UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: NEUROCIÊNCIAS

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

TESE DE DOUTORADO

TRANSTORNOS DE ANSIEDADE E LINGUAGEM EM CRIANÇAS E ADOLESCENTES: ESTUDOS DE NEUROPSICOLOGIA E NEUROIMAGEM FUNCIONAL

Rudineia Toazza

Orientadora: Profa. Dra. Gisele Gus Manfro

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Neurociências, como requisito parcial para obtenção do título de Doutor.

Porto Alegre
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...bem antes de servir para comunicar, a linguagem serve para viver. Se nós colocarmos que à falta de linguagem não haveria nem possibilidade de sociedade sem possibilidade de humanidade, é precisamente porque o próprio da linguagem é, antes de tudo, significar.

-Émile Benveniste

Para meus pais, ***Valdir*** *e* ***Maria Helena Toazza****.*

Por me ensinarem que todos os objetivos podem ser alcançados e os obstáculos superados;

Por abrirem mão dos seus próprios sonhos para que a nossa educação fosse prioridade.

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Aos meus ***amigos e amigas*** *que torceram genuinamente por mim, pelo carinho, apoio e compreensão dos momentos de ausência.*

Sumário

ABREVIATURAS E SIGLAS

ACP – Análise de Componentes Independentes

BOLD – *Blood-oxygen-level dependent*/ nível de oxigenação do sangue dependentes

CPF – Córtex Pré-Frontal

dlCPF - Córtex Pré-Frontal Dorsolateral

dmCPF – Córtex Pré-Frontal Dorsomedial

DSM - *Diagnostic and Statistical Manual of Mental Disorders*/Manual Diagnóstico e Estatístico dos Transtornos Mentais

FAS – Tarefa de Fluência Verbal que utiliza as letras F, A e S

TAG – Transtorno de Ansiedade Generalizada

TASe – Transtornos de Ansiedade de Separação

TASo – Transtorno de Ansiedade Social

TDAH – Transtorno de Déficit de Atenção e Hiperatividade

TP – Transtorno do Pânico

vmCPF - Córtex Pré-Frontal Ventromedial

ROI – *Regions of Interest*/Regiões de Interesse

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RESUMO

Os transtornos de ansiedade são o grupo de transtornos psiquiátricos mais comum na infância e adolescência. Embora avanços significativos tenham sido feitos para identificar os melhores tratamentos para crianças ansiosas, ainda pouco se sabe sobre a base neural subjacente a esses transtornos. Nesse sentido, essa tese teve por objetivo investigar a linguagem como pano de fundo para o entendimento dos transtornos de ansiedade através de avaliações neuropsicológicas e de neuroimagem funcional, levando em consideração que pobres habilidades de linguagem podem levar a reações negativas no meio de convívio, vieses de interpretação e comportamento de esquiva em situações sociais. Os três artigos aqui apresentados são resultado de dois projetos. O primeiro artigo é resultado da fase de avaliação (linha de base) de crianças (6 -12 anos) participantes de um ensaio clínico randomizado para ansiedade infantil. O segundo e terceiro artigos são resultados de um seguimento de pacientes e indivíduos de comparação - adolescentes e adultos jovens (15 – 20 anos) - acompanhados pelos últimos 5 anos, oriundos de amostra comunitária.

O primeiro artigo (*Verbal fluency and severity of anxiety disorders in young children*) teve como objetivo investigar o desempenho em testes de fluência verbal fonêmica com a gravidade dos sintomas de ansiedade em crianças. Os resultados mostraram uma associação entre sintomatologia ansiosa e o número de *clusters* (agrupamento de palavras que começam com as mesmas duas primeiras letras, diferem em uma única vogal, produzem rima ou homônimos). Os resultados replicam e avançam no entendimento de achados anteriores mostrando que a fluência verbal é consistentemente associada com a gravidade dos transtornos de ansiedade desde a

infância. Além disso, nesse trabalho nós mostramos que essa associação é independente de sintomas comórbidos de Transtorno de Déficit de Atenção/Hiperatividade. Nossos dados reforçam a importância da fluência verbal como um marcador de gravidade para a ansiedade e incentivam o desenvolvimento de novos estudos com o objetivo de investigar mecanismos acerca da relação entre fluência verbal e ansiedade e suas implicações terapêuticas. O segundo artigo (*Anxiety-related down-regulation of thalamic regions in the processing of sad and angry emotional narratives*) teve como objetivo investigar, através de ressonância magnética funcional, o processamento de narrativas de cunho emocional em comparação com conteúdo neutro, em adolescentes e jovens adultos com transtorno de ansiedade, em relação a um grupo de comparação com desenvolvimento típico. Os resultados mostraram uma hipoativação no tálamo, modulado pela emoção no contraste triste vs. neutro e raiva vs. neutro, em indivíduos com transtorno de ansiedade. Além disso, observou-se efeito da emoção em outras dez áreas cerebrais relacionadas com a linguagem e atenção (junção temporoparietal esquerda, giro pré-frontal medial direito, giro frontal inferior esquerdo, giro frontal medial esquerdo, cingulado posterior/precuneo, lobo parietal inferior esquerdo, giro temporal medial esquerdo, giro lingual esquerdo, giro temporal medial esquerdo, giro frontal inferior direito). Os resultados sugerem que pacientes com transtornos de ansiedade apresentam menor ativação talâmica relacionada a emoções negativas do que indivíduos de comparação, implicando um processamento aberrante de informações negativas nesses indivíduos. O terceiro artigo (*Amygdala-based intrinsic functional connectivity and anxiety disorders in adolescents and young adults*) teve como objetivo investigar a conectividade intrínseca de sub-regiões da amigdala em pacientes com transtorno de ansiedade quando comparados ao grupo sem o transtorno, através de exame de neuroimagem funcional em repouso. Os resultados mostraram conexões aberrantes entre a amigdala basolateral esquerda e cinco regiões cerebrais*:* giro

pré-central direito, cingulado direito, precuneo bilateralmente e giro frontal superior. Essas diferenças entre os grupos se refletem em uma maior coativação entre a amigdala e essas cinco regiões em indivíduos ansiosos, enquanto que em indivíduos não ansiosos ou essa coativação da amigdala com essas regiões não é significativa ou é inversa (como acontece no giro cingulado direito e precuneo direito). Os dados mostraram importante disfunção na conectividade intrínseca entre a amigdala basolateral esquerda com o córtex motor, em áreas envolvidas na regulação emocional e rede de modo padrão. Os resultados desse conjunto de estudos trazem informações importantes sobre a fisiopatologia da ansiedade em crianças e jovens utilizando métodos de Neuropsicologia e neuroimagem funcional. Espera-se que um melhor entendimento desses mecanismos psicológicos e biológicos em jovens e crianças leve a ideias para o desenvolvimento de novas terapêuticas para pacientes que sofrem com esses transtornos ao longo de toda a vida.

O conjunto de dados encontrados nesses três artigos nos mostram alterações significativas em indivíduos com transtornos de ansiedade a partir de diferentes enfoques de avaliação: Neuropsicologia, Neuroimagem funcional (com realização de tarefa e em repouso). Esses achados iniciais demonstram as potencialidades que a neurociências oferece para o entendimento dos transtornos mentais ao compreender o cérebro humano em suas diferentes redes funcionais, além de fornecer ideias para avanços na personalização terapêutica e desenvolvimento de novas estratégias de tratamento.

ABSTRACT

Anxiety disorders are the most common group of psychiatric disorders in childhood. Although significant advances have been made to identify the best treatments for children with anxiety disorders, little is known about the underlying neural basis for these disorders. This thesis used language as a background for the understanding anxiety disorders throughout neuropsychological and functional neuroimaging studies, taking into consideration that poor language skills can lead to negative reactions, interpretation biases and avoidance behavior in social situations. The three papers presented here are the results of two projects. The first paper is the result of the baseline assessment phase of children (ages 6 – 11) participating in a randomized clinical trial for childhood anxiety. The second and third papers resulted from a cohort of anxiety disorder patients and comparison adolescents and young adults (ages 15 – 20) followed for the past five years from a community sample. The first paper, (*Phonemic verbal fluency and severity of anxiety disorders in young children*) aimed to investigate if performance in a phonemic verbal fluency task is associated with the severity of anxiety symptoms in young children with anxiety disorders. The results showed an association between anxiety symptoms and the number of *clusters* (grouping of words that start with the same first two letters, differ in one vowel, produce rhyme or homonyms). The results replicate and extend previous findings showing that verbal fluency is consistently associated with the severity of anxiety disorders since childhood. Moreover, in this paper we showed that this association was independent from comorbid symptoms of Attention Deficit/Hyperactivity Disorder. We conclude verbal fluency is consistently associated with the severity of anxiety symptoms, as replicated by our current findings. Our data reinforce the importance of verbal fluency

as a marker of severity for anxiety and encourage the development of further studies aiming to investigate biological mechanisms for this association as well as its therapeutic implications. The second manuscript (*Anxiety-related down-regulation of thalamic regions in the processing of sad and angry emotional narratives*) aimed to investigate emotional processing through functional magnetic resonance imaging using narratives with emotional content as compared with neutral content in adolescents and young adults with anxiety disorders, relative to a comparison group. The results demonstrated a hypoactivation of a thalamic region modulated by emotion in sad vs. neutral and angry vs. neutral contrasts in individuals with anxiety disorders if compared to typically developing controls. In addition, there was an effect of emotion activation in ten others brain areas related to language and attention (left temporoparietal junction, right medial prefrontal gyrus, left inferior frontal gyrus/pars orbitalis, left superior/middle frontal gyrus, posterior cingulate/precuneus, left inferior parietal lobe, left middle temporal gyrus, left lingual gyrus, left middle temporal gyrus, right inferior frontal gyrus). The results suggest patients with anxiety disorders have lower activation in a thalamic region during negative emotions, implicating aberrant information processing in this region in anxious adolescents. The third article (*Amygdala-based intrinsic functional connectivity and anxiety disorders in adolescents and young adults*) aimed to investigate the intrinsic connectivity of amygdala subregions in patients with anxiety disorder when compared to the group without the anxiety disorder by examination functional neuroimaging at rest. The results showed aberrant connections between the left basolateral amygdala and five brain regions: right precentral gyrus, right cingulate gyrus, precuneus bilaterally, and right superior frontal gyrus. Between-group differences are reflected in a higher co-activation between the amygdala and these five regions in anxious subjects, whereas in non-anxious individuals that co-activation is not significant or is negatively correlated (as in cingulate

gyrus and right precuneus). The data showed significant dysfunction in the intrinsic connectivity between the left basolateral amygdala with the motor cortex in areas involved in emotional regulation and default mode network. The results from this set of studies provide important information on the pathophysiology of anxiety disorders in children, adolescents and young adults by using neuropsychological methods and functional neuroimaging. It is hoped that better understanding of these mechanisms might shed light on ideas for the development of new therapies for patients suffering from these disorders throughout life.

Results from these three articles show significant changes in individuals with anxiety disorders using different evaluation approaches: Neuropsychology and Functional neuroimaging (with task and at rest). These early findings demonstrate the potential neurosciences offers to the understanding of mental disorders and provide ideas for therapeutic advances in customization and development of new treatment strategies.

1. INTRODUÇÃO

Os transtornos de ansiedade são caracterizados por medo e ansiedade disfuncionais (Blackford & Pine, 2012). Constituem o grupo de transtornos psiquiátricos mais comum da infância, com quase uma em cada três crianças sofrendo com os sintomas em algum momento durante o seu desenvolvimento (Blackford & Pine, 2012; Salum, Desousa, do Rosário, Pine, & Manfro, 2013). O medo e a ansiedade são respostas normais e adaptativas a uma ameaça real ou potencial, sendo que vários deles podem aparecer e desaparecer durante o desenvolvimento das crianças. No entanto, algumas apresentam medos persistentes ou estão constantemente marcadas pelo aparecimento de novos medos, gerando sofrimento e prejuízo e, em alguns casos, perda da funcionalidade (Blackford & Pine, 2012; Salum, Desousa, do Rosário, Pine, & Manfro, 2013). Nesses casos, caraterizados como transtornos de ansiedade, é comum a persistência até a idade adulta, muitas vezes gerando condições crônicas de difícil tratamento. Transtornos de ansiedade em adultos estão associados a altas taxas de ocorrência de outras doenças, mortalidade cardiovascular, mortalidade por suicídio e condições médicas crônicas (Agarwal et al., 2010; Salum, Desousa, do Rosário, Pine, & Manfro, 2013).

Conforme o Manual Diagnóstico e Estatístico de Transtornos Mentais – quinta edição (DSM – 5), os transtornos de ansiedade são classificados em: Transtorno de Ansiedade de Separação (TASe), Transtorno de Pânico (TP), Transtorno de Ansiedade Social (TASo), Transtorno de Ansiedade Generalizada (TAG) e Fobia Específica (FE) (APA, 2013). Esses transtornos compartilham o mesmo construto subjacente da ansiedade e exibem altas taxas de comorbidade entre si, com evidências que respondem similarmente ao tratamento, especialmente no que se refere ao TASe, TASo, TP e TAG (Pine, Cohen, Gurley, Brook, & Ma, 1998).

Ainda há poucos estudos dedicados a compreender as características dos indivíduos com estes transtornos em relação às funções neuropsicolinguísticas, comportamentais e suas bases neurais. Esta tese de Doutorado visa contribuir para o entendimento das bases neurais dos transtornos de ansiedade através de estudos de Neuropsicologia e de neuroimagem funcional.

Nesta introdução discute-se aspectos relevantes da Neuropsicologia e da neuroimagem funcional relacionados aos transtornos de ansiedade. Ela é dividida em duas seções: (1) Bases neurocognitivas dos transtornos de ansiedade (com ênfase nos achados de déficits em fluência verbal); (2) Bases neurais dos transtornos de ansiedade. A seção (2) subdivide-se em dois aspectos principais: (2.1.) A linguagem como uma forma de estudar correlatos emocionais em transtornos de ansiedade; (2.2.) A conectividade funcional intrínseca e os transtornos de ansiedade.

1.1. Bases neurocognitivas dos transtornos de ansiedade: o papel da fluência verbal

A maioria dos estudos sobre cognição dos transtornos de ansiedade investigou a relação de como as funções cognitivas são afetadas frente a ameaças (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Salum et al., 2013). No entanto, alguns estudos têm sugerido que algumas funções cognitivas podem estar comprometidas mesmo em situações onde não há um contexto emocional (Mogg, Garner, & Bradley, 2007; Salum et al., 2013; Toazza et al., 2014).

Um trabalho desenvolvido no nosso grupo mostrou que indivíduos com transtorno de ansiedade, clinicamente diagnosticados, oriundos de uma amostra comunitária de

escolas públicas (idade entre 12 – 17 anos; emparelhado para sexo), tiveram pior desempenho em uma tarefa tempo-dependente de fluência verbal fonêmica. Além disso, o desempenho nessa tarefa específica foi negativamente correlacionado com a gravidade dos transtornos de ansiedade e com a quantidade de diagnósticos de ansiedade presentes. Fomos capazes de demonstrar que não houve diferenças estatisticamente significativas para as demais funções neuropsicológicas avaliadas como: a atenção, percepção, memória, aritmética, linguagem e praxias (Toazza et al., 2014). Esse achado nos fez voltar o interesse para a relação entre fluência verbal e ansiedade.

O teste de fluência verbal oral foi desenvolvido pela primeira vez por Arthur Benton há mais de 40 anos para um teste de avaliação de linguagem em afásicos (alteração de linguagem causada por lesão cerebral) com o nome de *Association Word*. O protocolo mais usado normalmente é o teste que utiliza as letras F, A e S (FAS) dos autores Spreen and Strauss, criado em 1998, e está incluído em diferentes baterias de testagem neuropsicológica (Barry, Bates, & Labouvie, 2008a). A tarefa exige a eficiência para criar, planejar e executar uma sequência de ações não-automáticas, em um tempo limitado. O indivíduo é orientado a gerar palavras que comecem com uma letra específica (denominada fluência verbal fonêmica), num determinado período de tempo, como por exemplo: FAS e CFL.

A tarefa FAS, como outros paradigmas baseados em fluência, é considerada uma tarefa complexa, pois é fundamentada em vários processos das funções executivas. Dentre esses, estão incluídos um conjunto de habilidades como memória de trabalho, inibição e flexibilidade cognitiva. Neste sentido, podemos supor que os testes de fluência verbal envolvem: (1) manutenção e atualização constante da memória de trabalho, (2) escolha do estímulo certo ou regra para orientar a produção da tarefa, (3) inibição de respostas incorretas ou já evocadas, (4) flexibilidade cognitiva para gerar novas respostas

e não reprodução dos mesmos padrões de comportamento anterior, e (5) monitoramento contínuo da evocação das palavras (Barry, Bates, & Labouvie, 2008b).

Nesse sentido, pesquisadores dessa área observaram que as palavras evocadas nessa tarefa tendem a ser produzidas em grupos fonêmicos, ou seja, palavras foneticamente relacionadas. *Cluster* é a definição utilizada para denominar grupos de palavras geradas sucessivamente que ou começam com as mesmas duas primeiras letras (fábula e favela), ou diferem apenas em sons de vogais (faca e foca), ou produzem rima (fogão e feijão), ou eram homônimos (acento e assento). Já os *Switches* foram definidos como transições entre os clusters e/ou entre as palavras isoladas, sugerindo uma mudança de estratégia cognitiva (como por exemplo, **fogão, feijão**, *ferro, ferida, fedor*, figo, firma, filho – 3 *clusters* e duas mudanças de estratégia de evocação) (Dell, Schwartz, Martin, Saffran, & Gagnon, 1997; Troyer, Moscovitch, & Winocur, 1997). De acordo com alguns autores, os *switches* (ou comutação) refletem fortemente um componente executivo associado à função do lobo frontal, enquanto, os *clusters* ou agrupamentos, refletiriam funções de memória semântica que podem estar associada ao lobo temporal (Troyer et al., 1997).

Nosso estudo prévio mostrou uma forte associação com grande tamanho de efeito entre fluência verbal e transtorno de ansiedade, que no conhecimento dos autores, ainda não havia sido investigado em amostras infantis. Os resultados desse estudo mostraram que adolescentes com transtornos de ansiedade tiveram um pior desempenho em uma tarefa de fluência verbal, dependente de tempo, com critérios fonêmicos-ortográficos em comparação aos adolescentes sem diagnóstico de transtorno de ansiedade e aos adolescentes com transtorno externalizantes (transtorno opositor desafiante, transtorno de déficit de atenção e transtorno de conduta). Essa associação foi particularmente importante para o número de *switches*, o que reforçou um envolvimento dos aspectos

executivos nos transtornos de ansiedade. (Toazza et al., 2014). Como os estudos de neuroimagem que avaliaram pacientes com lesão cerebral, relacionaram a tarefa de fluência verbal diretamente com a função do córtex pré-frontal (Tupak et al., 2012), os achados deste nosso estudo sugeriram que as regiões pré-frontais estão envolvidas na fisiopatologia da ansiedade em jovens (Toazza et al., 2014).

1.2 Bases neurais dos transtornos de ansiedade

Assumindo o modelo neurocientífico de processamento de informação (Pine, 2007), os transtornos mentais são resultado de alterações no processamento de informações que são suportados por circuitos neurais específicos. Esses circuitos estão alterados como resultado de relações causais complexas que envolvem diversos fatores, incluindo tanto genes, quanto ambientes. Essas alterações em viés específicas de processamento de informações resultam nas disfunções que são entendidas como transtornos mentais. Um esquema deste modelo pode ser encontrado abaixo.

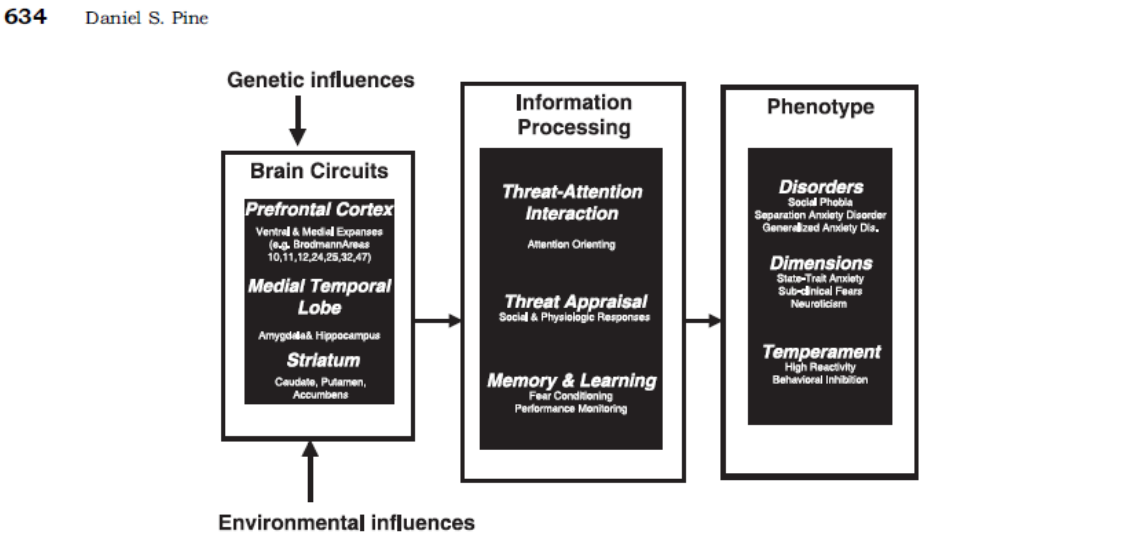

Figure 1 The current framework. This displays relationships among functional aspects of brain circuits, psychological processes, and clinical phenotypes

Parte dos substratos neurobiológicos conhecidos para os transtornos de ansiedade foram definidos através de pesquisas em modelos animais (Blackford & Pine, 2012). A literatura em animais sugere ainda que a amigdala é uma estrutura fundamental para a aquisição, expressão e consolidação do medo (Blackford & Pine, 2012). Um estudo de Timbie e Barbas, mostra que uma via robusta originada da amigdala está conectada com o tálamo e córtex orbitofrontal, formando uma rede estrutural tripartida, sugerindo que os transtornos de ansiedade podem estar associados com uma regulação deficiente da ativação do tálamo como um ponto específico nesta rede de ativação tripartida (Timbie & Barbas, 2015a).

A amigdala, é um grande complexo nuclear situado na porção dorsomedial do lobo temporal, ligada ao hipocampo. Ambos formam uma parte essencial do sistema límbico (Angel, 2012). As funções da amigdala estão principalmente relacionadas com as emoções e modulação da interpretação de estímulos, que também intervém na modulação do humor (Angel, 2012). A informação sensorial atinge a amigdala através do tálamo. O tálamo estimula a amigdala para ativar as respostas comportamentais e autonômicas via projeções para o tronco cerebral, hipotálamo, e outras estruturas límbicas (Agarwal et al., 2010). O estímulo sensorial é então regulado pelo córtex pré-frontal medial, córtex órbito-frontal e córtex cingulado anterior através de uma regulação *top-down* (de cima para baixo). Essa regulação permite modulação da resposta aos estímulos de acordo com a atividade dirigida a objetivo (Etkin & Wager, 2007).

Na figura abaixo, Figura 9 do artigo de Agarwal e colaboradores**,** uma ilustração das principais alterações no córtex pré-frontal, córtex cingulado anterior, caudado, ínsula, amigdala e hipocampo nos transtornos psiquiátricos e, em destaque, nos transtornos de ansiedade (Agarwal et al., 2010).

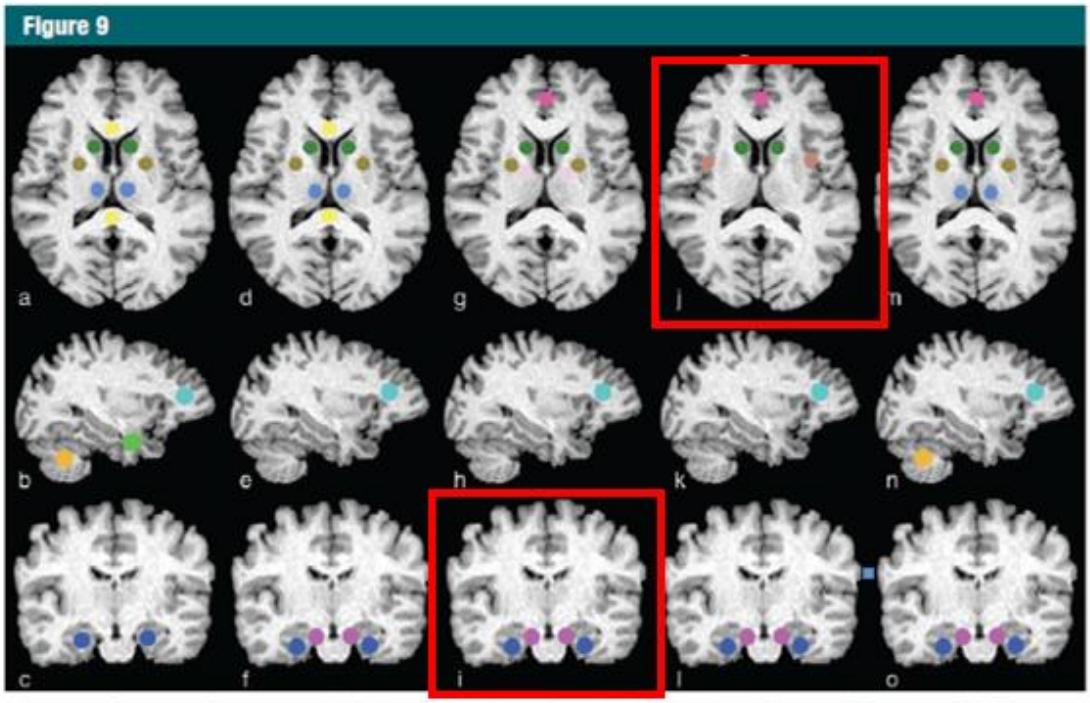

Figure 9: Summary of most reported findings on axial (top row), sagittal (middle row), and coronal (bottom row) MR image sections. ***(a–c)*** Findings of schizophrenia: The prefrontal cortex (turquoise), striatum (caudate: dark green, putamen: olive), thalamus (light blue), medial temporal lobe (light green), cerebellum (orange), and corpus callosum (yellow) are key areas of functional, structural, and neurochemical alterations. ***(d–f)*** Findings of bipolar disorder: The prefrontal cortex, anterior cingulate cortex (dark pink), striatum, corpus callosum, and limbic structures such as the hippocampus (dark blue) and amygdala (purple) are frequently reported as altered on MR images. ***(g–i)*** Findings of major depressive disorder: The prefrontal cortex, anterior cingulate cortex, striatum, basal ganglia (light pink), and limbic structures such as the amygdala and hippocampus are the most reported areas of abnormal structure, function, and neurochemical features. ***(j–l)*** Findings of anxiety disorders: The prefrontal cortex, anterior cingulate cortex, caudate, insula (dull orange in *j*), amygdale, and hippocampus are major sites of abnormality. ***(m–o)*** Findings of attention deficit–hyperactivity disorder: The prefrontal cortex, anterior cingulate cortex, cerebellum, striatum, amygdala, and hippocampus are the most reported areas of alteration.

Enquanto a atividade da amigdala está associada diretamente com o aumento dos sintomas de ansiedade, a atividade do córtex pré-frontal está associada à diminuição dos sintomas ansiosos, sugerindo que o córtex pré-frontal é a estrutura que controla a atividade da amigdala e age como um mecanismo regulatório essencial para os sintomas de ansiedade. Estudos mostraram que a ativação da amigdala e do córtex pré-frontal ventrolateral está aumentada em indivíduos com transtornos de ansiedade em relação a controles saudáveis (Pine, 2007).

Em resumo, a amigdala, tálamo e o córtex pré-frontal são componentes essenciais da neurocircuitaria do medo humano, estando interligados na modulação de respostas a situações ameaçadoras. Os resultados dos estudos sugerem que os transtornos de ansiedade podem ser caracterizados por disfunção cerebral, resultando em "muito gás e freios insuficientes" (*too much gas and not enough brakes*), ou seja, o sistema de produção do medo é muito forte e o sistema de regulação do medo é muito fraco (Blackford & Pine, 2012). Abaixo a ilustração mostrada no artigo citado, de Blackford e Pine, sobre a relação da amigdala e as diversas porções do córtex pré-frontal.

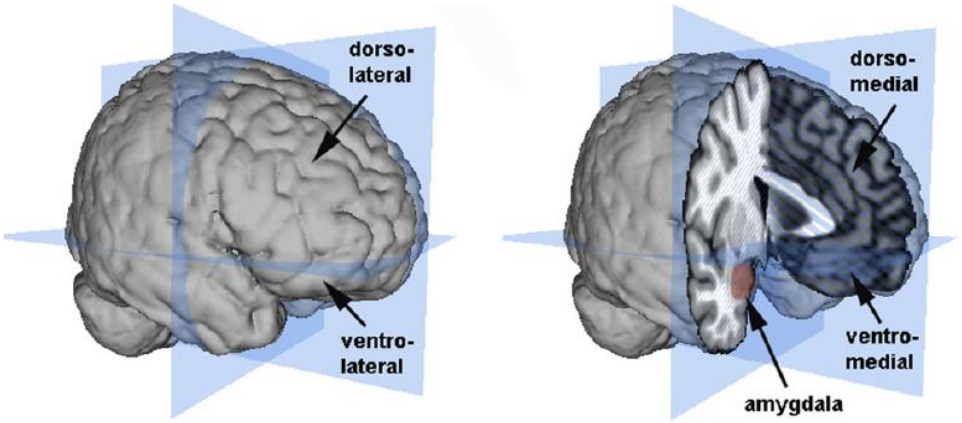

Fig. 1. Illustration of the amygdala and the major divisions of the PFC. The planes (*in blue*) show the major dorsal/ventral and anterior/posterior divisions of the brain. The lateral PFC is shown on the left and the medial PFC and amygdala are shown on the right. Brain images and surface constructions were created using Mango (Research Imaging Center, UTHSCSA; http://ric.uthscsa.edu/mango/mango.html) and a Montreal Neurological Institute standard brain.

Dentro os métodos disponíveis para testar aspectos teóricos e transpor achados empíricos de modelos animais para humanos podemos encontra a ressonância magnética funcional. Trata-se de uma técnica que mede alterações hemodinâmicas neurais. O aumento do fluxo sanguíneo causado pela atividade cognitiva leva a um excedente relativo de oxigênio no sangue local. O sinal medido em ressonância magnética funcional depende desta alteração na oxigenação e é referido como o nível dependente de oxigenação do sangue, ou o sinal *BOLD* (Poldrack, Nichols, & Mumford, [s.d.]). O

processamento de informações, num determinado circuito cerebral, traduz-se por uma intensificação da atividade neuronal, com consequente influxo de sangue oxigenado. O gradiente de oxihemoglobina/desoxihemoglobina das vênulas locais provoca uma distorção do campo magnético local (Rosen, Buckner, & Dale, 1998) que pode ser medido pelo aparelho.

O método para análise de imagens de ressonância magnética funcional mais comumente utilizado se vale de comparações estatísticas da média entre um grupo de pacientes com um determinado transtorno neuropsiquiátrico *versus* um grupo controle com voluntários sem transtorno (entre-sujeitos), ou ainda, avaliação estatísticas das mudanças de padrões nas imagens dos mesmos sujeitos estudados em diferentes condições ao longo do tempo (dentre-sujeitos). Através da computação das comparações estatísticas entre os grupos para cada *voxel*, pode-se produzir mapas tridimensionais que mostram a localização cerebral e a extensão dos agrupamentos de *voxels* que apresentam diferença estatística significativa entre os grupos no limiar significativo escolhido (Busatto, Almeida, Cerqueira, & Gorenstein, 2006).

Quanto ao tipo de paradigma, as análises funcionais podem ser divididas em: (1) ativação embasada em tarefas (onde se avalia a ativação cerebral frente a estímulo específicos); (2) ativação embasada no estado de repouso (*resting state funcional conectivity).* Uma das áreas promissoras para entendimento da ansiedade através de estudos de neuroimagem funcional é o estudo de narrativas emocionais. Deste modo utiliza-se a linguagem como um pano de fundo para entender o efeito da manipulação de aspectos emocionais em pacientes com e em transtorno de ansiedade.

1.2.1. A linguagem como uma forma de estudar correlatos emocionais em transtornos de ansiedade

Novas perspectivas sobre modelos de processamento e integração de texto no nível do discurso sugerem que há necessidade de combinações de informações linguísticas com outras não linguísticas, para permitir que o leitor incorpore informações pragmáticas e conecte o texto com seu conhecimento de mundo (Mason & Just, 2006). Pesquisas com neuroimagem sugerem que cinco redes paralelas estejam envolvidas no processamento da linguagem. Essas redes incluem: (1) Rede de processamento semântico (áreas temporais médio e superior direita); (2) Rede de monitoramento de coerência (pré-frontal dorsolateral bilateral); (3) Rede de integração de texto (frontal anteroinferior esquerda/ temporal esquerda); (4) Rede de imagens espaciais (sulco intraparietal bilateral, predominantemente esquerda) e, (5) Rede da Teoria da Mente para a compreensão de uma narrativa (Junção temporoparietal bilateral, predominantemente direita) (Mason & Just, 2009; 2006). Conforme figura esquemática abaixo:

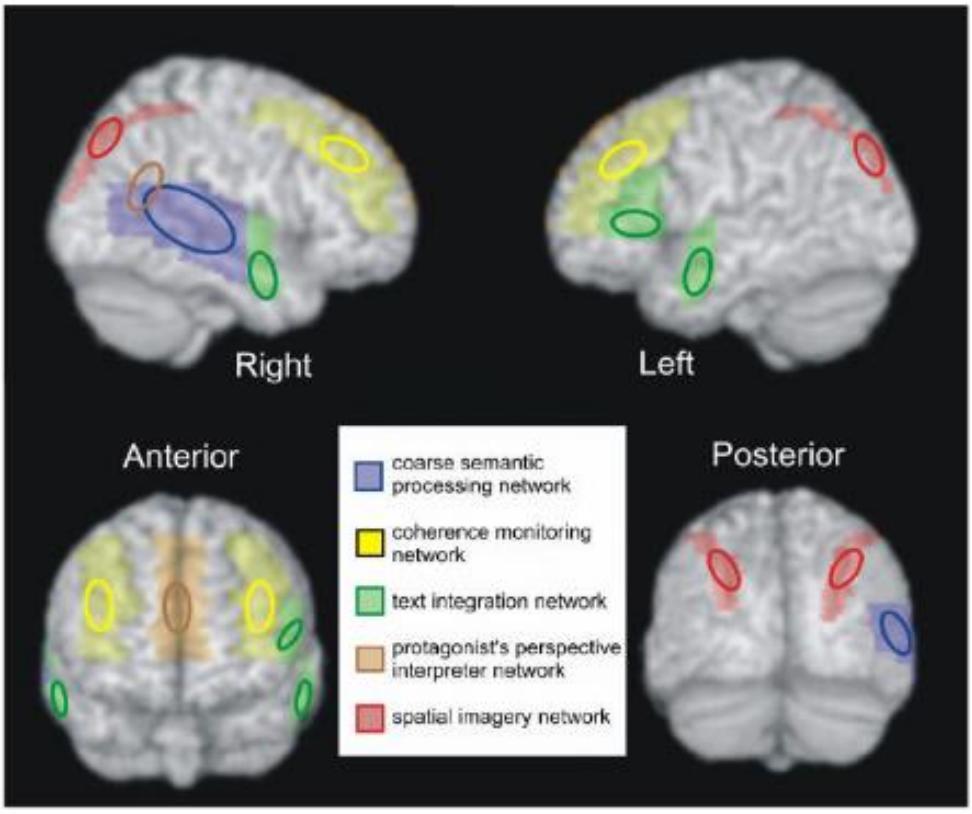

Figure 1. A Schematic representation of the Parallel Networks of Discourse. Shaded regions represent surface rendered anatomical regions as described in the text. A rough localization within anatomical regions are represented by colored ellipses.

O funcionamento adequado dessas redes é particularmente importante em nossa vida e interações sociais. Especialmente em um momento que, com o avanço da tecnologia, grande parte da nossa comunicação diária se faz pela escrita ou interpretação das emoções através de texto - seja por *e-mail*, no uso das redes sociais ou mensagens pelo celular. Dificuldades de linguagem são prevalentes em indivíduos com transtorno de ansiedade, especialmente em indivíduos com transtorno de ansiedade social. Tem sido sugerido que a falta de habilidades sociais de linguagem podem levar a reações negativas no meio de convívio, vieses de interpretação e comportamento de esquiva em situações sociais (Rapee & Spence, 2004).

A compreensão e expressão das emoções regula a interação social, fornecendo informações cruciais para a adaptação às exigências de um ambiente social determinado

(Mason & Just, 2011b). O discurso auxilia na inferência das mensagens emocionais expressas pelo interlocutor e uma ampla rede de estruturas corticais e subcorticais contribui para a decodificação dessas informações (Mason & Just, 2011a).

A compreensão do discurso inclui componentes como processamento de palavras e sentenças, mas também processamento cognitivo de níveis superiores, i.e., atenção, memória, geração de inferências, resolução de problemas, Teoria da Mente e interpretação social. Para se construir uma coerência entre as frases e compreender o discurso é necessário fazer inferências, ativar e integrar informações que não tenham ficado claramente expostas (Kuperberg, Lakshmanan, Caplan, & Holcomb, 2006).

As narrativas baseadas em estados mentais e motivação tem sido bastante utilizadas na literatura para avaliação do processamento emocional, pois exigem que o leitor compreenda os objetivos e as intenções do protagonista, entenda e aprecie a história e realize o processamento emocional, se colocando no lugar do protagonista (Kuperberg et al., 2006). Para tanto, as técnicas de neuroimagem são particularmente adequadas para estudar a participação dos diversos subconjuntos de processos cognitivos que constituem o processamento do discurso num determinado episódio que exija compreensão. Processamento do discurso é uma habilidade complexa que serve como um bom protótipo de "pensamento geral", pois é necessário que muitos níveis de processamento estejam organizados para processar o estímulo e fazer uma representação integrada do texto lido. Entende-se que compreender um texto curto não é muito diferente do que observar e compreender eventos diários no mundo, portanto, o processamento do discurso deve envolver um conjunto de redes corticais de uso geral (Mason & Just, 2011b).

Logo, estudos utilizando paradigmas relacionados ao processamento emocional podem ter grande potencial para desvendar os mecanismos cerebrais envolvidos em pacientes com transtornos neuropsiquiátricos. Por meio de dois exames de ressonância

magnética funcional independentes, pesquisadores investigaram a ativação cerebral em uma tarefa de palavras de cunho ameaçador e neutras. Os resultados mostraram aumento da ativação da amigdala, córtex pré-frontal medial e linguagem de processamento de áreas corticais com palavras de cunho ameaçador em comparação a palavras neutras, sugerindo que, as palavras emocionais podem ser capazes de desencadear aumento do processamento cortical e subcortical (Hoffmann, Mothes-Lasch, Miltner, & Straube, 2015).

1.2.2. A conectividade funcional intrínseca e os transtornos de ansiedade

Inicialmente, os estudos de ressonância magnética funcional eram unicamente baseados em tarefas, utilizando paradigmas de ativação como os descritos acima. Nos últimos anos têm havido um interesse crescente em aplicar técnicas de ressonância magnética funcional em condições de repouso (Biswal, 2012; Joo, Lim, & Lee, 2016). Os estudos de condição de repouso (*resting state functional connectivity)* surgiram com a observação de pesquisadores de que algumas áreas do cérebro aumentavam a ativação durante uma tarefa, enquanto que outras aumentavam a atividades após a finalização de uma tarefa. Esses achados levam pesquisadores a sugerir que algo acontece durante o período de "repouso", e portanto, convencionou-se chamar de conectividade intrínseca do cérebro. Esta observação levou a investigação desses períodos de repouso ao longo de diversos minutos e foi observado que existia padrões nas correlações de ativação do sinal *BOLD* entre as regiões cerebrais. O estudo desses padrões de correlação deu suporte a toda uma linha de pesquisa de conectividade intrínseca, hoje bastante difundida no meio acadêmico.

Nesses estudos existem três principais técnicas de análise comumente utilizadas para avaliar a conectividade funcional intrínseca (Joo et al., 2016): (1) *Seed-based, (2)* análise de componentes independentes (ACP) e (3) teoria dos grafos. Na análise baseada em *seed*, o pesquisador seleciona uma região de interesse (*ROI*) e extrai a série temporal de ativação nessa região, que é, então, correlacionado com as séries temporais dos *voxels* do cérebro, procurando regiões que sejam correlacionadas umas com as outras (Fox & Raichle, 2007). Na ACP, não é necessário selecionar uma região de referência, todo o conjunto de dados pode ser decomposto em cursos de tempo e mapas espaciais. A ACP procura componentes de ativação que são estatisticamente independentes umas das outras (Beckmann, DeLuca, Devlin, & Smith, 2005). Na teoria dos grafos, estuda-se a relação entre diversos parcelamentos do cérebro (nós), definindo as regiões mais ou menos centrais ou importantes para a rede de conexões (Stam et al., 2009).

As redes mais comumente observadas e descritas na literatura são as descritas por Sylvester e colaboradores (Sylvester et al., 2012), são elas: (1) *Rede cíngulo-opercular*: inclui cingulado, insula, córtex pré-frontal e tálamo. Esta rede está envolvida no controle cognitivo, processamento de afeto negativo e dor (Liao et al., 2010; Shackman et al., 2011). (2) *Rede fronto-parietal*: inclui porções bilaterais anteriores do córtex pré-frontal dorsolateral, lóbulo parietal inferior, porções do giro cingulado medial e porções do precuneo. É referida como papel importante no controle executivo, monitoramento para detectar desvios de conduta, assinalando assim a eventual necessidade de ajuste de estratégia (Seeley et al., 2007). (3) *Rede de modo padrão*: incluem cingulado, precuneo, córtex parietolateral, córtex pré-frontal medial, giro temporal inferior, giro parahipocampal e córtex frontal superior. Envolvida nas funções introspectivas, planejamento futuro, auto-monitoramento e regulação emocional (Raichle et al., 2001). (4) *Rede de atenção ventral*: inclui o córtex pré-frontal ventrolateral, junção temporo-

parietal e porções dos giros temporais superiores. Esta rede está envolvida no processamento de atenção automática a estímulos, sugerindo envolvimento em funções como cognição social (Corbetta, Patel, & Shulman, 2008).

Além dessas redes anteriormente descritas, é muito comum na literatura de ansiedade investigar a conectividade intrínseca da amigdala, dada a importância que essa região tem para o medo e ansiedade. Na última década, surgiram estudos mostrando conectividade funcional intrínseca aberrante na amigdala, em adultos (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Geiger et al., 2015; Roy et al., 2009; Yoon, Han, Yoon, Kim, & Yi, 2015) e adolescentes ansiosos (Hamm et al., 2014; He, Xu, Zhang, & Zuo, 2016; Liu et al., 2015; Roy et al., 2009; Sylvester et al., 2012) conforme descrito na tabela abaixo:

CRIANÇAS E ADOLESCENTES				
AUTOR E ANO	**N/IDADE**	**TIPO DE ANÁLISE**	**DIAGNÓSTICO**	**RESULTADOS**
Roy et al., 2013	35 adolescentes (12–17)	*seed-based*	TAG	Disfunção na conectividade da amigdala com córtex pré-frontal medial, ínsula e cerebelo. Correlação positiva entre severidade da ansiedade entre amigdala, insula e giro temporal superior.
Sylvester et al., 2013	80 crianças (3 - 6)	*seed-based*	TA: TAG, TASe, FS, FE e agorafobia.	Redução da conectividade da rede de atenção ventral (córtex pré-frontal ventrolateral, junção temporoparietal e porções dos giros temporais superiores).
Hamm et al, 2014	33 crianças (13 - 16)	*seed-based*	TAG, ASo, FS	Hiperconectividade entre amigdala direita e ínsula; Hiperconectividade entre amigdala esquerda e córtex pré-frontal ventromedial e cingulado posterior.
Liu et al., 2015	26 adolescentes (12–17)	*seed-based*	TAG	Hipoconectividade da amygdala com dorsolateral córtex pré-frontal; Amigdala contralateral estendendo para o hipocampo; Hiperconectividade com ínsula, cerebelo, estriado, giro temporal superior; Amigdala ipsilateral estendendo para o parahipocampo.
ADULTOS				
AUTOR E ANO	**IDADE**		**DIAGNÓSTICO**	**RESULTADOS**

Etkin et al., 2009	64 adultos (32.5 ± 2.0)	*seed-based*	TAG	Hiperconectividade entre amigdala basolateral e córtex frontoparietal, córtex pré-frontal medial, ínsula e cingulado.
Kim et al., 2010	84 adultos (19.6 ±0.9)	*seed-based*	TAG e DM	Baixa ansiedade: Hipoconectividade entre amigdala ventral e córtex pré-frontal medial; Alta ansiedade: Hiperconectividade entre amigdala ventral e córtex pré-frontal medial.
Pannekoek et al., 2015	140 adultos	*seed-based*	TA, depressão maior (DM)	TA e DM: hipoconectividade entre precuneo bilateral, córtex intracalcarino, giro lingual, posterior cingulado, giro precentral direito, giro frontal inferior e giro frontal medial.
Geiger et al., 2016	33 adultos	ACP	TASo	Hipoconectividade no giro orbitofrontal; Hiperconectividade no giro frontal medial; Hiperconectividade entre giro orbitofrontal esquerdo e amigdala esquerda.
He et al., 2016	280 adultos (18–83.5)	*seed-based*	Estado ou traço de ansiedade	Hiperconectividade entre amigdala amigdala esquerda e córtex sensoriomotor, rede de atenção dorsal; Hiperconectividade entre amigdala direita, córtex frontoparietal e rede de atenção ventral.

Conforme vimos acima, vários estudos prévios foram capazes de mostrar, através de ressonância magnética funcional, importantes informações sobre a conectividade intrínseca nos transtornos de ansiedade envolvendo várias áreas de conectividade aberrante entre a amígdala e diversas regiões do córtex. Entretanto, os estudos em adolescentes e jovens adultos ainda são escassos e contraditórios. Uma das possíveis razões para a inconsistência dos achados é o fato de que a maioria dos estudos não investiga de foram separada as sub-regiões da amígdala. No entanto, é sabido que diferentes núcleos da amígdala apresentam conectividades funcionais diferentes (Roy et al 2013). Portanto, o estudo pormenorizado da conectividade das sub-regiões da amígdala ainda guarda um potencial inexplorado para o entendimento dos transtornos de ansiedade em crianças.

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2. OBJETIVOS

3.1 Objetivo Geral

3.1.1 ARTIGO 1: Investigar se a gravidade dos transtornos de ansiedade está relacionada com desempenho em uma tarefa de fluência verbal fonêmica em crianças com diagnóstico de transtornos de ansiedade.

3.1.2 ARTIGO 2: Investigar diferenças no processamento emocional entre adolescentes e adultos jovens com e sem transtornos de ansiedade.

3.1.3 ARTIGO 3: Investigar diferenças na conectividade de diferentes sub-regiões da amigdala com todo o entre adolescentes e adultos jovens com e sem transtorno de ansiedade.

3.2 Objetivos Específicos

3.2.1 ARTIGO 1:

1. Avaliar a correlação entre desempenho numa tarefa de fluência verbal fonêmica e sintomas de ansiedade em diferentes informantes (crianças, pais e avaliação clínica);

2. Investigar se as associações entre fluência verbal fonêmica e sintomas de ansiedade são independentes de sintomas comórbidos de desatenção e hiperatividade;
3. Investigar se essas associações podem estar relacionadas de forma específica a formação de *clusters* ou de *switches.*

3.2.2 ARTIGO 2:

1. **Processamento emocional *versus* linguístico:** Avaliar se há diferenças na ativação cerebral entre narrativas de conteúdos emocionais de alegria, raiva, tristeza (processamento linguístico e emocional) em comparação com narrativas de conteúdo neutro (processamento linguístico) em toda a amostra;
2. **Análise de grupo**: Avaliar se há diferenças na ativação cerebral de processamento de narrativas (independente do conteúdo emocional) entre os sujeitos com transtorno de ansiedade em comparação com sujeitos sem transtorno de ansiedade;
3. **Interações**: Avaliar interações entre processamento emocional e diferença entre os grupos de indivíduos com e sem transtorno de ansiedade.

3.2.3 ARTIGO 3:

1. Investigar diferenças na conectividade intrínseca de subdivisões da amigdala com todo o cérebro entre indivíduos com e sem transtorno de ansiedade.

4. HIPÓTESES

4.1 ARTIGO 1: Nossa hipótese é que os déficits de fluência verbal estariam consistentemente associados com a gravidade dos sintomas de ansiedade em crianças pequenas, independentemente do informante e dos sintomas de Transtorno de Déficit de Atenção e Hiperatividade (TDAH).

4.2 ARTIGO 2: Neste artigo temos duas hipóteses, além de um objetivo exploratório:
(1) **Processamento emocional *versus* linguístico:** Não há hipótese específica para esse objetivo (análise exploratória);
(2) **Análise de grupo**: todos os participantes apresentariam uma ativação diferenciada no tálamo, amigdala, córtex pré-frontal dorsomedial, pólo temporal, córtex cingulado posterior e junção têmporo-parietal no processamento de narrativas emocionais se comparadas a narrativas neutras.
(3) **Interações**: pacientes com transtornos de ansiedade apresentaria uma rede de ativação diferenciada no tálamo, amigdala e córtex pré-frontal ventromedial para narrativas com conteúdo emocional negativo, quando comparadas com emoções neutras;

4.3 ARTIGO 3: Nossa hipótese é que indivíduos com transtorno de ansiedade apresentariam conectividade funcional intrínseca aberrante entre subdivisões da amigdala com regiões do córtex pré-frontal medial, ínsula, cerebelo e giro temporal superior.

5. ARTIGO 1

Verbal fluency and severity of anxiety disorders in young children

ACEITO na revista *Trends in Psychiatry and Psychotherapy*

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in Psychiatry and Psychotherapy

onbehalfof+mksantanna+gmail.com@manuscriptcentral.com Hoje em 1:55 PM

Para rudineiatoazza@yahoo.com.br

08-Mar-2016

Dear Mrs. Toazza:

It is a pleasure to accept your manuscript entitled "Phonemic verbal fluency and severity of anxiety disorders in young children" in its current form for publication in the Trends in Psychiatry and Psychotherapy.

Thank you for your fine contribution. On behalf of the Editors of the Trends in Psychiatry and Psychotherapy, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Marcia Kauer-Sant'Anna
Editor-in-Chief, Trends in Psychiatry and Psychotherapy
mksantanna@gmail.com

Brief communication

Phonemic verbal fluency and severity of anxiety disorders in young children

Short title: Verbal fluency and anxiety severity

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INTEGRITY OF RESEARCH AND REPORTING

Ethical standards

This study was approved by the Ethics Committee of the Hospital de Clínicas - Porto Alegre (number 11-0249) and all parents signed an informed consent.

Contributors

All authors had full access to all data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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ABSTRACT

Introduction: Previous studies have implicated impaired verbal fluency to be associated with anxiety disorders in adolescents. Our objective is to replicate and extend previous evidence by investigating if the performance in phonemic verbal fluency tasks is related to the severity of anxiety symptoms in young children with anxiety disorders. We also aim to investigate whether putative associations are independent from co-occurring Attention Deficit/Hyperactivity Disorder (ADHD) symptoms. **Methods:** Sixty children (6-12 years) with a primary diagnosis of an anxiety disorder participated in this study. Severity of symptoms was measured using the clinician-based, parent-rated and self-rated validated scales. Verbal fluency was assessed using a simple task that measures the number of words evoked in 1-minute with the letter F, from which we quantified the number of isolated words, number of clusters (groups of similar words) and number of switches (transitions between clusters and/or between isolated words). **Results:** There was a significant association between the number of clusters and anxiety scores. Further analysis revealed associations were independent from co-occurring ADHD symptoms. **Conclusion:** We replicate and extend previous findings showing that verbal fluency is consistently associated with severity in anxiety disorders in children. Further studies should explore the potential of cognitive training on symptoms from anxiety disorders.

Keywords: anxiety disorders; executive function; verbal fluency; language

INTRODUCTION

Anxiety disorders are common, significantly impairing, and typically emerge during childhood and adolescence (1,2). The study of disrupted information processing in cognitive functions associated with anxiety has the potential to further elucidate the pathophysiology of anxiety disorders. While many processes have been implicated in both adult and pediatric anxiety (1,3), recently deficits in specific aspects of executive functions have been received particular attention (4,5).

A study by our group has recently reported that children and adolescents from 10 to 17 years of age with anxiety disorders had poorer performance in a task of phonemic verbal fluency than a comparison group of typically developing children. This result seems to reflect specific deficits in executive function, therefore, involvement of the prefrontal cortex in anxious children (5). However, this result has not been replicated in the literature so far and there is no specific study with young children. In addition, given the well-known associations between Attention Deficit/Hyperactivity Disorder (ADHD) and executive functions, including lower performance in verbal fluency tasks(6,7), as well as the observation that ADHD symptoms frequently co-occur in children with anxiety disorders (1), it is important to investigate whether associations between verbal fluency and severity of anxiety disorders is not due to co-occurring ADHD symptoms.

Here we investigated if the performance in phonemic verbal fluency tests is related to the severity of anxiety symptoms in young children with anxiety disorders. We also investigate whether putative associations are independent from co-occurring ADHD symptoms. We hypothesize that verbal fluency deficits will be associated with anxiety severity in young children independently from ADHD symptoms.

METHODS

Participants

This study was approved by the Ethics Committee of the Hospital de Clínicas - Porto Alegre (number 12-0254) and all parents signed an informed consent. Sixty children (6 - 12 years) with a primary diagnosis of an anxiety disorder participated in this study. We included participants with the following characteristics: (a) Primary diagnosis of Generalized Anxiety Disorder (GAD), Separation Anxiety Disorder (SeAD) or Social Anxiety Disorder (SoAD) according to standard diagnostic procedures described below. Exclusion criteria were: (a) other psychiatric disorder judged by the clinician to cause more impairment or distress than GAD, SeAD or SoAD; (b) patients with previous psychiatric treatment including lifetime history of any type of psychotherapy or psychiatric medication; (c) intellectual disability defined as a score bellow the 5^{th} percentile on Raven's Progressive Matrices assessment (8,9). Participants were recruited by media advertisements and were prescreened with a brief telephone interview. Potentially eligible children and their parents were invited for a full diagnostic interview with a team of trained clinicians.

Diagnosis

All children underwent a comprehensive psychiatric diagnostic evaluation with the Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime (K-SADS-PL)(10). The K-SADS-PL is a semi-structured interview used for the diagnosis of childhood psychiatric disorders based on the DSM-IV(11) criteria. The K-SADS-PL has been adapted to Brazil and presented good psychometric properties(12). Interviews were performed by doctoral or master degree level clinicians and weekly supervised by a senior clinician.

Measures of psychopathology severity

Pediatric Anxiety Rating Scale (PARS): This is a clinician-rated measure of anxiety. The scale rates anxiety severity, frequency, distress, avoidance, and interference, in accordance to standardized methods(13) (PARS, 2002). We used the scores on the 50 rated symptoms evaluated as present/absent varying from 0 to 50.

Screen for Child Anxiety Related Emotional Disorders (SCARED): The SCARED(14,15) is a 41-item measure of pediatric anxiety investigating symptoms of generalized anxiety (9 items), separation anxiety (8 items), social anxiety disorder (7 items), panic/somatic (13 items), and school phobia (4 items). Items are rated on a 3-point scale, and total scores range from 0 to 82 with higher scores reflecting higher anxiety levels. There is a self-report version and a parent-report equivalent version of the SCARED. The SCARED has been translated to Brazilian-Portuguese and this version presented good psychometric properties(16,17). The SCARED total score, rather than subscale scores, was used as the dependent measure as suggested by the literature (18).

Swanson, Nolan, and Pelham Questionnaire (SNAP-IV): The SNAP-IV has 18 items about ADHD symptoms and 8 additional items about oppositional-defiant symptoms. The questionnaire is a parent rated 4-point scale, and total scores range from 0 to 78. The SNAP-IV was successfully adapted to Brazilian Portuguese and functioned well (19).

Verbal Fluency

The verbal fluency was assessed using the number of words evoked in 1 minute with the letter F, a task which is part of the Brazilian Brief Neuropsychological Assessment Battery – NEUPSILIN (child version) (20). The following variables were evaluated: (1) number of valid words (total words minus repetition and rule violation); (2) number of clusters (groups of successively generated words that began with the same first two letters (e.g., fire and financial), differed only in single vowel sounds (e.g., sit and set), produced rhyme (e.g., strip and ship), or were homonyms – starting and ending with the same sounds (e.g., see and sea)); (3) number of switches (transitions between clusters and/or between isolated words – which do not form clusters); (4) Isolated words (words that did not form clusters) (21,22).

This analysis provides, beyond the overall score, an evaluation of verbal fluency task, making it possible to investigate the cognitive processes underlying this neuropsychological function (21). The clusters provide information about categorization and switches evaluate cognitive flexibility (22).

Statistical analysis

First, we used multivariate general linear models (MGLM) with isolated words, clusters and switches as dependent variables (which indicate the overall valid performance on verbal fluency) and each anxiety symptomatic score (clinical, child and parent) separately as independent variables. Post-hoc univariate analyses were performed to identify which aspect of the verbal fluency task (isolated words, clusters or switches) was associated with anxiety symptomatic scales. Second, we repeated the models co-varying for SNAP-IV scores in multiple linear regressions. A significance level of 5% was considered.

RESULT

The mean age of the sample was 9.3 years (SD=1.6) and with 51.7% of were female. Mean scores for each symptomatic scale were as follows: PARS (mean=20.1, SD=7.06), SCARED-Parent (mean =38.7, SD=12.11), SCARED-Child (mean =32.8, SD=13.2), and SNAP-IV (mean =27.6, SD=15.88). Main diagnoses were: Separation Anxiety Disorder (n=48, 80%), Generalized Anxiety Disorder (n=44, 73.3%), Social Anxiety Disorder (n=16, 26.7%), Panic (n=6, 10%) and Agoraphobia (n=12, 20%). Comorbidities include: Attention Deficit/Hyperactivity Disorder (n=13; 21.7%), Major Depression (n=8, 13.3%), Enuresis (n=5, 8.3%) and Oppositional Defiant Disorder, ODD (n=7, 11.7%).

Descriptive statistics for verbal fluency analysis were as follows: total number of words (mean=7.2, SD=3.66, min=0, max 21), valid words (mean=6.8, SD=3.6, min=0, max=21), isolated words (mean=2.9, SD=2.14, min=0, max=10), number of switches (mean=3.8, SD=2.52, min=0, max=13), number of clusters (mean=1.72, SD=1.15, min=0, max=4) and mean cluster size (mean=2.2, SD=1.15, min=0, max=5).

Associations with anxiety scores

In the multivariate model there were significant associations between overall verbal fluency performance and anxiety severity for clinician-rated measures, PARS (Pillai's Trace=0.152, F(3,55)=3.28, p=0.028), parent-rated measures, SCARED-P (Pillai's Trace=0.141, F(3,54)=2.94, p=0.041) and trend-level significance for child-rated measures, SCARED-C (Pillai's Trace=0.108, F(3,52)=2.09, p=0.112). Post-hoc analysis for each of the measures revealed there were significant associations between the number of clusters for PARS (F(1,57)=6.73, p=0.012, η_p^2=0.106), SCARED-P (F(1,56)=8.52, p=0.005, η_p^2=0.132) and SCARED-C (F(1,54)=6.10, p=0.017, η_p^2=0.102); but no

significant associations emerged for isolated words and number of switches (all *F*s >1.8, all p-values >0.05). A correlation matrix for all measures is depicted in Table 1. The table shows moderate to small correlations effect size (*r*s ~0.3) between number of clusters and anxiety severity.

Analysis co-varying for ADHD symptoms

Multiple regression models for number of clusters co-varying for ADHD symptoms revealed that associations remained significant for PARS (β=-0.291, t=2.22, p=0.030), SCARED-P (β=-0.324, t=2.43, p=0.018), and SCARED-C (β=-0.290, t=2.15, p=0.036). In contrast, no associations between ADHD symptoms and number of clusters emerged (all p-values>0.05); but ADHD symptoms were significantly associated with the number of isolated words and number of switches (see Table 1).

DISCUSSION

Our results showed that severity of anxiety symptoms as assessed by clinicians, children and parents were negatively correlated with the number of clusters in a phonemic verbal fluency task and that these associations cannot be explained by co-occurring ADHD symptoms. These results replicate and extent our previous findings showing verbal fluency to be implicated in anxiety disorders even earlier in life.

In accordance with previous evidence (5) and other studies with adults (23,24), anxiety was significantly associated with verbal fluency performance. Our current analysis revealed that for young children this association was explained by a lower number of clusters. This contrasts with our previous study that reported the presence of lower number of switches in anxious as compared to typically developing adolescents.

Clustering relates more strongly to semantic memory, especially lexical storage of words; whereas switching relates more strongly to executive functions, search processes and cognitive flexibility (21,22). One possibility for anxiety to be associated with lower switching in adolescents and young adults and with clustering in children might be a result of an interaction between anxiety severity and developmental period. In our sample whereas switching significantly increases with age ($r=0.316$, $p=0.019$), no associations between clustering and age were noted ($r=-0.006$, $p=0.970$). Therefore, we hypothesize that whereas verbal fluency deficits are evident on clustering strategies in children (the strategy that prevails in children), anxiety is more strongly related to switching in adolescents (the strategy that prevails in adolescents and young adults). Another possibility is that whereas in young children deficits in verbal fluency might affect more general processes marked by clusters (25); in adolescents it might affect more specialized processes marked by switches (5,26) . Lastly, the effects of time pressure, which might be specifically salient to anxiety disorder patients, might have different effects on verbal fluency components according to the developmental period.

Moreover, interestingly, we found that ADHD symptoms were independently related to switches, but not clusters. Previous research has found ADHD in adults to be related to fewer switching (6,7), but other failed to find those association with switching in children. Nevertheless, these differential findings on clusters involving anxiety disorders and switching involving ADHD provide an interesting avenue for further research aiming to differentiate anxiety from ADHD in young children.

We conclude verbal fluency is consistently associated with the severity of anxiety symptoms, as replicated by our current findings. Our data reinforce the importance of verbal fluency as a marker of severity for anxiety and encourage the development of further studies aiming to investigate neurobiological measures and therapeutic

implications of our current findings. Interventions targeting mechanisms that support verbal fluency task (executive function, memory, language) in early ages may be the way to minimize future problems related to anxiety disorders.

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Table 1 - Univariate correlations between symptom scales and verbal fluency

	Symptom scales			Verbal fluency			
	SCARED (parent)	**SCARED (child)**	**SNAP (parent)**	**Total**	**Isolated words**	**Clusters**	**Switches**
Symptom scale							
PARS (clinician)	**0.630*** **	**0.436*** **	0.247	-0.124	0.107	**-0.325***	-0.085
SCARED (parent)	—	**0.593*** **	**0.345** **	-0.207	-0.005	**-0.363** **	-0.180
SCARED (child)		—	**0.276***	-0.233	-0.027	**-0.319***	-0.151
SNAP-IV (parent)			—	**-0.390** **	**-0.415*** **	-0.227	**-0.468*** **
Verbal fluency							
Total valid (n)				—	**0.580*** **	**0.741*** **	**0.822*** **
Isolated words (n)					—	0.050	**0.878*** **
Clusters (n)						—	**0.493*** **
Switches (n)							—

Note: PARS (Pediatric Anxiety Rating Scale); SCARED (Screen for Child Anxiety Related Emotional Disorders); SNAP-IV (Swanson, Nolan, and Pelham Questionnaire)
* p<0.05; **p<0.01; ***p<0.001

6. ARTIGO 2

Anxiety-related down-regulation of thalamic regions in the processing of sad and angry emotional narratives

Anxiety-related down-regulation of thalamic regions in the processing of sad and angry emotional narratives

Running title: Thalamic regions in the processing of emotions

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Keywords: anxiety disorders; language; emotional processing; fMRI

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ABSTRACT

Introduction: The objective of this functional magnetic resonance imaging (fMRI) study was to investigate how adolescents and young adults with anxiety disorders, relative to healthy comparison, processed narratives with emotional versus neutral content. **Methods**: Twenty-eight adolescents and young adults (14-22 years, mean 17,29), 50% female, public school, 14 with Anxiety Disorders (Generalized Anxiety, Social Anxiety and Panic Disorder) and 14 comparison subjects completed the fMRI study. Psychiatric diagnosis was assessed using standardized structured interviews (K-SADS-PL and symptomatic scales) performed by trained clinicians. The task consisted of 48 narratives divided into four categories according to the emotion involved in the outcome of the narrative: angry, happy, sad, or neutral. Mixed analyses of variance tested the effects of Group (anxiety, comparison), Emotion (angry, happy, sad, neutral) and Group by Emotion interaction on whole brain BOLD signal. **Results:** A significant Group by Emotion interaction was detected in a thalamic cluster ($F(3,78)=9.083$, $p<0.001$, $\eta p2=0.435$). This interaction was due to sad *vs.* neutral and angry *vs.* neutral contrasts, for which participants with anxiety disorders showed significantly lower thalamic activation relative to comparison subjects. In addition, Emotion showed a main effect in ten brain clusters, including language-related areas and attention-related anterior and posterior areas of the brain. Overall this effect reflected greater activation to emotional vs. neutral narratives. No main effect of Group was detected. **Conclusions:** Participants with anxiety disorders exhibited a weaker modulation of thalamic activation to negative vs. neutral narratives compared to healthy comparisons. These results suggest the implication of abnormal thalamic processing of negative information in the pathophysiology of anxiety disorders in adolescents and young adults.

INTRODUCTION

"Junior played with his dog, Toto, every evening. One day Junior came back from school and Toto was not waiting for him at the gate. Junior cried a lot when he learned Toto had been run over by a car." Modern life introduced 'texting', by the use of cell phones and social media, as one of the main instruments of communication among adolescents and young adults. By such means, several emotions we experience daily are transmitted using narratives such as the one described by Junior and his dog Toto. The present study builds on this type of narrative-based socio-emotional processing to investigate individual differences in brain processing of emotional material, both in adolescents and young adults with a typical development and those with anxiety disorders.

Previous studies of children and adolescents with anxiety disorders have focused on brain response to emotional stimuli, such as emotional faces or words (for a review, see [1]). These studies have found evidence of an association between anxiety disorders and increased activation of a limbic-prefrontal network involving the amygdala and various portions of the prefrontal cortex (PFC)[1]. In addition to the involvement of amygdala-PFC circuits in anxiety disorders, the paraventricular nucleus of the thalamus has been recently implicated as central structure to information processing related to fear and anxiety in animal research [2, 3, 4, 5, 6]. Recent work propose the thalamus, amygdala and PFC form a tripartite network, which is thought to mediate stress-induced changes in mood and behavior [2].

Very little is known on the effects of emotional content in narrative processing even in typically developing adolescents. The comprehension of narratives involves higher levels of cognitive processes involved in narrative comprehension [7, 8]. Narrative comprehension may stimulate readers' thoughts about protagonist cognitive and affective

states. Recent fMRI studies, have investigated the neural pathways of emotional responses to narratives in healthy individuals. These studies suggest emotional material is associated with higher activations in the thalamus, amygdala, dorsomedial prefrontal cortex, temporal pole, posterior cingulate cortex and temporoparietal junction [9, 10, 11].

Lastly, to our knowledge no previous study has focused specifically in language processing in subjects with anxiety disorders. Given that this study builds on this specific processing to investigate individual differences in the processing of emotions, it is important to investigate whether there are group differences with respect to processing of narratives in the absence of any emotional content. Impairments in language comprehension and pragmatics often coexist with disordered emotional processes. Also, language comprehension can be an important predictor of disorders in the presence of manifestations of socio-emotional alterations [12, 7].

The current study compared the neural correlates of emotional processing during an event-related fMRI study of narratives in adolescents and young adults with anxiety disorders and participants without anxiety disorders. Narratives told stories with angry, sad and happy outcomes, and stories with neutral outcomes (in terms of the emotions of the protagonist). Two hypotheses were tested: *First*, based on the vast literature with faces and threat words[1] and new evidence from animal research [3, 4, 5, 6], we predicted that thalamic, amygdala and ventromedial PFC activation to negative vs. neutral narratives would be different in patients with anxiety disorders than comparison subjects. *Second*, we predicted that emotional material will be associated with differential activations in the thalamus, amygdala, dorsomedial prefrontal cortex, temporal pole, posterior cingulate cortex and temporoparietal junction [9, 10, 11]. In addition, we aim to investigate language processing in subjects with anxiety disorders (as a *third* objective), but no

specific predictions are proposed with respect to the direction of the results given the absence of previous literature on this issue. Therefore this objective is exploratory.

METHODS

Participants

This study is part of a larger study focusing on the multidimensional evaluation and treatment of anxiety in children and adolescents followed for the past five years [13]. Details of the sampling procedures can be found elsewhere [13]. From the full cohort, we recruited 76 adolescents and young adults for assessment. A total of 32 were excluded due to pregnancy, recent tattoo, use of dental appliances, and claustrophobia and 44 participated in the fMRI acquisition. From those, 11 participants were excluded due to excess head movement during the fMRI acquisition (higher than 0.5 mm in any direction) [14] and 5 were excluded due to use of psychiatric medication or substance abuse/dependence. Parents or guardians from adolescents provided written informed consent for participating in the study; the adolescents provided written assent. Adults provided written informed consent for participating in the study (Table 1). The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (HCPA) (GPPG/HCPA, project number 12-0254) and the Research Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (CEP-PUCRS).

Measures

Psychiatric diagnosis

Psychiatric diagnosis was ascertained using two instruments: the Schedule for Affective Disorder and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL, for adolescents) [15] and the MINI (Mini-International Neuropsychiatric Interview) [16] for adolescents younger than 18 years of age and young adults (18 to 22 years of age), respectively.

The K-SADS-PL is a semi-structured interview used for the diagnosis of childhood psychiatric disorders based on the DSM-IV criteria. The K-SADS-PL has been adapted for use in Brazil and show good psychometric properties [17]. Severity of primary diagnoses were based on the Clinical Global Impression – Severity scale (CGI-S) that was rated independently for each psychiatric disorder to establish primary psychiatric diagnosis. The MINI (Mini-International Neuropsychiatric Interview) is a structured diagnostic interview, developed jointly by psychiatrists and clinicians, for DSM-IV and CID-10 psychiatric disorders. The MINI has been validated for Brazilian-Portuguese language [18], with good psychometric properties [19, 20]. Only non-medicated children were included in the study.

Symptom scales

SCARED

The Screen for Child Anxiety Related Emotional Disorders (SCARED) [21, 22] is a 41-item self-report measure of pediatric anxiety. It has been translated to Brazilian-Portuguese and the Brazilian version has good psychometric properties [23, 24]. Items are rated in a 3-point scale, and total scores range from 0 to 82 with higher scores reflecting higher anxiety levels.

CDI

The Children's Depression Inventory (CDI) is a 27-item self-report measure of pediatric depressive symptoms [25]. This scale has been translated to Brazilian-Portuguese and studies investigating the psychometric properties of the Brazilian version developed a shortened 20-item version of the instrument that shows good psychometric properties [25]. Items are rated in a 3-point scale. Total scores range from 0 to 60 with higher scores reflecting higher depressive symptomatology levels.

BDI

The Beck Depression Inventory (BDI) is a self-report measure of depressive symptoms [27]. The scale has been translated to Brazilian Portuguese with good psychometric properties [28]. This scale consists of 21 items, including symptoms and attitudes, rated on a scale of 0 to 3. Total scores range from 0 to 63 with higher scores reflecting higher levels of depression.

GAD-7

The Generalized Anxiety Disorder Scale, 7–items (GAD-7) is a valid and efficient tool to screen for GAD and assess its severity in clinical practice and research [29]. It has been translated to Brazilian-Portuguese and has been validated with good psychometric properties [30]. The scale has seven items, which are rated on a scale of 0 to 3. Total scores range from 0 to 21 with higher scores indicating higher GAD severity.

LSAS

The Liebowitz Social Anxiety Scale (LSAS) is a measure that assesses fears of social interaction and performance [31]. It has been translated and validated to Brazilian-Portuguese [32]. The scale has 24 items (13 questions relate to performance anxiety and 11 concern social situations), divided into two subscales (fear and avoidance). The scale is rated from 0 to 3. Total scores range from 0 to 72 with higher scores indicating higher social anxiety severity.

Emotional Narratives

The experimental paradigm was developed by the authors of this paper: speech and language therapists (RT), linguists (AB), psychiatrists (GGM), psychologists (DAS) and medical students (SMF). A group of three authors wrote 80 stories. The stories were then evaluated by 10 judges, which classified each story by each emotional type, quality of the story (poor, average or good) and intensity of emotion (weak, medium or strong). A total of 26 stories with ambivalent ratings were redrafted and judged again for inclusion in the study.

The experimental design included three sets of 12 emotional narratives (angry, happy and sad outcomes), totalizing 36 narratives, and 12 neutral narratives, for a total of 48 narratives. The narratives followed the structure of narratives used in previous studies [33, 34, 35]: three sentences, including two context-building sentences and a final, critical sentence, which conveyed either an emotion related to the protagonist, or a neutral outcome. The stories narrated ordinary events in which the protagonist expresses an emotion in the last critical sentence, (sadness, anger or happyness); the emotion resulted from the events described in the first two sentences. Below is an example of a, sad outcome:

Context

Junior played with his dog, Toto, every evening.

One day Junior came back from school and Toto was not waiting for him at the gate.

Critical

Junior cried a lot when he learned Toto had been run over by a car.

The neutral narratives had the same structure and size as the emotional narratives; however, the critical sentence and the context sentences merely narrated neutral everyday

events (going to work, having a snack). The neutral narrative did not include textual clues that suggested an emotional reaction. After presentation of the story, a probe question about the narrative was presented to ensure participants were paying attention to the narratives. The probe question was presented as a Yes/No question, which inquired about facts or details in the story. The question did not inquire about the emotional content of the narrative. The context sentences had aproximately 10 words and were presented for 5 seconds each. After the context sentences, there was a 4-second pause, during which a fixation cross was presented in the center of the screen. The critical sentence of approximately 10 words was also presented for 5 seconds. After the critical sentence, the probe appeared for 4 seconds and participants had to respond to the Yes/No question using mouse buttons. The inter-trial interval was randomly jittered among 5, 4 or 2-seconds. In addition, a total of six 30-s baseline periods were inserted in the experimental paradigm at the middle and at the end of each run. The thirty-second baseline was explicitly modeled in the analysis; the intertrial intervals were not explicitly modeled.

The 48 narratives were randomized and divided into three, 16-story runs; the sequence of presentation of the runs, and the order of the stories, were pseudo randomized. The narratives were presented using *E-Prime® Extensions for fMRI (EEfMRI)* software for studies with functional magnetic resonance imaging; stories and the fMRI scanner were synced using the first scanner trigger pulse Figure 1 shows an illustration of the experimental design. All contrasts used the critical sentence of each narrative as the event of interest.

Figure 1 around here

Acquisition

Structural scan and task-based FMRI were based on the following parameters: T2* EPI BOLD; three fMRI runs: 26 interleaved axial slices, 4.0 mm slice thickness with a 0.4 mm gap, 240mm x 240mm FOV and matrix size of 128 x 128, TE 30ms, TR 2000ms, flip angle of 90° for a total 233 volumes (7 minutes and 46 seconds).

fMRI data analysis

Single subject imaging processing was performed using AFNI and the following steps: slice-time correction, despiking, motion correction, and spatially normalized to the MNI152 template (T1 image as reference); images were blurred with a 6mm-FWHM Gaussian kernel.

Group analysis

Group level analysis used a mixed analysis of variance using 3dMVM [36], with one between-group factor with 2 levels (group: anxiety vs. non-anxiety), one within-subjects factor with 4 levels (emotion: neutral, angry, happy and sad) as well as group by emotion interaction. Results were thresholded voxel-wise at $p<0.005$. The 3dClustSim cluster correction was applied to control for multiple tests across the whole brain gray matter (2-sided, using third nearest neighbor clustering, NN3 and $\alpha=0.05$), which resulted in 54 voxels (1.458 μl).

Decomposition of significant Group by Emotion interactions: Clusters showing significant group by emotion interactions were decomposed by Emotion, investigating group differences in mean beta activations (*versus* baseline) for each type of emotion versus neutral narratives. Exploratory analyses examined correlations between mean beta values from these clusters and self-reported measures of anxiety and depression.

Post-hoc analysis for significant main effects of emotion: Clusters that showed significant main emotion effects were investigated using repeated measures analysis of variance to compare the activation levels in mean beta activation (*versus* baseline) in each cluster for each of the four conditions (anger, sadness, happiness and neutral), after Bonferroni adjustment for multiple comparisons. We also conducted one sample t-tests against baseline (a mean activation of 0).

RESULTS

Results from the ANOVA revealed a thalamic cluster of activation when testing the effects of group by emotion interaction (63 voxels, 1701 μL, MNI coordinates 15, 5.5, 10) (Figure 2). Emotion showed a main effect in ten brain clusters, including language-related areas and attention-related anterior and posterior areas of the brain. Group had no significant main effects ($p>0.05$). Decomposition of the group by emotion interaction and post-hoc tests for main emotion effects are discussed below.

Decomposition of the group by emotion interaction in the thalamic cluster

The group by emotion interaction ($F(3,78)=9.083$, $p<0.001$, $\eta p2=0.259$) was decomposed using contrasts for each emotional narrative versus the neutral narratives in this specific thalamic cluster (Figure 2, panel A). The interaction was significant for sad versus neutral comparisons ($F(1,26)=20.011$, $p<0.001$, $\eta p2=0.435$) and angry versus neutral comparisons ($F(1,26)=7.05$, $p=0.013$, $\eta p2=0.213$), but not for the happy versus neutral comparisons ($F(1,26)=3.42$, $p=0.076$, $\eta p2=0.116$) (Figure 2, panel B).

Exploratory analysis correlated activity in this specific thalamic cluster using sad versus neutral contrasts with self-reported measures of anxiety and depression. We found significant correlations between thalamic activation in sad vs. neutral contrast and depressive symptoms in the pooled sample measured by CDI ($r=-0.585$, $p=0.001$) and BDI ($r=-0.461$, $p=0.014$); as well as for anxiety symptoms measured by SCARED ($r=-0.387$, $p=0.042$), GAD-7 ($r=-0.420$, $p=0.026$) and LSAS-Fear ($r=-0.418$, $p=0.030$). No significant associations were found for LSAS-Avoidance. Nevertheless, only CDI showed trend-level associations with thalamus in sad versus neutral contrasts in both groups separately ($r_{comparison}=-0.462$, $p=0.097$; $r_{anxiety}=-0.485$, $p=0.079$) (Figure 3).

Post-hoc tests for main emotion effects

Main Emotion effects were found in several frontal-temporal regions. These regions mapped to a language-related network: left inferior frontal gyrus and left temporoparietal junction; an attention-related network, including left medial frontal gyrus and left inferior parietal lobe [37, 38, 8, 39] and a somatic information processing region, the posterior cingulate gyrus [10, 40, 38] (Figure 4).

Overall emotional narratives were associated with higher activation than neutral narratives in left-hemisphere language-related areas, and in attention-related anterior and posterior areas of the brain [10, 40, 38], including left temporoparietal junction and left inferior frontal gyrus [37, 38, 39]. There was also more activation in anterior and posterior areas known to be engaged in comprehension of connected discourse, including areas of fronto-medial cortex (medial prefrontal gyrus, superior frontal gyrus) and posterior areas of the brain (inferior parietal lobe and posterior cingulate gyrus) [10, 40, 38].

The closer inspection of each emotion effects (each emotion versus neutral contrasts), revealed a posterior-anterior medial line of activation specific to angry narratives, including the posterior cingulate/precuneus and the medial prefrontal gyrus [10] (Table 2) – which were not found for other types of emotion, with significantly different results. A description of the clusters of activation for emotional versus neutral narratives can be seen in Figure 2 and Table 2.

DISCUSSION

To our knowledge, the present study is the first to use narratives that convey emotions as a tool to investigate emotional processing in patients with anxiety disorders. We found a group by emotion interaction showing that during the processing of narratives with negative valences (sad and angry), showing anxiety disorders were associated with significant down-regulation of activation in a midline anterior thalamic cluster. This is partially consistent with our "a priori" hypothesis that predicted differential activations in thalamus amygdala and PFC. In addition, we were partially able to confirm our second hypothesis. We showed pronounced emotion effects in the dorsomedial prefrontal cortex, temporal pole, posterior cingulate cortex and temporoparietal junction, but contrary to our predictions, amygdala activation was not detected in this study. We also revealed emotion-related clusters of activation in language-related and attention-related areas of the brain. Our third exploratory research question was exploratory and revealed no differences in narrative processing to be related to anxiety disorders in the absence of emotion.

In the present study, the main difference between anxiety and control participants was in decreased thalamic activity while reading sad and angry narratives if compared to neutral narratives. Thalamic hypoactivation and smaller thalamic volume has been associated with anxiety disorders in adults [41, 42]. Down-regulation of activation in anxiety disordered patients, in comparison to controls, and smaller cortical volume may be associated with impaired processing of sensory information [43]. Recent studies show that a robust pathway originated at the amygdala is connected to the thalamus and to the orbital cortex, forming a tripartite structural network. This finding suggest that, anxiety disorders might be associated with a down-regulation in activation of the thalamus as an specific node in this tripartite network [2]. These thalamic dysfunctions might be more

evident in such types of narrative-based paradigms, given that they require first integration from several sources of information in order to elicit emotional processing. In counterpart, threatening faces and word paradigms might elicit more rapidly the amygdala and PFC regulatory regions, which are easily salient to the threat detection systems and might demand less integration from thalamic regions.

Emotion effects across all participants revealed frontal-temporal activation. The left middle temporal and left inferior frontal gyrus are well-known in their association with language-related processes [37, 38, 8, 39]; they are also postulated to be part of a network of brain areas involved in text integration [8, 44]. The medial prefrontal gyrus and the inferior parietal lobe have been reported as areas associated with specific comprehension processes, including interpreting the perspective of the protagonist of a story [8]. Together, these networks, which were equally activated in anxiety and control groups are associated with the processing of connected text. The activation of these areas reveals a modulation of brain activity by emotion in a network associated with higher-level comprehension processes, which include coherence monitoring and inference-making [8]. Finally, the posterior cingulate region, in addition to being part of the putative extended language network [38] has been associated with understanding social interactions and self-involvement [45, 46]. In sum, the above-reported results suggest that comprehension of narratives was associated with engaging similar language-related brain networks in anxiety patients and controls.

There are caveats to the present results. First, our small sample size might have limited our ability to identify more subtle differences in brain activation with this task. Nevertheless, even with a small sample size we were able to detect strong between group differences. Second, the study population included three-highly co-ocurring anxiety disorders (generalized anxiety, separation anxiety and social anxiety), thus it was not

possible to separate the effects of the disorders. In addition, the sample included comorbidities, which are common to anxiety disorders and limit our ability to draw inferences that are specific to anxiety. However, this sample is part of a cohort who was recruited from the community and, therefore, represents a typical clinical profile of anxiety disorder patients. In addition, additional analysis excluding depressive patients revealed the very same results (data not showed; available upon request).

In summary, the results reported lower response in the thalamic region in anxiety disorder patients, relative to controls, for sad or angry narratives if compared to neutral. These results highlight the importance of the thalamus as a region of interest for anxiety disorders research, as a part of a tripartite network formed by the amygdala and the PFC. Further systematic observation of the interaction between those three regions might help elucidate neural mechanisms of emotion processing in anxiety disorders.

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CONTRIBUTORS

RT, AB, GAS, GGM and *PPS* conceptualized the study. *RT, DDS, RDM, DMR, RSR, ABM, SMF,* performed the data collection. *JAP* was responsible for the clinical judgement of the magnetic resonance images *GAS*, *ARF* and *AB* performed the data analysis. *RT, AB,*

GAS, ARF, GGM and *ME* contributed substantially to the interpretation of the data and in the writing of the final version of the manuscript. All authors had full access to all of the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1 - Sample description

	TDC (n=14)		**Anxiety** (n=14)		**Total** (n=28)	
	n	%	n	%	n	%
Sex (female)	8	57.1	6	42.9	14	50.0
MINI/K-SADS diagnosis						
Separation Anxiety	-	-	2	14.3	2	7.1
Social Anxiety	-	-	4	28.6	4	14.3
Generalized Anxiety	-	-	10	71.4	10	35.7
Specific Phobia	-	-	1	7.1	1	3.6
Depression	-	-	3	21.4	3	10.7
Manic episode	-	-	1	7.1	1	3.6
Psychotic episode	-	-	1	7.1	1	3.6
Attention Deficit/Hyperactivity	-	-	5	35.7	5	17.9
Oppositional Defiant	-	-	3	21.4	3	10.7
Post-traumatic Stress	-	-	1	7.1	1	3.6
	Mean	SD	Mean	SD	Mean	SD
Age (years)	17.64	2.50	16.93	2.27	17.29	2.37
Anxiety symtpoms						
GAD-7	4.43	4.80	7.71	5.18	6.07	5.18
SCARED	19.71	12.00	26.71	11.83	23.21	12.23
LSAS-Fear	14.23	6.51	24.50	13.03	19.56	11.48
LSAS-Avoidance	12.83	9.03	20.86	11.49	17.15	11.01
Depressive symptoms						
CDI	4.71	3.79	8.14	4.38	6.43	4.38
BDI	5.71	6.11	10.29	5.98	8.00	6.37

Note: Typical development comparison (TDC); Generalized Anxiety Disorder Scale, 7–items (GAD-7); Screen for Child Anxiety Related Emotional Disorders (SCARED); Liebowitz Social Anxiety Scale (SLAS); Children's Depression Inventory (CDI); Beck Depression Inventory (BDI).

Statistical comparisons (TDC vs. Anxiety): age ($t_{df=26}$=0.792, p=0.436), gender ($\chi^2_{df=1}$=0.143, p=0.705), LSAS-Fear ($t_{df=25}$=2.56, p=0.017), CDI ($t_{df=26}$=2.21, p=0.036), LSAS-Avoidance ($t_{df=24}$=1.95, p=0.062), BDI ($t_{df=26}$=2.0, p=0.056), GAD-7 ($t_{df=26}$=1.74, p=0.094) and SCARED ($t_{df=26}$=1.55, p=0.132).

Table 2 - Results from the multivariate model and post-hoc tests investigating emotion effects

Emotion effect (4 levels)

Voxels	µL	Region	BA	Peak MNI coordinates			Mean activation (% signal change) Post-hoc tests (Bonferroni adjustment)				ANOVA (df1=3, df2=81)	One sample t-test (test value=0, df=27)
				x	y	z	Neutral	Anger	Sad	Happy		
969	26136	Left Temporoparietal Junction	40, 22	-42	-56.5	22	0.007^{a}	0.291^{b}	$0.188^{b,c}$	0.13^{c}	F=20.3, p<0.001, η_p^2=0.429	ANG, SAD, HAP
839	24111	Right medial prefrontal gyrus	9, 10	3	54.5	25	-0.039^{a}	0.188^{b}	$0.06^{a,c}$	-0.045^{a}	F=16.7, p<0.0001, η_p^2=0.381	ANG
504	13635	Left inferior frontal gyrus, pars orbitalis	45, 47	-54	24.5	13	0.052^{a}	0.361^{b}	$0.259^{b,c}$	$0.194^{c,d}$	F=14.84, p<0.001, η_p^2=0.355	ANG, SAD, HAP
182	4914	Left superior, middle frontal gyrus	6	-51	3.5	52	0.115^{a}	0.405^{b}	0.329^{b}	0.294^{b}	F=12.9 , p<0.001, η_p^2=0.324	NEU, ANG, SAD, HAP
164	4428	Posterior cingulate, precuneus	31	-9	-47.5	34	-0.041^{a}	0.18^{b}	$0.012^{a,c}$	$-0.066^{a,c}$	F=8.8 , p<0.001, η_p^2=0.246	ANG
148	3996	Left inferior parietal lobe	39	51	-53.5	22	-0.005^{a}	0.228^{b}	$0.162^{b,c}$	$0.058^{a,c}$	F=11.4 , p<0.001, η_p^2=0.296	ANG, SAD
122	3267	Left middle temporal gyrus	21, 22	48	-26.5	-5	0.092^{a}	0.275^{b}	$0.214^{b,c}$	$0.149^{a,c}$	F=9.5 , p<0.001, η_p^2=0.261	NEU, ANG, SAD, HAP
104	2808	Left lingual gyrus	17, 18	18	-83.5	-5	0.881^{a}	1.062^{b}	1.02^{b}	1.031^{b}	F=10.1 , p<0.001, η_p^2=0.271	NEU, ANG, SAD HAP
65	1836	Left middle temporal gyrus	21	-57	15.5	-35	-0.017^{a}	0.338^{b}	$0.219^{b,c}$	$0.12^{a,c}$	F=15.6 , p<0.001, η_p^2=0.366	ANG, SAD, HAP
59	1593	Right inferior frontal gyrus	44, 46	57	21.5	31	-0.031^{a}	0.204^{b}	0.204^{b}	$0.092^{a,b}$	F=10.1 , p<0.001, η_p^2=0.273	ANG, SAD

Note: Different letters represent statistically significant differences. Angry (ANG); Sad (SAD); Happy (HAP); Neutral (NEU).

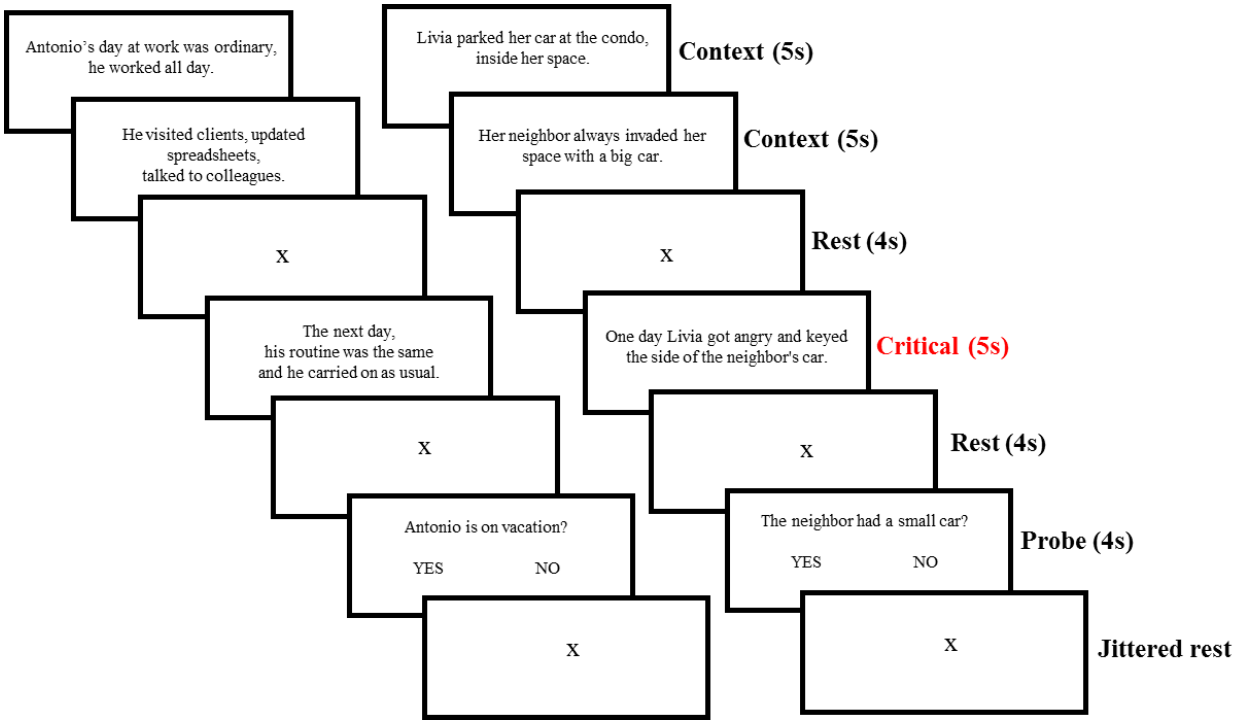
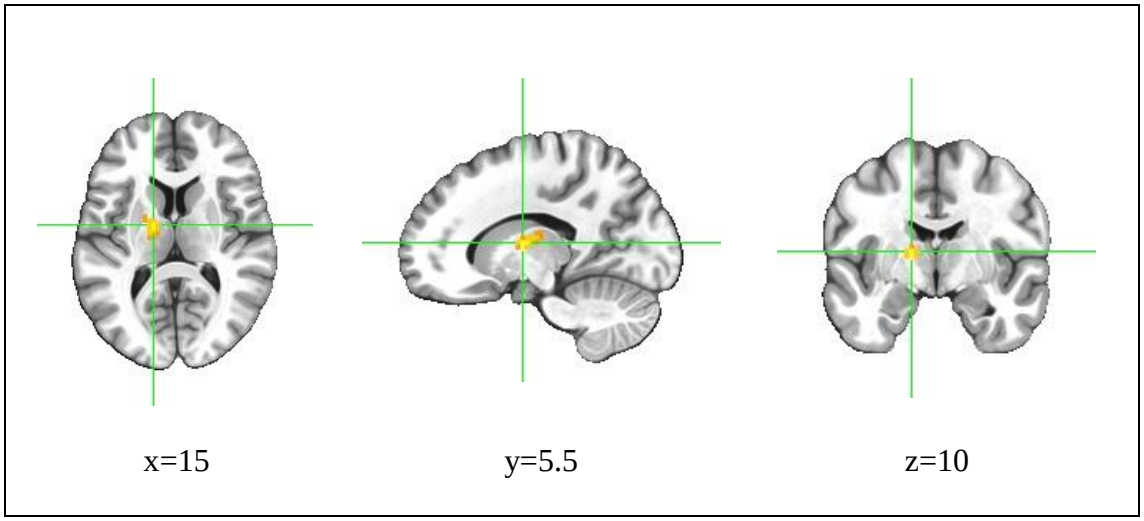


Figure 1 – Examples of neutral (left) and angry (right) trials from the emotional narratives paradigm.

Figure 2 – Group by emotion interaction showing thalamic differences between participants with anxiety disorder and comparison group



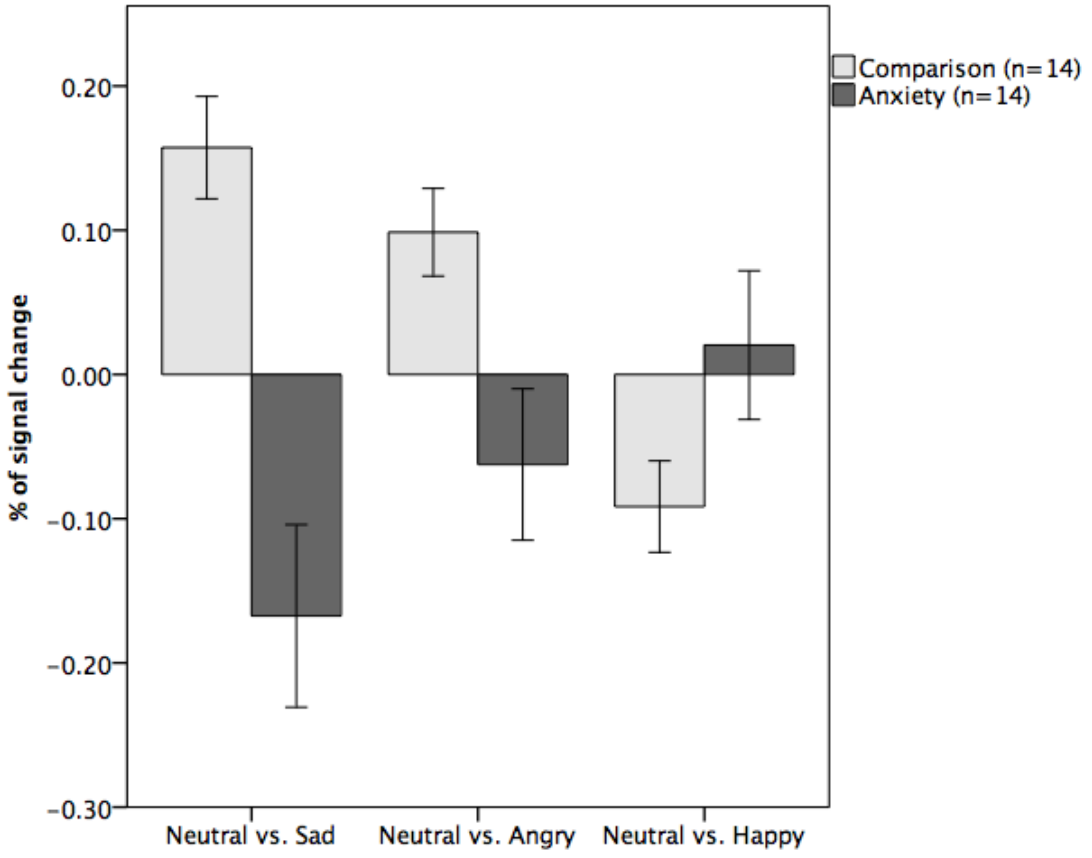


Figure 3 – Correlations between self-report measures and thalamic signal in sad versus neutral contrast, sad and neutral trials (Child Depression Inventory, CDI)

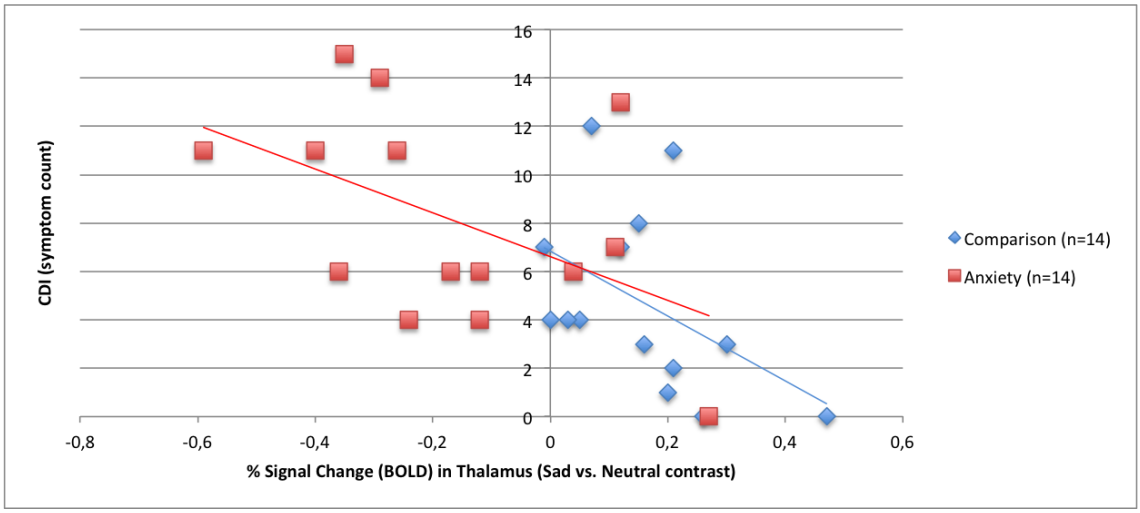
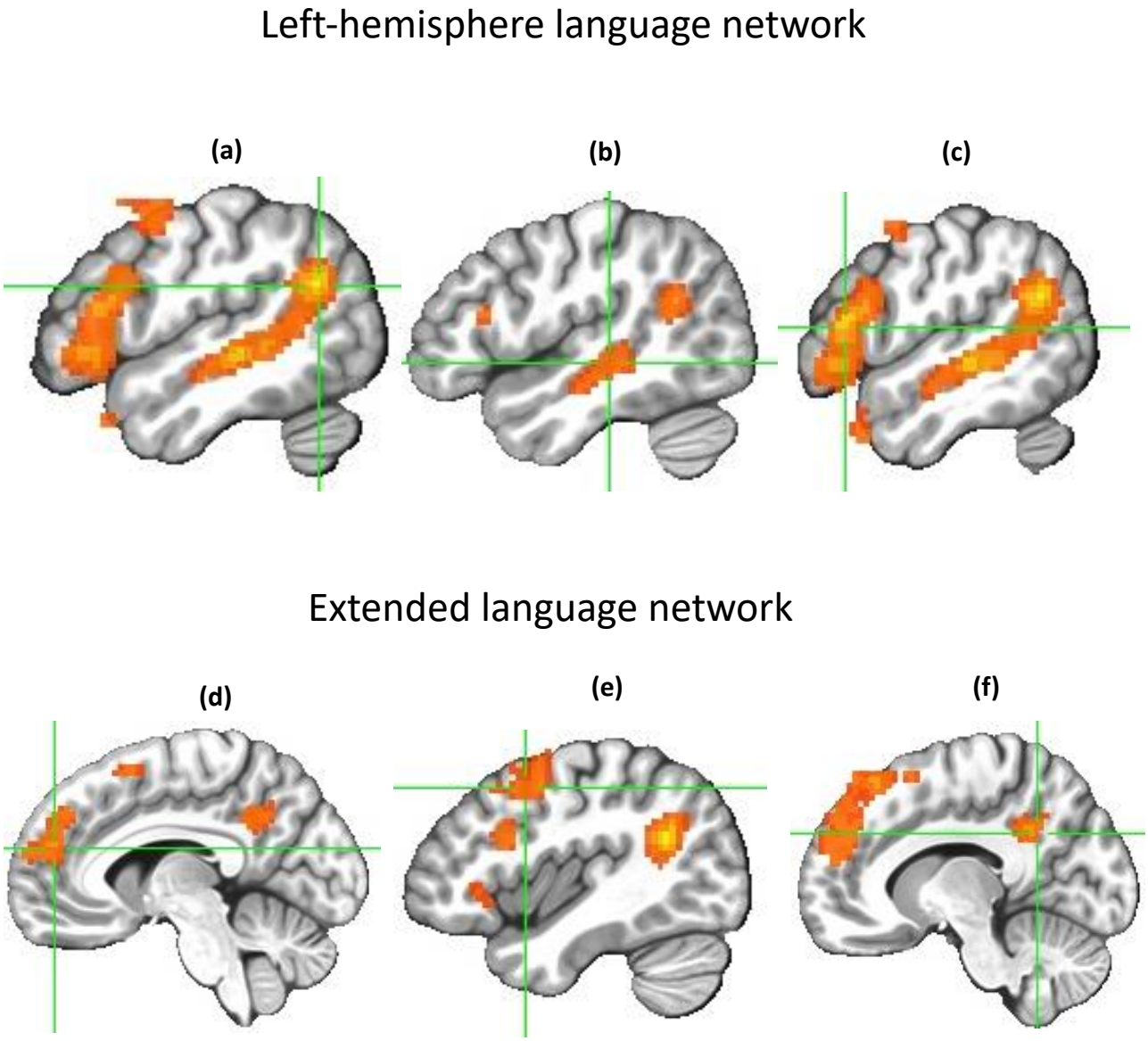
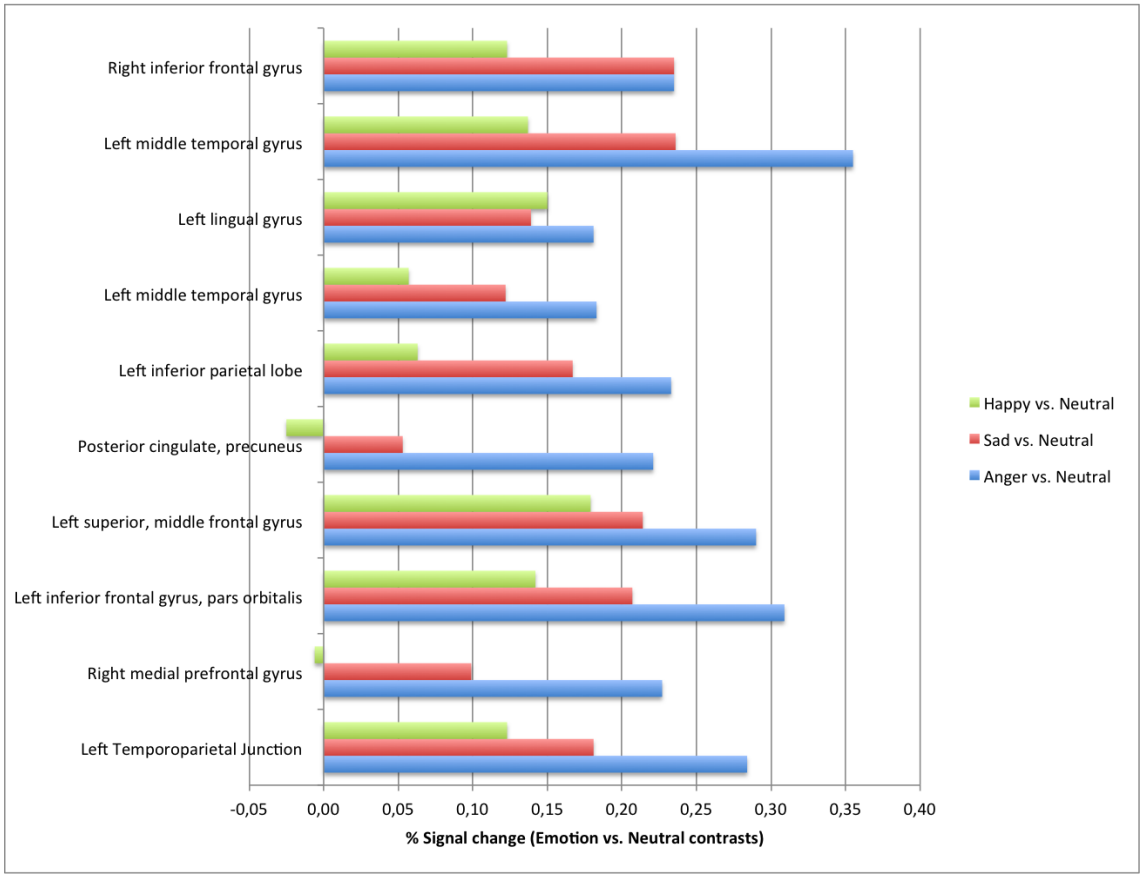


Figure 4 – Language-related brain regions showing significant emotion effects across all participants



Note: (a) Left temporoparietal Junction (x: -42, y: -56.5, z: 22); (b) Right middle temporal gyrus (x: 48, y: -26.5, z: -5); (c) Left inferior frontal gyrus, pars orbitalis (x: -54, y: 24.5, z: 13); (d) Right medial prefrontal gyrus (x: 3, y: 54.5, z: 25); (e) Left superior, middle frontal gyrus (x: -51, y: 3.5, z: 52); (f) Posterior cingulate, precuneus (x: -9, y: -47.5, z: 34).



Supplemental Figure S1 – Emotion versus neutral contrasts for the clusters that showed significant main emotion effects

7. ARTIGO 3

Amygdala-based intrinsic functional connectivity and anxiety disorders in adolescents and young adults

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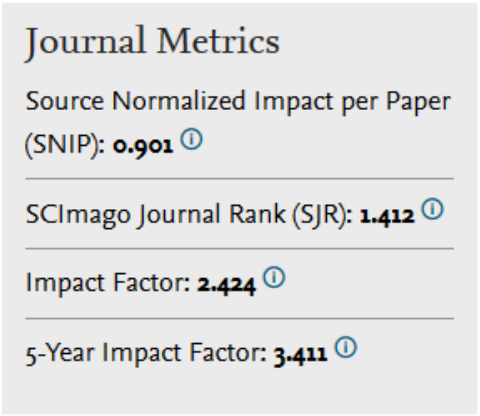

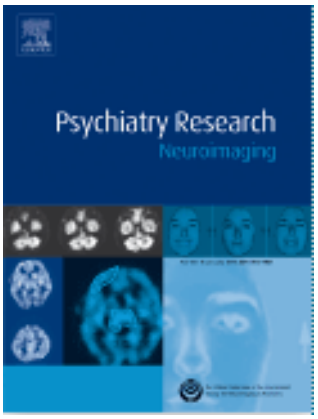

Amygdala-based intrinsic functional connectivity and anxiety disorders in adolescents and young adults

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Keywords: anxiety disorders; fMRI; resting state; functional connectivity

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ABSTRACT

Introduction: Anxiety disorders are the most prevalent psychiatric disorder in adolescents and young adults. Nevertheless, its pathophysiology is still poorly understood. The present study investigated the functional connectivity of intrinsic amygdala-based networks of participants with and without anxiety disorders. **Methods**: Resting state functional resonance neuroimaging data were obtained from 18 participants with an anxiety disorder (Separation Anxiety, Generalized Anxiety and Social Anxiety Disorder) and 19 healthy comparison individuals, matched for age and gender. Psychiatric diagnosis was assessed using standardized structured interviews. The comparison between groups was carried out using functional connectivity maps from six seed regions defined using probabilistic maps bilaterally within the amygdala (basolateral, superficial and centromedial amygdala). **Results:** We found significant between-group differences in five clusters, which showed aberrant functional connectivity with the left basolateral amygdala. The right precentral gyrus, right cingulate gyrus, left and right precuneus, and right superior frontal gyrus showed higher correlations with the left basolateral amygdala in subjects with anxiety disorders as compared with the comparison subjects. Further analyses showed positive correlations between the left basolateral amygdala and the five clusters in anxious subjects (evidence for 'hyperconectivity'); for the comparison subjects, the correlations between the amygdala and the five clusters were either non-significant, or negative (evidence for 'hypoconecivity'). **Conclusions:** The present study suggests there is an intrinsic disruption in the communication between left basolateral amygdala and a network of brain regions involved with emotion regulation, and with the default mode network in adolescents and young adults with anxiety disorders.

1. INTRODUCTION

Anxiety disorders are characterized by dysfunctional fear and anxiety. Disorder onset usually presents during childhood and frequently persists until adolescence and early adulthood (Pine, 2007; Salum et al., 2014) being the most prevalent group of psychiatric disorders is in this age range (Kessler et al., 2007; Merikangas et al., 2010). Anxiety disorders are typically comorbid with each other, and the presence of comorbidity is rather the rule rather than the exception (Rutter, 2011; Salum et al., 2014). The understanding of the neural correlates of anxiety disorders in adolescents is essential to treat and reduce morbidity of these disorders over the lifespan.

The investigation of the neural bases of anxiety disorders in increasingly relying on noninvasive brain imaging procedures. Disruptions in brain circuits involving the amygdala have been identified in adolescents and adults with anxiety disorders (Blackford & Pine, 2012; Pine, 2007; Salum et al., 2014). Amygdala-based disruptions in functional connectivity were shown in studies using resting-state data in anxious adults (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Geiger et al., 2015; Kim, Gee, Loucks, Davis, & Whalen, 2011; Roy et al., 2009) and adolescents (Hamm et al., 2014; He, Xu, Zhang, & Zuo, 2016; Liu et al., 2015; Roy et al., 2013; Sylvester et al., 2012).

Studies involving resting state functional connectivity have show, particularly, anxiety-related disruption in amygdala-based networks and regions of the prefrontal cortex (Kim et al., 2011), cingulate cortex (Etkin et al., 2009), striatum, insula, superior temporal gyrus, brainstem and cerebellum (Roy et al., 2013). These results are consistent with emotion regulation models, suggesting dysfunction in the integration of interoceptive information, disruption of fear learning and more widespread disruption of amygdala networks than observed previously in task-based studies (Etkin et al., 2009; Kim et al., 2011; Roy et al., 2013)

Nevertheless, with few exceptions (Etkin et al., 2009; Roy et al., 2013), most of the previous evidence on disrupted circuits associated with anxiety disorders in adolescents and young adults does not consider the heterogeneity within nuclei constituting of the human amygdala. The investigation of amygdala subdivisions revealed distinct patterns of connectivity within this structure and throughout the brain, thus suggesting that resting state fMRI can be used for more fine-grained investigations of human amygdala function than previously reported (Roy et al., 2009; Stoddard et al., 2015). The amygdala is a collection of nuclei that can be grouped into three main nuclear subdivisions: basolateral (BLA), centromedial (CMA), and superficial (SFA) (Roy et al., 2009). The subdivision of the amygdala has been corroborated by structural and functional evidence from tractography (Saygin, Osher, Augustinack, Fischl, & Gabrieli, 2011), task-based (Bzdok, Laird, Zilles, Fox, & Eickhoff, 2013), and resting state studies (Etkin et al., 2009; Kim et al., 2011; Roy et al., 2013). Importantly, the subdivisions of the amygdala have been linked to different biological functions (Roy et al., 2013).

Here, we aim to study the intrinsic connectivity of human amygdala using probabilistic maps from the amygdala subdivisions in participants with and without anxiety disorders. Based on previous evidence (Etkin et al., 2009; Kim et al., 2011; Roy et al., 2013), we hypothesized that subjects with anxiety disorders would present significant alterations in intrinsic functional connectivity of the amygdala subdivisions with regions of medium prefrontal cortex, insula, cerebellum and superior temporal gyrus. However, given the paucity of studies concerning this issue, the whole-brain analysis is used to explore other areas of disrupted connectivity.

2. METHODS

2.1 Participants

This study is part of a larger study focusing on the multidimensional evaluation and treatment of anxiety disorders in children and adolescents followed for the past five years. More details of the sampling procedures can be found elsewhere (Salum et al., 2011). From the full cohort, we recruited 76 right-handed adolescents and young adults for assessment in this present study. A total of 32 were excluded due to pregnancy, recent tattoo, use of dental appliances, and claustrophobia; thus 44 individuals participated in the fMRI acquisition. From those, 8 participants were excluded due to excess head movement during the fMRI acquisition (in excess of 0.5 mm in any direction) (Power, Barnes, Snyder, Schlaggar, and Petersen, 2012). A total of 36 participants, 22 participants with an anxiety disorder and 19 healthy comparisons participated in the study.

2.2 Ethical Statements

The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (HCPA) (GPPG/HCPA, project number 12-0254) and the Research Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (CEP-PUCRS). Adolescents' parents or guardians and the adult participants provided written informed consent for participating in the study and the adolescents also provided written assent.

2.3 Measures

2.3.1 Psychiatric diagnosis

Psychiatric diagnosis was ascertained using two instruments: (1) the MINI (Mini-International Neuropsychiatric Interview) (Sheehan et al., 1998) for young adults (18 to 22 years of age); (2) the K-SADS-PL (Kiddie Schedule for Affective Disorder and

Schizophrenia for School-Age Children – Present and Lifetime Version) (Kaufman et al., 1997), for adolescents (17 or bellow).

The K-SADS-PL is a semi-structured interview used for the diagnosis of childhood psychiatric disorders based on the DSM-IV criteria and was translated and adapted to be used in Brazil showing good psychometric properties (Petresco et al., 2009). The MINI (Mini-International Neuropsychiatric Interview) is a structured diagnostic interview, developed jointly by psychiatrists and clinicians, for DSM-IV and ICD-10 psychiatric disorders and was validated for Brazilian-Portuguese language (Amorim, Lecrubier, Weiller, Hergueta, & Sheehan, 1998), with good psychometric properties (Lecrubier et al., 1997; Sheehan et al., 1997). The severity of primary diagnoses were based on the Clinical Global Impression – Severity scale (CGI-S) that was rated independently for each psychiatric disorder in order to establish primary psychiatric diagnosis. Only non-medicated children were included in the study.

2.4 Image acquisition

MR images were acquired using the 3.0T GE Healthcare Signa HDxt scanner. Structural scans had the following parameters: T1 weighted, TE/TR = 6.13/2.18ms. Resting state fMRI had following parameters: T2* EPI BOLD;: 26 interleaved axial slices, 4.0 mm slice thickness with a 0.4 mm gap, 240mm x 240mm FOV and matrix size of 128 x 128, TE=30ms, TR=2000ms, flip angle of 90° for a total 233 volumes (7 minutes and 46 seconds). Resting State fMRI scans were acquired while participants passively viewed a fixation cross. During the scan, participants were instructed to rest with their eyes open while viewing a white cross centrally project against a black background.

2.5 Image processing

Single subject imaging processing was performed using AFNI's (Cox, 1996) *afni_proc.py* script with the following preprocessing steps: removal of the first 3 TRs, despiking, slice-time correction, motion correction, band-pass filtered (0.01-0.1Hz) and spatially normalized to the MNI152 template using nonlinear warping (T1 image as reference) and voxels resampled to be $3x3x3mm^3$ in size. Images were then blurred with a 6mm-FWHM Gaussian kernel. A multiple regression was then calculated with the functional data, were the average cerebral spinal fluid signal, a localized white matter signal (radius = 25mm) (Jo, Saad, Simmons, Milbury, & Cox, 2010), the six motion parameters and their derivatives' were used as nuisance regressors. Within the multiple regression, time points with large motion (>0.9) were censored. The residual of the multiple regression was used in the connectivity analysis.

2.6 Regions of interest (ROI) selection

The three amygdala ROI subdivision were generated from a probabilistic cytoarchitectonic-based atlas in MNI space, 50% probability threshold can identify basolateral, superficial, and centromedial ROIs that each generates an iFC network consistent with each subdivision's functional connectivity (Roy et al., 2013).

2.7 Single-subject connectivity maps

The six amygdala ROIs (three subregions of right amygdala and three of left) were resampled to match the voxel size of the normalized functional data. We calculated the mean BOLD time-series within each amygdala ROI and then used 3dTCorr1D to generate a voxel-wise Pearson's correlation map for each ROI. We used Fisher's r to z transformation to prepare the maps for group analysis.

2.8 Group analysis

Group-level analysis of each connectivity map was done by analysis of covariance using *3dMVM* (Chen, Adleman, Saad, Leibenluft, & Cox, 2014), with one between-group factor with 2 levels (group: anxiety vs. non-anxiety), including sex (male or female) and sex by group interaction as covariates in each of the three amygdala regions from both sides (left and right). The group-level formula was Fisher's z = group + sex + sex by group interaction + intercept + error.

Results were thresholded voxel-wise at $p<0.001$. Cluster correction was used to control for multiple tests across whole brain gray matter via 3dClustSim (2-sided, using third nearest neighbor clustering, NN3). To account for six seed analysis, the cluster correction alpha level was Bonferroni corrected to 0.008 (<0.05/6), yielding threshold of 108 voxels (549 μl). Within each cluster that differed between groups, a mean Fisher's z was calculated at the single subject level for *post-hoc* analysis.

3. RESULTS

3.1. Sample Description

There were no significant differences in age (mean=17.9, SD=2.47 vs. mean=16.7, SD=2.33, t=1.55, df=35, p=0.129) or gender (42.1% vs. 50%, χ^2=0.023, p=0.879) between anxiety and comparison groups. Main diagnoses in the anxiety group was: Separation Anxiety Disorder (n=4, 22%), Generalized Anxiety Disorder (n=13, 72.2%), Social Anxiety Disorder (n=5, 27.8%). Comorbidities in the anxiety group included: Attention Deficit/Hyperactivity Disorder, ADHD (n=6; 33%), Major Depression (n=5, 27.8%), and Oppositional Defiant Disorder, ODD (n=3, 16.7%).

3.2. Resting State Results

There were five regions of the brain that showed aberrant functional connectivity with the left basolateral amygdala in the anxious group relative to the comparison group. Subjects with anxiety showed higher connectivity with the left basolateral amygdala and the right precentral gyrus, right posterior cingulate gyrus, left precuneus, right precuneus and right superior frontal gyrus relative to comparison subjects.

Further analysis showed that correlations between the left basolateral amygdala and the five regions of the brain were positive in anxious subjects (evidence for 'hyperconectivity'). Also, we found negative correlations between amygdala and right cingulate gyrus and right precuneus for comparison subjects (evidence for 'hypoconectivity'), as shown in Table 1. No other statistically significant group differences were found for the other five amygdala seeds.

Supplemental analysis

Supplemental analyses were performed excluding subjects with ADHD, ODD and Major Depression from the analysis, and between-group differences in all cluster remained significant (all p-values <0.05; data not shown, available upon request).

DISCUSSION

Our results showed aberrant functional connectivity between the left basolateral amygdala and a distributed network of regions in the brain of participants with anxiety disorders. Specifically, we found hyperconectivity between the left basolateral amygdala and the right precentral gyrus, right posterior cingulate gyrus, left precuneus, right precuneus and right superior frontal gyrus in subjects diagnosed with anxiety disorders. On the other hand, we found hypoconectivity between amygdala and right posterior

cingulate gyrus and right precuneus for comparison subjects; correlations with the other clusters were not significant for this group.

Our finding of an aberrant amygdala connectivity in patients with anxiety disorder corroborates the literature and the identification of increased connectivity between the limbic system and frontal-parietal cortical regions (Roy et al., 2013). Contrary to our expectations we were not able to find any findings related to medium prefrontal cortex, insula, cerebellum and superior temporal gyrus (Roy et al., 2013). Nevertheless, the whole-brain approach to connectivity identified five brain regions whose connectivity is increased in anxiety disorders showing a significant effect of size (Cohen's d>1.5).

Hyperconectivity between amygdala and superior frontal cortex, precuneous and cingulate gyrus which is consistent with previous findings (Etkin, 2009) and also consistent with theoretical propositions (Sylvester et al., 2012). Evidence of increased connectivity between a posterior frontoparietal cingulate cortex – precuneous network was specifically associated with cognitive anxiety, potentially reflecting increased spontaneous negative cognition (Bijsterbosch, Smith, Forster, John, & Bishop, 2014). A recent study that investigated the individual differences in trait anxiety using amygdala structural connectivity and probabilistic tractography showed that higher trait anxiety was associated with aberrant connections between amygdala and regions implicated in extinction learning and memory encoding and environmental context recognition, including posterior cingulate cortex gyrus (Greening & Mitchell, 2015). Also, some theories predict dysfunctions in the *the default mode network*, hypothesized to perform functions such as self-referencial activities, future planning, self-inspection, and emotion regulation (Raichle et al., 2001). Nevertheless, hyperconectivity between the amygdala and the right precentral gyrus were not previously described and, if replicated, deserve attention from further studies.

Sampling and methodological differences between our study and Roy's study, the most comparable study in terms of the age range and statistical approach conducted so far, could be outlined. Whereas Roy et al studied adolescents with Generalized Anxiety Disorder (GAD); our study included subjects with GAD (72%), but also included subjects with social anxiety and separation anxiety disorder. Therefore, one possibility for different findings is that mechanisms which characterized GAD specifically might differ from those that characterized anxiety disorders as a group. Methodologically, despite using the same seed strategy as the one described by Roy and colleagues, pre-processing and analysis strategy were different between both studies and might partially account for the non-replication findings.

There are limitations to the present study. First, our small sample size might have limited our ability to identify more subtle differences in brain activation using resting sate fMRI. *Second*, the study population included three-highly co-ocurring anxiety disorders (generalized anxiety, separation anxiety and social anxiety), thus it was not possible to separate the specific effects of each disorder. In addition, the sample included comorbidities, which are common to anxiety disorders and limit our ability to draw inferences that are specific to anxiety. However, this sample is part of a cohort who was recruited from the community and, therefore, represents a typical clinical profile of anxiety disorder patients. Furthermore, additional analysis excluding patients with comorbidities yielded similar results.

The present work extends findings for anxiety disorders in adolescents and young adults and provide new insight into the functional connectivity of the human amygdala, supporting the hypotheses that adolescents and young adults with anxiety disorders exhibit alterations in amygdala circuits underlying emotional processes involved in fear

learning known to be deficient in adults. In sum, these results suggest a disruption of amygdala networks in adolescent with anxiety disorders.

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CONFLICT OF INTEREST AND FUNDING SOURCES

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Figure 1 – Clusters showing group differences in left basolateral amygdala-based connectivity between anxiety and comparison subjects

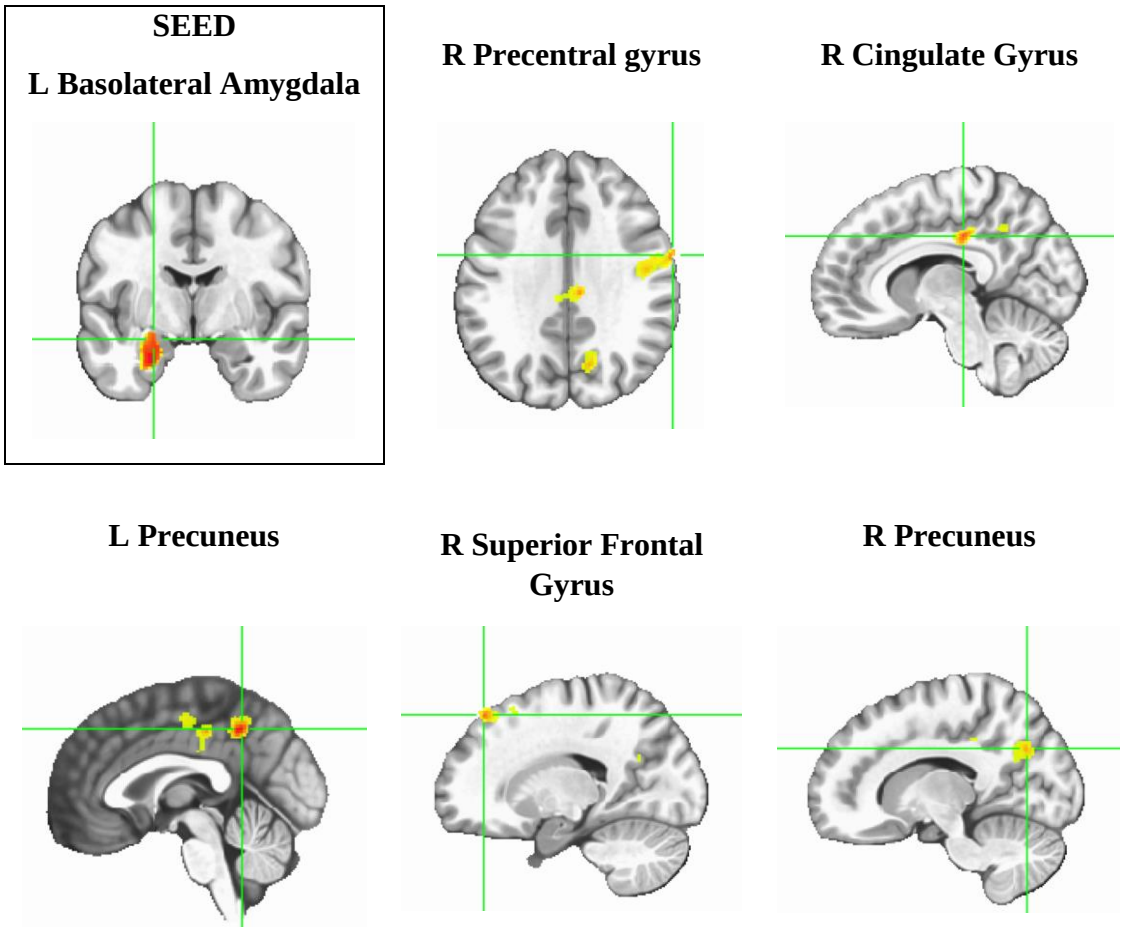

Table 2 - Group Differences in amygdala-based connectivity between anxiety and comparison subjects in left basolateral amygdala

Cluster	Voxels (n)	Volume (μL)	Cluster labels	BA	Peak MNI Coordinates			Mean Fisher's z				Independent Samples t test (ANX vs. COMP)		
					x	y	z	Comparison		Anxiety				
								Mean	SD	Mean	SD	t	p-value	Cohen's d
1	479	2567	Right Precentral gyrus	6	-63.9	0.5	32.9	-0.0092	0.1353	**0.2146***	0.1602	4.60	<0.001	1.51
2	417	2235	Right Cingulate Gyrus	31	-7.9	25	36.4	**-0.0995***	0.1246	**0.1269***	0.1089	5.87	<0.001	1.93
3	306	1640	Left Precuneus	7	0.9	49.5	45.1	-0.0525	0.1315	**0.1997***	0.1215	6.05	<0.001	1.99
4	305	1635	Right Superior Frontal Gyrus	8	-18.4	-39.8	53.9	-0.0156	0.1185	**0.1936***	0.1028	5.72	<0.001	1.88
5	143	767	Right Precuneous	7	-13.1	68.8	32.9	**-0.0999***	0.1453	**0.1421***	0.157	4.87	<0.001	1.60

Note: BA, Broadman Area; MNI, Montreal Neurological Institute; SD, Standard Deviation; ANX, Anxiety; COMP, Comparison;

* Means significantly different from 0 in one sample t-test.

8. DISCUSSÃO GERAL

Esta tese de Doutorado teve como objetivo estudar mecanismos fisiopatológicos associados aos transtornos de ansiedade utilizando estudos neuropsicológicos e de neuroimagem funcional. Foram apresentados três artigos produzidos a partir de dois projetos. O primeiro artigo como resultado de uma avaliação com crianças participantes de um ensaio clínico randomizado para ansiedade infantil. O segundo e terceiro artigos como resultados de um seguimento de pacientes adolescentes e adultos jovens acompanhados pelos últimos 5 anos.

No **artigo 1** (*Verbal fluency and severity of anxiety disorders in young children*) o objetivo principal foi investigar se o desempenho em testes de fluência verbal fonêmica estaria relacionado com a gravidade dos sintomas em crianças com transtorno de ansiedade, e, se essa associação seria independente dos sintomas de Transtorno de Déficit de Atenção e Hiperatividade (TDAH), uma comorbidade frequente.

Os resultados mostraram que a gravidade dos sintomas de ansiedade foram negativamente correlacionados com o número de *clusters* (agrupamentos) e que essa associação não pode ser explicada por sintomas de TDAH. Estes dados replicam um trabalho anterior de nosso grupo que mostram uma forte associação entre transtornos de ansiedade e pior desempenho em tarefas de fluência verbal.

Essa replicação reforça a importância da fluência verbal como um marcador de gravidade para a ansiedade e incentiva o desenvolvimento de novos estudos que investiguem de maneira mais aprofundada medidas neurobiológicas e implicações terapêuticas desses resultados atuais. Intervenções que visem mecanismos envolvidos na fluência verbal, tal como funções executivas, memória e linguagem, em idades precoces,

podem ser um caminho para minimizar problemas futuros e morbidades associadas aos transtornos de ansiedade.

O **artigo 2** (*Anxiety-related down-regulation of thalamic regions in the processing of sad and angry emotional narratives*) – entendido como o principal artigo da tese - teve como objetivo principal investigar o processamento emocional em pacientes com transtorno de ansiedade. Este artigo é o primeiro – para nosso conhecimento- a usar narrativas que transmitam emoção para estudar transtornos emocionais. O paradigma construído para utilização na ressonância magnética funcional foi desenvolvido pelo nosso grupo de pesquisa e foi capaz de revelar aspectos emocionais das narrativas e diferenças entre indivíduos ansiosos e não ansiosos quanto ao processamento emocional.

Os nossos resultados mostraram que o tálamo esteve consistentemente associado a um processamento aberrante de narrativas que veicularam conteúdo negativo (tristeza e raiva) se comparados ao conteúdo neutro – uma interação processamento emocional vs. grupo. Este achado é parcialmente consistente com a nossa hipótese inicial de que esperávamos ativações diferenciadas no tálamo, amigdala e córtex pré-frontal condizente com a literatura nessa área. Além disso, demonstramos efeitos pronunciados, relacionados com o processamento emocional no córtex pré-frontal dorsomedial, pólo temporal, córtex cingulado posterior e junção temporoparietal. Contudo, contrariamente às nossas hipóteses, a ativação na amigdala não foi detectada neste estudo. Nós também mostramos agrupamentos relacionados com processamento emocional em áreas relacionadas com a linguagem e processamento da atenção. Não detectamos diferenças no processamento de narrativas de cunho neutro relacionadas com o transtorno de ansiedade.

Uma possibilidade de termos encontrado disfunções talâmicas mais evidentes, e não ativação na amigdala, conforme era o esperado, pode ser devido ao tipo de tarefa.

Disfunções talâmicas podem ser mais evidentes em paradigmas baseados em narrativas, tendo em vista que exigem uma integração de diversas fontes de informação (linguísticas e não linguísticas) a fim de provocar o processamento emocional. Paradigmas com faces ou palavras de cunho ameaçador, em contrapartida, podem provocar modulações regulatórias mais rápidas em regiões como o córtex pré-frontal e amigdala, os quais são facilmente salientes para os sistemas de detecção de ameaças e podem exigir uma menor integração de regiões do tálamo. Outra possibilidade e, também, uma limitação deste estudo, é o nosso pequeno tamanho amostral, que pode ter restringido nossa capacidade de identificar diferenças mais sutis na ativação cerebral com essa tarefa. Além disso, a amostra incluiu comorbidades, que apesar de comuns aos transtornos de ansiedade, possam ter limitado nossa capacidade de fazer inferências específicas para a ansiedade.

Em suma, os resultados desse estudo destacam a importância do tálamo como uma região de interesse para a investigação da circuitaria dos transtornos de ansiedade - como parte de uma rede tripartite formada pela amigdala e o córtex pré-frontal. Além disso, a observação sistemática da interação dessas três regiões pode ajudar a elucidar os mecanismos neurais de processamento emocional nos transtornos de ansiedade. Esses resultados são especificamente relevantes num momento onde grande parte das informações emocionais são veiculadas através de narrativas, como mensagens de texto.

No **artigo 3** (*Amygdala-based intrinsic functional connectivity and anxiety disorders in adolescents and young adults),* o objetivo foi investigar a conectividade intrínseca da amigdala humana, usando mapas probabilísticos de cada subdivisão (basolateral, centromedial and superficial) em indivíduos com transtornos de ansiedade em comparação com indivíduos sem o transtorno. Baseado em evidências prévias de Roy e colaboradores (Roy et al., 2013), nós hipotetizamos que sujeitos com transtornos de ansiedade apresentam alterações significativas na conectividade funcional intrínseca,

relacionada com as subdivisões da amigdala e conexões com regiões do córtex pré-frontal, ínsula, cerebelo e giro temporal superior. Entretanto, dado os escassos estudos nessa área, foi usada uma análise de todo cérebro para explorar outras áreas possivelmente relacionadas.

Nossos resultados mostram uma conectividade funcional aberrante entre a amigdala basolateral esquerda e cinco regiões no cérebro relacionadas à ansiedade. Essa associação significa que encontramos correlações positivas entre a amigdala basolateral esquerda e giro precentral direito, giro cingulado direito, precuneo bilateral e giro frontal superior direito nos indivíduos com transtorno de ansiedade (uma hiperconectividade entre a amigdala e essas cinco regiões). Nos indivíduos comparação a maioria dessas correlações não é significativa e, além disso, encontramos uma correlação inversa entre a amigdala basolateral esquerda e duas regiões (cíngulo e precuneo direitos). Não encontramos diferenças estatisticamente significativas entre os sexos.

Corroborando com a literatura, nós encontramos conectividade intrínseca aberrantes em pacientes com transtornos de ansiedade. Contudo, contrariando às nossas hipóteses, nós não encontramos nenhuma conexão com córtex pré-frontal medial, ínsula, cerebelo e giro temporal superior (Roy et al., 2013). Apesar disso, os resultados encontrados apresentam forte associação com transtornos de ansiedade e com grande tamanho de efeito (Cohen's $d>1.5$).

O presente trabalho estende os escassos resultados prévios da literatura em adolescentes e jovens adultos com transtorno de ansiedade e fornece novos conhecimentos sobre a neuroanatomia funcional da amigdala, suportando a hipótese de que adolescentes e jovens adultos com esses transtornos exibem alterações nos circuitos relacionados com esta estrutura cerebral, bem conhecidos por alterações no aprendizado do medo em adultos. Em suma, esses dados convergem com estudos prévios e estudos

utilizando modelos animais, que suportam a validade deste déficit na psicopatologia e no neurodesenvolvimento.

O conjunto de dados encontrados nesses três artigos nos mostram alterações significativas em indivíduos com transtornos de ansiedade a partir de diferentes enfoques de avaliação: Neuropsicologia, neuroimagem funcional com realização de tarefa e neuroimagem funcional em repouso. Os estudos em crianças e adolescentes, especialmente os estudos em neuroimagem funcional ainda estão emergindo na literatura, com muitas lacunas a serem preenchidas, sobretudo no que se refere aos estudos de conectividade cerebral. Os poucos estudos publicados nessa área ainda apresentam resultados inconsistentes e com grande variabilidade metodológica, o que dificulta a generalização dos achados.

Desde os primeiros resultados encontrados no mestrado (Toazza et al., 2014) e associado aos resultados encontrados nesse estudo, a investigação de aspectos da linguagem nos transtornos de ansiedade mostrou-se frutífera para revelar déficits associados a estes transtornos mentais. Esses achados vão ao encontro das teorias propostas por Sylvester e colaboradores (Sylvester et al., 2012) que propõem o envolvimento de 4 redes funcionais envolvidas em diferentes aspectos da cognição que estariam alterados em indivíduos com transtornos de ansiedade.

(1) *Rede cíngulo-opercular*: inclui cingulado, insula, córtex pré-frontal e tálamo. Acredita-se que esta rede esteja envolvida no controle cognitivo, processamento de afeto negativo e dor (Liao et al., 2010; Shackman et al., 2011).

(2) *Rede fronto-parietal*: inclui porções bilaterais anteriores do córtex pré-frontal dorsolateral, lóbulo parietal inferior, porções do giro cingulado media e porções do precuneo. É referida como tendo papel importante no controle

executivo, monitoramento para detectar desvios de conduta, assinalando assim a eventual necessidade de ajuste de estratégia (Seeley et al., 2007).

(3) *Rede de modo padrão*: incluem cingulado, precuneo, córtex parietolateral, córtex pré-frontal medial, giro temporal inferior, giro parahipocampal e córtex frontal superior. A hipótese é que essa rede esteja envolvida na realização de funções introspectivas, planejamento futuro, auto-monitoramento e regulação emocional (Raichle et al., 2001).

(4) *Rede de atenção ventral*: inclui o córtex pré-frontal ventrolateral, junção temporoparietal e porções dos giros temporais superiores. Estaria envolvida no processamento de atenção automática a estímulos, sugerindo envolvimento em funções como cognição social (Corbetta et al., 2008)

Indivíduos com transtorno de ansiedade exigiriam um controle executivo adicional para processar a emoção na presença de estímulos de cunho emocional, quando comparados a sujeitos do grupo de comparação (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009), por uma dificuldade nessa modulação emocional (Cisler, Olatunji, Feldner, & Forsyth, 2010). Além disso, está bem descrito na literatura, também, que esses pacientes apresentam um viés para estímulos ameaçadores (Pine, 2007), alteração em alguns aspectos da função executiva, tais como o controle inibitório (Micco et al., 2009) e flexibilidade cognitiva (Toren et al., 2000). Essas alterações vão ao encontro de diferentes resultados levantados por nossos artigos nos indivíduos com transtornos de ansiedade, tais como alteração na fluência verbal, alteração no processamento emocional para estímulos negativos e alteração na conectividade intrínseca em repouso.

Um estudo recente mostra diferenças de conectividade intrínseca de repouso entre a amígdala esquerda e regiões envolvidas no processamento de emoções faciais,

aprendizado do medo e dor, quando comparando homens e mulheres. Esses dados sugerem que a conectividade intrínseca da amígdala é bem regulado pelos efeitos do cortisol e, por isso, diferiria entre homens e mulheres (Kogler et al., 2016). A influência do sexo nos estudos com transtornos de ansiedade é uma questão importante de ser discutida, levando em consideração que alguns artigos sugerem uma maior predominância de mulheres com esses transtornos. No nosso conjunto de artigos não encontramos diferenças estatisticamente significativas, no primeiro e no segundo artigo os resultados se mantiveram com a mesma magnitude quando controlados para o sexo, no terceiro, foi incluído como covariável e não encontramos diferenças. Entretanto, fica como uma sugestão para estudos futuros investigar a relação do cortisol nos estudos com neuroimagem.

A infância e adolescência são um período crítico e importante para o desenvolvimento do cérebro humano e mudanças sutis durante esta fase podem ser exponencialmente ampliadas por diferentes influências, tais como transtornos psiquiátricos. Essas influências podem gerar consequências em longo prazo persistindo até a vida adulta. Os estudos em ressonância magnética na última década têm nos oferecido uma oportunidade sem precedentes para estudar o neurodesenvolvimento *in vivo*.

Em suma, os dados dessa tese trazem contribuições importantes para o entendimento dos transtornos de ansiedade sob a perspectiva da Neurociência, Neuropsicologia, Psiquiatria e Fonoaudiologia. Esses achados iniciais demonstram as potencialidades que a neurociência oferece para o entendimento dos transtornos mentais ao compreender cérebro humano em suas diferentes redes funcionais. E fornecem ideias para avanços na personalização terapêutica e desenvolvimento de novas estratégias de tratamento.

9. CONCLUSÕES E CONSIDERAÇÕES FINAIS

Os resultados encontrados nesses três artigos que compõe esta tese nos trazem importantes contribuições sobre o desenvolvimento da ansiedade e dos circuitos neurais envolvidos em crianças e adolescentes. Os dados nos mostram alterações significativas na fluência verbal e alterações na conectividade cerebral, tanto em repouso quanto baseado em tarefas, presentes nos transtornos de ansiedade, trazendo novas informações que são importantes para o entendimento da origem dessas alterações.

A tese contribui também com o desenvolvimento de um paradigma para avaliação de processamento emocional utilizando narrativas. Esse produto pode ser utilizado por outros investigadores interessados em estudar processamento emocional.

Identificar no cérebro trajetórias comportamentais que possam estar associadas à ansiedade pode nos informar sobre aspectos mecanísticos do desenvolvimento dos transtornos, assim como sugerir novas metas para tratamento. Os resultados desses estudos podem sugerir novos caminhos para o desenvolvimento de tratamentos, além de novas terapias farmacológicas ou comportamentais poderem capitalizar-se sobre achados da neurociência almejando habilidades de regulação emocional em crianças ansiosas.

As contribuições podem ser no âmbito de novas terapias de funções relacionadas com a fluência verbal, as quais envolvem habilidades de linguagem e memória, podendo evitar a piora de sintomas de ansiedade. No campo da Fonoaudiologia e Neuropsicologia, nos ajudam a entender os mecanismos linguísticos, trazendo possibilidades de novas terapêuticas em diferentes quadros clínicos, levando em consideração a alta comorbidade de quadros com lesão ou disfunção cognitiva e transtornos psiquiátricos.

10. PERSPECTIVAS FUTURAS

Como pesquisadora, minhas perspectivas futuras são seguir com linhas que envolvam Neuropsicologia, linguagem e Neurociências em um possível pós-doutorado. Dentro dessa perspectiva tenho a intenção de avançar os conhecimentos nesta área integrando novos métodos que envolvam novas terapias, com medidas comportamentais e neurobiológicas antes e após tratamento, para entender mecanismos de funcionamento das alternativas terapêuticas disponíveis.

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11. ANEXOS

Artigos em colaboração publicados ou submetidos durante o Doutorado

- ***Impulsivity-based thrifty eating phenotype and the protective role of n-3 PUFAs intake in adolescents.***

  Aceito para publicação na Translational Psychiatry (FI: 4.360)

- ***A role for the cingulate cortex in dyslexia: activation and underconnectivity in a study of Brazilian children.***

  Submetido para Developmental Neuropsychology (FI: 2.241)

- ***Interaction Between Stress Responsiveness and Insulin Sensitivity on Eating Behavior In Adolescents***

  Submetido para Obesity Research & Clinical Practice (FI: 1.177)

- ***Intrauterine growth programming of adolescent feeding behavior and related brain mechanisms***

  Dados ainda não submetidos

- ***Interaction between perceived maternal care, anxiety symptoms and the neurobehavioral response to palatable foods in adolescents***

  Submetido na Stress (FI: 2.715)

- ***Assessment of Anxiety Severity and Impairment in Community and Clinical Contexts***

  Dados ainda não submetidos

Impulsivity-based thrifty eating phenotype and the protective role of n-3 PUFAs intake in adolescents.

Roberta Sena Reis[1], Roberta Dalle Molle[1], Tânia Diniz Machado[1], Amanda Brondani Mucellini[3], Danitsa Marcos Rodrigues[2], Andressa Bortoluzzi[2], Solange Mara Bigonha[4], Rudineia Toazza[2], Giovanni Abrahão Salum[3], Luciano Minuzzi[5], Augusto Buchweitz[6,7,9], Alexandre Franco[6,7,8], Maria do Carmo Gouveia Pelúzio[4], Gisele Gus Manfro[2,3], Patrícia Pelufo Silveira[1,2]

ABSTRACT

Impulsivity and poor inhibitory control are important behavioral traits moderating non-adaptive feeding in intrauterine growth restriction (IUGR) children. We hypothesized that IUGR affects brain responses to palatable foods in a brain fMRI task and DHA could moderate the association between IUGR and brain responses and/or behavioral responses to palatable foods, decreasing non-adaptive behaviors. A brain fMRI in a task facing the visualization of palatable foods, neutral foods and neutral items had brain activation as the outcome, serum DHA and birth weight ratio (BWR) as continuous predictors (multiple regression). In the contrast Palatable Food>Neutral Items we found an activation in the right superior frontal gyrus with BWR as the most important predictor; the lower the BWR (indicative of IUGR) the greater the activation of this region involved in impulse control/decision making facing the viewing of palatable food pictures vs. neutral items. At the behavioral level, a GLM model predicting External Eating using the Dutch Eating Behavior Questionnaire showed a significant interaction between DHA and

IUGR status; in IUGR individuals, the higher the serum DHA, the lower External Eating. In conclusion, we suggest that IUGR moderates brain responses when facing stimuli related to palatable foods, activating an area related to impulse control. Moreover, higher intake of n-3 PUFAs can protect IUGR individuals of developing inappropriate behaviors, especially decreasing intake in response to external food cues in adolescents/young adults.

Keywords: n-3 PUFAs, intrauterine growth restriction, feeding behavior.

Developmental Neuropsychology

A role for the cingulate cortex in dyslexia: activation and underconnectivity in a study of Brazilian children.

Abstract

The goal of the present study was to investigate functional and connectivity differences in dyslexic children relative to typical readers in the brain. The results show (1) more activation of the anterior cingulate cortex for typical readers; and (2) decreased connectivity in dyslexic's occipitotemporal (visual word form area) region and the posterior cingulate cortex. The results suggest executive control processes associated with typical reading development, and impaired connectivity between a key area for reading and the brain's posterior cingulate cortex. The results are discussed in the light of noninvasive brain imaging evidence on atypical brain function in dyslexia.

Keywords

Dyslexia; fMRI; Visual Word Form Area; Cingulate Cortex

INTERACTION BETWEEN STRESS RESPONSIVENESS AND INSULIN SENSITIVITY: EFFECT ON SATIETY AND EATING BEHAVIOR IN ADOLESCENTS

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ABSTRACT

Introduction: The modern lifestyle is associated with daily exposure to stressful events. Increasing exposure to stress often induces higher consumption of so-called comfort foods and decreased intake of fruits and vegetables. Thereby, obesity can be related to behavioral and metabolic modifications in response to stress. **Methods:** Twenty-four teenagers were assessed throughout the Dutch Eating Behaviour Questionnaire (DEBQ) in order to evaluate feeding behavior. Blood was collected after fasting to quantify insulin levels. Salivary samples were collected for cortisol measurement before, shortly after, and 30 minutes after the exposure to an acute stressor. Meal-induced satiety was evaluated by measuring subjective feelings of hunger before and after a standard snack. **Results:** There was a significant interaction between the stress responsiveness and insulin sensitivity in our sample with an effect on eating behavior. We found an increase in emotional eating in response to stress in insulin-resistant adolescents, but not in those having normal insulin sensitivity [Wald = 4.394; df = 1; P = 0.036]. This interaction was specific for emotional eating and did not affect meal-induced satiety scores. **Conclusion:**

This study demonstrates that the metabolic profile related to insulin sensitivity plays a decisive role in eliciting the cortisol effect on emotional eating. This finding emphasizes the development of non-eating strategies to deal with stress as important tool in the management of obesity, which is often seen in concomitance with insulin resistance. The chronic and recurrent use of comfort foods to relieve stress symptoms can be a perpetual driver of obesity in these individuals. Adding new strategies to cope with stress, allied with reasonable nutritional guidance, appears to be essential to controlling weight gain in this population.

Keywords: stress, insulin, satiety, eating behavior, obesity.

Interaction between perceived maternal care, anxiety symptoms and the neurobehavioral response to palatable foods in adolescents

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Abstract

Introduction: Studies in rodents have shown that early life trauma leads to anxiety and increased stress responses to threatening situations and modifies food intake in a new environment. However, these associations are still to be tested in humans **Objectives:** We aimed at verifying complex interactions among anxiety diagnosis, maternal care and baseline cortisol on food intake in a new environment in humans. **Methods:** A community sample of 32 adolescents and young adults was evaluated for psychiatric diagnosis using standardized interviews, maternal care using the Parental Bonding Instrument (PBI), caloric consumption in a new environment (meal choice at a snack bar) and salivary cortisol. They also performed a brain fMRI task including the visualization of palatable foods vs. neutral items. **Results and discussion**: We found a three-way interaction between anxiety diagnosis, maternal care and baseline cortisol levels on total calories consumption (snack) in a new environment. This interaction means that for those with high maternal care there were no significant associations between cortisol levels and food

intake in a new environment. However, for those with low maternal care, which have an anxiety disorder (affected), cortisol was associated with higher food intake; whereas for those with low maternal care, which did not have an anxiety disorder (resilient), cortisol was negatively associated with lower food intake. In addition, higher anxiety symptoms was associated with a decreased activation in superior and middle frontal gyrus when visualizing palatable vs. neutral items only in those reporting high maternal care. These results in humans mimic experimental research findings and demonstrate that a combination of anxiety diagnosis and maternal care moderate the relationship between HPA axis functioning and feeding behavior in adolescents and young adults.

Keywords: poor maternal care; feeding behavior; obesity; anxiety; functional fMRI

Title Page

Title: Intrauterine growth programming of adolescent feeding behavior and related brain mechanisms

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Abstract

Low birth weight for a given gestational age (SGA) is associated with an increased preference for palatable foods from the beginning of life to adulthood, which could contribute to increased risk for developing obesity and related chronic diseases. We aimed at investigating the effect of SGA on adolescents' feeding behavior, as well as verify changes in brain resting state connectivity in regions that may be related to the behavioral changes. Participants were classified in SGA or controls and had anthropometric data and feeding behavior (food choice task, in which everyone received a monetary value to purchase a snack; and Dutch Eating Behavior Questionnaire - DEBQ) assessed. Resting state fMRI was also performed, and connectivity between brain regions related to reward and self-control were analyzed. We showed that healthy SGA adolescents have a different behavior for buying a snack and lower resting state connectivity for a network of areas that includes the orbitofrontal cortex, dorsolateral prefrontal cortex, dorsal striatum, and amygdala. We also observed that the restrictive eating dimension of the DEBQ increases with the decrease of birth weight ratio and the increase of body mass index. This study confirms the hypothesis that SGA is associated to changes in feeding behavior. It also introduces the idea that SGA individuals, when compared to controls, have a different brain resting state connectivity for a network of areas related to reward and decision-making.

Keywords: small for gestational age; feeding behavior; functional connectivity; resting state fMRI

Capítulos de livro em colaboração publicados durante o Doutorado

SALLES, J. F.; HAASE, V. G.; MALLOY-DINIZ, L. F. (Org.). **Neuropsicologia do desenvolvimento**: infância e adolescência. Porto Alegre: Artmed, 2016.

- ***Aspectos Neuropsicológicos nos Transtornos de Ansiedade na infância e na adolescência***

  Autores*: Rafaela Behs Jarros;* ***Rudineia Toazza****; Gisele Gus Manfro.*

- ***Ambulatório de aprendizagem do projeto ACERTA (Avaliação de Crianças Em Risco de Transtorno de Aprendizagem): métodos e resultados em dois anos***

  Autores*: Adriana Correa Costa;* ***Rudineia Toazza****; Ana Bassôa; Mirna Wetters Portuguez; Augusto Buchweitz.*