_____
Krogsrud et al., 2015	159 (69 male)	Time 1: 4-9 (mean age: 6.2 ± 1.1); Time 2: 5-11 (mean age: 7.8 ± 1.1)	Longitudinal	TBSS	FA, MD, AD, RD	iFOF; sFOF; sLF; UF	éFA, êMD, êRD; êAD in the sLF only	GLM, Correlation, t-tests
Kumar et al., 2012	30 (18 male)	8-24; mean age: 17.7 ± 4.6	Transversal	ROI	AD, RD	FWM	êAD, êRD	Pearson correlation

Study	N	Age ranges	Design	Method of analysis	DTI Indexes	Region	Results	Statistics
Li & Noseworthy, 2002	16	13-15 (mean age: 14); 22-35 (mean age: 36)	Transversal	Voxelwise	FA	MFG	éFA	t-tests
Lobel et al., 2009	72	3 weeks-19 years	Transversal	ROI	FA, MD	SFG; IFG	éFA, êMD	Spearman correlation
Moon et al., 2011	87 (37 male)	4-17; mean age: 11.2 ± 3.6	Transversal	ROI	FA, MD	SFG	é FA, êMD	Linear regression
Muftuler et al., 2012	126 (67 male)	6-11; mean age: 8.1 ± 1.3	Transversal	TBSS	FA, MD	iFOF; sLF; UF	éFA, êMD	GLM
Qiu et al., 2008	75 (40 male)	6-8 (n=24, mean age: 7.4 ± 0.3); 9-11 (n=27, mean age: 10.3 ± 0.5); 18-26 (n=24, mean age: 22.8 ± 2.3)	Transversal	TBSS/ROI	FA, MD, RD, AD	FWM	éFA (older children *vs* adults), êMD, êRD, êAD	ANOVA
Qiu et al., 2010	92 (42 male)	11-13 (n=32); 16-18(n=32); 23-25(n=28)	Transversal	Voxel based	FA, MD	FWM	éFA, êMD	Linear correlation + t-test
Schneider et al., 2004	52 (30 male)	Birth -16 (mean age: 6.6)	Transversal	ROI	MD, FA	FWM	êMD, éFA	Multiexponential regression
Simmonds et al., 2014	128 (61 male)	8-29; mean age: 14.9 ± 4.2	Longitudinal	TBSS	FA, AD, RD	FWM; sFOF; sLF; UF	éFA, êRD, êAD; éAD in UF	Mixed-effects regression
Snook et al., 2005	60 (28 male)	8-12 (n=32, mean age: 11.1 ± 1.3); 21-27 (n=28, mean age: 24.4 ± 1.8)	Transversal	ROI	FA, MD	SFG	éFA, êMD	Linear regression + t-tests
Snook et al., 2007	60 (28 male)	8-12 (n=32, mean age: 11.1 ± 1.3); 21-27 (n=28, mean age: 24.4 ± 1.8)	Transversal	ROI, Voxel based	FA, MD	sLF; sFOF	éFA, êMD	Linear regression + t-tests
Taki et al., 2013	246 (119 male)	5-18	Transversal	Voxel based ROI	FA, MD	sLF; UF; iFOF	éFA, êMD	Correlation
Tamnes et al., 2010	168 (81 male)	8-30; mean age: 17.7 ± 6.1	Transversal	ROI	FA, MD, AD, RD	SFG; MFG; IFG; OFC	éFA, êMD, êRD, êAD	Regression
Uda et al., 2015	52 (33 male)	2 months-25 years; mean age: 105.8 ± 82.9 months	Transversal	ROI	FA, MD, AD, RD	sLF	éFA, êMD, êAD, êRD	Regression ANOVA

AD: axial diffusivity; **DLPFC:** dorsolateral prefrontal cortex; **DTT:** diffusion tensor tractography; **FA:** fractional anisotropy; **FWM:** frontal white matter; **IFG:** Inferior frontal gyrus; **iFOF:** inferior fronto-occipital fasciculus; **MD:** mean diffusivity; **MFG:** middle frontal gyrus; **OFC:** orbitofrontal cortex; **RD:** radial diffusivity; **ROI:** region of interest; **SFG:** superior frontal gyrus; **sFOF:** superior fronto-occipital fasciculus; **sLF:** superior longitudinal fasciculus; **TBSS:** tract-based spatial statistics; **UF:** uncinate fasciculus

4. Discussion

The purpose of this review was to systematize current knowledge on the developmental pathway of the frontal cortex in terms of its microstructural connectivity, as shown by DTI metrics. Overall, the findings suggest an extended developmental course of frontal white matter and its main association tracts (superior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior fronto-occipital fasciculus and uncinate fasciculus), as shown by variations in white mater-related indexes (FA, MD, RD and AD). The majority of the studies suggest that FA (increase), MD, RD and AD (decrease) values present a cumulative rate of variance with age, since neonatal ages until adulthood, although existing some periods of steadiness.

The results of this systematic review document that frontal white matter maturation continues far beyond the 3rd decade of life, which is consistent with other MRI derived measures, such as reduction of cortical thickness and increasing of white matter volume observed throughout adolescence and early adulthood (Tamnes et al., 2010). Specifically, a tendency to increased anisotropy (FA) and decreased diffusivity (MD and RD) as a function of age was observed in the frontal white matter and association tracts. The lowest FA values and the highest MD and RD values were observed in infants and children and contrastingly, the highest FA and lowest MD and RD in young adults. Comparing to other regions of the brain, the lowest FA was found in the neonatal frontal lobes (Schneider, 2004) and the highest being observed in the adolescents (>144 months), while the highest MD being observed in the newborns and the lowest in adolescents, in comparison with all other brain areas (Schneider, 2004). Additionally, adult FA in the frontal lobes was lower than in all other regions of the brain (e.g. temporal, parietal and occipital lobes) reflecting the protracted myelination of the frontal lobes (Schneider, 2004).

As mentioned earlier, higher values of anisotropy (e.g., FA) are commonly associated with axonal organization, bundles density, coherence of fibers' orientation (Beaulieu, 2002; Ding et al., 2013; Scholz et al., 2009), while lower values of diffusivity seem to be related with intact healthy axons

(as measured through MD and AD; Beaulieu, 2002; Preziosa et al., 2017; Sun et al., 2008) and myelin growth (as measured through RD; Alexander et al., 2007; Song et al., 2003; Sun et al., 2006). Overall, the studies converge in showing that FA, MD, AD and RD may be sensitive to different aspects of white matter maturation.

It is important to note that although greater axonal organization and coherence are events commonly associated to anisotropic diffusion, it is difficult to attribute particular microstructural features that are underlying changes in FA of the frontal white matter, as it is not possible to know whether FA variation occurs due to the improved coherence of the fiber tracts or if it is dependent only on the myelination process. Still, healthy cellular membranes are thought to be the major basis of anisotropic water diffusion in white matter fibers and myelination is thought to modulate the degree of anisotropic diffusion, even though the currently available methods do not allow dissociating these two microstructural features (Beaulieu, 2002). The understanding of reduced AD measures is however more controversial. Lower AD values are associated with injured axons (Beaulieu, 2002; Sun et al., 2008), but may also be related with regulation of axon branching across development (Gallo, 2010; Gibson & Ma, 2011), increasing number of white matter tracts, and increasing axonal caliber across development and consequent inter-axonal space decrease (Beaulieu, 2002; Hagmann et al., 2006; Pierpaoli et al., 1996). Decreased RD values reflect higher myelination, as myelin sheaths seem to modulate anisotropic diffusion by creating barriers to the displacement of water molecules (Beaulieu, 2002). Additionally, disruptions of the myelin sheaths have been related to increasing RD (Song et al., 2002).

In accordance, the process of myelination, axonal density and organization and coherence of fibers' orientation within the frontal white matter lasts until the thirties, as shown by the linear increase of FA and decrease in MD and RD, but not so consistently AD until this age range. Nevertheless, the rate of change in anisotropic and diffusion measures appears to be lower in the interval period between

6 and 11 years old (Muftuler et al., 2012; Qiu et al., 2008; Uda et al., 2015). This deceleration of change with age (between 6 and 11 years) suggests that this developmental period might represent a rather stable period of frontal white matter development comparing to the first years of life, till 4 years old, (Krogsrud et al., 2016) and adolescence. A possible explanation for white matter growth to slowdown in this interval may be associated with the increase of GM (Giedd et al., 1999). In fact, a relation between these two events might eventually exist since a peak in GM growth was noted around age 12 (followed by a decline) and the cerebral volume remained quite unchangeable after age 6, as shown by MRI studies (Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Lenroot & Giedd, 2006). In addition, it was observed that frontal cortical thickness was negatively correlated with the white matter volume and FA values, and positively associated with MD, AD and RD values. This pattern is likely to be associated with GM pruning and myelination and consequent enhanced fiber organization throughout development (Tamnes et al., 2010).

Moreover, distinct timings of maturation, as measured through anisotropy and diffusivity, were found for the main frontal association tracts. Apparently some of the tracts exhibited fully maturation, as shown by DTI indexes, either in terms of axonal organization (increased FA and decreased MD and AD) or myelin growth (decreased RD) between adolescence and early adulthood, such as the inferior fronto-occipital fasciculus (Asato et al., 2010; Bava et al., 2010; Eluvathingal et al., 2007; Lebel & Beaulieu, 2011), the superior fronto-occipital fasciculus (Simmonds et al., 2014), and superior longitudinal fasciculus (Asato et al., 2010; Bava et al., 2010; Lebel et al., 2008). Moreover, the development course of the uncinate fasciculus continues beyond the 28 years old (Lebel et al., 2008; Simmonds et al., 2014) possibly associated to axonal organization and fibers' cohesion (Simmonds et al., 2014).

The analyzed studies suggest that whereas the cellular myelin growth process of all frontal association tracts, as shown by decreased RD, is nearly completed by the end of adolescence (i.e., the fronto-occipital, fronto-parietal and fronto-temporal connections; Asato et al., 2010; Lebel & Beaulieu,

2011; Simmonds et al., 2014); the axonal organization process continues throughout adulthood (Lebel & Beaulieu, 2011; Lebel et al., 2008). This suggests that myelination is likely to be an ongoing process, which occurs since neonatal ages until late adolescence/early adulthood, while fibers' organization and refinement of axonal connectivity might occur at later ages, as it is possibly a more complex and demanding process, highly dependent on the individual' interplay with the environment. Therefore, while differences in FA and AD but not in RD may indicate a developmental pattern characterized by higher axonal organization or increases in axon caliber, changes in RD values may reflect an age-related change in myelination.

The current systematic review offers a characterization of the developmental pathway of frontal lobe white matter, as shown by DTI measures, since neonatal ages until early adulthood. Significant alterations of the DTI indexes measured in the frontal white matter of healthy individuals were observed along the brain development, in parallel with alterations in the fibers' pathways and connectivity. These cellular and brain tissues remodeling suggest that frontal brain regions are in constant reorganization and refinement, in response to continuing adaptation to environmental stimulation.

Moreover, once that the diffusion parameters showed substantial sensitivity to age-dependent changes, the observed changes in the DTI metrics across the lifecycle suggest that distinct and possibly non-linear periods of white matter development exist and that changes in frontal white matter microstructure may be qualitatively distinct in different developmental periods. In fact, frontal white matter maturation seem to follow a hierarchical trajectory because changes appear to be more prominent in infancy, between birth and 6 years old, followed by a plateau during childhood-early adolescence (range: 6-10), returning to rise between adolescence and early adulthood. These developmental changes in the frontal white matter maturation occur in parallel with improvements in cognitive abilities (Gogtay et al., 2004). In fact, brain development between adolescence and early adulthood is associated with increasingly new, complex and highly demanding experiences, such as

advanced education, employment, new social relations, new familiar responsibilities, that will ultimately influence and be influenced by brain structural and functional architecture, such as the case of higher level executive functions.

Associations between DTI metrics in the frontal regions and performance in executive functions-related tasks were previously reported, showing an association between better performance and higher anisotropy values and/or lower diffusivity (Madsen et al. 2010; Nagy, Westerberg & Klingberg, 2004; Urger et al., 2015). Specifically, higher FA and lower RD values in inferior frontal gyrus of young adolescents were associated to better scores in response inhibition, as measured by the Stop Signal task (Madsen et al., 2010). Working memory capacity was also positively correlated with FA in the superior and inferior frontal gyri in a group of adolescents (Nagy et al., 2004). Finally, anisotropic values were positively associated with executive set-shifting and attentional tasks, and diffusivity values were negatively correlated with inhibitory control, suggesting a link between maturation processes and improved cognitive functions (Urger et al., 2015). These findings suggest that the microstructural changes occurring across frontal white matter development, and in particular during adolescence, may be the neural substrate of mature executive functions.

In fact, changes in frontal white matter structure along adolescence seem to occur very fast, once that the alterations observed in the DTI indexes are noticed even when little time elapsed between the first and the second evaluations (Bava et al., 2010). In addition to great developmental improvements, adolescence is also a vulnerable period for external factors to negatively affect brain maturation, including the frontal cortex. Therefore, it is often a period for the onset of psychopathological disorders and emergence of maladaptive behaviors (for a review see López-Caneda et al., 2014 in binge drinking) that might be predicted by disrupted developmental processes or interfere with the optimal process of brain maturation.

DTI derived measures are not intrinsic properties of the tissues but instead, assumptions driven

by a conglomerate of knowledge from several fields (mathematics, physics, engineering, computer science and neurosciences) that might limit the associations between DTI data and biological variables. Building on this, Jones & Cercignani (2010) discussed several limitations of the methodology. These limitations can be encountered in each step of the DTI pipeline; i.e., image acquisition, pre-processing, tensor estimation or extraction of scalar measures. For example, distortions caused by eddy currents and head motion, defining the most suitable model to diffusion tensor estimation, or the best method to extract the quantitative measures; e.g., ROI, histogram, voxel-based analysis or TBSS. Anyhow, what seems to be the most noticeable handicap of all is the reliability degree that one may have, when interpreting the DTI metrics (FA, MD, RD and AD) in the light of cerebral development and its association with biological processes (Jones & Cercignani, 2010; Soares, Marques, Alves, & Sousa, 2013). Taking into account all of these factors and the basic assumption that DTI is grounded in the manipulation of proton spins and not a direct measure of axonal connectivity, it seems to be worth reflecting on the DTI metrics and accept the fact that errors might be present in the data, as much as in other imaging methods. However, until know, DTI appears to be the most reliable imaging method to assess white matter structure in vivo (Neil et al., 2002).

Finally, while restricting our review to DTI, reports that used other diffusion imaging methods (e.g., DSI or HARDI) were excluded, which could add further important information about frontal white matter maturation. Future reviews should focus in the remaining diffusion methods.

This systematic review highlights the extended development course of frontal white matter, evidenced by DTI measures, and documents that brain development is a continuous long-lasting process, driven by specific developmental events (e.g. synaptic refinement, pruning, myelination), designed to sustain the optimal functioning of neuronal networks that support cognition and behavior. Detailed knowledge about the frontal white matter maturation timing is not only important to understand the development of cognitive functions that are dependent on frontal structures but it may be also

useful in signaling individuals at risk for developmental disorders.

5. References

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Chapter III

Gray Matter Abnormalities in the Inhibitory Circuitry of Young Binge Drinkers: a Voxel-Based Morphometry Study

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Abstract

Binge drinking (BD) is defined as a pattern of high alcohol intake in a short time followed by periods of abstinence. BD is very common in adolescence, a stage of life characterized by maturation of self-regulatory processes. It has been widely demonstrated the role of the prefrontal–striatal networks in self-regulatory processes in adolescents.

Herein, we presented the results of a voxel-based morphometry (VBM) study with a group of 36 young college students, 20 participants classified as binge drinkers (BDs) in order to look for morphometric alterations in prefrontal areas associated with self-regulatory processes. The results showed increased gray matter (GM) densities in the left inferior frontal gyrus in BDs. Additionally, BDs males have higher GM densities in the left inferior frontal gyrus when compared with alcohol abstinent males. BDs females showed higher GM densities in the left and in the right middle frontal gyrus in comparison to alcohol abstinent females. Finally, GM density in the BDs middle frontal gyrus was positively correlated with the BIS sub-factor self-control.

These findings suggest the existence of abnormalities for BDs in the core brain regions associated with self-regulatory processes.

Keywords: Binge Drinking; Gray matter; Inhibitory control; Self-regulation; Impulsivity; Adolescence; College-students; Voxel-Based Morphometry

1. Introduction

Binge drinking (BD) is a common pattern of consumption among college students and is characterized by repeated episodes of large amounts of alcohol intake (a minimum of four drinks for women and five for men, in a brief period of time, ± 2h) at least once per month, followed by periods of abstinence. This pattern of high alcohol ingestion leaves individuals particularly susceptible to several risky behaviors (Courtney & Polich, 2009; NIAAA, 2015).

According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), nearly 60% of US college students (age range 18–22 years) reported alcohol consumption and 40% exhibited a BD pattern in the past month. This highly risky behavior is seriously harmful and causes the death of approximately 1,825 US college students each year (NIAAA, 2015; SAMHSA, 2016). In Europe, a growth of this abusive pattern of consumption among young people was noted between 1995 and year 2000, and its prevalence rate has remained quite unchangeable over the past two decades (ESPAD, 2015). Men reported higher BD frequency (36%) compared to 19% of women, and the greatest percentage of frequent BD involved young consumers; i.e., 33% of individuals ranging in age from 15 years to 24 years reported a BD pattern in the past month (Eurobarometer, 2010).

Despite the growing prevalence of BD among young people, research on the putative neural abnormalities related to BD, either as a precursor and/or as an effect of alcohol abuse, is still scarce. Yet, data gathered up to now from electrophysiological, functional neuroimaging and neuropsychological assessments revealed atypical functioning of several regions including the dorsolateral prefrontal cortex (DLPFC), the inferior, middle and superior frontal gyri, the anterior cingulate cortex and the parietal and temporal lobes. These functional impairments have been associated with a multiplicity of deficits in executive functioning-related abilities as attention, cognitive flexibility, working memory, planning, decision-making and inhibitory control (see Hermens et al., 2013 and Lopez-Caneda et al., 2014 for a review).

There are conflicting findings from structural neuroimaging studies with binge drinkers (BDs). While some studies reported regions of enlarged gray matter (GM) such as the striatum, the DLPFC, the cingulate cortex, the temporal gyri and the middle frontal gyrus (Doallo et al., 2014; Heikkinen et al., 2017; Howell et al., 2013; Wilson et al., 2015) other studies showed decreased GM in the temporal gyri, the superior and middle frontal gyri and the pars triangularis (Luciana, et al., 2013; Wilson, et al., 2015). In addition to the aforementioned results, which may be related to a neurotoxic effect of alcohol, GM reduction in the cingulate cortex and inferior and middle frontal cortex was found in prospective BDs before alcohol initiation, suggesting that premorbid changes might be related with future alcohol misuse (Cheetham et al., 2014; Squeglia et al., 2014; 2017; Wilson et al., 2015).

Of particular importance is to understand how this behavior might be enhanced as a result of disruptions in the normative brain development or how this BD pattern affects the optimal brain maturation and integrity. Adolescence tends to be a critical period for the beginning of abusive alcohol consumption (Casey & Caudle, 2013; Crews & Boettiger, 2009). In this development period individuals tend to increasingly engage in social behaviors in order to attain social conformity and the use of alcohol as a coping strategy to deal with negative emotions and achieving a feigned state of well-being (Laghi et al., 2015; Lorant, et al., 2013). This is also a period of great physiological changes including intracellular events (e.g., loss of overproduced synapses/ increase of myelin sheaths) essential to brain maturation and advancements in complex cognitive functions such as inhibitory control and self-regulation, allowing individuals to deal with risk-taking choices (Casey & Caudle, 2013). Notably, the ability to regulate behavior and suppress inappropriate emotions or actions seems to be in development during adolescence, fact that has been associated with structural immaturity in several cortical and subcortical regions (Bari & Robbins, 2013; Bava & Tapert, 2010; Crews & Boettiger, 2009), rendering individuals more prone to risky environmental factors (e.g. drugs or alcohol misuse) and impulsive behaviors.

Taking this developmental, neurofunctional and neurocognitive results into consideration, we hypothesized that BDs would show morphological alterations within core brain regions associated with self-regulatory processes (i.e., superior and inferior frontal gyri, orbitofrontal cortex, anterior cingulate, nucleus accumbens and caudate). In order to test this hypothesis, we performed a voxel-based morphometry (VBM) study in a group of young college students that met the criteria for binge alcohol consumption and a group of alcohol-abstinent controls (AACs) employing as regions of interest (ROI) brain area associated with self-regulatory processes (Crews & Boettiger, 2009; Koob &Volkow, 2010).

2. Method

2.1. Participants

Potential participants were recruited through an online survey with college students, which included items regarding the use of alcohol (frequency of alcohol consumption, number of drinks consumed on each day of the past week, speed of drinking, etc.) and other drugs (type of drug, frequency of consumption, etc.).

Participants who met on this survey either BD or alcohol-abstinent control (AAC) criteria, were invited to a clinical interview covering several aspects related to their current alcohol and drug consumption, personal and family history of alcoholism, medical or psychopathological disorders, as well as the assessment of their laterality, impulsivity and psychopathological symptomatology. All the participants (regardless of whether they had been pre-classified as BDs or AACs) underwent the same assessment.

Sociodemographic and substance use data were collected through a questionnaire that, besides sociodemographic information, included items 10, 11 and 12 from the Alcohol Use Questionnaire (AUQ) (Townshend & Duka, 2002), assessing speed of drinking (average number of drinks consumed per hour), number of times getting drunk in the past 6 months, and percentage

(average) of times getting drunk during drinking episodes. Additionally, a diary of alcohol ingestion, questions about consumption of alcohol and other psychoactive substances (type of substance, frequency of consumption, etc.) and the Portuguese version of the Alcohol Use Disorder Identification Test (AUDIT) (Cunha, 2002) were administered. Total AUDIT score reflects the subject's level of risk due to harmful alcohol intake: scores in the range of 8-19 reveal hazardous drinking, while scores of 20 or above warrant further diagnostic evaluation for alcohol dependence (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001).

Personal and family history of alcoholism plus medical or psychopathological disorders information was collected through a semi-structured interview including: a Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA), Individual Assessment Module (IAM) and Family History Assessment Module (FHAM), designed by the Collaborative Study on the Genetics of Alcoholism (Bucholz et al., 1994). In addition, in order to assess the psychopathological symptomatology, the Portuguese version of the Symptom Checklist-90-Revised (SCL-90-R) (Derogatis, 2002; Almeida, 2006) was used. This self-report questionnaire is used to evaluate a range of current psychological symptoms and distress providing a Global Score index (GSI), which is a measure of the overall psychological distress and nine primary symptom dimensions (interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism).

The Edinburg Handedness Inventory (Oldfield, 1971) was used to assess participants' laterality. Impulsivity was assessed through the Barratt Impulsiveness Scale 11 (BIS11) (Patton et al., 1995). BIS is a self-report questionnaire intended to evaluate personality and behavioral aspects of impulsiveness providing a full-scale score plus second and first order subscores reflecting subtraits of impulsiveness. The Portuguese version was used (Cruz & Barbosa, 2012).

Exclusion criteria for both groups were defined as the following: be left-handed; scores ≥ 20 in the AUDIT; GSI ≥ 90 or scoring in at least 2 symptomatic dimensions of the SCL-90-R; uncorrected

sensory deficits; personal history of traumatic brain injury or neurological disorder; regular (i.e. on a weekly basis) consumption of cannabis, personal history of regular or occasional use of other drugs (opiates, hallucinogens, cocaine, ecstasy, amphetamine compounds or medically prescribed psychoactive substances); Alcohol Use Disorder (AUD), i.e. alcohol abuse/dependence, based on DSM-V criteria; personal and/or family history of any neurological or DSM-V axis I disorder in first-degree relatives, family history of alcoholism in first-degree relatives; and magnetic resonance imaging (MRI) contraindications.

Finally, 36-college students ranging in age between 18 and 23 years old were included in the study, being 20 participants classified as BDs (10 women) and 16 AACs (10 women). Participants were classified as BDs if they consumed a minimum of four drinks or five for men in a brief period of time (approximately 2h), at least once per month, for the last ten months (minimum). Participants assigned to the AAC group were completely alcohol abstinent, i.e. do not drink alcohol at all, neither now nor in the past. The demographic and drinking characteristics of the two groups are shown in Table 1.

Prior to the MRI assessment, participants were asked to abstain from practicing BD during the three preceding days, consuming drugs and alcohol 12h before the scanning and to avoid smoking and drinking tea or coffee for at least 3h in advance.

All participants gave written informed consent after the procedure had been carefully explained and received a financial stipend for their participation. The research was conducted in accordance to the ethical principles for medical research involving human subjects of the World Medical Association present in the Declaration of Helsinki (Williams, 2008). The local Bioethics Committee approved the protocol.

2.2. Magnetic Resonance Image acquisition

The neuroimaging assessment was conducted with clinically approved Siemens Magneton TrioTim 3T MRI scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a 32-channel receive-only head coil. Sagittal high-resolution 3D T1 weighted anatomical images were acquired using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with the following parameters: repetition time (TR) = 2700 ms, echo time (TE) = 2.33 ms, flip angle = 7°, 192 slices with 0.8 mm thickness, in-plane resolution = 1 x 1 mm2, and 256 mm field of view (FoV).

2.3. Image Processing

Before running the postprocessing protocol, all MRI scans were visually controlled to discard for critical head motion or brain lesions. All the images were normalized to the ICBM 152 average SPM template in Montreal Neurological Institute (MNI) space. Data was processed using SPM12 pipeline and statistical tools (Wellcome Trust Centre for Neuroimaging, University College London, UK; http://www.fil.ion.ucl.ac.uk/spm/) executed in MatLab R2015a (MathWorks, Natick, MA) with the VBM module. VBM is an automated processing technique applied to the entire brain allowing the characterization of shape and neuroanatomical configuration of different brains. Local composition of brain tissues is compared based in a voxelwise approach (Ashburner & Friston, 2000; Mechelli et al., 2005). Images were segmented into GM, white matter and cerebrospinal fluid using an extension of the standard unified segmentation model in SPM12. White and GM segmentations were co-registered across participants using the DARTEL algorithm (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra; Ashburner, 2007; Ashburner & Friston 2009) and smoothed with a 8 mm FWHM Gaussian filter to reduce errors from between-subject variability in local anatomy and to improve the normality of the data. For the purpose of this study only GM segmentations were analyzed.

2.4. Region of Interest Definition

For this purpose, a review on the prefrontal-striatal network underlying self-regulatory mechanisms involved in the addiction circuitry (Bari & Robbins, 2013; Bava & Tapert, 2010; Crews & Boettiger, 2009; Koob, 2011; Koob & Volkow, 2010; Goldstein & Volkow, 2011) was performed. The brain regions that consistently emerged were the superior and middle frontal gyri, the frontal superior orbital gyrus, the anterior cingulum, the caudate nucleus and the nucleus accumbens. Therefore, a mask (see figure 1) was generated with the WFUpickatlas toolbox version 3.0.5b (http://www.ansir.wfubmc.edu) based on the Talairach Daemon database running on MatLab R2015a (MathWorks, Natick,MA) to include cortical and subcortical areas of the inhibitory circuitry.

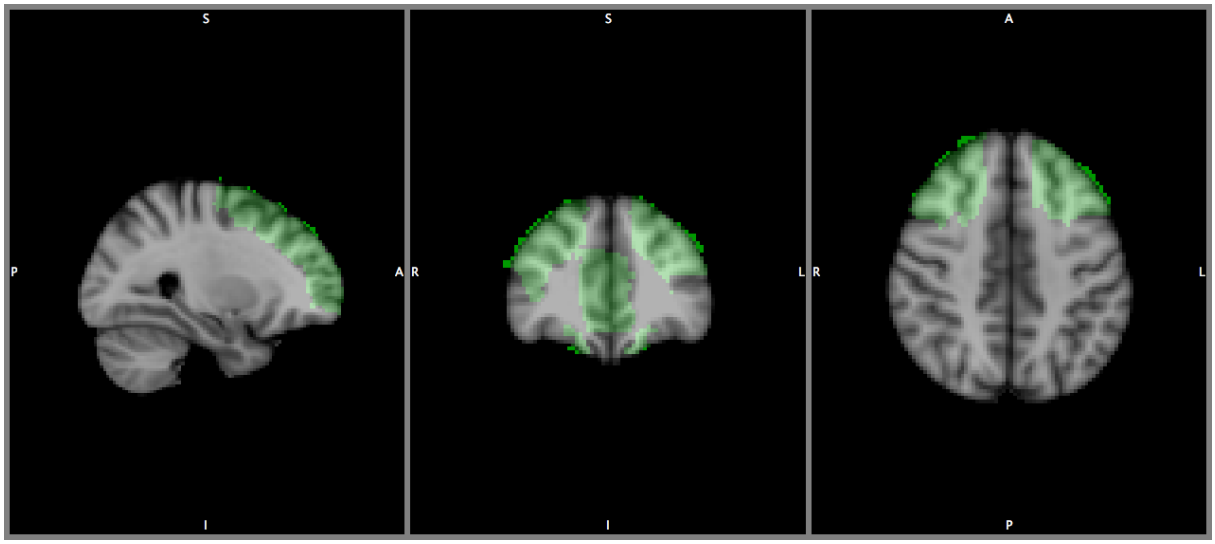

FIGURE 1 | Illustrates the selected ROI for VBM analysis

2.5. Statistical Analysis

For statistics, two-way analysis of variance was performed. Gender and Group were included as between subject factors and age and scores on the BIS as covariates. For statistical threshold criteria significant results were considered after Monte Carlo correction for multiple comparisons $p<0.05$. The correction was determined over 1000 Monte Carlo simulations using AlphaSim tool, distributed with REST toolkit (http://resting-fmri.sourceforge.net/; Song et al., 2011) and mask set to the

corresponding ROI previously generated. Anatomical labeling was obtained using the anatomical automatic labeling atlas (AAL), (Tzourio-Mazoyer et al. 2002).

In addition, Pearson correlations (with bootstrap corrections) were performed to analyze the relationship between GM volumes and both alcohol-related measures: number of times of binge drinking per month, number of months with BD pattern, grams of alcohol consumed per week, speed of drinking (grams/h during BD episodes), AUDIT scores and BIS scores.

Table 1. Demographic and Behavioral data for BDs and AACs[1]

	BD **N = 20** **Mean (SD)**	**AAC** **N = 16** **Mean (SD)**	**t (34)**
% Male	50%	37,5%	
% Female	50%	62,5%	
% Caucasian	100	100	
Age	20.45 (1.60)	21.00 (1.71)	.99
Age of onset of BD	17.45 (1.08)	–	
AUDIT (total score)	11.20 ± 3.25	.62 ± 1.20	-13.43***
Number of times of BD per month	3.57 ± 1.87	0	-8.54***
Number of months with BD pattern	35.90 ± 14.03	0	-11.44***
Grams of alcohol consumed per week	151 ± 44.27	0	-14.78***
Speed of drinking (gr/h during BD episodes)	34.50 ± 8.26	0	-18.69***
Percentage of times getting drunk when drinking	43.25 ± 20.41	0	-9.48***
Tobacco Smokers	7	0	
Occasional users of Cannabis	2	0	
BIS (Total Score)	64.80 ± 5.83	63.56 ± 6.05	-.62

3. Results

3.1. ROI based Gray Matter Differences between BD and AAC

3.1.1. Main effect of Group and Gender

[1] **AUDIT**: Alcohol Use Disorders Identification Test; **BIS**: Barratt Impulsiveness Scale; **BD**: binge drinking; **AAC**: alcohol-abstinent control; **SD**: standard deviation

All p-values reported are for 2-tailed independent samples t-tests; * P≤0.05; *** P≤0.000

Increased GM densities in the left inferior frontal gyrus were observed in BDs (MNI coordinates: -44,24,33; F=16.89, K=59, p<0.0001 uncorrected; AlphaSim correction; p<0.05, cluster size>29), compared to AACs. Figure 2 illustrates the regions where significant peak-level densities differences were observed between BDs and AACs.

Increased GM densities were observed in males as compared to females in the right rectus gyrus (MNI coordinates: 5,63,-17; F=25.74, K=392, p<0.001 uncorrected), in the right superior frontal-orbital (MNI coordinates: 33,65,-6; F=21.13, K=97, p<0.001 uncorrected), and in the left middle frontal gyrus (MNI coordinates:-35,42,24; F=18.64, K=100, p<0.001 uncorrected) (AlphaSim correction - p<0.05, cluster size>29).

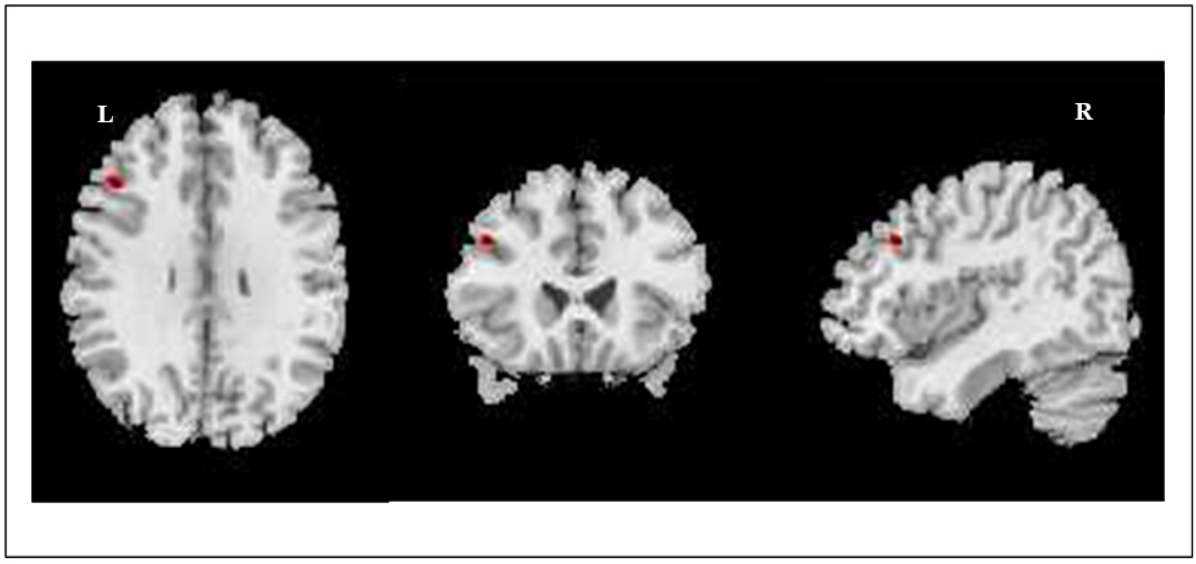

FIGURE 2 | Image illustrates the regions where significant differences in GM densities (peak-level) were observed between BDs and AACs. BDs > AACs. Left inferior frontal gyrus, MNI coordinates: -44,24,32, K=59, p<0.0001

3.1.2. Interaction effect Group x Gender

A group-by-gender interaction effect was observed in the left middle frontal gyrus (MNI coordinates: -45,51,15; F=20.12, K=301, p<0.001 uncorrected), in the right middle frontal gyrus (MNI coordinates: 38,57,12; F=14.87, K=29, p≤0.001 uncorrected), and in the right frontal superior-orbital

region (MNI coordinates: 9,60,-20; F=18.45, K=179, p<0.001 uncorrected) (AlphaSim correction - p<0.05, cluster size>29). Post-hoc tests revealed that BDs males displayed higher GM densities than AACs males in the left inferior frontal gyrus (MNI coordinates: -42,24,32; t=3.95, K=91, p<0.001 uncorrected) while BDs females displayed higher GM densities in the left (MNI coordinates: -45,50,14; t=3.75, K=125, p<0.001 uncorrected) and right middle frontal gyrus (MNI coordinates: 39,62,3; t=3.86, K=82, p<0.001 uncorrected) when compared to AACs females. Figure 3 and table 2 illustrates the regions where significant peak-level densities differences were found between BDs and AACs for both genders.

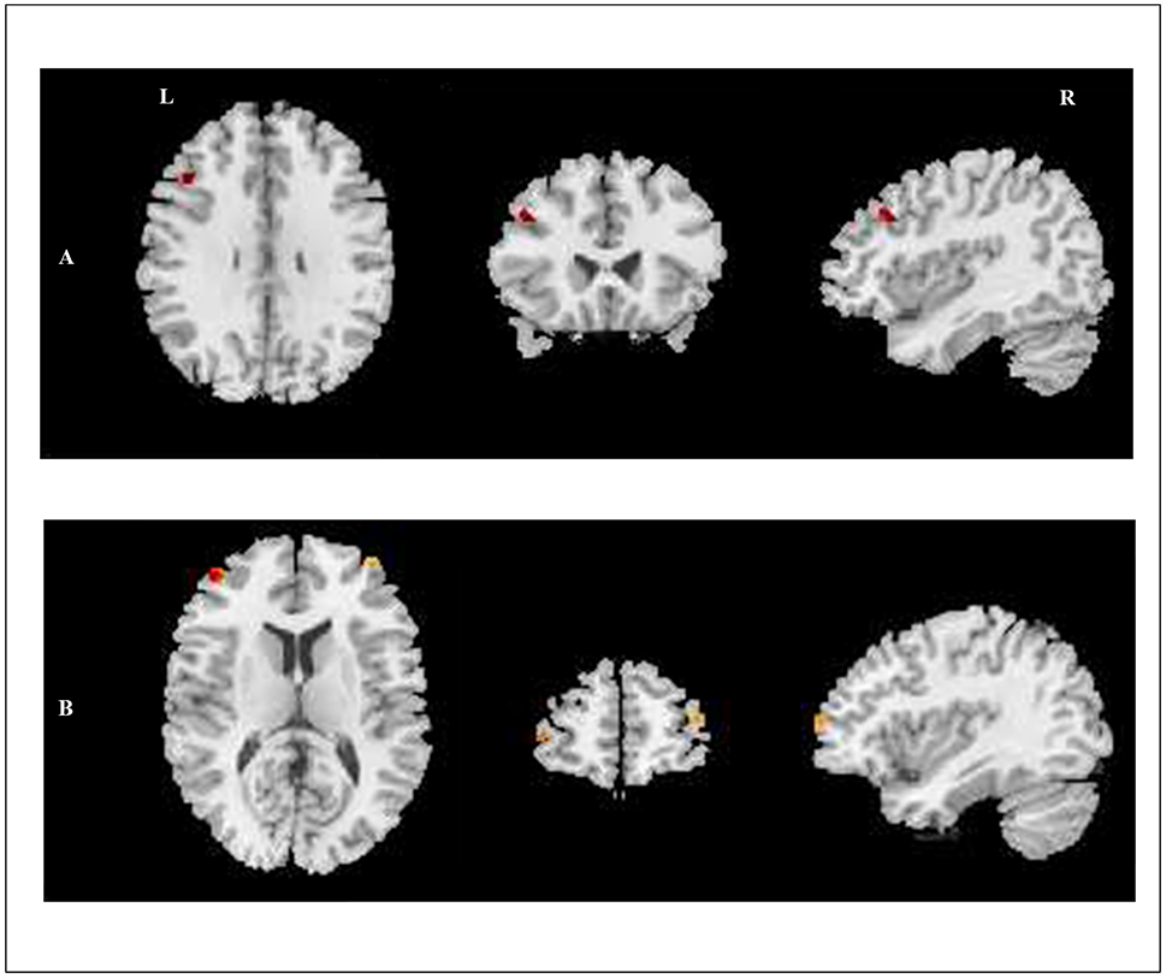

FIGURE 3 | Illustrates the regions where significant differences in GM peak-level densities (peak-level) were found between BDs and AACs for both genders. BDs > AACs. Panel (A) represents the region

where significant differences in GM densities were observed between males' BDs and AACs: the Left inferior frontal gyrus (MNI coordinates: -42,24,32, K=91, p<0.001). Panel (B) illustrates the regions where significant differences in GM densities were observed between females' BDs and AACs: the Left (MNI coordinates: -45,50,14, K=125, p<0.001), and Right (MNI coordinates: 39,62,3: K=82. p<0.001) middle frontal gyri

Table 2. Regions of significant differences in gray matter densities (BDs >AACs)

Anatomical Label	Direction of difference	MNI Coordinates x y z	Cluster size (K_E)	Peak *t* score	*p* value
Males					
L_inferior_frontal	BDs	-42 24 32	91	3.95	0.000
Females					
L_middle_frontal	BDs	-34 40 24	63	4.08	0.000
R_middle_frontal	BDs	39 62 3	82	3.86	0.000

MNI: Montreal Neurological Institute, L: left, R: right.

Results reported were generated from statistical parametric maps at a threshold of P < 0.001 whole brain uncorrected and Monte Carlo correction for multiple comparisons p < 0.05 using AlphaSim tool.

Anatomical localizations were performed using the Automatic Anatomical Labeling (AAL) atlas (see Method for more details).

3.2. Correlations

Pearson correlations within the BD group revealed positive associations between GM densities in the left (r = 0.45 p<0.05) and in the right middle frontal gyrus (r = 0.49, p<0.05) and scores in the sub-factor self-control of the BIS. No significant correlations between GM densities and alcohol-related measures were observed. Figure 3 illustrates the main associations between brain data and BIS results in the BD group.

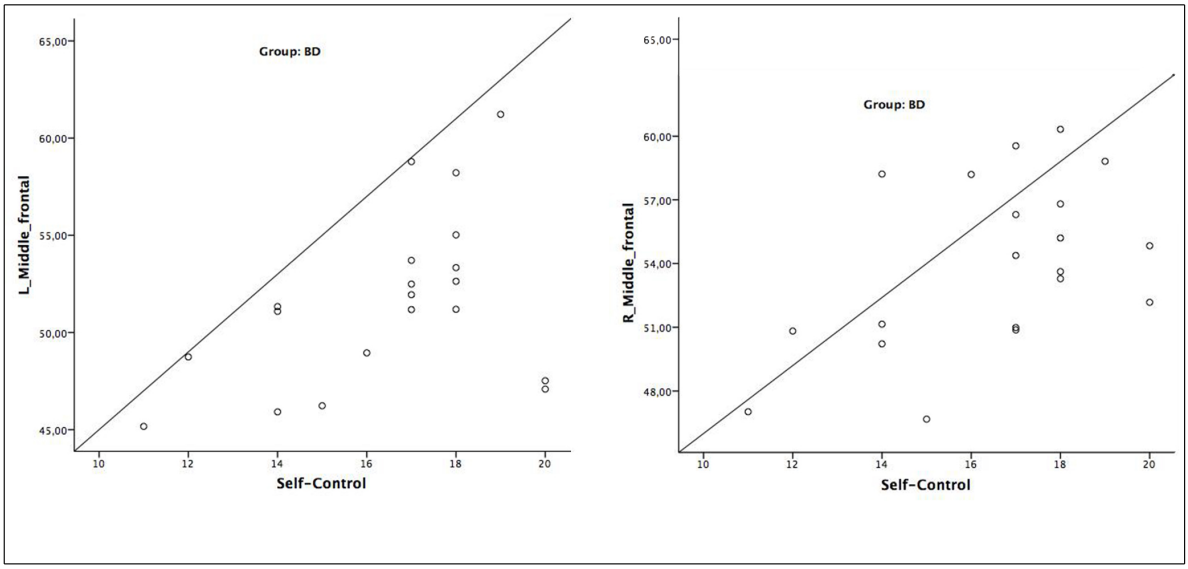

FIGURE 4 | Image shows the positive associations between brain data (GM densities) and BIS (self-control) scores in the BD group.

4. Discussion

The main objective of this study was to assess the existence of morphological abnormalities associated with BD patterns in core brain regions for self-regulatory processes. Overall we found that female and male BDs displayed increased GM densities in the bilateral middle frontal gyrus and in the left inferior frontal gyrus, respectively, when compared with their gender AACs counterparts. Moreover, GM densities in the middle frontal gyrus were positively associated with scores in the BIS sub-factor self-control in the BD group.

Increased GM densities in the inferior and middle frontal gyrus are consistent with previous studies, either using similar (cortical thickness) or the same morphometric methodologies (e.g., VBM) Squeglia et al., 2012; Doallo et al., 2014). In particular, thicker frontal areas were previously reported in female BDs (age range: 16 to 19 years old) in comparison with non-BDs females (Squeglia et al., 2012). Doallo et al. (2014), also found higher GM volume in the DLPFC of young BDs, when compared to light consumers in a similar age cohort. These authors interpreted these findings as a consequence of high alcohol intake during this developmental period (i.e. adolescence and early adulthood), but, due

to the cross-sectional nature of the studies, they also alert for the possibility that the encountered abnormalities may be considered a risk factor for heavy substance use (e.g. diminished efficiency in information processing and problem solving abilities, in addition to decreased ability in weighting risks *vs* benefits), rather than a consequence of BD (Squeglia et al., 2012; Doallo et al., 2014). Wilson et al. (2015) and Gropper et al. (2016) reported a deleterious effect of alcohol exposure in the ventral diencephalon, middle temporal gyrus and hippocampus, yet no significant effects on other areas such as the frontal and parietal cortices were found. However, using a method that assesses microstructural features directly related to neuronal morphology - Orientation Dispersion Imaging - Morris et al. (2017) showed diminished dendritic complexity and organization in the DLPFC of a BD cohort. Nevertheless, no significant correlation between these measures and alcohol use severity was found, suggesting that these neuronal abnormalities in BDs might be a premorbid marker and not a direct consequence of high alcohol intake.

Given these results, it is possible that the frontal morphologic changes observed in our BD group may be related with premorbid disruptions in regions associated with self-regulatory processes (Bava & Tapert, 2010; Crews & Boettiger, 2009; Koob, 2011). In fact, abnormalities in the middle frontal gyrus have been frequently associated to difficulty in regulate behavior in face of failure and undercontrol, prospectively predicting substance use (Heitzeg et al., 2014; Japee, Holiday, Satyshur, Mukai, & Ungerleider, 2015), whereas inferior frontal gyrus changes have been previously correlated with deficits in response inhibition shown by alcohol-dependent individuals (Aron, Robbins, & Poldrack, 2004; Swick, Ashley, & Turken, 2008; Wiers et al., 2015).

Consistent with data reported above, positive associations between GM densities in the middle frontal gyrus and scores in the BIS sub-factor self-control were found in our BD group, but not with any other alcohol-related measures. In the same line, Cho et al. (2013) found a relationship between larger

middle frontal gyrus GM density and higher scores in impulsivity related measures in a group of young healthy adults.

On the other hand, and contrasting with our findings, recent longitudinal studies showed decreased volume and cortical thickness in the inferior (Squeglia et al., 2014; 2017; Wilson et al., 2015) and in the middle frontal gyrus (Squeglia et al., 2017; Wilson et al., 2015) of young adolescents prior to alcohol use onset and associated with alcohol initiation and BD. These authors associated their findings to premorbid characteristics such as a delay of GM growth that should be happening at these ages as a part of the normative neurodevelopmental process (Squeglia et al., 2014; 2017; Wilson et al., 2015). Building on this assumption, and given that the age range observed in those studies is younger (12-14; 12-17; 14-17) than ours (18-23), we may speculate that the typical neurodevelopmental timing/schedule is also postponed in our sample, leading to increased GM occurring at later ages in the BD group (18-23 years old), contrasting with the developmental process following its normal course in age-matched abstainers.

Inconsistent data with other volumetric studies (e.g. Luciana et al., 2013) can be due to the different morphometric analyses used. While some of the studies analyzed cortical thickness (Luciana et al., 2013; Squeglia et al., 2017), others evaluated volumes or densities (Doallo et al., 2014), which limit the generalization of the findings, as these measures are not directly comparable. In fact, volume seems to be more closely related to surface area than to cortical thickness. Surface area and cortical thickness are changeable along development but not necessarily following the same direction or rate of variance than volume or densities (Tamnes et al., 2017; Winkler et al., 2009; 2010). Finally, different age ranges are associated with distinct neurodevelopment periods and can therefore represent an additional confounding factor.

Future studies should take the advantage of longitudinal designs with more than two follow up assessments and the combination of morphometric, genetics and behavioral measures in order to

disentangle whether structural abnormalities reflect vulnerability factors or consequences of high alcohol consumption.

The gender-related differences found may be associated with specific inhibitory process (i.e., cognitive *vs* motor). In particular, the middle frontal gyrus has been more associated with the capacity to take control over thoughts and emotions in a self-oriented long-term perspective (Cho et al., 2013), while the left inferior frontal gyrus has been found to play a crucial role in inhibiting automatic inappropriate motor responses (e.g., the go-no go task) (Swick et al., 2008). Therefore, BDs females might be less efficient in cognitive self-regulation (Cross, Copping, & Campbell, 2011), while BDs males experience more difficulty in inhibiting automatic motor responses (Cross et al., 2011).

Conclusion: This study suggests frontal GM abnormalities in BDs college students, which is likely to impact self-regulatory processes. The pattern of increased regional GM density suggests that developmental factors may contribute to brain alterations in BDs.

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Chapter IV

White matter Abnormalities in the Dorsolateral Prefrontal Cortex of Young Binge Drinkers: a Diffusion Tensor Imaging Study

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Abstract

Binge drinking (BD) is associated with ineffective self-regulatory processes and poor response inhibition. Research on the structural white matter connectivity of core regions involved in self-regulatory and inhibitory control processes, as the prefrontal-striatal network, is scarce. Therefore, in this study, we used a ROI-based approach Diffusion Tensor Imaging (DTI) analysis to evaluate a preclinical sample of 20 binge drinkers (BDs) adolescents and 16 alcohol-abstinent controls (AACs). Overall, the results showed gender specific white matter microstructural alterations [axial diffusivity (AD) and mean diffusivity (MD)] in the inferior frontal gyrus and middle frontal gyrus of BDs, when compared to AACs.

Specifically, BDs males presented higher AD and MD in the right middle frontal gyrus in comparison with AACs males. Contrastingly, female BDs displayed lower AD and MD values in the right inferior frontal gyrus when compared to female AACs. Additionally, the number of months with a binge pattern of consumption was positively correlated with the MD of the right middle frontal gyrus, while the AD of the right inferior frontal gyrus was negatively associated with the number of binge drinking episodes per month.

The results of this study suggest abnormal white matter structural connectivity in regions of the prefrontal cortex, which is possibly related with altered myelination processes and axonal integrity in brain regions associated with self-regulation and inhibitory processes.

Keywords: Binge Drinking; White matter; Inhibitory control; Self-regulation; Development; Myelination; Diffusion Tensor Imaging

1. Introduction

The large intake of alcohol in a short time followed by alcohol-free periods is known as binge drinking (BD) and is widely observed among adolescents and college students (Courtney & Polich, 2009; NIAAA, 2015). In fact, this highly prevalent behavior among adolescent college students (30 to 40%) has been associated to several hazardous consequences (e.g. car crashes, assaults) and may ultimately lead to death (NIAAA; SAMHSA, 2015). This pattern of heavy alcohol intake has been linked to the affectation of several systems, including the cognitive.

Despite the high prevalence of heavy alcohol consumption among youth, further research on the putative neurocorrelates of BD is of particular importance, since this abusive alcohol consumption seems to initiate during adolescence (Casey & Caudle, 2013; Crews & Boettiger, 2009). Increased social relations and peers influence seems to trigger the BD behavior among adolescents, in an attempt to obtain social conformity and feeling integrated. Additionally, adolescents use alcohol to achieve an untruthful well-being, and as a strategy to deal with negative emotions (Laghi et al., 2016; Lorant, et al., 2013).

Adolescence is a period of great physiological (e.g. synaptic refinement/ increased axonal organization) and cognitive improvements, such as self-regulatory and inhibitory processes, which warrant the ability of dealing with risky choices (Casey & Caudle, 2013). However, the self-regulation and the capacity to suppress inappropriate behaviors seem to be underdeveloped in adolescents, and have been associated with immature prefrontal cortex structure and functioning (Bari & Robbins, 2013; Bava & Tapert, 2010; Crews & Boettiger, 2009). In particular, atypical functioning of several regions comprising the dorsolateral prefrontal cortex (DLPFC), the cingulate cortex, the inferior, middle and superior frontal gyri, the temporal cortex and the parietal cortex has been shown in binge drinkers (BDs), in addition to several deficits in executive control-related functions such as attention, cognitive

flexibility, working memory, planning, decision-making and inhibitory control (see Hermens et al., 2013 and Lopez-Caneda et al., 2014 for a review).

Specifically, recent structural neuroimaging findings revealed reduced white matter in widespread areas of the prefrontal cortex (PCF) such as the superior frontal gyrus, but also in other brain regions as the middle temporal and lingual gyrus, cerebellum, and corpus callosum in young individuals with a BD pattern of alcohol consumption compared to non-consumers (Lisdahl et al., 2013; Luciana, Collins, Muetzel, & Lim, 2013; Squeglia et al., 2014; 2015).

Although research on the brain structural connectivity of young binge drinkers (BDs) is still scarce, some studies revealed lower fractional anisotropy (FA) in the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus, uncinate fasciculus, corpus callosum, corona radiata, internal and external capsule and in the caudate nucleus (Jacobus et al., 2009; Jacobus, Squeglia, Bava, & Tapert, 2013; Luciana et al., 2013; McQueenyet al., 2009) associated with binge drinking behavior. Jacobus et al. (2013) showed decreased FA values in the inferior fronto-occipital and superior longitudinal fasciculus, from baseline to 3-year post-baseline assessment between young alcohol abusers (age range: 19-22) and non-heavy alcohol users. These results suggest that white matter integrity was affected by heavy alcohol consumption during the adolescent brain developmental course (Jacobus et al., 2013). There is also some evidence that alcohol differentially affects males and females' brain structure (Hommer, Momenan, Kaiser, & Rawlings, 2001; Squeglia et al., 2012; 2015).

Therefore, considering this evidence of abnormal white matter structural connectivity in BD as well as our previous evidence of gray matter abnormalities in the prefrontal-striatal regions related to self-regulation and inhibitory processes (see Sousa, Sampaio, Marques, Gonçalves & Crego, 2017b), we predicted that BDs would show structural connectivity alterations within the dorsolateral prefrontal regions (i.e. inferior and middle frontal gyri). Furthermore, we expected to find gender-specific prefrontal

abnormalities in the BDs. In order to assess these hypotheses, we conducted a ROI-based DTI analysis of the dorsolateral prefrontal cortex in a group of 20 college students with BD pattern and in 16 paired alcohol abstinent controls (AACs).

2. Method

2.1. Participants

Thirty-six college students aged 18 to 23 years old were enrolled in the present study. Twenty participants (10 women) composed the BD group as they consumed a minimum of four drinks or five for men in a brief period of time (approximately 2h), at least once per month, for the past ten months (minimum). The Sixteen participants (10 women) who assigned the AAC group were alcohol abstinent, i.e. do not drink alcohol at all, neither now nor in the past. Table 1 shows the demographics and alcohol-related characteristics for the two groups.

The recruitment of the college students was conducted through an online survey including alcohol-use-related items such as frequency of alcohol consumption, the number of drinks consumed per day in the previous week, the speed of drinking, and other drugs consumption (e.g. type of drug, the frequency of consumption, etc.).

The participants, who fulfilled the BD or alcohol abstinent criteria, were requested for a clinical interview. Sociodemographic information plus issues related to the actual alcohol and other drugs consumption, history of own alcoholism and relatives, presence of psychopathological or medical conditions, handedness and impulsiveness, were assessed through the Alcohol Use Disorder Identification Test (AUDIT) (Cunha, 2002), a diary of alcohol ingestion, a self-report questionnaire to collect substance use data (e.g. type of substance, frequency of consumption, etc.), the semi-Structured Assessment for the Genetics of Alcoholism (SSAGA), Individual Assessment Module (IAM) and Family History Assessment Module (FHAM) (Bucholz et al., 1994), the Portuguese version of the Symptom

Checklist-90-Revised (SCL-90-R) (Derogatis, 2002; Almeida, 2006), the Edinburg Handedness Inventory (Oldfield, 1971) and the Barratt Impulsiveness Scale 11 (BIS11) (Cruz & Barbosa, 2012; Patton et al., 1995).

Information about the BDs past and actual alcohol-consumption, specifically, the number of BD episodes per month, the number of months since they enrolled in the BD pattern, the grams of alcohol consumed per week, and the grams of alcohol they consumed in the BD episodes, gathered in the clinical interview, in addition to the AUDIT scores. These measures (alcohol related measures) were selected to be further correlated with the white matter indexes, described below.

Participants (either BDs or AACs) were excluded if they presented some of the following criteria: AUDIT scores $\geq$ 20; SCL-90-R Global Score Índex (GSI) $\geq$ 90 or at least 2 symptomatic dimensions; uncorrected sensory deficits; being left-handed; history of traumatic brain injury or neurological disorder; history of regular or occasional use of other drugs (opiates, hallucinogens, cocaine, ecstasy, amphetamine compounds or medically prescribed psychoactive substances); regular cannabis consumption (once per week or more); alcohol abuse or dependence, based on DSM-V criteria; personal and/or first-degree relatives history of neurological or DSM-V axis I disorder; first-degree relatives history of alcoholism; and further conditions affecting magnetic resonance imaging (MRI) acquisition.

Before the MRI assessment the participants were requested to abstain from binge drinking in the three previous days, consuming drugs and alcohol in the 12 preceding hours and to avoid smoking and drinking tea or coffee for at least 3h in advance.

After a careful explanation of the procedure, all the participants provided an informed consent. The research was conducted according to the Declaration of Helsinki (Williams, 2008) and was approved by the local Bioethics Committee.

2.2. Magnetic Resonance Image acquisition

A sagittal high-resolution T1 weighted anatomical sequence (3D magnetization prepared rapid acquisition gradient echo) was acquired using a clinically approved Siemens Magneton TrioTim 3T MRI scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a 32-channel receive-only head coil, with the following parameters: repetition time (TR) = 2700 ms, echo time (TE) = 2.33 ms, flip angle = 7°, 192 slices with 0.8 mm thickness, in-plane resolution = 1 x 1 mm^2, and 256 mm field of view (FoV). The Diffusion Weighted Imaging (DWI) was performed using a spin-echo echo-planar imaging (SE-EPI) sequence: TR = 9300 ms, TE = 93 ms, FoV = 2048 × 2048 mm, acquisition matrix = 128 × 128, 65 2-mm axial slices with no gap, 64 non-collinear gradient directions with b=700 s/mm^2, and one b=0 s/mm^2 acquisition. Before data pre-processing, the raw MRI and DWI acquisitions were visually inspected to discard the possibility of any brain lesions and/or critical head motion or artifacts that could compromise the analysis.

2.3. Region of Interest Definition

For the region of interest definition, we performed a review on the brain regions of the prefrontal-striatal network underlying self-regulatory and inhibitory control mechanisms involved in addiction (Bari & Robbins, 2013; Bava & Tapert, 2010; Crews & Boettiger, 2009; Koob, 2011; Koob & Volkow, 2010; Goldstein & Volkow, 2011). The brain regions that emerged in this review were previously used (see Sousa et al., 2017b), and included the superior, middle and inferior frontal, the frontal superior orbital, the anterior cingulate, the caudate nucleus and the nucleus accumbens.

2.4. Image Processing and Analysis

Image preprocessing was performed with tools provided with the FMRIB Software Library (FSL v5.0.8; http://fsl. fmrib.ox.ac.uk/fsl/). The analysis pipeline was carried out as follows: (a) motion and

eddy current corrections were applied to diffusion-weighted images (DWI) using FMRIB's Diffusion Toolbox (FDT); (b) brain extraction tool (BET) was applied to the T1 images, and first b0 volume of each subject, to strip skull and generate a mask. The mask was applied to the remaining volumes to remove non-brain voxels; (c) gradient vectors were rotated with FSL's fdt_rotate_bvecs script using the affine transformations previously applied to register each volume; (d) tensor fitting and scalar maps computation were performed with the Diffusion Toolkit (DTK) provided with the TrackVis software package (v0.6.2.2; http://trackvis.org/dtk) which implements the reconstruction of the diffusion tensors and fiber tractography; (e) After tensor estimation, streamline tractography and scalar maps of FA, MD, AD, and RD were generated; (f) label-map extraction. The inferior frontal gyrus and middle frontal gyrus white matter extraction were performed using the Talairach labels computed with the FSL software; (g) the ROIs were registered into each subject DTI space using FSL; (h) spatial registration of DTI and ROI fiber tracking; and (i) estimation of the FA, MD, RD, and AD parameters using TrackVis software (http://trackvis.org/dtk). FA represents the degree of anisotropic or unidirectional water diffusion in the brain. High FA values are commonly associated with axonal organization and coherence of fibers' orientation (Beaulieu, 2002; Ding et al., 2013; Scholz, Klein, Behrens, & Johansen-Berg, 2009). MD indicates the overall displacement of water molecules in a voxel. Low MD values are associated to fiber' integrity and myelination (Beaulieu, 2002; Preziosa et al., 2017). AD represents the rate of water diffusion parallel to the axonal tracts and seems to be related with axonal damage (Beaulieu, 2002; Sun, Liang, Cross, & Song, 2008). RD represents water diffusion perpendicular to the fiber direction, which is possibly constrained by cellular structure and myelination. (Alexander, Lee, Lazar, & Field, 2007; Sun et al., 2006).

2.5. Statistical Analysis

For statistics, GLM Univariate analyses of variance were performed for males and females independently using the Statistical Package for Social Sciences (IBM SPSS for MAC v20). Group was included as between subject factor and age and scores on the BIS as covariates. As aforementioned, considering results from our previous study (see Sousa et al., 2017b), we conducted independent analysis by gender. Finally, Pearson correlations (with bootstrap corrections) were performed to analyze the relationship between the white matter indexes, and alcohol-related measures: number of times of binge drinking per month, number of months with BD pattern, grams of alcohol consumed per week, speed of drinking (grams/h during BD episodes) and AUDIT scores.

Table 1. Demographic and Behavioral data for BDs and AACs

	BD N = 20 Mean (SD)	AAC N = 16 Mean (SD)	t (34)
% Male	50%	37,5%	
% Female	50%	62,5%	
% Caucasian	100	100	
Age	20.45 (1.60)	21.00 (1.71)	.99
Age of onset of BD	17.45 (1.08)	—	
AUDIT (total score)	11.20 ± 3.25	.62 ± 1.20	-13.43***
Number of times of BD per month	3.57 ± 1.87	0	-8.54***
Number of months with BD pattern	35.90 ± 14.03	0	-11.44***
Grams of alcohol consumed per week	151 ± 44.27	0	-14.78***
Speed of drinking (gr/h during BD episodes)	34.50 ± 8.26	0	-18.69***
Percentage of times getting drunk when drinking	43.25 ± 20.41	0	-9.48***
Tobacco Smokers	7	0	
Occasional users of Cannabis	2	0	
BIS (Total Score)	64.80 ± 5.83	63.56 ± 6.05	-.62
BIS (Attention)	10.65 ± 2.23	10.63 ± 2.16	-.03
BIS (Cognitive Instability)	6.10 ± 1.41	5.50 ± 1.51	-1.23
BIS (Motor)	11.80 ± 2.76	10.00 ± 2.03	-2.17*
BIS (Perseverance)	7.05 ± 1.05	7.31 ± 1.40	.64
BIS (Self-Control)	16.50 ± 2.46	17.38 ± 2.58	1.04
BIS (Cognitive Complexity)	12.70 ± 1.95	12.75 ± 1.88	.08

AUDIT: Alcohol Use Disorders Identification Test; **BIS**: Barratt Impulsiveness Scale; **BD**: binge drinking; **AAC**: alcohol-abstinent control; **SD**: standard deviation

All p-values reported are for 2-tailed independent samples t-tests; * P≤0.05; *** P≤0.000

3. Results

3.1. ROI based White Matter Differences between BDs and AACs analyses

Higher values of AD ($F(3,12) = 12.36$, $p < 0.01$) and MD ($F(3,12) = 9.30$, $p = 0.01$) were observed in the right middle frontal gyrus of males BDs in comparison to AACs males. Figure 1

illustrates the regions where significant white matter AD and MD differences were observed between male BDs and AACs. In the female group, lower AD ($F(3,16) = 11.22$, $p < 0.01$) and MD values ($F(3,16) = 4.83$, $p = 0.04$) were observed in the right inferior frontal gyrus of BDs when compared to its peers AACs (See Figure 2).

3.2. Correlations between White Matter indexes and Alcohol-Related measures in the BD group

Associations between the diffusion metrics and the alcohol-related scores were observed in the BD group. Specifically, in males, the MD of the right middle frontal gyrus was positively associated with the number of months with BD ($r = 0.66$ $p = 0.038$). Additionally, in females, the AD of the inferior frontal gyrus was negatively correlated with the number of times of binge drinking per month ($r = -0.65$ $p = 0.043$).

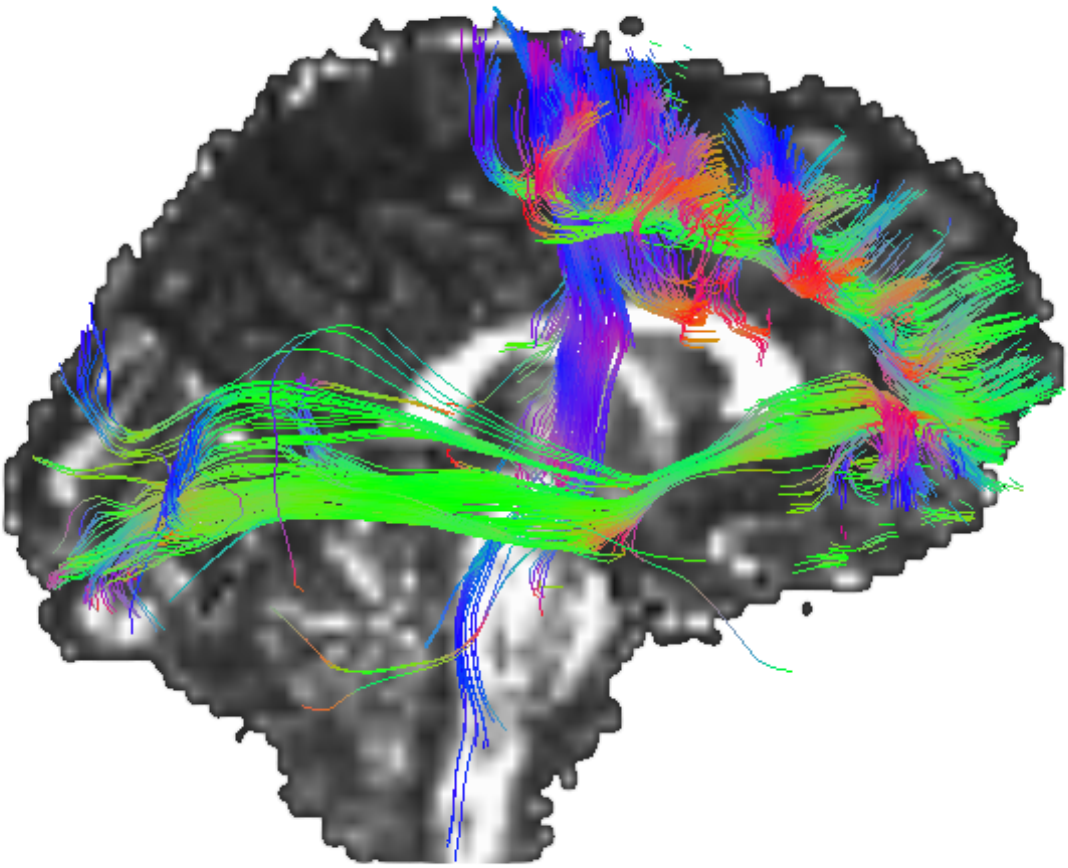

FIGURE 1 | illustrates the right middle frontal bundles where significant white matter AD and MD differences were observed between male BDs and AACs

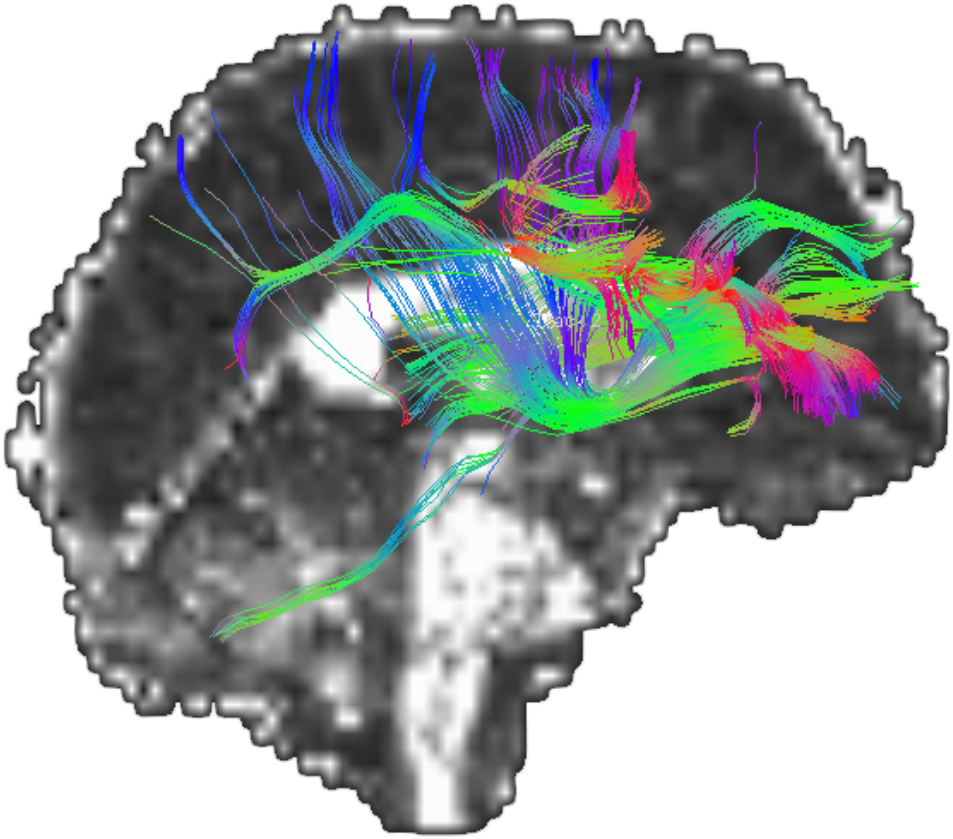

FIGURE 2 | illustrates the right inferior frontal bundles where significant white matter AD and MD differences were observed between female BDs and AACs

4. Discussion

In this study, we analyzed the white matter structural connectivity of core regions related with self-regulatory processes and inhibitory control in a group of BDs, comparing with AACs. We observed higher diffusivity measures (as shown by MD and AD indexes) in the males BDs right middle frontal gyrus, when compared to AACs males. Lower right inferior fontal gyrus diffusivity (as shown by AD and MD values) was observed in the female BDs, compared to its counterparts female AACs. Furthermore, a pattern of positive correlations between the MD of the right middle frontal gyrus and the duration of the BD pattern in the male BD group was observed, whereas the AD of the right inferior frontal gyrus was negatively associated with the frequency of BD episodes per month in the female BDs.

Overall, the results document alterations in the inferior and middle frontal white matter structural connectivity in the BD group that were associated to alcohol-related measures. These results are consistent with some of the tract-based studies conducted in BD, showing alterations in the major frontal main white matter association tracts (e.g. the superior longitudinal fasciculus and inferior fronto-occipital fasciculus), when compared to non-BDs (Jacobus et al., 2009; 2013; Luciana et al., 2013; McQueeny et al., 2009). Moreover, this is in line with a longitudinal study conducted by Jacobus et al. (2013) showing decreased FA in the inferior fronto-occipital and superior longitudinal fasciculus of BD. The authors associated their findings with the detrimental effects of high alcohol intake during the adolescent brain development that affected thus the white matter integrity (Jacobus et al., 2013).

In fact, during adolescence, dramatic neuromaturational processes such as tuning neuronal communication and tissue organization (e.g. synaptic pruning and intense myelination) occur (Giedd & Rapoport, 2010; Giedd et al., 1999). This cerebral rearrangement plays an important role in the brain maturation and seems to be activity-dependent. Specifically, the frontal white matter development, as shown by diffusion tensor imaging (DTI), seems to be a protracted process lasting until the 3rd decade of life with the time elapsing between adolescence and early adulthood appearing particularly relevant

in terms of frontal myelination and microstructural organization (see Sousa, Amaro Junior, Crego, Gonçalves & Sampaio, 2017a for a review). In accordance, complex cognitive functions that rely on the prefrontal cortex (PFC) seem to be underdeveloped during adolescence, which is associated with the structural immaturity of the PFC. This is likely to predispose individuals to take risky choices and to enroll in harmful behaviors such as alcohol misuse (Bari & Robbins, 2013; Bava & Tapert, 2010; Crews & Boettiger, 2009). In fact, reduced white matter in PCF' areas (e.g. superior frontal gyrus), in addition to other widespread regions such as the middle temporal gyrus, cerebellum, lingual gyrus, and corpus callosum was observed in BDs compared to non-consumers (Lisdahl et al., 2013; Luciana, Collins, Muetzel, & Lim, 2013; Squeglia et al., 2014; 2015). which is consistent with our findings and with our MRI study, showing that college students who continuously enrolled in high alcohol intake displayed gray matter disruptions in frontal regions related to self-regulatory processes (e.g. inferior and middle frontal gyri) (Sousa et al., 2017b). Abnormalities of the middle frontal gyrus have been frequently associated to difficulty in regulate behavior in face of failure and undercontrol, and prospectively predicting substance use (Heitzeg et al., 2014; Japee, Holiday, Satyshur, Mukai, & Ungerleider, 2015). The inferior frontal gyrus changes have been previously correlated with deficits in response inhibition shown by alcohol-dependent individuals (Aron, Robbins, & Poldrack, 2004; Swick, et al., 2008; Wiers et al., 2015) and with stimuli salience (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010).

Moreover, we found different results for males and females, and different pattern of associations between alcohol-related measures and white matter indexes. In fact, it is likely that the MD differences between male BDs and male AACs might be mediated by pubertal factors. In fact, some authors highlighted the important role of testosterone in the optimal white matter development (Menzies, Goddings, Whitaker, Blakemore, & Viner, 2015). Specifically, adolescents with lower testosterone levels were shown to exhibit higher MD values in widespread white matter regions including the frontal tracts (Menzies et al., 2015), suggesting that adolescent males with more

advanced pubertal development exhibited lower MD values in comparison to their less-developed peers (Menzies et al., 2015). Nevertheless, these differences might be secondary to alcohol consumption and may differentially affect males and females' brain (Squeglia et al., 2015), which need to be deeper investigated in future studies.

Thus, our MD and AD findings suggest that our male BDs may exhibit a delay in the right middle frontal gyrus white matter maturation processes in comparison with its age-matched abstainers peers, which might precede the BD behavior. Nevertheless, the observed MD changes might be an effect of high alcohol consumption, since this index was the only in showing positive correlations with the duration of the BD pattern. Further studies are necessary in order to test these hypotheses.

The lower AD in the right inferior frontal gyrus of the female BDs were associated with more binge episodes per month. Therefore, these findings suggest that the females' white matter alterations in the inferior fontal gyrus, and the lower MD values found in the female BDs right inferior frontal gyrus might be associated with differentiated right inferior frontal gyrus developmental pathways between female BDs and AACs females, suggesting an overdeveloped right inferior frontal gyrus white matter (e.g. increase myelination and axonal branching) in the BD group, compared to the AAC group.

The current analysis revealed white mater abnormalities in the same frontal regions (i.e. inferior and middle fontal gyri) as we previously described for gray matter in the BD group, compared to AACs (see Sousa et al., 2017b). In fact, differences between males and females BDs were evident in both studies. However, the deep understanding of these differences is not possible to disentangle through the current cross-sectional analysis.

In summary, our cross-sectional study revealed white matter alterations within dorsolateral prefrontal regions in the BD group. However, from the current findings we cannot establish neuromarkers as precursors or effects of the BD behavior. Future longitudinal studies should try to disentangle whether frontal abnormalities subjacent to self-regulatory processes are due to disruptions

in the optimal brain development or if is the alcohol misuse that leads to the alterations in the optimal brain development.

Therefore, to conclude, the alterations in frontal structural connectivity disclosed by the analysis of this subclinical sample, eventually related with myelination processes and axonal integrity in brain regions related with self-regulation processes, might be an important contribution in defining biomarkers that could be predictive of alcohol abuse disorders or effects of high alcohol ingestion.

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CHAPTER V

1. General Discussion

Adolescence is an important phase of transition in the individuals' life that brings new experiences and challenges, which might influence and/or be influenced by the great brain restructuring and improvement of high-level cognitive skills, still immature in this period.

Adolescent self-control seems to be less efficient in correctly inhibit actions/emotions or thoughts that might have negative outcomes, because the self-regulation processes are still immature (Casey & Caudle, 2013).

An underdeveloped prefrontal cortex appears to be the reason why adolescents have a diminished self-control capacity, since self-control processing relies on the prefrontal brain structures that seems to be the latest regions of the human brain reaching maturation, being fully matured closest to the thirties.

Overall, there is extensive evidence that during adolescence the brain undergoes dramatic reshaping and restructuring, and therefore might be highly vulnerable to adverse environmental factors, such is the case of drugs and alcohol misuse. Among substance abuse clusters, high alcohol ingestion (binge drinking) has recently received significant attention, due to its high prevalence rates among college students and disastrous consequences.

An immature inhibitory control has been referred in addiction models as having a major role in substance use/abuse, since an inefficient inhibitory control seems to be highly associated to adolescents' tendency to engage in risky-driven behaviors, such is the case of alcohol misuse (Crews & Boettiger, 2009; Bava & Tapert, 2010; Bari & Robbins, 2013).

Under this assumption, the main goals of our investigation were: 1) o assess the gray and white matter morphometry associated with binge drinking, in the prefrontal-striatal network involved in inhibitory control and impulsivity processes; 2) to analyze the associations between the morphometric measures, both in gray and white matter, and two behavioral measures: the Barratt Impulsiveness

Scale (BIS) and the intensity and frequency of alcohol consumption (e.g., number of binge episodes per month). Therefore, we conducted 3 studies. In the first study, we performed a systematic review of the literature. Twenty-seven articles were analyzed in order to gather information about the normative developmental trajectory of prefrontal white matter, since birth until adulthood, as shown by diffusion tensor imaging. The findings suggested a protracted process of frontal white matter maturation, which is observed since birth until adulthood, lasting until approximately the fourth decade of life. Specifically, increased myelination, axonal density, and fibers' organization, as measured through increased FA and decreased MD and RD, but not so consistently AD, was observed. However, the developmental rate across the distinct stages (e.g. infancy, adolescence) was not constant. Specifically, white matter drastically increases in the youngest years (until 4 years of age); a more steady moment is observed in pre-pubertal ages (± 6 to 11 years old), sprouting again during adolescence. It seems that the first 4 years of life and adolescence are the sharpest periods of white matter development. These findings suggest that frontal brain regions are in constant reorganization and refinement, to continuously adapt to environmental exigencies, and that changes in frontal white matter microstructure may be qualitatively distinct in different developmental periods, maybe because white matter development is activity/experience-dependent.

A factor that might be contributing for the white matter development to slowdown in pre-pubertal ages may be gray matter growth (Giedd et al., 1999). In fact, a relation between these two events possibly exists, once that gray matter growth peaks at approximately age 12, and since then it begins to decline until the twenties, even though the cerebral volume remains quite constant after age 6, as shown by MRI studies (Giedd et al., 1999; Giedd, 2004; Gogtay et al., 2004; Lenroot & Giedd, 2006). Additionally, negative and positive associations were observed between frontal cortical thickness (a measure of gray matter) and white matter volumes and FA values, plus MD, AD and RD values, respectively. These findings might suggest a relation between gray matter pruning, myelination and

enhanced fibers' organization throughout development (Tamnes et al., 2010).

Moreover, frontal white matter maturation seems to parallel improvements in cognitive abilities, such as inhibitory control (Gogtay et al., 2004). Adolescent enhanced performance in inhibitory related tasks is associated with higher FA and lower RD values measured in frontal regions (Madsen et al., 2010), and the adult level of performance is not attained before late adolescence (Huizinga, Dolan, & van der Molen, 2006), suggesting a link between structural maturation processes and enhanced cognitive functions.

Taking into account the developmental background of the adolescent brain, the aim of our second study was to understand if adverse environmental factors (e.g. binge drinking) might disturb the integrity of frontal regions (gray and white matter), specifically those subserving the inhibitory control process. Therefore we conducted the first structural analysis study targeting young binge drinkers' gray matter. The main goal was to assess the existence of morphological abnormalities in brain regions associated to self-regulatory processes in individuals with a BD pattern of alcohol consumption, using VBM. The findings revealed increased gray matter densities in the middle and inferior frontal gyri, of female and male BDs, respectively, compared to their alcohol-abstinent peers. Additionally, increased gray matter densities within the BD group were associated to lower self-control.

The results disclosed by this analysis point to a disruption in the optimal gray matter developmental trajectory in young BDs, compared to non-binge. In fact, as shown by previous developmental studies, between adolescence and early adulthood (±12-20 years, a decrease in gray matter should be noted, largely driven by the pruning process of unnecessary synaptic connections. Likewise, it seems that this process might be altered in our BD group, since they displayed more frontal gray matter than their counterparts' non-consumers. Additionally, longitudinal studies with youngest binge consumers (12-14; 12-17; 14-17 years) showed decreased gray matter when compared to non-consumers. Particularly, these findings were associated to premorbid characteristics, such as a delay of

gray matter growth that should be happening at those ages as a part of the normative neurodevelopmental process (Squeglia et al., 2014; 2017; Wilson, Malone, Thomas, & Iacono, 2015).

Considering these results in the frontal gray matter, the third study analyzed white matter analysis in the same brain regions. The results revealed microstructural gender-specific alterations in the BDs inferior frontal gyrus and middle frontal gyrus, as measured through AD and MD indexes, compared to alcohol-abstinent controls. Binge-drinkers males presented higher AD and MD in the right middle frontal gyrus, in comparison with alcohol-abstinent controls males. Contrastingly, female BDs displayed lower AD and MD values in the right inferior frontal gyrus compared to female alcohol-abstinent controls. Additionally, the changes in the frontal white matter of young BDs (measured through MD) were associated to alcohol-related measures.

Over the normative developmental course, as shown by our systematic review, white matter increases and its microstructure becomes more organized, as measured through FA, MD, RD and AD indexes. The current analysis however showed disruptions of these processes such as myelination and axonal branching and organization (as measured through MD and AD indexes), in the BDs, when compared to its alcohol-free counterparts.

Overall, our results of white and gray matter abnormalities in BD might be associated to premorbid characteristics of the binge-drinkers' brain (e.g., delayed developmental schedules) or it may be consequence of the maladaptive behavior (i.e., binge drinking).

In fact, co-twins studies revealed premorbid characteristics of the binge drinkers' brains, such as gray matter reductions in frontal regions previous to alcohol initiation, that might have been previous to the behavior onset and possibly genetics-driven (Wilson et al., 2015), which might highlight that early developmental factors might be associated with prospective disruptive behaviors. In addition, another study has found that individuals who showed difficulties in suppressing an action related to a reward at 4 years of age, still had the same difficulties in suppressing their response to positive social cues 40

years later, but not for neutral cues (Casey et al., 2011), suggesting that specific environmental cues (e.g. enjoyable stimuli) may present additional difficulties to individuals with a diminished self-control, in suppressing inadequate responses (Casey & Caudle, 2013).

Summing up, our empirical studies revealed abnormalities of the frontal gray and white matter in the BDs' brains, in comparison to alcohol-abstinent controls. These gray and white matter changes are associated to a weak and underdeveloped inhibitory control. This is likely to prompt them to risky choices (e.g. high alcohol intake), and may be a factor contributing to the binge onset.

Finally, this work suggests that the access to risky environmental factors (e.g. alcohol) in such an important period of brain remodeling as adolescence, might adversely impact brain structure. Frontal gray matter alterations, which were associated to self-regulatory processes and impulsiveness but not with alcohol-related measures, might be a premorbid factor, already present in the young binge drinkers brain even before their alcohol initiation, in a way triggering the behavior. In fact, as shown by previous addiction models, impulsiveness is an important characteristic of the initial phase of the addiction circuit, that is the phase of compulsive use of the substance or bingeing. On the other hand, white matter alterations are likely to be seen as consequences of the binge drinking behavior, since correlations were found between alcohol-related measures, but not with self-control scores. Longitudinal studies are needed to further explore these hypotheses.

2. Limitations and Future Directions

The main limitation of our investigation is its cross-sectional nature and being conducted with a small sample size. In fact, from our findings we cannot establish a relation between the structural abnormalities of frontal regions found in our binge drinkers, compared to their non-binge peers, and the binge drinking behavior. We may argue that such abnormalities might be related to abnormal self-regulation processes, in addition to alcohol-related consequences but in fact we are not able to draw a

connection between the structural findings and the adverse behavior (i.e. binge drinking). In this sense, in order to disentangle whether structural abnormalities reflect vulnerability factors or consequences of high alcohol consumption, future studies should take the advantage of longitudinal designs with more than two follow up assessments and the combination of morphometric, genetics and behavioral measures. Additionally, the inclusion of an alcohol-related inhibitory paradigm such as the stop-signal task or the think-no-think task, in a functional MRI study would be of interest in order to study the relationship between the structural functioning of the prefrontal cortex and striatal regions and behavioral performance in alcohol-related inhibitory tasks.

Also, graph analysis of functional brain networks (De Vico Fallani, Richiardi, Chavez, & Achard, 2014) would be of interest in order to analyze the communication between prefrontal and striatal nodes, in an attempt to understand how these regions interact across adolescence and how they contribute to risk-taking behaviors.

Additionally, several software packages are available to process diffusion based tractography (e.g. streamline/probabilistic/voxel-based). The tractography is a virtual 3D reconstruction of all brain fiber pathways, or within a specific seed or region of interest. Specifically, in the present work, we used a streamline tractography, which is based on the assumption that in one voxel only onset of fibers can be present. However this method does not account for regional fiber crossing, branching and curving (Feigl et al., 2014). Therefore, more sophisticated analysis using probabilistic methods that consider fiber crossing and branching should be conducted in future investigations (Wedeen et al., 2008).

To further improve our investigation and taking the currently available data, we already conducted a preliminary analysis of two important resting state networks that we hypothesized to be additionally altered in young BDs. We conducted functional resting-state connectivity analyses of the executive control network (ECN) and default mode network (DMN), which we found to be abnormal in BDs, compared to AACs. A brief description of this work will be found in the end of this chapter.

Moreover, it worth note that current magnetic resonance imaging assessment although being very informative about gray matter volumes, densities and cortical thickness, still do not provide measures that could be associated to cellular processes underlying the development of gray matter (e.g. dendritic arborization and pruning). More recent measures (e.g. orientation dispersion index) however start to be applied to explore dendritic microstructure but studies are still scarce.

Finally, and despite the above-mentioned limitations, to our knowledge this is the first cross-sectional study using a completely alcohol-abstinent sample as the control group, thus empowering the brain differences between BDs and completely alcohol-free college students and providing additional contribution in defining potential biomarkers that could be predictive of alcohol misuse among young college students at risk.

3. Conclusions

The pattern of increased regional GM density, which is likely to impact self-regulatory processes, and the increased frontal white matter, which seems related to alcohol consumption, suggests that disrupted developmental factors may contribute to the initiation of risky behaviors during adolescence, and that adverse environmental factors might impact the normative development of the frontal white matter during adolescence.

These findings may shed light in the identification of potential neurostructural factors that might be precursors of BD behavior or neurotoxic candidates, which might change the neurodevelopmental trajectories of gray and white matter and impact self-regulatory processes.

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Functional Resting State Analysis - Preliminary Results

Binge Drinking and Functional Brain Connectivity: a Resting-State fMRI Study

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Abstract

The intake of large amounts of alcohol in a short time (habitually in weekends) followed by a period of abstinence is known as binge drinking and is a very common pattern of consumption among young people. Understanding what significant exposure to alcohol during adolescence where dramatic neuromaturational processes such as tuning neuronal communication and tissue organization (e.g, synaptic pruning and intense myelination) occur is of particular importance although literature about the effects in the functional brain connectivity during this developmental state is still scarce.

Herein we assessed the architecture of the resting state networks (RSNs) brain functional connectivity using functional magnetic resonance imaging (fMRI) in a group of young adults that met the criteria for binge drinking (10 women, mean age: 20), and alcohol-abstinent controls - (N=14, 8 women, mean age: 20). Specifically, we analyzed a task-positive network, the executive control network (ECN) and a task negative network, the Default Mode Network (DMN).

Significantly decreased functional connectivity in the left middle frontal region of the ECN was observed in the binge-drinking group, when compared with the alcohol-abstinent control group. When we analyzed the effects of gender on this pattern of functional connectivity, males from the binge-drinking group displayed decreased functional connectivity in the right thalamus and in the right angular region of the ECN, as well as increased functional connectivity in the left middle orbitofrontal region of the DMN, when compared with alcohol-abstinent control males. Increased functional connectivity in the

right precuneus cluster of the DMN was observed in the female binge-drinking group when compared to alcohol-abstinent control females.

These results suggest alterations of functional connectivity in the binge-drinkers within the ECN and the DMN. The pattern of decreased functional connectivity within the ECN may be related to a diminished capacity to refrain the urge for high alcohol intake that characterize alcohol use disorders. Likewise, the increased functional connectivity within the DMN, particularly in the orbitofrontal regions, may be associated to reward system abnormalities. The alterations in brain functional connectivity disclosed by the analysis of this subclinical sample might be an important contribution in defining biomarkers that could be predictive of alcohol abuse disorders.

1. Introduction

The intake of large amounts of alcohol, in a short time (habitually in weekends) followed by a period of abstinence is known as binge drinking and is a very common pattern of consumption among adolescents and college students (NIAAA, 2014; Courtney & Polich, 2009).

Understanding the consequences of significant exposure to alcohol during adolescence when dramatic neuromaturational processes such as neuronal communication tuning and tissue organization occur is of particular importance. In fact this period of structural and functional immaturity is related to a diminished capacity of the inhibition system in addition to a hyperactive reward system, leading to an imbalance at the circuit level interactions and adolescents more prone to take risky choices, (Crews & Boettiger, 2009; Bava & Tapert, 2010; Bari & Robbins, 2013).

Recently, the study of the resting-state networks (RSNs) have provided an important tool to address the mechanism underlying neurocognitive and socio-emotional disorders, which allows the characterization of circuit-level functional alterations within brain networks that are continuously sharing information playing a fundamental role in cognitive performance. Although the effects of alcohol abuse

in the functional adolescent brain connectivity are still scarce, some studies with adults point to abnormalities in the RSNs related with inhibition and reward mechanisms. Heavy drinkers or individuals with alcohol abuse disorder showed decreased activation within the left executive control network (LECN) and default mode network (DMN) (Spagnolli et al, 2013; Weiland et al., 2014) while oppositely increased connectivity within the left fronto-parietal network and DMN was observed in individuals with alcohol dependence (Jansen et al, 2015; Zhu et al, 2015). Considering these results in adults, we assessed the resting state fMRI in a group of 20 participants with binge-drinking pattern of consumption and 14 alcohol abstinent controls. We hypothesized altered functional connectivity within the ECN and DMN regions related to impulsivity in a pre clinical sample of binge drinkers and that this changes would be more prominent in male binge-drinkers.

2. Method

Participants: 20 young adults that met the criteria for binge drinking (binge drinkers; 10 women, 10 men; mean age: 20 years) and 14 ACC (10 women, 6 men; mean age: 20 years) were included in this study.

Image Acquisition: T1 weighted scans were obtained with a 3D volumetric magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence on a Siemens 3T magnet with TR = 2700ms; TE = 2.33ms; slice thickness = 0,80mm; Voxel size =1x1x1mm; flip angle = 7°; FOV = 256x256.

The resting state fMRI scan was performed using a blood oxygen level dependent (BOLD) sensitive echo-planar imaging (EPI) with the following parameters: 39 interleaved axial slices, repetition time (TR) = 2000 ms, echo time (TE) = 29 ms, flip angle (FA) = 90°, slice thickness = 3, slice gap = 3.75 mm, in-plane resolution = 3 x 3 mm2, field of view (FoV) = 222 mm and 210 volumes.

Statistical Analysis: Two-sample t-tests of the RSNs maps were performed, as implemented in the SPM12 (Statistical Parametrical Mapping, version 8, http://www.fil.ion.ucl.ac.uk), to analyze functional connectivity differences between binge-drinkers and alcohol-abstinent controls. SPM maps were generated for between group differences in regions where functional connectivity was significantly lower/higher in binge-drinkers when compared to alcohol-abstinent controls. For statistical threshold criteria significant results were considered after Monte Carlo correction for multiple comparisons $p < 0.05$. The correction was determined over 1000 Monte Carlo simulations using AlphaSim tool, distributed with REST software tool (http://resting-fmri.sourceforge.net/), and mask set to the corresponding RSN. Anatomical labeling was obtained using the anatomical automatic labeling atlas (AAL) (Tzourio-Mazoyer et al. 2002).

3. Results

Group differences in regions with altered functional connectivity were found in the ECN and DMN. Specifically, figure 1 illustrates a decreased functional connectivity in the left middle frontal region of the ECN in the binge-drinking group, when compared with the alcohol-abstinent control group. When we analyzed the male subgroup, we observed a decreased functional connectivity in the right thalamus and angular region of the ECN and an increased functional connectivity in the left middle orbitofrontal region of the DMN in males' binge-drinkers (see figure 2). Finally, an increased functional connectivity in the right precuneus of the DMN in the female binge-drinking group when compared with the female alcohol-abstinent control group is illustrated in figure 3.

[2]Anatomical Label	Direction of difference	MNI Coordinates[a] x y z	Cluster size (K_E)	Peak *t* score	*p* value [b] (uncorrected)
ECN					
Frontal_Mid_L	Decreased in BD	-40 22 50	10[c]	3.75	0.000

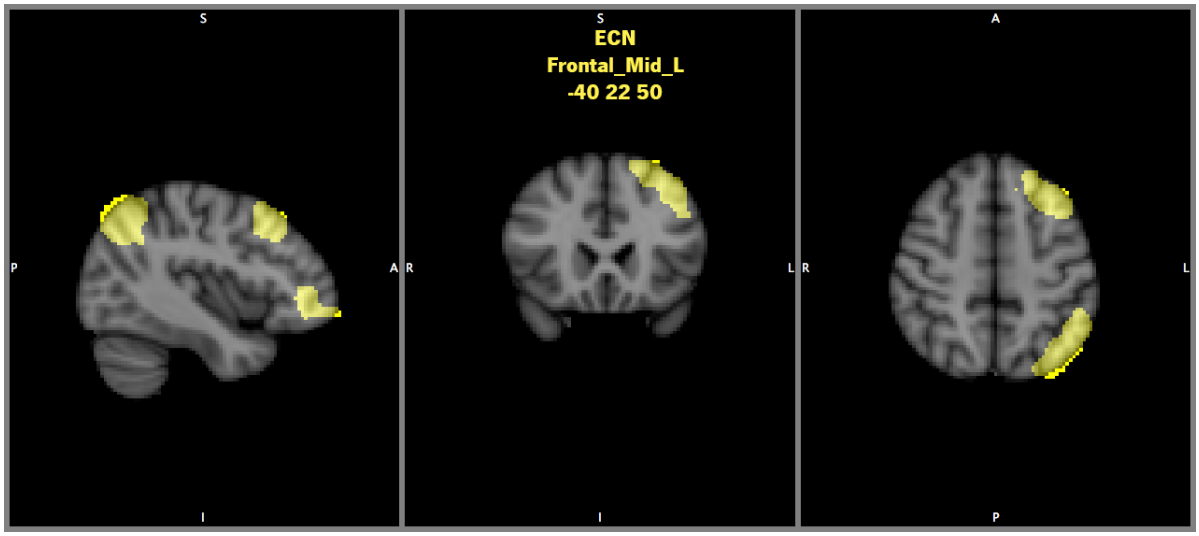

FIGURE 1 | Illustrates the differences in the ECN functional connectivity between the binge drinking group and the alcohol-abstinent control group

[2]

[a] Montreal Neurological Institute coordinates of the voxel of maximal statistical significance within each region
[b] Statistical significance set to p<0.001, uncorrected for multiple comparisons at voxel level
[c] Cluster size calculation set to minimum of 9 voxels, AlphaSim p<0.05

[3]Anatomical Label	Direction of difference	MNI Coordinates[a] x y z	Cluster size (K_E)	Peak *t* score	*p* value [b] (uncorrected)
DMN					
Frontal_Med_Orb_L	Increased in BD	-10 60 0	28[c]	4.65	0.000
ECN					
Angular_R	Decreased in BD	54 -52 34	15[d]	5.08	0.000
Thalamus_R	Decreased in BD	12 -6 14	12[d]	4.67	0.000

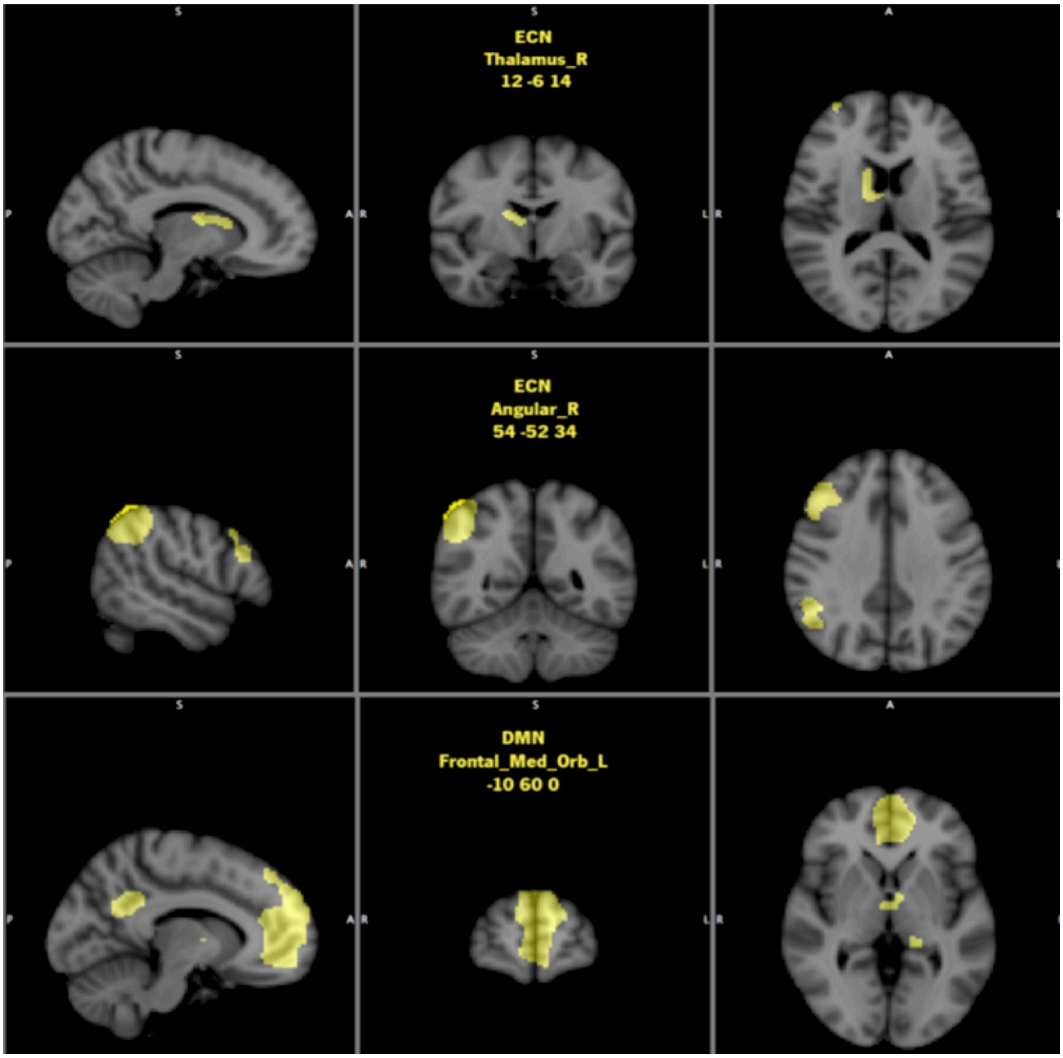

FIGURE 2| Illustrates the differences in the DMN and ECN functional connectivity between males binge drinkers and males alcohol-abstinent controls

[3] [a] Montreal Neurological Institute coordinates of the voxel of maximal statistical significance within each region
[b] Statistical significance set to p<0.001, uncorrected for multiple comparisons at voxel level
[c] Cluster size calculation set to minimum of 13 voxels, AlphaSim p<0.05
[d] Cluster size calculation set to minimum of 11 voxels, AlphaSim p<0.05

[4]Anatomical Label	Direction of difference	MNI Coordinates[a] x y z	Cluster size (K_E)	Peak *t* score	*p* value [b] (uncorrected)
DMN					
Precuneus_R	Increased in BD	6 -52 28	16[c]	5.08	0.000

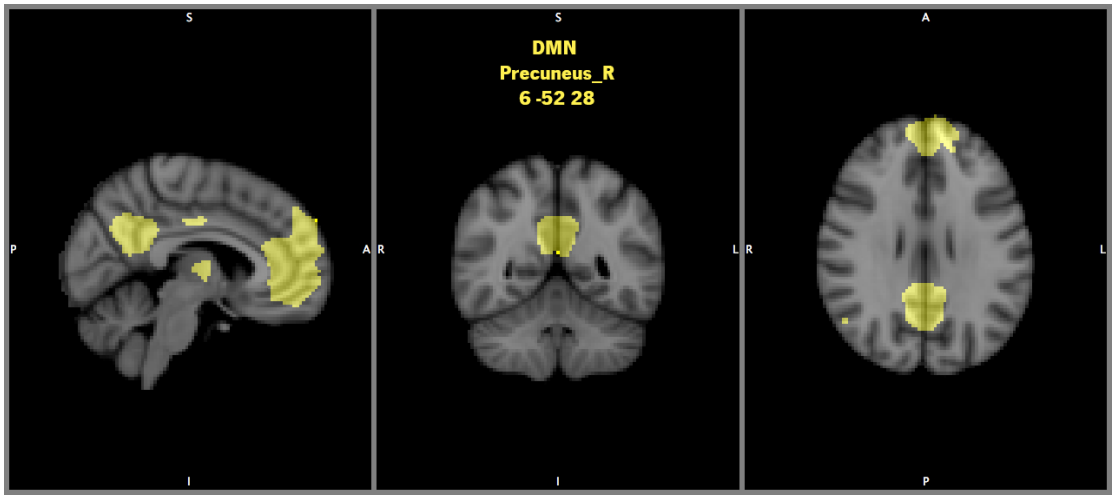

FIGURE 3| Illustrates the differences in the DMN functional connectivity between females binge drinkers and females alcohol-abstinent controls

4 [a] Montreal Neurological Institute coordinates of the voxel of maximal statistical significance within each region
[b] Statistical significance set to p<0.001, uncorrected for multiple comparisons at voxel level
[c] Cluster size calculation set to minimum of 13 voxels, AlphaSim p<0.05

4. Discussion

In this study, we analyzed the functional connectivity of the ECN and DMN in a group of binge-drinkers compared with the alcohol-abstinent control group. We observed significantly decreased functional connectivity in the left middle frontal region of the ECN in the binge-drinking group, when compared with the alcohol-abstinent control group. Furthermore, when analyzed the effects of gender, we observed that males from the binge-drinking group displayed decreased functional connectivity in the right thalamus and in the right angular region of the ECN, contrasting with increased functional connectivity in the left middle orbitofrontal region of the DMN, when compared with alcohol-abstinent control males. Finally, we observed increased functional connectivity in the right precuneus cluster of the DMN in the binge-drinking female group, when compared to alcohol-abstinent control females.

Overall, these results suggest alterations of functional connectivity in the binge-drinkers within the ECN and the DMN. The pattern of decreased functional connectivity in specific regions within the ECN, as the left middle frontal region, an important region related with inhibitory control, may be related to a diminished capacity to refrain the urge for high alcohol intake that characterize alcohol use disorders. Specifically the middle frontal and angular regions of the ECN play an important role in top-down inhibitory control that seems impaired in binge-drinkers (Japee et al., 2015; Garavan et al, 1999). Likewise, the increased functional connectivity within the DMN, particularly in the orbitofrontal cortex and precuneus, may reflect reward system abnormalities described in binge-drinkers, which might be related to an heightened response to alcohol related-cues, enhancing the subjective value of alcohol consumption (Schoenbaum & Shaham, 2008; DeWitt et al, 2015).

Finally, the alterations in brain functional connectivity disclosed by the analysis of this subclinical sample might be an important contribution in defining biomarkers that could be predictive of alcohol abuse disorders.

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