UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: PSIQUIATRIA

TESE DE DOUTORADO

TRANSTORNOS MENTAIS COMUNS NA INFÂNCIA: ESTUDO DE MECANISMOS GENÉTICOS E NEUROPSICOLÓGICOS

Giovanni Abrahão Salum Júnior

Orientadora: Profa. Dra. Gisele Gus Manfro

Co-orientador: Prof. Dr. Luis Augusto Paim Rohde

Porto Alegre, Agosto de 2012

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

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A well-known scientist (some say it was Bretrand Russel) once gave a public lecture on astronomy. He described how the earth orbits around the sun and how the sun, in turn, orbits around the center of a vast collection of stars called our galaxy. At the end of the lecture, a little old lady at the back of the room got up and said: "What you have told us is rubbish. The world is really a flat plate supported on the back of a giant tortoise." The scientist gave a superior smile before replying, "What is the tortoise standing on?" "You're very clever, young man, very clever," said the old lady. "But it's turtles all the way down!"

—Hawking, 1988

All models are wrong, but some are useful.

—Box, 1979

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Para meu avô Abrahão Salum Netto.
Por me ensinar a relatividade das verdades
e a importância das pessoas.

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SUMÁRIO

ABREVIATURAS E SIGLAS

TDAH	Transtorno de Déficit de Atenção/Hiperatividade
TOD/TC	Transtorno Opositor Desafiante/Transtorno de Conduta
RDoC	*Research Domain Criteria*
NIMH	*National Institute of Mental Health*
TOC	Transtorno Obsessivo Compulsivo
TAG	Transtorno de Ansiedade Generalizada
TEPT	Transtorno de Estresse Pós-Traumático
LgLg	Homozigose para o alelo longo do transportador da serotonina
TEA	Transtornos do Espectro Autista
PLD	Potencial de Longa Duração
DLD	Depressão de Longa Duração
BP	*Basic Processing*
IB-EF	*Inhibitory Based Executive Function*
DM	*Diffusion Model*
ADHD	*Attention Deficit/Hyperactivity Disorder*
ODD/CD	*Oppositional Defiant Disorder/Conduct Disorder*
TDC	*Typically Developing Children*
2C-RT	*Two Choice Reaction Time*
CCT	*Conflict Control Task*
GNG	*Go/No-Go*
RT	*Reaction Time*
DAWBA	*Development and Well-Being Assessment*
CBCL	*Child Behavior Checklist*
FHS	*Family History Screen*
MINI	*Mini International Neuropsychiatric Interview*

WISC	*Weschler Intelligence Scale for Children*
Q	Variabilidade *trial-to-trial* no tempo de não decisão
Ter	Média do tempo de não decisão
a	Separação dos limiares de resposta
e	Variabilidade *trial-to-trial* na eficiência de processamento
v	Média da eficiência de processamento
MANCOVA	*Multivariated Analysis of Covariamce*
ANCOVA	*Analysis of Covariance*
IQ	*Intelligence Quotient*

RESUMO

A psiquiatria moderna tem avançado no sentido de não mais apenas focar-se em descrever as síndromes psiquiátricas clínicas, mas também no intuito de entender os mecanismos pelos quais processos biológicos e psicológicos disfuncionais podem levar a trajetórias atípicas de desenvolvimento. Os quatro artigos desta tese se inserem dentro deste contexto e procuram buscar os mecanismos genéticos (artigo #1) e neuropsicológicos (artigos #2, #3 e #4) envolvidos em transtornos mentais comuns na infância. Eles se utilizam dos dados de dois grandes estudos de base comunitária e que integram tanto avaliações psiquiátricas como uma série de avaliações neuropsicológicas e biológicas. O primeiro artigo teve a intenção de estudar o papel moderador do estágio puberal no risco conferido por variações genéticas na região promotora do gene transportador da serotonina para os sintomas de depressão na adolescência. Este estudo mostrou que, apenas nos adolescentes pós-púberes (mas não em pré-púberes e púberes), as variantes de alta expressividade desse polimorfismo (LgLg) são protetoras para os sintomas de depressão na adolescência. Esse artigo avança na compreensão mecanística dos sintomas depressivos por demonstrar que variações genéticas podem apenas exercer seus efeitos de risco e proteção após períodos de regulação da sua expressão, como os que ocorrem de forma programada na puberdade. O segundo artigo aborda a orientação da atenção para estímulos ameaçadores (faces de raiva) e recompensadores (faces de felicidade). Em uma grande amostra de sujeitos da comunidade (n=2512), este estudo mostrou que sintomas de internalização na infância (como ansiedade e depressão), estão associados a vigilância para estímulos ameaçadores. Além disso, o efeito dos sintomas internalizantes foi diferente dentro de cada grupo de psicopatologia. Enquanto crianças com transtornos do estresse (ansiedade generalizada, depressão e estresse

pós-traumático) mostraram vigilância para estímulos ameaçadores, as crianças com fobias evitaram esses estímulos. Este estudo avança na compreensão mecanística destes transtornos, no sentido de demonstrar que o tipo da psicopatologia pode interferir na direção da orientação da atenção. O terceiro artigo tem a intenção de estudar os mecanismos de processamento básico (p.ex, preparação motora, eficiência de processamento) e de controle inibitório (capacidade de inibir um estímulo quando há uma forte tendência para executá-lo) no Transtorno de Déficit de Atenção/Hiperatividade (TDAH). Esse artigo avança no entendimento dos mecanismos relacionados ao TDAH ao demonstrar que uma pior eficiência de processamento é um déficit específico do TDAH e não encontrado em qualquer outro transtorno psiquiátrico investigado. Além disso, desafia a hipótese executiva do TDAH, embasada no controle inibitório, ao mostrar que todos os achados nessas tarefas foram explicadas por déficits em processamento básico. O quarto artigo é desenhado para testar a hipótese de dimensionalidade do TDAH. Este estudo confirma que déficits neuropsicológicos encontrados como sendo característicos do TDAH estão associados com desatenção e hiperatividade/impulsividade em sujeitos de desenvolvimento típico. Desta maneira provê uma evidência fisiopatológica de que mecanismos de processamento básico estão envolvidos em todo o espectro de problemas atencionais e de hiperatividade/impulsividade. A compreensão dos mecanismos de doença na psiquiatria moderna é imperativa. A combinação das neurociências à clínica psiquiátrica apresenta-se como uma alternativa promissora de avançar o conhecimento, sem perder os referenciais teóricos que movem o campo adiante.

ABSTRACT

Modern psychiatry is focused not only in describing psychiatric clinical syndromes, but also in understanding the biological and psychological dysfunctional mechanisms that may lead to atypical developmental trajectories. The four papers of this thesis are based on this concept and look for finding genetic mechanisms (paper #1) and neuropsychological mechanisms (papers #2, #3 and #4) involved in psychiatric disorders in childhood. These studies were performed in two large community-base studies that integrate both clinical assessment and a variety of neuropsychological evaluations and biological measures. The first paper had the objective to study the role of puberty as a moderator for the risk conferred by genetic variations in the promoter region of the serotonin transporter for depressive symptoms in adolescence. This study showed high expression variations of this polymorphism (LgLg) are protective for depressive symptoms only in post-pubertal adolescents (but not in pre-pubertal and pubertal). This study advances in the comprehension of depressive symptoms suggesting that genetic variations may exert their risk and protective effects only after some periods of regulation of their expression, like the programed regulation period that takes place during and after puberty. The second paper focus on attention orienting to threats (angry faces) and rewards (happy faces). In a large sample of subjects from the community (n=2512), this study showed that internalizing symptoms in childhood (such as symptoms of anxiety and depression) were associated with vigilance to threating stimuli. Besides that, the effect of internalizing symptoms was different in each different psychopathological group. Whereas in children with distress-related disorders (generalized anxiety, depression and post-traumatic stress disorder) internalizing symptoms increased vigilance toward threats, in children with fear-related disorders internalizing symptoms were associated

with threat avoidance. This study adds to the understanding that different forms of psychopathology may influence the direction of attention orienting for being towards or away threats. The third paper aimed to study basic processing mechanisms (*e.g.*, motor preparation, processing efficiency) and inhibitory-based executive function (ability to inhibit a stimulus when there is a strong tendency to execute it) in Attention Deficit/Hyperactivity Disorder (ADHD). This paper helps understanding the mechanisms associated with ADHD in demonstrating that poorer processing efficiency in tasks with no executive component is a specific deficit of ADHD, which is not found in any other psychiatric disorder. Besides that, it challenges the inhibitory-based executive hypothesis of ADHD in showing that all executive deficits were fully explained by deficits in basic processing. The forth paper intends to test the hypothesis that ADHD is a dimensional disorder taking evidence from deficits in basic processing. This study corroborates that the same neurocognitive deficits in basic processing that were found to be characteristic to ADHD were also associated with attention and hyperactivity/impulsivity in typically developing children. This study provides pathophysiological evidence that deficits in basic processing efficiency are involved in the whole spectrum of inattention and hyperactivity/impulsivity. The mechanistic comprehension in modern psychiatry is imperative. The combination of neurosciences to clinical psychiatry is a promising strategy to advance in scientific knowledge without loosing the theoretical references and helps moving the field forward.

1. APRESENTAÇÃO

Este trabalho consiste na tese de doutorado intitulada "Transtornos Mentais Comuns: Estudo de Mecanismos Genéticos e Neuropsicológicos", apresentada ao Programa de Pós-Graduação em Ciências Médicas: Psiquiatria da Universidade Federal do Rio Grande do Sul, em 14 de Agosto de 2012.

Os estudos oriundos dessa tese foram desenvolvidos dentro de dois grandes projetos de base comunitária, que integram avaliações de psiquiatria clínica, avaliações nutricionais, neuropsicológicas, de marcadores biológicas (genéticos e marcadores periféricos) e tem o intuito de avançar na descrição dos mecanismos fisiopatológicos envolvidos nos transtornos mentais comuns. O artigo #1 foi realizado em uma sub-amostra de pacientes avaliados pelo projeto "*Avaliação Multidimensional e Tratamento da Ansiedade em Crianças e Adolescentes"*. O desenho deste projeto foi publicado em formato de artigo e encontra-se na seção de anexos (artigo anexo #5) . Os artigos #2, #3 e #4, foram realizados em amostras de pacientes avaliados pelo projeto "*Coorte de Escolares de Alto Risco para Transtornos Psiquiátricos na Infância e Adolescência"*, um dos maiores projetos já realizados no país na infância e adolescência. Este projeto triou aproximadamente 10.000 famílias e está seguindo 2.512 crianças (1.500 de alto risco para o desenvolvimento transtornos psiquiátricos) com avaliações genéticas, neuropsicológicas, de marcadores biológicos e de neuroimagem estrutural e funcional. Uma descrição breve deste projeto também se encontra na seção de anexos.

Abaixo descreve-se brevemente o racional para a elaboração das questões de pesquisa de cada um dos artigos que compõe esta tese.

Desde muito cedo no desenvolvimento, genes e ambiente modelam os circuitos cerebrais de forma indissociada e são responsáveis, em última análise, pelas

manifestações das emoções e comportamentos em todo seu espectro (típico e atípico). No entanto, os mecanismos pelos quais esses genes atuam ao longo do desenvolvimento gerando trajetórias atípicas, isto é, crianças com problemas com seus sentimentos e comportamentos, ainda é pouco entendido. A puberdade é um marco na adolescência, caracterizada tanto pelo aumento da incidência da depressão quanto para o início da diferença de prevalência entre os gêneros. Levando esse fato em consideração, **o primeiro estudo** investiga a associação entre um polimorfismo encontrado na região promotora do transportador da serotonina e sintomas depressivos em crianças em diferentes estágios do desenvolvimento puberal (artigo #1). O intuito foi testar a hipótese de que a puberdade pudesse agir como um evento moderador do efeito conferido por essas variações genéticas no risco de sintomas depressivos.

Evoluções no campo da genética psiquiátrica, por sua vez, através de varreduras do genoma inteiro, evidenciaram que poucos genes se mostraram associados com transtornos psiquiátricos de forma consistente. Além disso, os genes consistentemente associados com transtornos psiquiátricos não são específicos para esses transtornos e são encontrados em condições psiquiátricas distintas (como autismo e esquizofrenia, por exemplo). Esses achados impulsionaram o campo a procurar alternativas na definição dos fenótipos, tendo em vista que não é provável que um único gene esteja associado a um transtorno psiquiátrico como um todo.

Desta forma uma série de pesquisadores dedicaram-se a alternativas de uma nova caracterização do "fenoma" em psiquiatria, isto é, as unidades mínimas das funções mentais que podem ser estudadas sob o ponto de vista empírico. Desta maneira, a psiquiatria deu um passo além da fenomenologia clássica e passou a procurar os mecanismos fisiopatológicos que constituem as síndromes psiquiátricas, e

que, na maioria dos casos, transcendem as barreiras diagnósticas dos sistemas classificatórios. Evidências do crescimento dessa corrente podem ser observados em iniciativas como o *Research Domain Criteria (RDoC)* do *National Institute of Mental Health* (*NIMH*), um novo sistema nosológico que tem a intenção de descrever "funções básicas do cérebro" e suas associações com mecanismos biológicos de doença, como genes, moléculas e sistemas cerebrais. Os demais estudos apresentados neste trabalho se inserem dentro deste contexto.

O **segundo estudo** dedica-se a estudar a orientação da atenção para estímulos ameaçadores e recompensadores no ambiente. Dentre outras funções, a atenção é um mecanismo essencial para selecionar do ambiente aquilo que merece prioridade de processamento do cérebro. Estímulos ameaçadores e recompensadores tem prioridade para serem processados em relação a estímulos neutros. Tanto bases teóricas quanto evidências empíricas demonstraram de forma consistente que indivíduos com transtornos de ansiedade tem "*vieses*" na atenção para estímulos ameaçadores. Isto é, o limiar desses indivíduos para direcionar a atenção para ameaças é diminuído em relação a indivíduos não ansiosos, e alguns autores sugerem que eles também possuem dificuldade de desfocar a atenção desses estímulos depois que ela foi capturada por eles. Este estudo então teve a intenção de avaliar se os vieses na atenção relacionados a ameaças (faces de raiva) e a recompensas (faces de felicidade) estão associados a sintomas internalizantes (ou emocionais) na infância, e, se essa associação varia de acordo com o tipo de transtorno psiquiátrico e com o tempo de exposição aos estímulos (artigo #2).

O terceiro estudo foca-se nos processos psicológicos envolvidos nos problemas atencionais na infância e adolescência. Em específico, na avaliação do processamento básico de informações (*encoding,* eficiência de processamento,

resposta/organização motora e aspectos estratégicos de resposta, como um estilo mais cauteloso ou impulsivo de resposta) e de função inibitória (capacidade de inibir uma ação quando a forte tendência para que ela seja realizada) – (artigo #3). Este estudo utilizou um método de análise sofisticado, chamado modelo de difusão, que permite a decomposição de todos esses componentes do processamento para análise detalhada em tarefas envolvendo tempo de reação. A intenção foi estudar se déficits no processamento básico de informações e no controle inibitório são específicos do Transtorno de Déficit de Atenção/Hiperactividade (TDAH) e não são encontrados em outros transtornos psiquiátricos comuns. Além disso, procurou entender se a comorbidade entre TDAH e Transtorno Opositor Desafiante/Transtorno de Conduta (TOD/TC), que é tanto comum, quanto grave, representa apenas os efeitos aditivos dos seus constituintes ou se essa comorbidade representa, no que se refere ao processamento básico e controle inibitório, uma entidade clínica distinta.

O **último estudo** (artigo #4) tem a intenção de estudar a hipótese de dimensionalidade do TDAH, isto é, de que o TDAH representa um extremo da distribuição sintomática de desatenção e hiperatividade, e não uma entidade qualitativamente distintas. Neste estudo pretendeu-se investigar se os processos disfuncionais que caracterizam o TDAH estariam relacionados dimensionalmente com a desatenção e hiperatividade/impulsividade em todo o espectro de apresentação dos sintomas, desde crianças com desenvolvimento típico até casos de TDAH clinicamente diagnosticados.

Esta tese está organizada na ordem que segue: Introdução, Objetivos, Artigo #1 (publicado na revista *Journal of Psychiatric Research*), Artigo #2 (publicado na revista *Psychological Medicine*), Artigo #3 (a ser submetido para publicação na revista *Biological Psychiatry*) e Artigo #4 (a ser submetido para publicação na revista

Journal of the American Academy of Children and Adolescent Psychiatry), Conclusões e Considerações Finais e Anexos. Os anexos contém uma breve descrição dos projetos que deram origem a esses estudos e outros artigos em fisiopatologia dos transtornos mentais produzidos pelo autor durante o período do doutorado.

2. INTRODUÇÃO

"Os transtornos mentais são doenças crônicas dos jovens"

The WHO World Mental Health Survey Consortium, 2008

Transtornos mentais, neurológicos e de uso de substância constituem 13% do total do ônus relacionado às doenças humanas, ultrapassando o ônus causado por doenças cardiovasculares e pelo câncer (Collins *et al*., 2011). Os estudos transversais em saúde mental demonstraram que mais de metade dos pacientes com transtornos mentais relatam o início dos seus sintomas na infância e quase dois terços relatam início dos sintomas antes da adolescência (Kessler *et al*., 2007, Kessler *et al*., 2005). Diversos estudos prospectivos também demonstraram que na trajetória do desenvolvimento, tanto uma continuidade homotípica (p.ex., um transtorno ansioso na infância preceder um transtorno ansioso na vida adulta) quanto uma comorbidade sequencial (p.ex., o transtorno de déficit de atenção na infância preceder transtorno de personalidade antisocial na vida adulta) são uma constante dentro dos transtornos mentais (Babinski *et al*., 1999, Ferdinand and Verhulst, 1995, Hofstra *et al*., 2000, 2002, Kim-Cohen *et al*., 2003). Dentre os casos adultos diagnosticados com transtornos psiquiátricos até a meia idade, cerca de 75% receberam o diagnóstico antes dos 18 anos e cerca de 50% antes dos 15 anos (Kim-Cohen *et al*., 2003). Além disso, os transtornos psiquiátricos de início precoce estão associados a fatores de risco mais graves na infância (Geller *et al*., 1998, Jaffee *et al*., 2002, Moffitt and Caspi, 2001) e piores prognósticos na vida adulta (Jaffee *et al*., 2002, Moffitt *et al*., 2002, Rosario-Campos *et al*., 2001, Weissman *et al*., 1999, Wickramaratne *et al*., 2000).

Os transtornos psiquiátricos na infância e adolescência são extremamente prevalentes, afetando aproximadamente 1 em cada 10 crianças brasileiras (Anselmi *et*

al., 2010, Fleitlich-Bilyk and Goodman, 2004). Além disso, são também a principal causa de incapacidade relacionada à saúde nessa faixa etária com efeitos duradouros ao longo da vida. No entanto, as demandas de saúde mental de crianças e adolescentes são largamente negligenciadas, especialmente em países de baixa renda (Kieling *et al*., 2011).

A investigação das bases neurobiológicas dos transtornos mentais é um dos principais focos das pesquisas em saúde mental na atualidade, especialmente nos períodos mais sensíveis para o neurodesenvolvimento, como a infância e adolescência. As pesquisas recentes em saúde mental estabelecem dois princípios organizadores: (1) a maioria das doenças crônicas tem rotas na infância; (2) os transtornos mentais são resultados de diferenças individuais nas funções do cérebro (Insel and Fenton, 2005, Insel and Quirion, 2005, Pine, 2007). A investigação e a avaliação de processos cerebrais envolvidos em trajetórias atípicas de desenvolvimento são o foco desta tese.

A introdução foi dividida em três seções. Na primeira serão apresentadas as principais ideias que contribuíram para as propostas de "mudança de paradigma" na psiquiatria moderna e que constituem a orientação principal das investigações desta tese. Na segunda, serão discutidos os modelos vigentes de causalidade em psiquiatria, com foco no papel dos genes, dos ambientes e de suas relações. Na terceira, será apresentada uma breve revisão acerca dos transtornos internalizantes e externalizantes na psiquiatria da infância e adolescência, com foco nos transtornos de ansiedade e no TDAH.

2.1. Mudanças de paradigma na psiquiatria moderna

A transição de uma psiquiatria embasada primariamente na fenomenologia para a busca dos mecanismos relacionados aos transtornos mentais pode ser encarada como uma mudança de paradigma na psiquiatria moderna. Nesta seção serão apresentados algumas considerações que permeiam essa mudança, iniciando por breves comentários relacionados aos modelos de explicação em psiquiatria, referindo-se, em especial, às ideias do psiquiatra contemporâneo Kenneth Kendler. Em seguida serão apresentadas algumas considerações acerca da nosologia psiquiátrica. Por fim, serão discutidas implicações dessas propostas para as revisões dos sistemas classificatórios vigentes e para o aparecimento de novos sistemas nosológicos, como o *Research Domain Criteria (Insel et al., 2010)*.

2.1.1. Modelos de Explicação em Psiquiatria

Em uma revisão acerca dos modelos explicativos para estudar e entender os transtornos psiquiátricos, Kenneth Kendler (Kendler, 2008), um dos psiquiatras mais influentes do nosso tempo, expõe suas visões acerca dos desafios que os cientistas encontram para formular modelos com o intuito de estudar transtornos psiquiátricos. Um dos principais problemas apontados por ele vem das visões tradicionais de ciência oriundas da física. Essas visões sugerem que iremos encontrar um número determinado de princípios que irão explicar toda a complexidade normal e patológica do comportamento humano. Kendler argumenta, entretanto, que esse modelo não é facilmente aplicável para a biologia e ciências sociais relevantes para a psiquiatria, e de que os processos causais em psiquiatria não podem ser entendidos como resultados de apenas uma perspectiva ou um conjunto de leis básicas. Advoga que uma abordagem em múltiplos níveis de causalidade (p.ex., biológico/genético, psicológico,

social e cultural/econômico), e focada em descrever os mecanismos de doença, tem maior chance de avançar o campo da psiquiatria no sentido de melhor entender suas causas. Como as moléculas formam as membranas, os neurônios formam os circuitos, esses circuitos neuronais são responsáveis por funções complexas que formam os indivíduos e os indivíduos formam a sociedade, certamente pode-se encarar causalidade em múltiplos níveis permeando diversas disciplinas.

Kendler propõe que para avançar no entendimento das causas das doenças psiquiátricas será necessário um processo contínuo de "decomposição e remontagem". Ele descreve que acha impossível que a partir do nível celular será possível montar os complexos mecanismos psicológicos envolvidos nos transtornos mentais (estratégia "*bottom-up"*). Afirma que uma abordagem "*top-down*" será mais promissora no entendimento desses fenômenos, esta abordagem consiste em partir de modelos teóricos oriundos da psicologia para os correlatos neurobiológicos envolvidos nesses processos. Salienta que isso não será um processo unidirecional, pois os psicólogos nem sempre entenderão o constructo da maneira biologicamente mais apropriada – portanto, a biologia e psicologia terão que se desenvolver de uma forma harmoniosa e conjunta.

O teórico ressalta que isso não quer dizer que estratégias mais "reducionistas" de ciência, isto é, que encaram que apenas através da biologia celular e molecular, não podem fornecer "*insights"* para o entendimento dos transtornos psiquiátricos. Terapias efetivas podem ser desenvolvidas a partir de pesquisas básicas (como a partir de genes associados a esses transtornos), sem se ter nenhuma noção acerca de como as variações genéticas produzem os sintomas associados. Além do mais, alternativas de tratamento efetivas, tais como a Terapia Cognitivo Comportamental, são oriundas

de constructos psicológicos que não levavam em consideração aspectos biológicos. No entanto, essas perspectivas irão nos deixar apenas com uma parte da história.

O modelo etiológico proposto é o "pluralismo de base empírica" ele (Kendler, 2012) que responde por ser um modelo aberto para as evidências científicas em diferentes níveis de causalidade que podem ser pensados em psiquiatria. De forma crítica, é embasado não naquilo que "gostaríamos que o mundo fosse", mas em como os fatores de risco ("*difference-makers for psychiatric illness"*) estão distribuídos nas populações. Esse modelo difere do modelo Bio-Psico-Social proposto por Engel (Engel, 1977), por não assumir que são essas as dimensões específicas relacionadas aos transtornos psiquiátricas e estar aberto para a evidências que estão por vir.

2.1.2. Nosologia e sistemas de classificação

Provavelmente mais do que qualquer outro indivíduo, Emil Kraepelin (1856-1926) forneceu as bases fenomenológicas que compõem a maneira pela qual hoje podemos ver as síndromes psiquiátricas (Kendler and Jablensky, 2010). Embora este autor seja muito conhecido por suas formulações diagnósticas e pela fenomenologia, pouco se sabe acerca da sua visão no que concerne a nosologia psiquiátrica, e acerca do seu desejo de encontrar a "verdadeira natureza" ou a "estrutura essencial" dos transtornos psiquiátricos.

As ambições nosológicas de Kraepelin eram embasadas em ideias enunciadas por outros autores como Griesinger (Griesinger, 1861) e Kahlbaum (Kahlbaum, 1863). No entanto, Kraepelin foi pioneiro em buscar alianças com "ciências auxiliares" - como a psicologia, neuropatologia, farmacologia e genética – como um meio para melhor entender os fenômenos psiquiátricos que observava na clínica. Além disso, Kraepelin foi o primeiro psiquiatra a ser treinado em uma nova disciplina

intitulada “psicologia experimental” pelo seu fundador Wundt (Wundt, 1874) e, precocemente, reconheceu o potencial da psicologia para complementar as observações clínicas e a patologia (Kendler and Jablensky, 2010, Kraeplin, 1887). Embora Kraepelin tenha buscado uma “classificação natural” das doenças psiquiátricas, ele também reconheceu precocemente que com o nível de conhecimento etiológico disponível no início do século XX isso não seria possível no seu tempo de vida. No entanto, mesmo há mais de 100 anos atrás, esse autor já enunciava as bases dos modelos etiológicos ainda usados atualmente, que entendem as doenças mentais como doenças multifatoriais, emergindo da “dificuldade de separar ação e interação de causas internas e externas” (Kendler and Jablensky, 2010).

As diversas doenças médicas são definidas em diferentes níveis de abstração, como por exemplo: patologia estrutural (p.ex., colite ulcerativa), apresentação sintomática (p.ex., cefaleias), desvios das normas populacionais (p.ex., hipertensão), e agente etiológico (p.ex., pneumonia pneumocócica). Os transtornos mentais, por sua vez, já foram definidos por uma variedade de conceitos (p.ex., sintomas, etiologia, prejuízos funcionais, etc.), no entanto, nenhuma definição especifica com delimitadores precisos para o conceito de doença mental é um consenso na literatura acerca do tema (Stein *et al.*, 2010).

A biologia tem lidado com problemas classificatórios desde sua origem. Na botânica, nos séculos XVI e XVII, por exemplo, diversas classificações foram propostas no intuito de capturar “*o plano de Deus para a criação*” (Sloan, 1972). Discussões acaloradas tomaram conta da comunidade científica por vários anos em virtude de diversas classificações partindo de características únicas e com agrupamentos completamente diferentes.

O filósofo inglês John Locke escreveu sobre as questões taxonômicas na botânica no seu trabalho de 1690 entitulado *Essay Concerning Human Understanding* (Locke, 1870). O trecho abaixo descreve as evoluções do século XIX acerca da questão taxonômica.

> *"...the very claim that a natural classification was a worthwhile goal of scientific investigation had...rested on the assumption that there is some 'natural' arrangement of organisms, and furthermore that this arrangement ultimately can be known by man... [However] the grouping of objects into different classes and kinds cannot claim to be based on the knowledge of some real essence or substantial form... There can be no possibility of weighting one character or structure as being more indicative of the real essence than any other."* (Sloan, 1972)

Na psiquiatria não foi diferente, e nos séculos XIX e XX diversos *experts* como Pinel, Griesinger, Kahlbaum, Krafft-Ebing, Wernicke, Kraepelin e Bleuler (Kendler, 2009) propuseram suas nosologias psiquiátricas baseando em pressupostos de características elementares dos transtornos psiquiátricos. Kraepelin (1987), por exemplo, focou-se no curso da doença enquanto que Bleuler (1950) assumiu que as diferentes manifestações da esquizofrenia por exemplo eram resultado de anormalidade específicas mais profundas. Nos termos de hoje podemos dizer que os autores se focaram em diferentes validadores.

Como afirma Kendler, se nossa tarefa como pesquisadores fosse determinar os elementos que compõe a tabela periódica, "os cientistas" que realizaram a classificação desses elementos não seriam exatamente determinantes do resultado final. No entanto, as doenças psiquiátricas, assim como as espécies, são constructos pouco definidos, que mudam radicalmente quando vistos sob ângulos diferentes. Elas

são extremamente vulneráveis ao "efeito do observador" (Kendler, 2009) e portanto, sujeitos a diversas classificações com pouco valor heurístico.

Para os que convivem com pacientes com problemas relacionados ao comportamento e às emoções, isso não torna esses fenômenos menos reais. Acerca da nosologia psiquiátrica Kendler faz algumas considerações:

> *"A defining feature of the mature sciences is their cumulative nature. ... For critics of psychiatric diagnosis who view them as social constructions, this is an incoherent Project. If there is no truth out there, we cannot expect to get closer to it. For those who adopt either realist or pragmatist perspectives on psychiatric nosology – that there are thing or inter-related sets of things out there in the real world that correspond to individual psychiatric illnesses - it is a more rational and, I would argue, vital task."(Kendler, 2009)*

2.1.3. Novos sistemas classificatórios

A revisão dos principais sistemas classificatórios impulsionou a discussão na psiquiatria sobre a continuidade versus a mudança. Isto é, os sistemas classificatórios estariam prontos para adotar classificações mais embasadas em mecanismos etiológicos e abandonar as orientações fenomenológicas? Abaixo descreve-se as principais evoluções dessas discussões e o surgimento de alternativas como o *Research Domain Criteria*, que se propõe a atuar de forma paralela aos sistemas clínicos no ambientes de pesquisas que consideram a psiquiatria biológica.

A resposta para a pergunta acima para os principais sistemas classificatórios vigentes é não, nossos sistemas ainda não estão prontos para fazer essa transição. Por essa razão, no seu esquema de revisão, a 5ª. edição *Diagnostic and Statistical Manual for the Mental Disorders* (www.dsm5.org; DSM-5 - agora descrito com um algarismo

arábico ao invés dos romanos, para permitir atualizações mais frequentes como 5.1), adotou o modelo de "iteração epistêmica". O termo iteração vêm da matemática e é utilizado para descrever processos computacionais que geram uma série de estimativas acerca de um determinado parâmetro. Com um número suficiente de iterações, cada estimativa melhora a estimativa predecessora até que o processo se estabiliza como uma estimativa acurada do parâmetro. O termo epistêmica diz respeito à "aquisição de conhecimento". Isso quer dizer que as formulações diagnósticas só irão mudar se houver evidência suficiente para que isso aconteça. Embora isso lentifique o processo, é considerado um método mais "seguro" no intuito de não desviar dos conhecimentos já adquiridos até o momento acerca desses fenômenos.

Dessa maneira, mesmo assumindo que o sistema é fortemente influenciado pelas bases históricas fenomenológicas da psiquiatria, como o processo de iteração epistêmica não depende do ponto de partida, ele sempre converge para o mesma solução "correta". Essa decisão foi tomada pela interpretação por parte dos organizadores de que o campo ainda não estava pronto para a substituição do modelo nosológico/classificatório vigente, por um modelo alternativo (Kendler and First, 2010).

Uma alternativa do modelo de "iteração epistêmica" é a "mudança de paradigma". Em outras palavras essa dicotomia representa o dilema "continuidade" *versus* "mudança". A mudança de paradigma se insere no contexto de que evidências acumuladas de que o sistema vigente não funciona deveriam resultar em uma revolução científica. Na psiquiatria alguns modelos foram propostos como (1) uma definição clínica baseada em protótipos clínicos (embasados no argumento de que o uso clínicos dos manuais classificatório não se baseia em critérios e sim em

protótipos); (2) um modelo dimensional de psicopatologia (como já é utilizado em paralelo com o sistema vigente e de alguma maneira está sendo incorporado no DSM-5); (3) uma perspectiva "*bottom-up*" de evidências empíricas. Este último está sendo adotado de forma paralela pelo *National Institutes of Mental Health* – o *Research Domain Criteria (RDoC)* (Kendler and First, 2010).

O RDoC surge como uma alternativa paralela de fornecer as bases para um novo sistema classificatório que tem o intuito de integrar a neurociência moderna com a pesquisa em psicopatologia (Insel *et al.*, 2010, Insel, 2009, Sanislow *et al.*, 2010) . Dentre vários problemas que podem ser levantados para o DSM-IV (Regier *et al.*, 2009), dois são de especial atenção para essa discussão: o elevado número de categorias diagnósticas (num total de 365)(APA, 1994) e os elevados índices de co-ocorrência de transtornos psiquiátricos (Kessler *et al.*, 2012, Kessler *et al.*, 2011).

É improvável que haja 365 mecanismos de doença específicos para as 365 categorias diagnósticas presentes no DSM-IV. Além disso, os elevados graus de co-ocorrência nos levam a acreditar que várias dessas entidades clínicas compartilham mecanismos de doença comuns, tanto genéticos, como ambientais (Hyman, 2007).

Evidências da genética são categóricas em afirmar que os subtipos de transtornos psiquiátricos não são resultado de genes de pequeno efeito (Craddock *et al.*, 2009, Kendler, 2006). Como afirmam os modelos vigentes, as doenças psiquiátricas são multifatoriais. Elas não apenas são influenciadas por um conjunto diverso de fatores de risco, mas esses fatores de risco atuam em conjunto para produzir diferentes combinações de fatores suficientes combinados. Os mesmos mecanismos podem estar implicados em diferentes transtornos psiquiátricos e vários mecanismos podem estar relacionados a um transtorno em especial.

Embora o RDoC surja como um importante marco de direcionamento da psiquiatria moderna para a busca dos mecanismos de doença, esse processo, como já dito, não se inicia com ele. Diversas outras iniciativas de abordagens transdiagnósticas de psicopatologia são correntes na pesquisa em psiquiatria atual (Nolen-Hoeksema and Watkins, 2011). Duas nomenclaturas tem sido bastante utilizadas: o conceito de fenótipos intermediários e de endofenótipos (Cannon and Keller, 2006, Gottesman and Gould, 2003). Enquanto fenótipos intermediários representam funções neurocognitivas, processos emocionais que estão ligados ao desenvolvimento de sintomas; endofenótipos referem-se a fenótipos intermediários que são herdáveis (Gottesman and Gould, 2003).

No entanto, o RDoC vem suprir uma necessidade de construir sistemas e teorias que não estão estritamente ligadas aos diagnósticos de uma forma organizada e uniforme. Os objetivos desses sistemas são restritos aos ambientes de pesquisa. Dentre os objetivos desse novo sistema pode-se destacar três em especial: (1) identificar os componentes comportamentais fundamentais que podem estar presentes em vários transtornos e que são mais "amigáveis" para alternativas na neurociência; (2) integração com os fundamentos da genética, da neurobiologia, do comportamento, do ambiente e da experiência que compõe os transtornos mentais; (3) avaliação de todo o espectro do comportamento (do normal ao patológico).

Um sistema classificatório satisfatório deve integrar a pesquisa sobre as dimensões fundamentais do comportamento, os circuitos cerebrais que implementam esses comportamentos, e os componentes genéticos e epigenéticos que modelam o seu desenvolvimento. Embora o objetivo final seja prover melhores práticas de saúde para os pacientes psiquiátricos, esse é um objetivo de muito longo prazo, e esse sistema tem ambições apenas no meio de pesquisa e sem implicações clínicas diretas.

2.2. Bases Etiológicas dos transtornos mentais

Uma relação complexa entre genes e ambiente ao longo do neurodesenvolvimento

Embora as discussões filosóficas e epistemológicas acerca dos rumos da psiquiatria estejam em um momento crítico, o modelo etiológico geral das influências genéticas e ambientais tem sido de extrema valia para o avanço do campo. Segundo este modelo, os transtornos mentais são um resultado de uma interação complexa entre diversos genes e diversos ambientes. Estudos mostram que todos os transtornos mentais possuem um componente genético e que também todos possuem um componente ambiental importante, de forma indissociada e complementar. O neurodesenvolvimento é um processo multifacetado, dinâmico que envolve múltiplas interações entre genes e ambientes resultando em mudanças em curto e longo prazo na expressão gênica, interações celulares, formação de circuitos cerebrais, estruturas neurais e comportamento ao longo do tempo. Esse processo é maleável e constantemente influenciado por uma série de fatores internos e externos e qualquer um desses processos pode causar um desvio da trajetória normal do desenvolvimento cerebral, com consequências moleculares, sistêmicas, envolvendo a pessoa como um todo (Levitt and March, 2008).

A suscetibilidade genética de cada indivíduo com um transtorno pode variar muito e a expressão desses genes em um transtorno específico pode também depender da exigência colocada pelas adversidades ambientais (Caspi *et al*., 2005, Moffitt, 2005, Moffitt *et al*., 2005). Sendo assim, identificar variáveis ambientais responsáveis por manifestações psicopatológicas é tão importante quanto reconhecer suas causas genéticas (Plomin *et al*., 2001, Plomin and Kosslyn, 2001). Um mesmo fator genético

pode levar a diferentes transtornos psiquiátricos, e estes podem ter genes comuns entre si (Caspi and Moffitt, 2006). Ao mesmo tempo, fatores ambientais também podem apresentar influência genética, uma vez que a herança biológica pode influenciar na escolha de algumas experiências de vida (Jaffee and Price, 2007). Ou seja, é preciso examinar também as origens das experiências de risco e não apenas focar nos seus efeitos (Rutter and Sroufe, 2000).

Em se considerando a complexidade desses processos, apenas avaliações multidisciplinares que se utilizem dos conhecimentos da ciência básica e clínica se utilizando de instrumentos de diversas disciplinas como a bioinformática, neurogenética, biologia celular e molecular, psicologia, neurologia, psiquiatria e epidemiologia do desenvolvimento são capazes de fornecer dados mais completos acerca do entendimento da origem, manutenção, curso, prevenção e tratamento dos transtornos mentais (Levitt and March, 2008). Dentro desse contexto, são indispensáveis o estudo da genética, neuroimagem, neuropsicologia e dos fatores de risco ambientais, em conjunto com uma avaliação clínica detalhada das crianças e de suas famílias.

Os estudos em genética tiveram um papel importante na busca de alternativas de descrição do fenótipo que foram discutidos acima. Mesmo estudos com varreduras de todo o genoma falharam em achar resultados consistentes que associassem variações comuns no genoma às doenças psiquiátricas de uma forma global. Além disso, mesmo em áreas em que algumas variações genéticas foram mais consistentemente replicadas, como estudos de sequenciamento em autismo e na esquizofrenia, os mesmos genes estiveram implicados com um tamanho de efeito muito pequeno (Talkowski *et al*., 2012).

A partir de então, uma série de estudos começaram a avaliar o papel das variações genéticas nos processos psicológicos e não mais as associações das variações genéticas com as síndromes psiquiátricas como unidade de análise. A figura abaixo ilustra o papel dos genes em determinar circuitos cerebrais, que por sua vez estão relacionados a alguns processos cerebrais específicos que se agrupam na população e quando disfuncionais estão associados aos transtornos psiquiátricos.

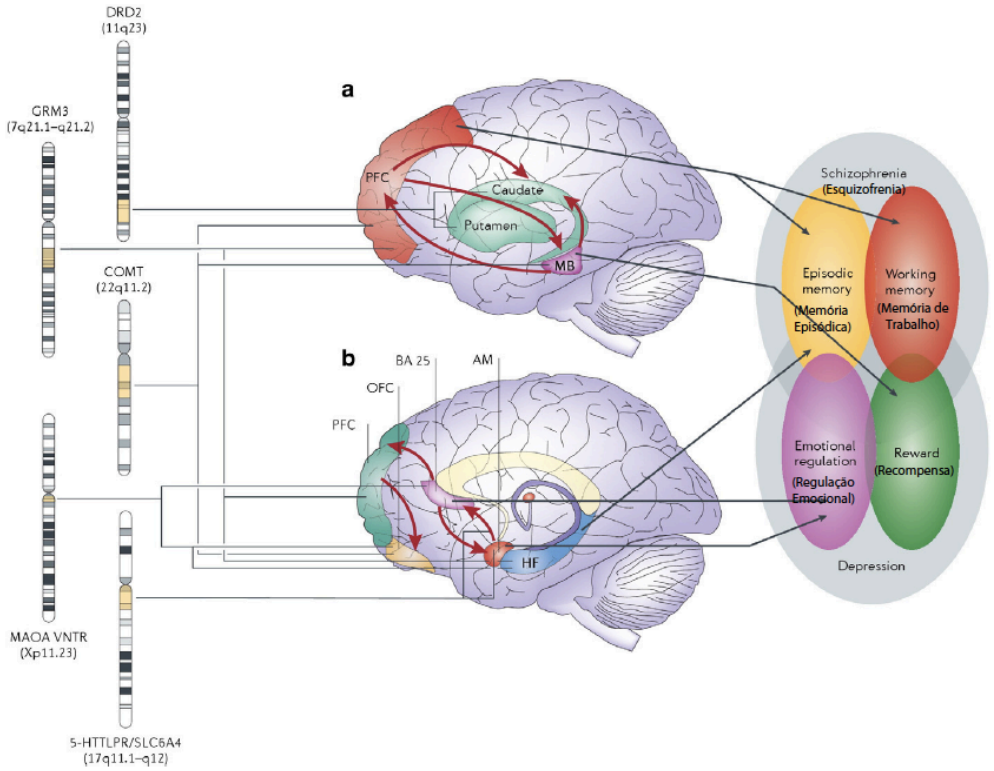

FIGURA 1 - A via complexa dos genes para o comportamento e o fenótipo da doença: mediação através da circuitaria cerebral. Múltiplas variações genéticas afetam, através da interação entre elas próprias e com o ambiente, múltiplos sistemas neuronais que estão ligados a vários domínios neuropsicológicos e do comportamento que estão disfuncionais, em proporções diferentes, nas doenças psiquiátricas. Estes circuitos, por sua vez, medeiam o risco para esquizofrenia e depressão e várias funções neuropsicológicas. Abreviações: BA, área 25 de Brodmann, HF, Formação Hipocampal; OFC, Córtex Orbito-frontal.

Figura traduzida de Tost e colaboradores (Tost et al., 2011)

Além disso, o campo avançou no sentido de incluir as diversas relações que o ambiente pode ter nessas variações do genoma e nas explicações de como estes fenômenos podem ocorrer. A figura abaixo também demonstra essa relação complexa ao longo do desenvolvimento.

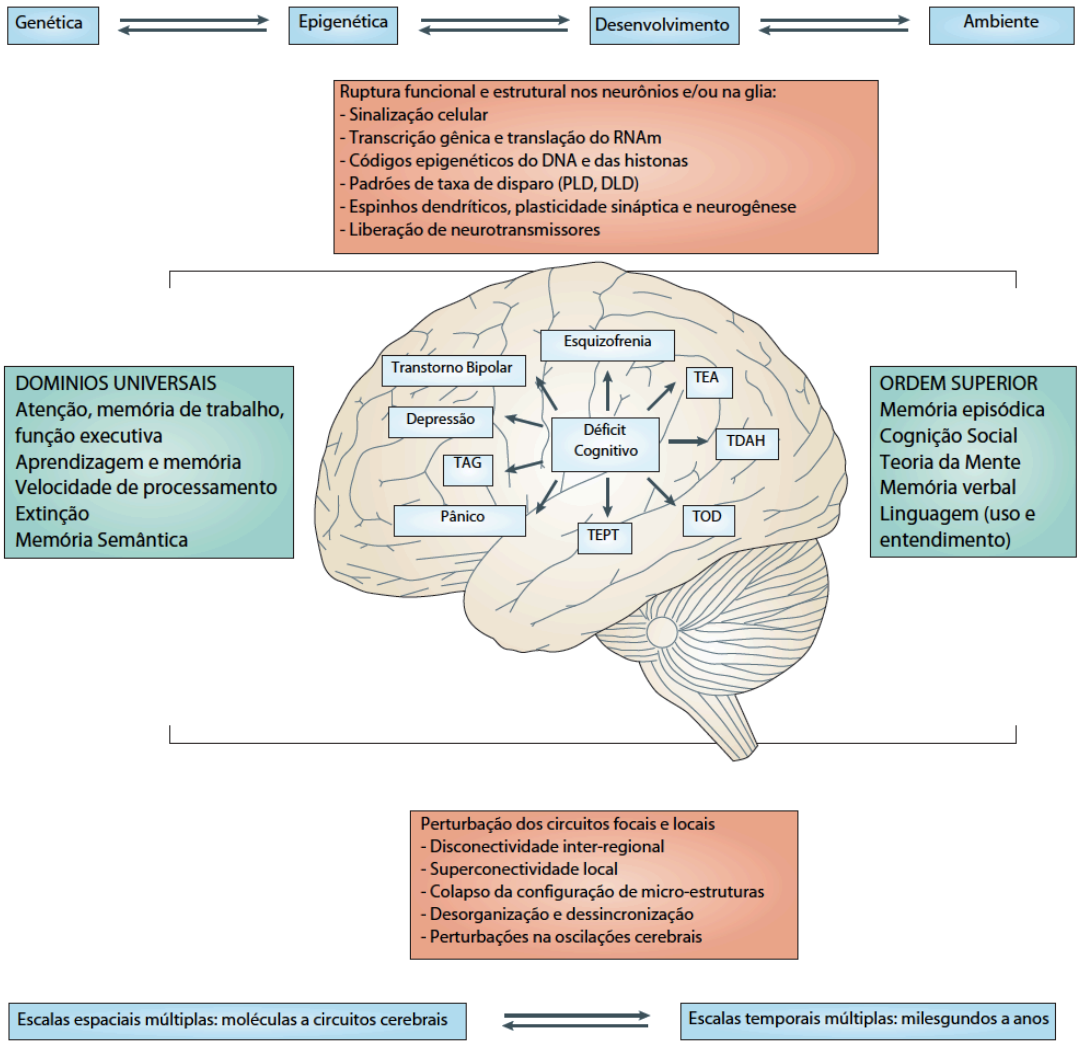

FIGURA 1 - Uma visão global da cognição e das suas alterações nos transtornos psiquiátricos. Transtornos psiquiátricos estão associados com padrões complexos e doença-específicos de disfunções cognitivas. Certos domínios podem ser considerados "Superiores" em termos de especialização e sofisticação natural. Eles estão bastante presentes em humanos se comparados a roedores e alguns estão também presentes em macacos e - refletindo convergência evolutiva - em pássaros, cetáceos e elefantes. Disfunções na cognição são provocadas por vários fatores genéticos, epigenéticos, desenvolvimentais e ambientais que interagem entre si. As mudanças são expressas tanto no nível do neurônio e da glia (desde alterações na transcrição gênica até mudanças no padrão de disparo neuronal) e no nível dos circuitos neurais (regiões cerebrais locais e interligadas). Disfunções por detrás dos déficits cognitivos são hierarquicamente e espacialmente diversas e atuam em uma escala temporal que vai desde milisegundos (por exemplo, disparo celular), horas (por exemplo, síntese protéica), até anos (arquitetura sináptica). Alguns fatores de susceptibilidade, como fatores germinativos e epigenéticos, podem ser passados para as gerações seguintes. Algumas causas de déficits cognitivo podem ser retificadas ou compensadas, mas mudanças nos circuitos do nível molecular ao nível sistêmico não são necessariamente reversíveis, portanto prevenção e identificação precoce são cruciais. TDAH, Transtorno de Déficit de Atenção/Hiperatividade; TEA, Transtornos do Espectro Autista; TAG, Transtorno de Ansiedade Generalizada; PLD, Potencial de Longa Duranção; DLD, Depressão de Longa Duração; TOC, Transtorno Obsessivo Compulsivo; TEPT, Transtorno de Estresse Pós-traumático.

Figura traduzida de Millan e colaboradores (Millan *et al.*, 2012).

2.3. Bases Fisiopatológicas dos transtornos mentais

O estudo dos processos mentais e dos correlatos neuroanatômicos e funcionais

Duas disciplinas são de especial interesse para a busca do entendimento da fisiopatologia dos transtornos mentais: (1) a neuropsicologia, que estuda os processos mentais que constituem unidades básicas de análise das funções do cérebro e a (2) neuroimagem, que tenta identificar correlatos neuroanatômicos e funcionais para essas funções e para explicar diferenças individuais no comportamento.

Estudos com **neuropsicologia** têm ajudado a entender os processos mentais relacionados aos transtornos psiquiátricos. Isso é de extrema importância, tendo em vista que é bastante provável que as variáveis biológicas, como genes e correlatos neuroanatômicos, estejam mais relacionados à unidades fenotípicas mais primárias do que a um transtorno como um todo.

Os estudos com **neuroimagem** são um dos focos principais dos estudos relacionados ao neurodesenvolvimento (Levitt and March, 2008). Na busca pelos substratos biológicos dos transtornos psiquiátricos, esforços consideráveis têm sido dirigidos na procura de evidências anatômicas e funcionais de anormalidades cerebrais em pacientes psiquiátricos. O aprimoramento de técnicas sofisticadas de neuroimagem, às quais possibilitam análises anatômicas, funcionais e moleculares do cérebro *in vivo*, representou um grande impulso para das especulações acerca dos mecanismos fisiopatológicos dos transtornos psiquiátricos.

A compreensão atual sobre o transtorno mental infantil deslocou-se de uma perspectiva estática, em que somente diagnósticos categóricos importavam, para um modelo em que uma abordagem dimensional explica melhor a heterogeneidade dos traços individuais na população (Hudziak *et al*., 2007). Portanto, tem sido crescentemente aceita uma fundamentação que enfatize uma perspectiva de

desenvolvimento como a chave para a compreensão das complexidades dos transtornos mentais (Levitt and March, 2008). Nesse contexto, a pesquisa sobre indivíduos em risco de desenvolverem transtornos mentais ou casos sub-clínicos adquire um papel essencial nos estudos voltados ao desenvolvimento de psicopatologia e resiliência em períodos sensíveis do neurodesenvolvimento.

Qual é o objetivo final de entender a fisiopatologia dos transtornos mentais?

O objetivo final é a oportunidade de desenvolver **estratégias de prevenção**. As intervenções preventivas universais, isto é, aplicadas em todas as crianças independentemente do seu status de risco, possuem uma limitação importante no sentido de provar sua efetividade, tendo em vista o baixo risco de desfechos negativos para a grande maioria das crianças tratadas. Por essa razão, os estudos de prevenção de transtornos mentais tem se voltado para as intervenções seletivas, isto é, aquelas realizadas em indivíduos selecionados, com alto risco para o desenvolvimento do transtorno. No entanto, não há critérios claros na literatura capazes de definir uma criança de risco para transtornos psiquiátricos.

Embora as grandes coortes em psiquiatria têm sido de fundamental importância para o estabelecimento de fatores de risco, tanto genéticos como ambientais (como suas possíveis associações), esses estudos são limitados pelo pequeno número de desfechos clínicos no seu seguimento, o que dificulta a avaliação de fatores proximais que possam ser alvos de intervenção clínica.

Só após a descrição clara de fatores de risco proximais, modelos médicos como os que já são utilizados com sucesso na cardiologia, por exemplo, serão possíveis de serem implementados na psiquiatria. Para que isso seja possível, a

descrição fisiopatológica é fundamental, no intuito de se entender as rotas biológicas relacionadas às trajetórias atípicas e como podemos modifica-las.

Esses objetivos foram também identificados como prioritários por um consórcio de pesquisadores, pacientes e clínicos que chama a atenção para as prioridades em pesquisa para melhorar a vida das pessoas com doenças mentais ao redor do mundo: Prioridade A - "identificar rotas causais, fatores de risco e proteção" e Prioridade B – "Avançar na prevenção e implementação de intervenções precoces" (Collins *et al*., 2011).

Esta tese foca-se em dois grupos de transtornos psiquiátrico extremamente prevalentes: **os Transtornos Internalizantes ou Emocionais** e os **Transtornos Externalizantes ou Comportamentais**.

Os Transtornos Internalizantes ou Emocionais compreendem os Transtornos de Ansiedade (Fobias, Ansiedade de Separação e Ansiedade Generalizada) e a Depressão. Embora os artigos da tese abordem também sintomas de depressão e alguns artigos os quadros de depressão serão considerados dentro do grupo de transtornos do estresse (que envolvem tanto ansiedade generalizada, depressão e transtorno do estresse pós-traumático), nesta tese, revisaremos o estado da arte no que se refere aos Transtornos de Ansiedade (TA) como um grupo.

Os Transtornos Externalizantes ou Comportamentais, compreendem o Transtorno de Déficit de Atenção/Hiperatividade (TDAH), Transtorno Opositor Desafiante e Transtorno de Conduta (TOD/TC). Embora o TOD e TC sejam usados como grupos de comparação nos artigos da tese, nesta introdução, revisaremos o estado da arte no que se refere ao TDAH.

As principais características desses transtornos serão apresentadas, tendo em vista que elas fornecem a base teórica por trás do desenvolvimento dos estudos que compõem esta tese.

2.4. Transtornos de Ansiedade (TA)

O Manual Diagnóstico e Estatístico dos Transtornos Mentais, 4ª. Edição Revisada (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision - DSM-IV-TR*) classifica os transtornos primários de ansiedade em: Transtorno do Pânico, Fobias Específicas, Transtorno de Ansiedade Social ou Fobia Social, Transtorno de Ansiedade Generalizada (TAG), Transtorno Obsessivo Compulsivo (TOC), Transtorno de Estresse Pós-Traumático (TEPT) e Transtorno de Estresse Agudo. O Transtorno de Ansiedade de Separação, classificado dentro da seção de transtornos primariamente diagnosticados na infância, também faz parte do grupo dos transtornos de ansiedade. Embora o TOC e TEPT sejam classificados dentro do grupo de transtornos de ansiedade na classificação diagnóstica atual, alguns autores defendem outros agrupamentos, tendo em vista que tanto um como outro apresentam especificidades importantes no que se refere às bases biológicas e ao tratamento (Hollander *et al*., 2008, Resick and Miller, 2009).

Poucos estudos se dedicaram a estudar diferenças dentre os transtornos de ansiedade. Embora uma boa parte dos pesquisadores acredite que esses transtornos compartilhem fatores etiológicos comuns, tanto genéticos quanto ambientais, é também consenso de que há diferenças do ponto de vista fisiopatológico entre eles. A elucidação dos mecanismos compartilhados e comuns entre os transtornos de ansiedade é também uma área de interesse para pesquisas futuras.

Estudos prospectivos demonstraram que cerca de 90% dos casos de transtornos de ansiedade na idade adulta já preenchiam critérios na infância e adolescência (Kim-Cohen *et al*., 2003). Alguns autores associam características de inibição do comportamento já aos quatro meses de idade a sintomas de ansiedade na infância, indicando que as manifestações clínicas podem ser realmente muito precoces (Kagan *et al*., 1999). Há que se considerar ainda que tanto uma continuidade homotípica (p.ex., um transtorno ansioso na infância preceder um transtorno ansioso na vida adulta) quanto uma comorbidade sequencial (p.ex. um transtorno ansioso na infância preceder depressão ou abuso de álcool na vida adulta) são freqüentes nos casos de ansiedade na infância (Kim-Cohen *et al*., 2003).

Sugere-se que as trajetórias de desenvolvimento anormais para os transtornos de ansiedade envolvam ações precoces de genes e ambiente resultando na desregulação de circuitos cerebrais que influenciam o processamento de estímulos aversivos. Embora alguns processos anormais estejam descritos, um deles merece uma maior atenção neste projeto: o viés atencional para ameaças e recompensas.

O limiar para um indivíduo com transtorno de ansiedade para ter sua atenção capturada por estímulo moderadamente aversivos no ambiente é menor do que para indivíduos sem transtornos de ansiedade. Por essa razão, podemos dizer que a atenção de indivíduos com transtornos de ansiedade está enviesada para estímulos ameaçadores no ambiente. O paradigma que utilizamos para avaliação desse processo mental chama-se "*dot-probe"* (Mogg *et al*., 1997). Esse processo mental é um dos principais candidatos para avaliação dos seus componentes biológicos (com a investigação dos paradigmas com neuroimagem funcional e de genética). A importância deste paradigma está no fato de que estudos recentes mostraram que tarefas cognitivas com o intuito de modificar esses vieses (treinamento atencional),

são capazes de melhorar sintomas de ansiedade, oportunizando uma nova forma de tratamento para transtornos de ansiedade na infância e adolescência. No entanto, os mecanismos biológicos e as indicações terapêuticas (isto é, quem pode se beneficiar do tratamento), ainda não estão claras (Hakamata *et al*., 2010).

Estudos mostram que duas regiões cerebrais estão mais envolvidas nesse processo mental: a amígdala cerebral e o córtex pré-frontal, principalmente a expansão ventral. Especula-se que disfunções nesse circuito sejam responsáveis por reações anormais de ansiedade e caracterizem os transtornos de ansiedade na infância e adolescência (Monk *et al*., 2006). Estudos nessa área visam estudar fenômenos como o medo condicionado, isto é, um processo pelo qual uma associação é formada entre um estímulo neutro, como uma luz ou um som e um estímulo aversivo, como um choque elétrico (Pine, 2007). Estes estudos também apontam para um importante papel da amígdala e algumas regiões do córtex pré-frontal, assim como para o *striatum* e o cíngulo anterior (Pine, 2007).

2.5. Transtorno de Déficit de Atenção/Hiperatividade (TDAH)

O Transtorno de Déficit de Atenção/Hiperatividade (TDAH) é atualmente classificado como um transtorno do neurodesenvolvimento. Ele é caracterizado pela presença de desatenção, hiperatividade e/ou impulsividade. Apresenta três subtipos principais: (1) Predominantemente Desatento; (2) Predominantemente Hiperativo/Impulsivo; (3) Subtipo Combinado. Assim como os TA e como qualquer outro transtorno psiquiátrico, o TDAH é resultado de interações complexas entre genes e ambiente. No entanto, como há uma concordância muito maior para os sintomas dos transtorno entre gêmeos monozigóticos do que em dizigóticos (estimativa de herdabilidade), estima-se que esse transtorno tem um componente

genético bastante importante, sendo que cerca de 60% da variabilidade de sintomas desse transtorno pode ser explicada por fatores genéticos (Larsson *et al*., 2012).

A prevalência do TDAH é bastante elevada, sendo um dos transtornos psiquiátricos mais prevalentes na infância e adolescência, com taxas de prevalência de aproximadamente 5% ao redor do mundo (Polanczyk *et al*., 2007). Além disso, uma boa parte dos pacientes persiste com sintomas mesmo na vida adulta.

No que se refere à neuropsicologia, diversas teorias acerca dos processos mentais envolvidos no TDAH foram elaboradas. A teoria mais difundida é a de um déficit único no controle inibitório, isto é, pacientes com TDAH teriam dificuldade de inibir uma ação quando há uma forte tendência para executá-la (Barkley, 1997). No entanto, uma série de outros estudos encontraram déficits em outros domínios das funções mentais, como déficits motivacionais, representados pelo conceito de *delay aversion* (aversão à espera) (Sonuga-Barke, 2005) e até mesmo em outros processamentos básicos como o processamento temporal (Castellanos *et al*., 2006) e oscilação entre mecanismos neurais relacionados a funções ativas e estados de conectividade intrínseca (Castellanos and Proal, 2012, Castellanos *et al*., 2005, Sonuga-Barke and Castellanos, 2007). Outros modelos mais complexos, como o modelo cognitivo-energético, são de especial importância (Sergeant, 2000), pois fornecem alternativas de integração de diversas dessas dimensões. Este modelo propõe que a eficiência do processamento de informações é determinada pela interação entre mecanismos computacionais da atenção, fatores de estado ou "*pools*" energéticos ("*arousal"*, ativação e "*effort"*) e um controle executivo. No entanto, uma série de questões ainda permanecem em aberto no TDAH, especialmente a especificidade desses achados e a relevância clínica da qualificação desses déficits.

Do ponto de vista da neuroimagem, Shaw e colaboradores (2007) destacaram a importância do acompanhamento do desenvolvimento da doença para uma melhor compreensão do processo psicopatológico. Os autores conduziram um estudo longitudinal com ressonância magnética estrutural em crianças com transtorno de déficit de atenção com hiperatividade (TDAH) em comparação com controles normais. Eles utilizaram a espessura cortical como uma medida de maturação cerebral e descreveram que as crianças com TDAH atingiram o pico de sua espessura cortical, em média, três anos após os controles (Shaw *et al*., 2007). Mais do que isso, seus resultados evidenciaram que, ao invés de um desvio do desenvolvimento típico, o TDAH reflete um atraso em processos de maturação. Portanto, para descobrir a origem das doenças mentais, pesquisadores precisarão entender a inter-relação de fatores genéticos e ambientais em fases específicas do desenvolvimento, seu impacto no desenvolvimento cerebral e, por fim, a progressão fenotípica resultante desta complexa interação (Kieling *et al*., 2008). Vale ressaltar que a visão dos transtornos mentais como transtornos do desenvolvimento não se restringe àqueles transtornos que se manifestam claramente já a partir da infância e da adolescência.

A justificativa para esta tese está no fato de que a maioria dos transtornos mentais tem rotas na infância e um curso crônico ao longo da vida. Por essa razão, o estudo da fisiopatologia dos transtornos mentais na infância é primordial. Este conhecimento pode representar um avanço importante para entender a complexa relação entre os diversos fatores de risco e psicopatologia. Desta forma, a combinação das neurociências à clínica psiquiátrica apresenta-se como uma alternativa promissora de avançar o conhecimento nessa área e, em longo prazo, podem ser determinantes

para o desenvolvimento de estratégias claras de prevenção de acordo com o modelo médico.

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4. OBJETIVOS

4.1. Objetivo Geral

Estudar do ponto de vista fisiopatológico e mecanístico processos mentais e mecanismos biológicos relacionados aos transtornos psiquiátricos comuns na infância.

4.2. Objetivos Específicos

A. Mecanismos relacionados aos transtornos emocionais

a. Investigar se os estágios do desenvolvimento puberal podem moderar a relação entre fatores de risco genéticos conhecidos (como o polimorfismo da região promotora do gene transportador da serotonina) em sintomas depressivos em adolescentes (artigo #1).
b. Estudar se os vieses na atenção relacionados a ameaças (faces de raiva) e a recompensas (faces de felicidade) estão associados a sintomas internalizantes ou emocionais na infância (artigo #2)
c. Estudar se essa associação varia de acordo com o tipo de transtorno psiquiátrico;
d. Estudar se essa associação varia de acordo com o tempo de exposição aos estímulos ameaçadores e recompensadores (artigo #2);

B. Mecanismos relacionados aos transtornos comportamentais

a. Estudar se déficits no processamento básico de informações (*encoding,* eficiência de processamento, resposta/preparação motora, estilo de resposta e variabilidade nesses processos) e controle inibitório (ser capaz de inibir uma ação mesmo há uma forte tendência para realizá-la) podem estar associados ao Transtorno de Déficit de Atenção/Hiperactividade (TDAH) detectados na comunidade (artigo #3);
b. Estudar se esses déficits são específicos do TDAH e não encontrados em outras formas de psicopatologia (artigo #3).

c. Investigar se a frequente comorbidade de TDAH e Transtorno Opositor desafiante/Transtorno de Conduta (TOD/TC) pode ser explicada pelos efeitos aditivos dessas duas condições clínicas, ou, representa uma condição clínica distinta no que se refere a esses processos mentais (artigo #3);
d. Investigar se os déficits de controle inibitório são independentes de déficits em funções de ordem inferior, como o processamento básico (artigo #3);
e. Testar o modelo dimensional do TDAH em contraste com o modelo categorial através de processos mentais que caracterizam esse transtorno. Investigar se déficits em processamento básico e controle inibitório estão associados à desatenção e hiperatividade/impulsividade, mesmo em crianças com desenvolvimento típico (artigo #4);

5. ARTIGO #1

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Letter to the Editor

Is puberty a trigger for 5HTTLPR polymorphism association with depressive symptoms?

Giovanni Abrahão Salum[1,2,3]*, Andressa Bortoluzzi[2,4], Patrícia Pelufo Silveira[4,5], Vera Lúcia Bosa, Ilaine Schuch[5], Marcelo Goldani[5], Carolina Blaya[2,6], Sandra Leistner-Segal7 and Gisele Gus Manfro[1,2,3,4,]

[1] Post Graduate Program in Medical Sciences: Psychiatry, Federal University of Rio Grande do Sul (UFRGS), Hospital de Clínicas de Porto Alegre (HCPA), Brazil

[2] Anxiety Disorders Outpatient Program for Child and Adolescent Psychiatry, Hospital de Clínicas de Porto Alegre (HCPA), Brazil

[3] National Institute of Developmental Psychiatry for Children and Adolescents (INPD/CNPq), Brazil

[4] Post Graduate Program in Neuroscience, Institute of Basic Sciences/ Health, Federal University of Rio Grande do Sul, Brazil

[5] Center for Child and Adolescent Health Studies (NESCA), Hospital de Clínicas de Porto Alegre (HCPA), Brazil

[6] Universidade Federal de Ciência da Saúde de Porto Alegre (UFCSPA), Brazil

[7] Medical Genetics Service, Hospital de Clinicas de Porto Alegre (HCPA), Brazil

*** Corresponding author**

Hospital de Clínicas de Porto Alegre (HCPA), Ramiro Barcelos 2350 – room 2201A, Porto Alegre 90035-003, Brazil. Tel./fax: þ55 51 3359 8094. E-mail address: gsalumjr@gmail.com (G.A. Salum)

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Dear Editor,

Adolescence is not only a critical period for depression onset, but also the period that gender became a risk factor for depression susceptibility (Hankin et al., 1998). Puberty is one of the most important landmarks of adolescence with clear consequences in emotion regulation, thinking and behavior. During puberty, steroid hormones trigger various brain circuits remodeling responses for functional and structural changes (Sisk and Zehr, 2005). The serotonin transporter gene promoter polymorphism (5HTTLPR) has been implicated as a moderator of the effects of psychosocial stressors in depression in several studies (Karg et al., 2011). Furthermore, there is clinical (Bridge et al., 2007) and animal (Ansorge et al., 2004) evidence for age-related developmental moderation of serotonergic pathways. The aim of this study was to test whether the 5HTTLPR polymorphism would be associated with depressive symptoms in adolescents in different stages of development. We hypothesized that low functional variants would be associated with higher depressive symptoms only in post-pubertal adolescents.

This sample was primarily designed in order to investigate anxiety disorders in the community and involves an oversampling of anxious adolescents. Detailed description of the sample selection can be found elsewhere (Salum et al., 2010). The current study addresses a sub-sample of 121 adolescents who accepted and have completed the whole evaluation protocol, including genetic evaluation. This study was approved by the ethical committee of Hospital de Clínicas de Porto Alegre. We collected separate informed consent from primary caretakers and assent from adolescents.

Psychiatric diagnoses were assessed throughout clinical and structured interview using the K-SADS-PL based on the DSM-IV criteria (Kaufman et al., 1997). We measured depressive symptoms with the Childhood Depressive Inventory (CDI) (Golfeto et al., 2002). Pubertal stage was evaluated with a self-report instrument (Morris and Udry, 1980) consisting of schematic drawings based on Tanner's Sexual Maturity Scale (Tanner, 1962). The ratings obtained with this instrument well correlated with the ratings based on physical examination

by physicians (Leone and Comtois, 2007). DNA was extracted from biological samples of saliva using the DNA 2006 Oragene® Kit (Laboratory Protocol for Manual Purification of DNA from 4.0 mL of Oragene® DNA saliva). The 5HTTLPR was analyzed into three groups classified in accordance with expression: LaLa vs. (LgLa or LaS) vs. (LgLg or LgS or SS).

We used a Generalized Linear Model using depressive scores as continuous dependent variable, and Tanner stages (pre- pubertal, pubertal and post-pubertal status) and 5HTTLPR as independent variables. We also tested their interaction. Confounders were defined based on conceptual theoretical relevance according to the current literature and/or using a broad statistical definition (association with dependent variables at a p 0.20). Variables evaluated included age, gender, ethnicity, socioeconomic status, major psychiatric diagnosis according to K-SADS-PL with a frequency higher than 10% and body composition variables. Interaction terms of the model were interpreted using pairwise contrasts, with a significance level of 5%. Model assumption was checked graphically.

Female gender (β= - 2.34, p = 0.012) and higher age (β = 0.536; p = 0.049) were associated with CDI scores and were controlled in the statistical analysis that also includes the diagnosis of any anxiety disorder (β = 1.56; p = 0.089). No main effects were found for 5HTTLPR (p = 0.209) or Tanner stages (p = 0.558). However, we found a significant interaction between pubertal status and 5HTTLPR (p interaction = 1.41×10^{-4}). Pairwise contrasts of CDI estimated marginal means between groups reveal that the group of low expression alleles of 5-HTTLPR is associated with depressive symptoms in post-pubertal adolescents, but no group differences between genotypes can be detected in pre-pubertal or pubertal adolescents (see Fig. 1).

We are limited by a small sample size and by a cross-sectional design. Moreover our external validity may be restricted considering our oversampling of anxious adolescents. In spite of that we were able to detect an association of 5HTTLPR polymorphism and depressive symptoms in post-pubertal adolescents. Our results are in agreement with previous findings regarding this gene and depression, in which lower functional variants are associated with

increased risk for depressive symptoms, especially when individuals are exposed to life stressors (Karg et al., 2011).

The specific association between 5HTTLPR and depressive symptoms in post-pubertal adolescents may represent differences in susceptibility to depression that may be only triggered after programmed hormonal changes during puberty. We hypothesize that this event may cause epigenetic changes in the serotonin receptor, and only after this hormonal regulation, LaLa subjects became protected against depressive symptoms if compared to other subjects with lower functional copies. Furthermore, another possibility is that puberty can be considered a period of stress that can be experienced differently according to functional copies of serotonin transporter gene. We believe that such findings may contribute to explain developmental serotonergic pathways to depression in adolescents. Further prospective studies are warranted.

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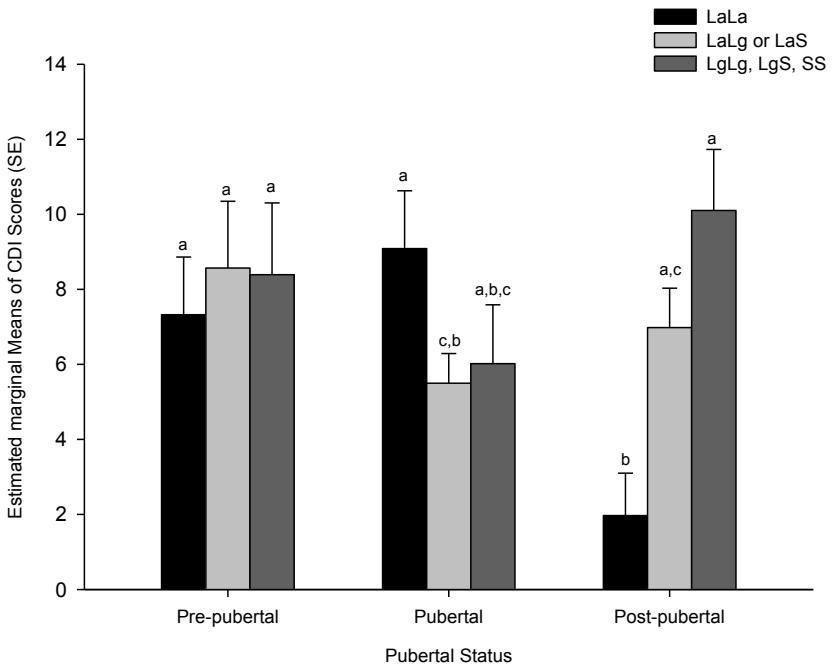

Figure 1 - 5-HTTLPR x Puberty interaction in adolescent's depressive scores

Note: Sample sizes in LaLa, LaLg or LaS and LgLg, LgS or SS groups are as follows: pre-pubertal (4/9/6), pubertal (13/23/14) and post-pubertal (16/26/10), respectively.

6. ARTIGO #2

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Threat Bias in Attention Orienting:

Evidence of Specificity in a Large Community-based Study

Giovanni Abrahão Salum, MD[1,2], Karin Mogg, PhD [3], Brendan Patrick Bradley, PhD[3], Ary Gadelha, MD[1,4], Pedro Pan, MD[1,4], Ana Carina Tamanaha, PhD[1,4], Tais Moriyama, MD[1,4,5], Ana Soledade Graeff-Martins, PhD[1,4,5], Rafaela Behs Jarros, MSc[2], Guilherme Polanczyk, PhD[1,5], Maria Conceição do Rosário, PhD[1,4], Ellen Leibenluft, MD[6], Luis Augusto Rohde, PhD[1,2,5], Gisele Gus Manfro, PhD[1,2] and Daniel Samuel Pine, MD[6]

Affiliations

[1] *National Institute of Developmental Psychiatry for Children and Adolescents (INCT-CNPq), Brazil*

[2] *Federal University of Rio Grande do Sul, Porto Alegre - Brazil*

[3] *Southampton University, Southampton – United Kingdom*

[4] *Federal University of São Paulo, São Paulo - Brazil*

[5] *University of São Paulo, São Paulo - Brazil*

[6] *National Institute of Mental Health Intramural Research Program, Bethesda – United States of America*

Address correspondence and reprint requests

Giovanni Abrahão Salum

Hospital de Clínicas de Porto Alegre

Ramiro Barcelos, 2350 – room 2202; Porto Alegre, Brazil – 90035-003;

E-mail:gsalumjr@gmail.com - Phone/Fax: +55 51 3359 8094

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Abstract

Background: Preliminary research implicates threat-related attention biases in pediatric anxiety disorders. However, major questions exist concerning diagnostic specificity, effects of symptom-severity levels, and threat-stimulus exposure durations in attention paradigms. This study examines these issues in a large, community school-based sample.

Methods: A total of 2,046 children (ages 6-to-12) were assessed using the Development and Well Being Assessment (DAWBA), Childhood Behavior Checklist (CBCL) and dot-probe tasks. Children were classified based on presence or absence of "fear-related" disorders, "distress-related" disorders, and behavior disorders. Two dot-probe tasks, which differed in stimulus exposure, assessed attention biases for happy-face and threat-face cues. The main analysis included 1774 children.

Results: For attention bias scores, a three-way interaction emerged among face-cue emotional valence, diagnostic group, and internalizing-symptom severity ($F=2.87$, $p<0.05$). This interaction reflected different associations between internalizing symptom severity and threat-related attention bias across diagnostic groups. In children with no diagnosis (n=1411; mean difference=11.03; SE=3.47, df=1, $p<0.001$) and those with distress-related disorders (n=66; mean difference=10.63; SE=5.24, df=1, $p<0.05$), high internalizing symptoms predicted vigilance towards threat. However, in children with fear-related disorders (n=86; mean difference=-11.90; SE=5.94, df=1; $p<0.05$), high internalizing symptoms predicted an opposite tendency, manifesting as greater bias ***away*** from threat. These associations did not emerge in the behavior-disorder group (n=211).

Conclusions: The association between internalizing symptoms and biased orienting varies with the nature of developmental psychopathology. Both the form and severity of psychopathology moderates threat-related attention biases in children.

Introduction

Pediatric anxiety disorders are extremely common (Fleitlich-Bilyk and Goodman, 2004, Merikangas *et al.*, 2010) and are associated with both concurrent and future negative health outcomes (Bittner *et al.*, 2007, Kim-Cohen *et al.*, 2003, Pine *et al.*, 1998). The study of psychological processes involved in anxiety disorders vitally informs attempts to identify and treat these conditions (Pine *et al.*, 2009). While many processes have been implicated in both pediatric and adult anxiety disorders, biases in attention orienting are one of the most consistently-observed information-processing correlates of anxiety (Guyer *et al.*, 2007, Shechner *et al.*, 2012). Moreover, evidence from experimental studies suggests that such biases either cause or maintain anxiety (Hakamata *et al.*, 2010, Hallion and Ruscio, 2011).

Studies in adults establish the ability of threats to uniquely influence attention orienting in anxious subjects (Bar-Haim *et al.*, 2007, Mogg and Bradley, 1998). Available findings suggest that the threshold for mild threats to influence orienting is lower in anxious than non-anxious adults, thereby eliciting pathological fear in inappropriate contexts (Guyer *et al.*, 2007). While various attention paradigms have been used to demonstrate such associations, findings appear most consistent with the dot-probe attention-orienting paradigm (Bar-Haim *et al.*, 2007). Imaging studies (Monk *et al.*, 2006, Monk *et al.*, 2008) and animal models (Nelson *et al.*, 2002) suggest that attention biases on the dot-probe paradigm reflect early-life perturbations in specific brain regions. These data, when coupled with longitudinal data linking pediatric and adult anxiety (Pine *et al.*, 1998), generate interest on biased orienting in pediatric anxiety disorders. While research has begun to examine this issue (Guyer *et al.*, 2007), major questions exist concerning the available findings.

Some questions relate to diagnosis. Attention biases on the dot-probe paradigm emerge in a range of pediatric anxiety disorders, including generalized anxiety disorder (Monk *et al.*, 2006, Taghavi *et al.*, 2003, Waters *et al.*, 2008), post-traumatic stress disorder (Dalgleish *et al.*, 2003, Pine *et al.*, 2005), separation anxiety disorder (In-Albon *et al.*, 2010), social phobia (Waters *et al.*, in press), anxiety disorders as a group (Hankin *et al.*, 2010, Roy *et al.*, 2008) and even non-diagnosed youth with high scores on trait-anxiety measures (Telzer *et al.*, 2008, Waters *et al.*, 2010a, Watts and Weems, 2006). Hence, one set of questions concerns the degree to which attention biases relate to specific anxiety disorders,

overall symptoms of anxiety irrespective of diagnostic status, or to broader categories of psychiatric diagnosis. Proposed nosological revisions recognize the distinction between fear and distress disorders within the broader anxiety-disorders group based on independent evidence from twin studies showing distinct genetic and environmental contributions (Kendler *et al.*, 2003, Lahey *et al.*, 2011), as well as distinct symptom structures (Watson, 2005, Watson *et al.*, 2008). When combined with other work on attention bias, this generates questions on the manner in which attention bias relates to anxiety symptoms in children with fear disorders, distress disorders, and children with other forms of psychopathology distinct from anxiety.

Beyond these diagnostic issues, other questions concern the relationships among attention bias, diagnosis, and severity of pediatric internalizing symptoms. While attention biases have been found to be associated with symptom-severity, results across studies have been mixed. Thus, some studies find larger biases **towards** threat in children with higher ratings on internalizing symptom scales (Dalgleish *et al.*, 2003, Hankin *et al.*, 2010, In-Albon *et al.*, 2010, Roy *et al.*, 2008, Waters *et al.*, 2010a, Waters *et al.*, 2010b, Waters *et al.*, 2008), although other studies find no relationship between the attention bias and the severity of symptoms in children with anxiety disorders (Monk *et al.*, 2006, Pine *et al.*, 2005). Of note, cross-study differences in diagnoses may explain these inconsistencies (Waters *et al.*, 2008). However, no study has directly examined the relationship between attention bias and symptom-severity across groups of diagnoses. Thus, questions remain on the degree to which diagnosis moderates the relationship between attention biases and levels of internalizing symptoms.

A final set of questions relate to methodological issues in studies of attention orienting using the dot-probe task. For example, different threat-stimulus exposure durations can be used to examine the time-course of attention biases. While cognitive models of anxiety disorders commonly predict that anxiety is associated with increased attention bias towards threat (Mogg and Bradley, 1998, Mogg *et al.*, 1997, Williams *et al.*, 1997), it has also been hypothesized that initial orienting of attention towards threat may be followed by subsequent attention avoidance at more protracted stimulus exposure durations (Mogg and

Bradley, 1998). Studies using the dot-probe task with varying stimulus exposure durations predominantly find a bias in anxious adults towards threat, with less consistent evidence of vigilant-avoidant patterns (Bar-Haim *et al.*, 2007); the few studies examining this issue in pediatric populations generate inconsistent findings (Perez-Edgar *et al.*, 2010, Waters *et al.*, 2010b). Other methodological concerns relate to sampling issues. Existing studies on anxiety-related orienting biases remain restricted to clinically-ascertained samples with high rates of comorbidity. Research on other biomarkers reveals the potentially biased nature of findings from clinical samples and the complex role of comorbidity in biological traits (Poustka *et al.*, 2010). Studies are needed in community-based samples.

The current study addresses these needs by examining the relationship among attention orienting, psychiatric diagnosis, and the severity of internalizing symptoms in a large school-base sample. Following the proposed nosological revisions for anxiety disorders, children are classified based on the presence or absence of distress, fear, and behavior disorders. We use the dot-probe task to test one specific hypothesis: relative to non-disordered individuals with low levels of internalizing symptoms, high levels of internalizing symptoms predict attention bias towards threat faces among individuals with distress disorders, fear disorders, or no disorder; attention bias is not expected in individuals with behavior disorders.

Methods and Materials

High Risk Cohort Study

This report is part of a large community school-based study that combines standardized evaluation from a psychiatric and cognitive neuroscience perspective, as well as genetics and neuroimaging, to inform preventive strategies in developmental psychiatry. Our study population in the screening phase of the study consisted of students in public schools with more than 1,000 students in the age-range assessed that are located close to the research centers in Porto Alegre and São Paulo, Brazil. This study was performed in multiple steps involving several evaluation teams and research protocols. These steps, described here briefly, included: (1) screening; (2) psychiatric assessments; and (3) cognitive

evaluation. This study was approved by the ethics committee of the University of São Paulo (IORG0004884, project IRB registration number: 1132/08). Written consent was obtained from all parents of participants, and verbal assent was obtained from all children. When appropriate, written assent also was obtained.

Participants

A total of 2512 provided data on psychopathology. These subjects were also selected to complete both a short and a long version of the dot-probe task. From the 2512 subjects, 2172 performed the short task, 2082 performed the long task, and 1936 provided complete bias score data in each task-format (bias scores were not calculated if >50% reaction time data were missing). The latter (n=1936) were allocated to four diagnostic groups (fear-related, distress-related, behavior, or no-disorder groups), leaving an additional 162 excluded subjects who did not satisfy selection criteria (see later for details, e.g. exclusion of children with any disorder from no-disorder group; or those with comorbidity precluding placement into one diagnostic group). This yielded 1774 subjects for the main data analysis. Except for being older (mean difference=0.39 years, SE=0.09, $p<0.001$), those included in the main analysis were similar to those not included regarding internalizing and externalizing symptoms and demographic variables (data not shown; all p-values>0.05).

Procedures

Community Screening and Sampling

A total of 57 schools from two cities (22 schools in Porto Alegre and 35 schools in São Paulo) participated in screening and enrollment procedures. Eligible subjects were those: (1) registered for school by a biological parent capable of providing consent and information about the children's behavior; (2) at an age range between 6-12 years of age; (3) have remained in the same school during the study period. For screening, 9,937 informant interviews based on the *Family History Survey* (FHS) were conducted (the child's biological mother in 88% of the families).

From this pool, we selected two subgroups: a random and high-risk stratum. For subjects in the random-selection stratum, a simple randomization procedure from school

directories was used, without replacement of non-available subjects. Selection for the high-risk stratum involved a risk-prioritization procedure, focused on individuals with a family history of a disorder and/or ongoing symptoms in one of the five targeted domains [Attention Deficit Hyperactivity Disorder (ADHD), Anxiety, Obsessive Compulsive Disorder (OCD), Psychosis and Learning Disorders], as detected during screening. Subjects in this second, high-risk stratum were oversampled and, if not available, replaced by the next subject listed in the high risk sampling frame. From 1,315 children selected in the first random stratum who fulfilled inclusion criteria, 958 (73%) completed the household evaluation. From the 2050 children selected in the second high-risk stratum who fulfilled inclusion criteria, 1554 (76%) completed the evaluation (see Figure 1 for further details on participation rates at each stage of the study).

[Figure 1 around here]

Attention Bias Assessment

Attention biases were measured with a visual dot-probe paradigm similar to the paradigm used in prior studies of pediatric (Monk *et al.*, 2006, Pine *et al.*, 2005, Roy *et al.*, 2008, Waters *et al.*, 2010a, Waters *et al.*, 2010b, Waters *et al.*, 2008) and adult (Bradley *et al.*, 1999, Mogg and Bradley, 1998, 1999, Mogg *et al.*, 2004) anxiety disorders. Tasks were presented in Eprime 2.0 (Psychology Software Tools, Pittsburgh) by testers blind to all clinical data.

Two versions of the dot-probe task were employed, one of which was shorter than the other. Both tasks used identical stimuli, which were photographs of face-pairs from different actors (half of each gender). Each face-pair showed an emotional face (angry or happy) presented side-by-side with a neutral face of the same actor.

In both tasks, each trial started with a central fixation cross (for 500 msec), followed by the face-pair (for 500 or 1250 msec), which was replaced with an asterisk (probe) that appeared on either the left or right side of the screen (for 1100 msec) in the spatial location previously occupied by one of the faces. Participants were instructed to press one of two response keys as quickly and accurately as possible to indicate whether the asterisk

appeared in the left or right hemi-field. Emotional faces and probes appeared on either the left or right side of the screen with equal frequency, so that in half the trials, the probe appeared in the same spatial location as the emotional face (congruent trials) and, in the other half of trials, the probe appeared in the opposite location to the emotional face (incongruent trials). Both tasks involved fully randomized presentation of each trial type. The inter-trial interval varied randomly from 750 to 1250 msec. This variation has been used consistently in prior studies of pediatric anxiety disorders, to facilitate subject engagement by minimizing the predictability of trial onset times. To maintain consistency with the prior research literature, the procedure also is included here. Subjects were provided with standardized instructions and also asked about their understanding of the task (*e.g., "What you should do if the probe appears at the left side of the screen? And if it appears at the right side?").*

The short version of the dot-probe task involved 80 trials comprising 32 "threat-neutral" trials (16 with probe congruent with angry face; 16 with probe incongruent with angry face), 32 "happy-neutral" trials (16 congruent; 16 incongruent), and 16 neutral-neutral trials. In this short version, each face-pair was presented for 500 msec. The long version of the dot-probe task involved 160 trials, half of which were the same as in the short version and used 500 msec face-cue presentations; while the other half used 1250 msec exposure duration. Each face-pair appeared once per exposure duration, with trial order being fully randomized.

The two tasks generated six bias measures: threat and happy-face bias scores in each task-format (500 msec short-task; 500 msec long-task, 1250 msec long-task). Response times (RTs) were excluded as errors from trials where the response was incorrect or did not occur before probe offset. RTs less than 200 msec or more than 2SD above each participant's mean were excluded as outliers (Roy *et al.*, 2008). Attention bias scores were not calculated if more than 50% RT data were missing due to errors or outliers (Roy *et al.*, 2008). Bias scores were calculated separately for each face-emotion, task-format and subject, using the conventional formula in which RTs from congruent trials are subtracted from RTs from incongruent trials. Positive values indicate attention bias towards threat; negative values indicate bias away from threat.

General testing procedures

The dot-probe was part of a large neuropsychological battery used in the project. Task administration was fully randomized across two blocks. As a pre-defined rule, the two dot-probe tasks occurred in different blocks. A total of 30.6% subjects received the two dot-probe tasks in a single day and 69.4% received both tasks administered on two different days. Previous exposure did not affect bias scores (data not shown; all p-values>0.05). The mean duration of sessions 1 and 2 were 64 and 62 minutes. Dot-probe short and long versions take approximately 5 and 10 minutes, respectively.

The tasks were administered by trained speech therapists on an Acer 14-inch laptop with a Intel Pentium Dual Core T4300 running in Windows 7 Premium software, set to 100% brightness. Children sat 50 cm from the screen, arranged at 90º with the base of the notebook. All task instructions were standardized.

Data quality was monitored by each tester, who noted whether administrative problems occurred, and registered their perception of the testing environment (e.g. ambient noise) and child's comprehension and co-operation, to provide an index of test-quality conditions for each task. These variables were not correlated with bias scores (p>0.20) and therefore were not considered for further analysis.

Psychiatric diagnosis and Symptom severity

The psychiatric diagnoses were assessed with the Development and Well-Being Assessment (DAWBA), a structured interview administered by trained lay interviewers, and further rated by trained supervised psychiatrists. The DAWBA was administered to biological parents who indicated they could provide accurate information in accordance with previously reported procedures (Goodman *et al.*, 2000). All lay interviewers were extensively trained by the research team and their work was submitted to constant supervision during the entire project. Nine psychiatrists performed the rating procedures. All were trained and constantly supervised by a senior child psychiatrist.

To examine relationships between attention biases and diagnostic categories using adequately powered analysis, anxiety disorders were divided into two groups: (1) a "fear-disorder" group that includes phobias and separation anxiety disorder, and (2) a "distress-disorder" group, that includes generalized anxiety disorder (GAD), depressive disorders (MD)

and post-traumatic stress disorder (PTSD). Decisions on disorder groupings were guided by proposed grouping for DSM-5. In DSM-5, such groupings considered independent evidence from twin studies (Kendler *et al.*, 2003, Lahey *et al.*, 2011), as well as from studies of symptom structure (Krueger, 1999, Trosper *et al.*, 2012, Vollebergh *et al.*, 2001, Watson, 2005, Watson *et al.*, 2008). This work specifically supported the suggestion of viewing GAD as an anxiety disorder that is somewhat distinct from phobia, clustering more closely to MD. Sample sizes were too small to support analyses examining associations with more narrowly-defined, specific psychiatric disorders. Diagnoses of 'other anxiety' (n=37), or obsessive compulsive disorder (n=3) were not included in these categories due to uncertainty about their classification as fear versus distress disorders (Watson, 2005, Watson *et al.*, 2008).

Beyond these two anxiety groups, subjects were arranged into two other groups: (3) children without any psychiatric diagnosis; (4) "behavior-related" psychiatric disorders including attention-deficit/hyperactivity disorder (the three subtypes and non-otherwise specified), oppositional defiant disorder and conduct disorder. Children with manic episodes (n=3), pervasive developmental disorders (n=9), tics (n=17), eating disorders (n=9), psychosis (n=1) and attachment disorder (n=2) were excluded from all groups. No cases of panic disorder, stereotypies, or selective mutism were identified. For all groupings, children who had a comorbid disorder in one of the other diagnostic categories were excluded (n=68), so that analyses would not be confounded by comorbidity. Hence, none of the children in the distress disorder group had a fear or behavior disorder; and vice versa for the other diagnostic groups. For the disorder-free group, we excluded those children who had any specific diagnosis (described above) and also those with a non-otherwise specified diagnosis (n=16).

Severity of internalizing symptoms was assessed using the broad-band total internalizing-scale score of the Child Behavior Checklist (CBCL) that has shown adequate diagnostic performance to predict both fear and distress disorders (Petty *et al.*, 2008). To facilitate hypothesis-testing while accounting for dissimilarly shaped distributions of internalizing-symptom scores across diagnostic groups, dichotomous groupings were made of children with "high" (>18) and "low/moderate" (0-18) scores. This cutoff score corresponds to the 90th percentile in the random sub-sample of this project. We decided to use

internalizing symptoms instead of more specific measures of anxiety due to previous evidence showing that attention biases may be related broadly to aspects of negative affect (Lonigan and Vasey, 2009). Raw scores were used since there is, as yet, no normative CBCL data for the Brazilian population.

Statistical analysis

Primary hypotheses were tested in a 2 x 3 x 4 x 2 mixed design Multivariate Analysis of Variance (MANOVA), which comprised two within-subjects independent variables: face-emotion valence [happy/threat] and task-format [500ms short-task; 500 ms long-task, 1250ms long-task]; and two between-subject independent variables: diagnostic status [none, fear, distress, behavior]; and level of internalizing symptom-severity [low/moderate, high]. To address specific hypotheses, significant results were clarified with General Linear Models (GLM), using pairwise contrasts and Least Significant Differences. We used type III sum of squares in order to account for different group sample sizes. Potential confounders were explored using zero-order correlations and ANOVA. All analyses used SPSS 18.0 with an alpha level of 0.05, two-tailed.

Results

The final sample comprised 1774 children; 86 were in the fear-disorder group (specific phobia, n=47; separation anxiety, n=30; social phobia, n=13; agoraphobia, n=2); 66 in the distress-disorder group (generalized anxiety disorder, n=25; major depression, n=28; posttraumatic stress disorder, n=6; other depression, n=4; undifferentiated anxiety/depression, n=4); 211 in the behavioral-disorder group (ADHD, n=165; oppositional-defiant, n=79; conduct, n=20; other disruptive disorder, n=6); and 1411 in the no-disorder group. Final sample characteristics and task parameters are depicted in tables 1 and 2.

[Table 1 around here]

[Table 2 around here]

The omnibus MANOVA of bias scores showed a significant three-way interaction among face-emotion valence, diagnostic group, and internalizing symptom severity, $F(3,1764)=2.87$, $p=0.035$, $\eta_p^2=0.005$, $\eta^2=0.001$. No other significant main effects or interactions emerged (see table 3). Since no statistically-significant results emerged involving task-format (task- and stimulus-duration; $Fs<2$, $ps>.15$), subsequent analyses used bias scores averaged across the three task-formats.

[Table 3 around here]

The three-way interaction included four levels of diagnostic group, two levels of symptom severity, and two levels of face-emotion valence. To decompose this complex interaction, we performed two GLMs, one for the happy-face valence and a second for the angry-threat-face valence. This analysis showed that the model for angry-threat-face trials revealed a two-way interaction between severity and diagnostic group (Omnibus test LR $\chi^2=21.10$; $df=7$; $p=0.004$). No such interaction emerged for happy-face trials (Omnibus test LR $\chi^2=1.92$; $df=7$; $p=0.964$). Thus, the findings related to diagnostic and symptom-level groups were specific to threat bias and not to happy bias.

Next, findings for threat bias only were further decomposed in post-hoc analyses. A linear model predicting threat bias revealed no effect of severity (Wald $\chi^2=0.2$, $df=1$; $p=0.655$), and a main effect of diagnosis (Wald $\chi^2=7.97$, $df=3$; $p=0.047$) which was subsumed under a significant severity-by–diagnosis interaction (Wald $\chi^2=15.3$, $df=3$; $p=0.002$). Post-hoc analyses showed distinct severity-by-bias associations in the four diagnostic groups. Relative to children in the no-disorder group with low internalizing symptoms, those with no disorder but high internalizing symptoms (mean difference=11.03; SE=3.47, $df=1$, $p<0.001$), and those with distress disorders with high internalizing symptoms (mean difference=10.63; SE=5.24, $df=1$, $p=0.043$) attended to threat stimuli. In contrast, relative to children in the no-disorder group with low symptoms, those in the fear group with high internalizing symptoms (mean difference=-11.90; SE=5.94, $df=1$; $p=0.045$) avoid threat stimuli (Figure 2).

[Figure 2 around here]

Supplementary analyses examined effects of potential confounders. There were no significant effects of face-emotion, diagnostic group and symptom-severity on errors, missing data or RT. There were no significant relationships between age, gender or sampling strategy (random/high-risk) and bias scores (ps>0.3). The overall three-way interaction between face-emotion, diagnostic group and symptom-severity remained significant when effects of age, gender and sampling-strategy were controlled, $F(3,1764)=2.785$, $p=0.04$, $\eta_p^2=0.005$, $\eta^2=0.001$.

Discussion

We examined the effect of severity of internalizing symptoms on attention biases in children with fear, distress, and behavioral disorders, compared with children without any psychiatric disorders, in a large school-based sample. The key finding was that levels of internalizing symptoms interacted with the nature of psychopathology. Namely, high internalizing symptoms predicted a similar pattern of attention bias towards threat cues in children with no psychiatric diagnosis and in those with distress disorders; i.e. higher symptoms were associated with increased attention bias *towards* threat. In contrast, in children with fear-related psychiatric disorders, higher symptom severity predicted greater attention bias *away* from threat. Internalizing symptom severity was unrelated to attention bias in children with behavior disorders. These findings were specific for threat stimuli (i.e. not found for happy faces) and irrespective of stimulus duration.

As hypothesized, our study replicates well-established findings of attention bias towards threats in anxious subjects, a finding central to many current cognitive models of anxiety. The findings add to an emerging pediatric literature indicating that high levels of internalizing symptoms are associated with increased attention bias towards threat in children free of psychiatric diagnosis (Waters *et al.*, 2010b). Our study also extends previous findings from clinical studies (Waters *et al.*, 2010a, Waters *et al.*, 2008, Waters *et al.*, in press) showing that symptom severity modulates the direction of attention biases. For example, Waters et.al found a greater bias towards threats in severe cases of pediatric anxiety

disorders, considered as a group (Waters *et al.*, 2010a) and in severe cases of GAD (Waters *et al.*, 2008). In the present study, attention bias towards threat similarly increased as a function of symptom-severity in the distress-disorder group, as well as in the no-disorder group.

The current study is the first to demonstrate symptom-by-diagnosis interactions across the categories of anxiety disorders examined here. Perhaps the most novel finding in the current study is to show that the positive relationship between emotional symptom severity and threat-related attention bias does not hold across all pediatric diagnostic groups. The novelty of our findings may reflect the relatively pure status of our samples, as there was no overlap between the main categories of psychiatric disorder. While previous research suggested that children with high levels of emotional distress sometimes show reduced attention and even avoidance to threat, relative to non-anxious children (Monk *et al.*, 2006, Pine *et al.*, 2005), the specific determinants of the direction of threat bias were uncertain. Moreover, these prior studies had not convincingly identified a threat-monitoring and a threat-avoiding clinical group, as found in the current study.

The present findings indicate that the combination of high emotional distress and a fear-related disorder is associated with an attention bias away from threat. This may reflect a form of cognitive threat avoidance seen in other clinical scenarios. In such scenarios, cognitive avoidance is thought to follow from an initial, vigilance response that occurs too rapidly to be detected with methods used in the current study. Such avoidance also may represent a complement of other behaviors seen in anxious patients. For example, much like cognitive avoidance that occurs after an initial vigilance response, behavioral phobic avoidance also may occur after an initial state of enhanced reactivity to a threat.

The increasing enthusiasm for research into attention biases in pediatric anxiety is supported by recent evidence suggesting that treatments designed to modify these biases attenuate anxiety symptoms in adults (Hakamata *et al.*, 2010) and children (Bar-Haim, 2010, Bar-Haim *et al.*, 2011). The current findings inform attempts to further refine these techniques. Virtually all available attention-related treatment trials train anxious subjects to shift their attention away from threats. Such an approach would be reasonable for children in the current study with high internalizing symptoms and either no diagnosis or a distress-

related anxiety disorder. However, one can question the reasonableness of this approach for children with both fear-related disorder and high symptoms. These children manifest a bias away from threat, relative to those with low symptoms, and one might expect further training designed to accentuate such a pre-existing bias to provide few clinical benefits.

Our results should be viewed in the light of limitations. First, we were not able to investigate each psychiatric disorder individually due to few available subjects with specific disorders, yielding insufficient statistical power. However, we were able to investigate diagnostic specificity by grouping psychiatric disorders that share common biological backgrounds (Lahey *et al.*, 2011) and symptom structures (Watson, 2005, Watson *et al.*, 2008). Second, since this is a study performed in the community, heterogeneity of testing procedures may introduce noise in analyses. However, variance of bias measures were comparable to published studies using the same task with youth at similar age-range (Monk *et al.*, 2006, Pine *et al.*, 2005, Roy *et al.*, 2008, Waters *et al.*, 2010a, Waters *et al.*, 2010b, Waters *et al.*, 2008). Finally, the diagnostic evaluation relied on information of trained lay interviewers. Nevertheless, all interviews were carefully revised by psychiatrists and this procedure produced satisfactory results for other studies (Goodman *et al.*, 2000).

The study also has notable strengths. This is the largest study so far performed that aims to investigate attention biases in children. In addition, the large-scale community nature of the study allowed us to disentangle contributions of pure (non-overlapping) classes of psychiatric disorders to attention bias. In addition, we were able to show the importance of this neuropsychological process to emotion-related disorders, and not to behavioral disorders. Future longitudinal studies are needed in order to investigate whether threat biases in attention orienting could predict poor outcomes considering both the form and severity of anxious manifestations. In addition, the importance of such findings to other areas, such as brain imaging, is worth noting. The role of diagnosis and symptom levels in moderating effects of the amygdala and prefrontal cortex in different anxiety disorders are of special interest for new investigations, given their associations in previous dot-probe studies (Monk *et al.*, 2006).

In conclusion, the association between the severity of internalizing symptoms and biased orienting to threat varies with the nature of developmental psychopathology. Both the

form and severity of psychopathology moderates threat-related attention biases in children, with specific relationships between symptoms and disorders. These results have potential implications to therapeutics and add to the body of evidence showing the implications of dysfunctional threat-related attention mechanisms to explain individual differences in pediatric anxiety disorders.

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Table 1 – Sample description

	Broad High Order Categories							
	None (n= 1411)		**Fear-related** (n=86)		**Distress-related** (n=66)		**Behavioral** (n=211)	
	n	%	n	%	n	%	n	%
Sampling strategy (high risk)	804	57.0%	56	65.1%	48	72.7%	138	65.4%
Gender (male)	740	52.4%	42	48.8%	24	36.4%	132	62.6%
Socioeconomic status								
Vey Low / Low	86	6.1%	3	3.5%	6	9.1%	12	5.7%
Medium	879	62.3%	56	65.1%	44	66.7%	141	66.8%
High	446	31.6%	27	31.4%	16	24.2%	58	27.5%
Any current medication*	18	1.3%	1	1.2%	2	3.0%	19	9.0%
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	9.79	1.94	9.60	1.78	10.30	1.95	9.71	1.79
CBCL Internalizing score	6.15	6.28	13.88	7.91	18.42	10.04	10.95	8.17

Note: CBCL, Child Behavior Checklist; SD, Standard Deviation. * Psychotropic medications in use for more than 1 month.

Table 2 – Description of attentional task measures as a function of diagnostic category, internalizing symptom severity, and task-format (500 msec short task; 500 msec long task; 1250 msec long task).

	High order diagnostic categories (n=1774)															
	None (n=1411)				**Fear-related** (n=86)				**Distress-related** (n=66)				**Behavioral** (n=211)			
	Low/Moderate (n=1338)		**High** (n=73)		**Low/Moderate** (n=62)		**High** (n=24)		**Low/Moderate** (n=35)		**High** (n=31)		**Low/Moderate** (n=178)		**High** (n=33)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Threat bias	2.9	27.9	13.9	32.6	8.4	27.4	-9.0	33.9	1.7	32.8	13.5	26.4	1.6	34.4	1.5	28.0
Short, 500ms	3.3	44.6	24.9	51.3	6.9	46.0	1.3	55.5	-5.4	54.5	2.1	47.4	.1	52.4	5.3	38.0
Long, 500ms	4.4	51.7	9.0	56.1	13.7	47.3	-9.8	55.3	-6.6	46.7	23.4	54.7	7.7	52.1	9.6	47.0
Long, 1250ms	.9	52.1	7.8	53.4	4.6	48.2	-18.6	43.7	17.0	44.9	14.9	39.7	-3.2	53.8	-10.4	58.5
Happy bias	2.1	28.5	.1	26.6	3.5	24.6	3.6	40.1	-.5	27.8	-1.2	26.3	3.9	32.3	1.7	32.7
Short, 500ms	.9	45.2	5.0	47.7	6.1	46.7	3.0	57.1	1.4	53.8	-2.2	44.5	1.2	51.2	1.3	50.8
Long, 500ms	3.2	55.2	-3.7	39.5	5.9	55.7	2.6	54.0	-6.6	54.8	-1.8	45.7	7.8	54.9	-6.4	55.2
Long, 1250ms	2.3	50.7	-1.2	40.5	-1.5	54.0	5.2	73.8	3.5	46.6	.4	57.4	.1	55.3	10.1	56.2
Mean RT	590.1	97.3	594.7	95.4	627.4	92.4	582.5	95.8	591.6	80.4	599.4	74.1	603.2	91.9	589.4	89.3
Short, 500ms	544.0	109.1	559.0	108.3	574.2	104.5	546.6	110.8	550.9	81.6	540.6	80.1	554.2	100.6	544.1	108.0
Long, 500ms	622.1	103.7	624.0	108.3	662.2	99.1	609.0	96.0	619.2	88.9	632.5	89.6	637.5	96.6	620.9	95.1
Long, 1250ms	604.3	103.9	601.0	110.1	646.0	100.4	591.7	106.8	604.7	88.4	625.1	90.8	618.0	101.4	603.3	97.5
% Errors	8.3	8.2	9.1	8.8	9.7	9.1	7.3	7.1	7.9	8.0	9.0	7.3	9.9	8.5	7.5	7.4
Short, 500ms	6.0	7.6	6.9	8.2	6.6	8.0	5.9	7.4	5.9	7.8	5.6	4.9	7.1	8.6	5.7	7.3
Long, 500ms	10.2	10.4	11.1	11.3	12.2	10.7	9.0	9.7	9.7	8.8	11.2	9.2	11.8	10.4	8.1	7.8
Long, 1250ms	8.8	9.8	9.2	10.0	10.4	10.9	6.9	8.8	8.3	9.0	10.2	10.0	10.7	10.2	8.6	10.3
% Missing	12.7	7.7	13.5	8.2	13.5	8.2	11.6	6.6	12.6	7.2	13.5	7.1	14.2	8.2	11.8	7.0
Short, 500ms	10.9	7.4	11.9	7.9	10.9	7.3	10.5	7.1	10.8	7.3	10.5	4.6	12.6	8.8	10.1	7.1
Long, 500ms	14.3	9.8	15.2	10.4	15.8	9.8	13.2	9.7	13.9	8.1	15.9	9.1	15.6	9.8	12.7	7.9
Long, 1250ms	10.3	7.4	10.6	7.6	11.1	8.1	8.9	6.2	10.3	6.8	11.4	8.2	11.6	7.9	10.0	7.7
% Outliers	4.3	2.5	4.4	2.6	3.8	2.1	4.4	2.5	4.6	2.3	4.5	2.1	4.4	2.4	4.3	2.1
Short, 500ms	4.1	2.4	4.1	2.5	3.6	2.1	4.2	2.1	4.3	2.3	4.7	1.7	3.8	2.1	4.6	2.6
Long, 500ms	4.1	2.2	4.1	2.1	3.6	2.2	4.3	2.9	4.7	2.4	4.0	2.5	3.9	2.2	4.0	2.1
Long, 1250ms	4.9	2.8	5.0	3.4	4.3	2.0	4.6	2.5	4.9	2.1	4.9	2.2	5.5	2.9	4.4	1.7
RT Variance	15646.0	7272.1	16777.1	7335.3	16130.4	7071.2	15848.8	7049.7	15092.7	5636.7	16646.2	7934.2	17295.1	7891.5	16403.0	7772.7
Short, 500ms	13744.4	8701.8	15235.2	8722.8	13542.0	9024.7	14922.3	8281.2	14258.1	8819.0	14768.3	7915.7	15836.6	9530.9	13955.0	7938.3
Long, 500ms	17189.7	8617.6	18326.5	9569.2	17527.2	7289.8	16361.2	6991.0	16340.9	6474.8	17248.5	9232.3	18593.8	8855.4	18113.2	9029.8
Long, 1250ms	16003.9	8822.6	16769.8	8763.4	17322.1	8550.7	16262.9	10799.8	14679.2	6076.7	17921.6	11607.1	17454.8	9004.9	17140.8	11312.5

Note: RT, Reaction Time; SD, Standard Deviation; ms, milliseconds.

Table 3 – Multivariate analysis of variance

Multivariated analysis of variance

Within subjects factors (Pillai's Trace)					
	Value	F	p-value	Effect size	
				η_p^2	η^2
valence	.001	$1.619_{1,1764}$	.203	0.001	<0.001
valence * diagnosis	.004	$2.353_{3,1764}$	.070	0.004	0.001
valence * symptoms	.000	$.377_{1,1764}$	.539	<0.001	<0.001
valence * diagnosis * symptoms	.005	$2.870_{3,1764}$	.035	0.005	0.001
task-format	.000	$.196_{2,1763}$	.822	<0.001	<0.001
task-format * diagnosis	.005	$1.352_{6,3568}$	.230	0.002	0.001
task-format * symptoms	.001	$.609_{2,1763}$	.544	<0.001	<0.001
task-format * diagnosis * symptoms	.003	$.983_{6,3528}$	.435	0.002	0.001
valence * task-format	.001	$.823_{2,1763}$	.439	<0.001	<0.001
valence * task-format * diagnosis	.003	$.943_{6,3528}$	.463	0.002	0.001
valence * task-format * symptoms	.002	$1.809_{2,1763}$	.164	0.001	<0.001
valence * task-format * diagnosis * symptoms	.002	$.488_{6,3528}$	.818	0.001	<0.001
Between subjects factors					
	F	p-value		η_p^2	η^2
diagnosis	$.684_{3,1765}$	.562		0.001	<0.001
symptoms	$.001_{1,1765}$	.974		<0.001	<0.001
diagnosis * symptoms	$2.238_{3,1765}$	.082		0.004	<0.001

Note: symptoms, internalizing symptoms (above or below the 90th percentile); diagnosis, diagnostic category (no psychiatric disorder, fear, distress and behaviour); valence, face-emotion valence (threat and happy); task-format (500ms short, 500ms long, 1250ms long). η_p^2, Partial Eta squared; η^2, Eta squared.

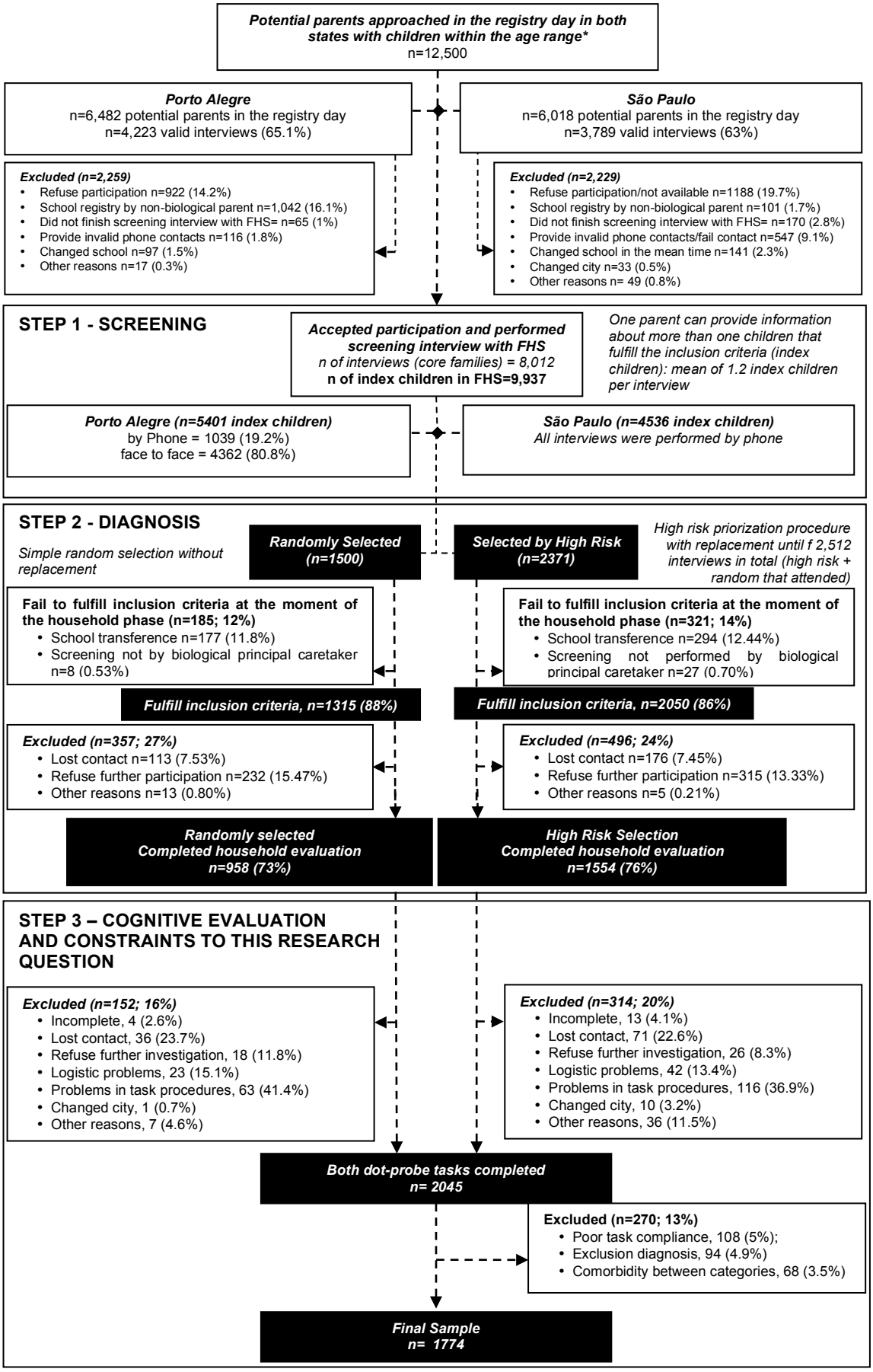

Figure 1 – Flowchart of participants in this report

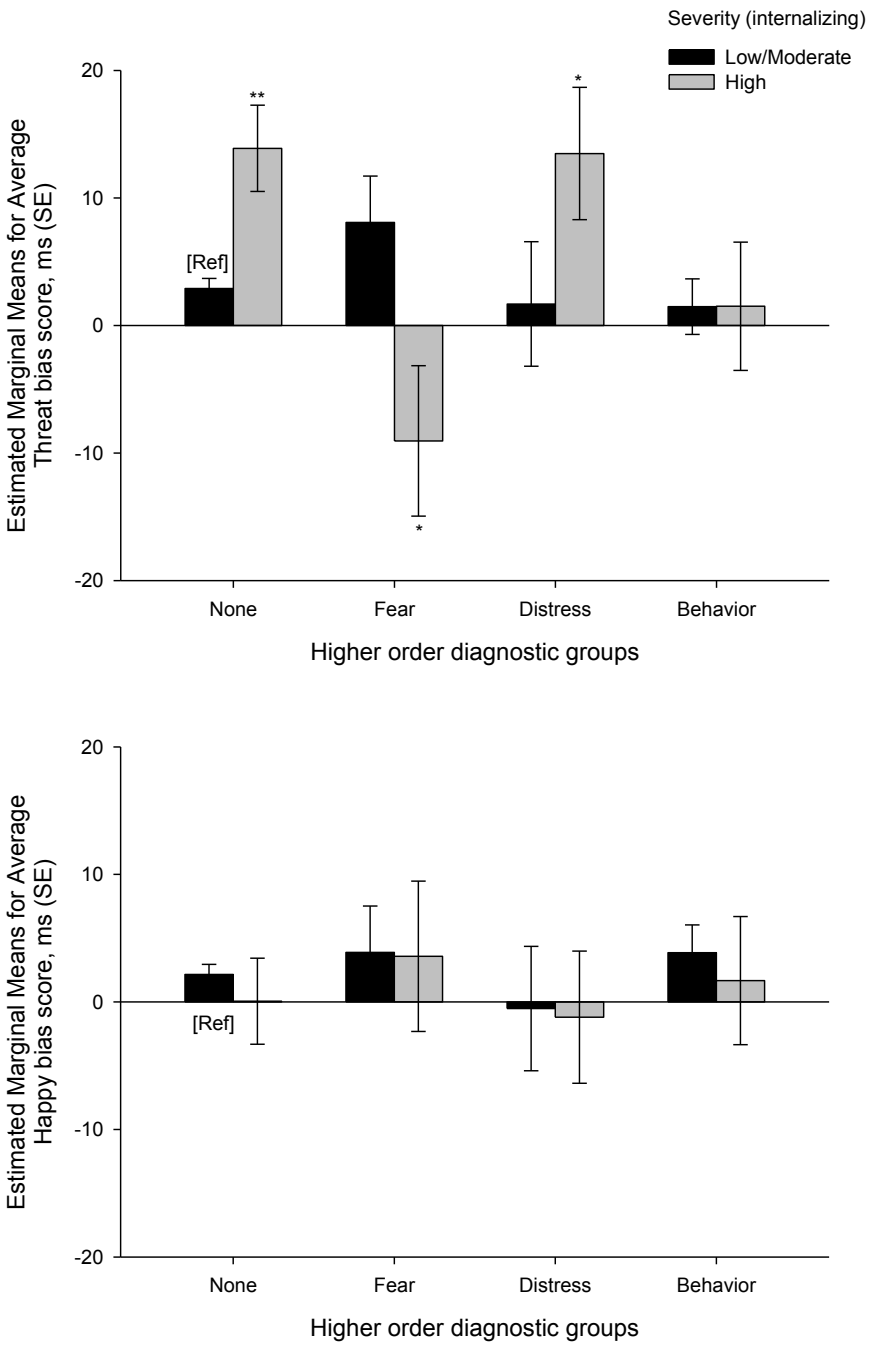

Figure 2 – Mean attention bias scores for threat faces (A) and happy faces (B) in the dot-probe task, as a function of diagnostic category and level of internalizing symptoms. Statistics: General Linear Models post-hoc tests using pairwise contrasts of the interaction term using Least Significant Differences. Error bars indicate ± 1 SE; [Ref], Reference category; *p<0.05; **p<0.001

7. ARTIGO #3

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Specificity of Basic Information Processing and Inhibitory Control in Attention Deficit/Hyperactivity Disorder (ADHD): Evidence from a Large Community Sample

Giovanni Abrahão Salum[1,2], Joseph Sergeant[3], Edmund Sonuga-Barke[4,5]Joachim Vandekerckhove[6], Ary Gadelha[1,7], Pedro Pan[1,7], Tais Moriyama[1,7,8,] Ana Soledade Graeff-Martins[1,8], Pedro Alvarenga[1,8], Maria Conceição do Rosário[1,7], Gisele Gus Manfro[1,2], Guilherme Polanczyk[1,8], Luis Augusto Paim Rohde[1,2,8]

[1] National Institute of Developmental Psychiatry for Children and Adolescents - CNPq, Brazil

[2] Federal University of Rio Grande do Sul, Brazil

[3] Vrije Universiteit, The Netherlands

[4] Southamptom University, United Kingdom

[5] Ghent University, Belgium

[6] University of California, United States

[7] Federal University of São Paulo

[8] University of São Paulo

Address correspondence and reprint requests

Giovanni Abrahão Salum

Hospital de Clínicas de Porto Alegre

Ramiro Barcelos, 2350 – room 2202; Porto Alegre, Brazil – 90035-003;

E-mail:gsalumjr@gmail.com - Phone/Fax: +55 51 3359 8094

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Luis Augusto Rohde was on the speakers' bureau and/or acted as consultant for Eli-Lilly, Janssen-Cilag, Novartis and Shire in the last three years (received less than US$10 000 per year, which less than 5% of LR's gross income per year). LR also received travel awards (air

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Abstract (word count =236)

Introduction: Both Inhibitory Based Executive Function (IB-EF) and Basic Information Processing (BIP) deficits are found in clinic referred Attention-Deficit/Hyperactivity Disorder (ADHD) samples. However, it remains to be determined: (1) whether such deficits occur in non-referred samples of ADHD; (2) whether they are specific to ADHD; (3) if the comorbidity between ADHD and Oppositional Defiant/Conduct disorder (ODD/CD) has additive or interactive effects; (4) if IB-EF deficits are primary in ADHD or due to BIP deficits.

Methods: We assessed 704 subjects (6-12 years old) from a non-referred sample using the Development and Well Being-Behavior Assessment (DAWBA) and classified them into five groups: Typical Developing Controls, TDC (n=378), Fear disorders (n=90), Distress disorders (n=57), ADHD (n=100), ODD/CD (n=40) and ADHD+ODD/CD (n=39). We evaluated neurocognitive performance with: 2-Choice Reaction Time (2C-RT), the Conflict Control Task (CCT) and Go/No-Go (GNG). We used a Diffusion Model to decompose BIP into processing efficiency, speed accuracy trade-off and encoding/motor-function as well as variability parameters.

Results: Poorer processing efficiency was found to be specific to ADHD. Faster encoding/motor-function differentiated ADHD from TDC and from fear/distress; whereas a more cautious (not impulsive) response style differentiated ADHD from both TDC and ODD/CD. The comorbidity between ADHD and ODD/CD reflected only additive effects. All ADHD-related IB-EF classical effects were fully moderated by deficits in BIP.

Discussion: Our findings challenge the IB-EF hypothesis for ADHD and underscore the importance of processing efficiency as key specific mechanism for ADHD pathophysiology.

Introduction

There is a large body of evidence showing that Attention Deficit/Hyperactivity Disorder (ADHD) is associated with deficits in both Basic Information Processing (BIP) (1-8) and Inhibitory-Based Executive Functions (IB-EF) (9-12). BIP encompasses low order bottom-up cognitive processes, such as encoding, search, decision and response organization and form necessary components for higher order cognitive operations (13, 14). IB-EF undermine top down cognitive process (from a higher order) linked to the ability to inhibit an inappropriate pre-potent or dominant response in favor of a more appropriate alternative (9).

This literature is limited in a number of important ways. *First,* nearly all studies are restricted to clinical samples and as a consequence are likely to be affected by referral biases (15). In particular, referral patients may be different from non-referred cases regarding important demographical and clinical characteristics (16-18) and are likely to have high levels of exposure to medication (19, 20). These factors could affect cognitive function in ways not specifically linked to ADHD. For instance, both medication and comorbidity also have proven to affect both BIP and IB-EF in previous studies (21-27).

Second, studies have often not addressed the issue of diagnostic specificity of neurocognitive deficits (25, 28, 29). Thus, certain features of the deficits in BIP and IB-EF showed in ADHD studies might be general markers of childhood psychopathology (25, 28, 29). For instance, IB-EF and BIP have been found to be impaired in children with other disorders such as Oppositional Defiant / Conduct Disorders (ODD/CD) (25, 30, 31) and autism (28, 32). Additionally, BIP and IB-EF are rarely studied in relation to anxiety and depressive disorders, despite evidences suggesting dysfunctional executive processes in emotional disorders (33-36).

Third, the impact of ADHD comorbid with ODD/CD on processing deficits needs to be studied more extensively (37). This comorbidity is extremely prevalent and represents a more severe clinical disorder with poorer long-term prognosis (16, 38-41). The available evidence regarding IB-EF in comorbid and non-comorbid ADHD groups is mixed: some studies suggest that the neurocognitive profile of comorbid ADHD and ODD/CD represents a substantially

different entity than only ADHD or ODD/CD (42-46) while others report additive effects only (21, 25, 28, 37, 47). However interactive effects are rarely formally tested (37).

Finally, the relationship between BIP and IB-EF in ADHD needs further study. Studies of IB-EF in ADHD often assume that BIPs such as encoding, decision-making and motor executions are intact. Thus, they rarely take into account possible between-group differences in BIP (3, 6). Despite that, previous evidence underscores the importance of taking bottom-up processes into consideration when investigating executive functions (2, 3, 6, 48-52). Furthermore, most of the literature analyzing both BIP and IB-EF does so with summary measures such as mean reaction time (RT) and indexes of RT variability, with some exceptions (8, 32, 53). For tasks with a single level, the distribution of RT needs to be decomposed to provide information on different basic processes components. This decomposition also allows one to test the specific nature of BIP deficits underlying ADHD. For instance, problems could be due to a general inefficiency in processing or reflect reduced willingness to spend time accumulating information before responding leading to a trading of accuracy for speed. Such process decomposition is also important to provide means of measuring high order function in the context of BIP deficits.

We report here a study using a large community sample of never medicated children with a variety of non-comorbid psychiatric disorders using classical IB-EF measures and advanced Diffusion Models to disentangle various BIP components (54, 55). Our objective was fourfold: (1) to investigate differences in BIP and IB-EF between TDC and participants with ADHD detected in the community; (2) to investigate if potential differences in ADHD neurocognitive performance are specific to ADHD; (3) to test if ADHD and ODD/CD affect additively or interactively information processing; (4) to test if IB-EF deficits as measured by the classical inhibitory parameters could be fully mediated by deficits in more basic BIP processes.

Our hypotheses were: (1) ADHD will be related to inefficient information processing, but not an impulsive response style or deficient encoding/motor organization when compared to TDC; (2) This will be specific to ADHD and not found in other psychiatric disorders; (3) The comorbidity between ADHD and ODD/CD will impact additively in these neurocognitive

functions; (4) Any associations between ADHD and classical IB-EF measures (as assessed by classical variables) will be fully accounted for by BIP deficits.

Methods

Participants

The sample is drawn from a large community school-based study. The ethics committee of the University of São Paulo approved the study. Written consent was obtained from parents of all participants, and verbal assent was obtained from all children.

The screening phase of the study included children from public schools situated close to the research centers in two Brazilian cities, Porto Alegre and São Paulo. We screened 9,937 parents using the *Family History Survey* (FHS)(56). From this pool, we recruited two subgroups: one randomly selected (n=958) and one high-risk sample (n=1524). Selection for the high-risk sample involved a risk-prioritization procedure, to identify individuals with current symptoms and/or a family history of specific disorders (see (57), for further details). Data for the main tasks used in this study was available for 1993 of these 2512 participants (79.3%). A total of 119 participants (4.7%) were excluded for representing outliers for the diffusion model analysis. Six non-overlapping groups were selected from the remaining sample (n=1881) based on current proposals for DSM-5. Such groupings considered independent evidence from twin studies (58, 59), as well as from studies of symptom structure (60-64).

(1) *Typical developing controls* (TDC): subjects without any psychiatric disorder and without any history of ADHD in any family member; (2) *ADHD:* individuals with any ADHD subtype; (3) *Fear disorders*: separation and social anxiety disorder, specific phobia or agoraphobia; (4) *Distress disorders*: generalized anxiety disorder, depression (major or not otherwise specified) or post-traumatic stress disorder; (5) ODD or CD (6) ADHD comorbid with ODD/CD.

Exclusion criteria were lifetime use of any psychiatric medication (n = 75; 4%), IQ below 70 (n = 38; 2%), mania (n=3; 0.2%), pervasive developmental disorder (n=11; 0.6%), tics (n=15; 0.8%), eating (n=8; 0.5%), obsessive-compulsive (n=5; 0.3%) or psychotic disorders (n =1; 0.1%).

Psychiatric diagnoses

Psychiatric diagnoses were made with the Development and Well-Being Assessment (DAWBA) (65), a structured interview applied by trained lay interviewers. The DAWBA was administered to biological parents in accordance with previously reported procedures (65). A team of 9 psychiatrists under supervision of a senior child psychiatrist rated data from these interviews. DAWBA is a reliable and clinically valid tool for assessing childhood psychiatric disorders (66).

Family History of ADHD (FH-ADHD)

Family history of ADHD was assessed using the ADHD module of the Mini International Psychiatric Interview – (MINI Plus) (67, 68) and the Family History Screen (FHS) (56).

Neurocognitive Tasks

Three tasks were used to assess BIP and IB-EF: a simple two-choice reaction time task (2C-RT), a conflict-control task (CCT) (69) and Go/No-Go task (GNG) (2).

2C-RT: This task measures the ability of the participant to perform extremely basic perceptual decisions about the direction an arrow on the screen is pointing with no or little executive component A total of 100 arrow stimuli were presented, half requiring left and half requiring a right button press.

CCT: This measures builds on the 2-CRT and includes a second inhibitory executive component requiring participants to occasionally suppress a dominant tendency to respond to the actual direction of an arrow and to initiate a response indicating the opposite direction. This requirement was indicated by a change in the color of the arrow (a “conflict” effect). There were 75 congruent trials with green arrows - participants had to press the button indicating the actual direction of the arrow and 25 incongruent trials (n=25), when red arrows were presented and participants had to respond in the opposite direction to that indicated by the arrows presented.

GNG: this also builds on the 2-CRT but also includes a different IB-EF component that require participants to completely suppress and withhold a dominant tendency to press

the buttons indicating the direction of the green arrows (Go stimuli; n=75) when a double-headed green arrow (No-Go stimuli; n=25) appear in the screen. This task consisted of 100 trials.

Inter-trial interval was 1500 msec and the stimulus duration was 100 msec for all three tasks. These three tasks were used to derive BIP variables using Diffusion Models (2C-RT and CCT), IB-EF measured in the context of BIP deficits (i.e., above and beyond deficits in BIP or measured independently from BIP) and classical IB-EF measures (CCT and GNG).

Basic Information Processing (BIP) derived from Diffusion Models

BIP variables were derived directly from Diffusion Models (55, 70) in both 2C-RT and in congruent trials of CCT. We use the following parameters for analysis: boundary separation ("*a"*), non-decision time ("*Ter"*), drift rate (*"v"*) as well as two parameters for variability from trial to trial for both extra-decisional processes ("Q") and decisional processes ("e"). The boundary separation indicates the relationship between speed and accuracy (i.e., speed-accuracy trade-off – a response caution or impulsive response style). The non-decision time encompasses encoding, motor function (preparation and execution). The drift rate reflects the rate at which an individual is able to acquire information from an encoded stimulus to make a forced choice response (71). Both non-decision time and drift rates fluctuate from trial to trial in the course of the experiment also providing parameters of BIP variability. The correlations between DM parameters in both tasks and between congruent and incongruent conditions of CCT task are given in supplementary Table 1.

Inhibitory-Based Executive Function (IB-EF)

IB-EF measured using Diffusion Models: since classical parameters of IB-EF assume an intact BIP (a controversial assumption) we used DM to investigate IB-EF in a way it is above and beyond potential pre-existing deficits in BIP. The IB-EF can be measured as the difference in mean non-decision time from congruent and incongruent trials ($v_{incongruent} - v_{congruent}$)(70). See supplemental material for further explanations.

Classical parameters: For CCT we used the % of correct responses in incongruent trials and for GNG the % of correct inhibitions on No-Go trials (2).

Intelligence

Intelligence quotient was estimated using the vocabulary and block design subtests of the Weschler Intelligence Scale for Children, 3rd edition – WISC-III (72) using the Tellegen and Briggs method (73) and Brazilian norms (74).

Statistical Analysis

Multivariate Analysis of Covariance (Pillai's Trace) were used to test overall group differences in BIP across all variables. The source of differences on specific dependent variables for BIP and differences in IB-EF were explored using ANCOVAS. These analyses tested the effect of group, site, gender, controlling for estimated IQ and age as covariates. Significant differences between groups were further checked using two simple contrasts in order to avoid multiple testing: (1) differences between TDC and other groups; (2) differences between ADHD and other groups of psychopathology.

Our first hypothesis (ADHD versus TDC differences), was tested using the first of these contrasts. For our second hypothesis (ADHD specificity), we predicted that (1) ADHD participants would differ significantly from TDC (contrast 1); (2) ADHD would differ from the other psychopathological groups (contrast 2) and (3) other psychopathological groups did not differ from TDC in the same direction as ADHD (contrast 1).

In order to investigate our third hypothesis (effects of the comorbidity between Attention and ODD/CD), a similar analytic strategy was followed with one difference. Instead of using non-overlapping diagnostic groups (as in the first and second hypotheses), we used "Any ADHD" and "Any ODD/CD" as dummy variables in order to test their interaction in the linear model ("Any ADHD" * "Any ODD/CD").

In order to test our fourth hypothesis, point-bi-serial correlations were calculated for classical indexes of the inhibitory tasks (CCT: % of inhibitions on the incongruent trials; GNG: % of correct inhibitions). Following this, partial correlations were calculated controlling for age, IQ, site and gender and for baseline BIP parameters.

Effect sizes were defined in terms of % of explained variance and 1, 9 and 25% were defined as small, medium, and large effects corresponding to 0.01, 0.06 and 0.14 partial eta square (η_p^2) values (75). Diffusion Model Analysis was performed using computer codes from hierarchical diffusion models for two-choice response times (76). All scores were z-transformed before analysis using Van der Waerden transformation (77). All tests were two-tailed.

Results

Differences in demographics, psychopathology and classical task measures among groups are depicted in Table 1. The *Distress* group had a higher percentage of females ($\chi^2(5)=14.2$, p=0.014; adjusted residuals = 2.8) than the TDC group. The ADHD group had lower IQ than the TDC (F(5,698)=3.8, p=0.002). The groups did not differ in age (F(5,698)=2.20, p=0.053).

Hypothesis 1: Do non-referred community cases of ADHD differ from TDC in BIP components and IB-EF?

Results from all MANCOVAs and post hoc ANCOVAs related to hypothesis 1 can be found in Table 1.

BIP: ADHD subjects had faster encoding and/or motor preparation/execution (lower "Ter"), poorer processing efficiency (lower "v"), higher variability in processing efficiency from trial to trial (higher "e") and a more cautious response style (higher "a") (Table 2, Figure 1) in the 2C-RT. ADHD group differed significantly from controls for also having poorer processing efficiency (lower "v") and faster encoding and/or motor function (lower "Ter") in the CCT.

IB-EF: ANCOVAs with IB-EF estimates measured above and beyond BIP in the CCT revealed no statistically significant group effects (Table 2).

Thus in the both 2C-RT and CCT, children with ADHD have shown poorer processing efficiency and faster encoding/motor function (Table 2, Figure 1). A more cautious response style and higher variability in deciding from trial to trial were only significant in 2C-RT task, but not in the CCT (Table 2, Figure 1). No findings for IB-EF were found.

Hypothesis 2: Are BIP deficits specific to ADHD?

Results from all MANCOVAs and post-hoc ANCOVAs related to hypothesis 2 can be found in Table 1.

BIP: Poorer processing efficiency in the 2C-RT differentiated ADHD group from all other groups indicating that this deficit was specific for ADHD. A faster encoding/motor function differentiated ADHD from both the Fear and Distress groups, but not from ODD/CD group. In addition, ADHD subjects had a more cautious response style whereas ODD/CD subjects had a less cautious or "impulsive" response style (Table 2, Figure 2). For the CCT, only poorer processing efficiency differentiated ADHD from Fear group (Table 2, Figure 2).

IB-EF: ANCOVAs with IB-EF estimates measured above and beyond deficits in BIP in CCT revealed no statistically significant group effects (Table 2).

Thus only processing efficiency was found to be specifically associated to ADHD in the 2C-RT.

Hypothesis 3: Does comorbidity between ADHD and ODD/CD represent a qualitatively different clinical entity with respect to these deficits in BIP and IB-EF?

BIP: MANCOVAs testing the interaction term between ADHD and ODD/CD as dummy variables for all BIP parameters in the 2C-RT and in CCT resulted in non-significant results (all p-values >0.05).

IB-EF: No interactive effect for IB-EF was found (all p-values>0.05).

Thus, the comorbidity seems to represent only additive effects of its constituents and not a distinct category in terms of BIP and IB-EF.

Hypothesis 4: Do classical parameters of IB-EF remain significant after controlling for deficits in BIP?

In the three-abovementioned hypothesis, we measured IB-EF using diffusion analysis, a way of measuring IB-EF above and beyond potentially pre-existing BIP deficits. With this rigorous analysis no evidence of IB-EF deficits were found in ADHD. Despite that, deficits in IB-EF measured with classical parameters such as % of correct responses in incongruent trials in the CCT and % of correct inhibitions in No-Go trials in GNG are

frequently reported in ADHD literature. Therefore this fourth hypothesis aim to investigate: (1) if we can find the same classical findings in our sample; and (2) if these potential differences in such parameters wouldn't just reflect the already dysfunctional underlying BIP that we found.

First we found that classical IB-EF measures were significantly associated with ADHD in both tasks, corroborating previous findings in the field (Table 4). Second, we conducted partial correlations in order to control for baseline BIP deficits (as measured by 2C-RT) and to investigate whether the associations found for classical IB-EF variables would be fully accounted by the lower order deficits in baseline BIP. After controlling for baseline BIP parameters, the association between ADHD and classical parameters of IB-EF in both tasks were no longer significant (Table 4). Moreover, mediation tests (Sobel Goodman) showed that about 50% of classical IB-EF Go/No-Go variable and 76% of the classical IB-EF CCT were mediated by processing efficiency (a BIP component) and only the mediated effects were significant. No evidence for direct effects was found in this analysis.

Discussion

In this study, we have demonstrated that some BIP components are impaired in ADHD subjects. Results revealed that children with ADHD differ from controls by having faster encoding and/or motor preparation/execution times and poorer processing efficiency in both tasks. Further, poorer processing efficiency in the 2C-RT task was the only parameter that met the criteria for being specific to ADHD and differentiated ADHD from all the other psychopathological groups. Overall evidence supports a correlated risk factors model for the comorbid group (ADHD+ODD/CD). All deficits frequently seen in ADHD subjects measured with classical IB-EF variables were fully accounted by pre-existing BIP deficits.

Our results challenge inhibitory theories that propose inhibitory deficits as an unique deficit of ADHD (9-11) but are consonant with studies suggesting that all between clinical group differences in inhibitory findings become non-significant after controlling for baseline measures in BIP (6), or following the introduction of incentives (50, 78, 79). They also concur with electrophysiological studies indicating that inhibitory control difficulties in ADHD are accompanied by altered response preparation and motor execution processes, which may

indicate dysfunctional processes in some BIP components during these tasks (80-82). These findings provide further evidence in supporting the thesis that non-executive deficits are primary in ADHD.

Findings for the relevance of processing efficiency are in agreement with a recent meta-analysis (71) documenting that poorer rate of accumulating information in DM is a critical parameter to explain individual differences related to ADHD. Children with ADHD are impaired in accumulating information in order to perform a very simple decision with respect to the direction that a given arrow is pointing to. An inefficient accumulation of information to reach very simple decisions may explain a variety of ADHD symptoms, since all the time children are required to contrast information accumulated in their given environments to a series of instructions about how to behave on them. Our study extends previous findings demonstrating that poorer processing efficiency is not shared with other forms of psychopathology.

Faster encoding and/or motor preparation/execution differentiated ADHD group from distress and fear groups in the 2C-RT. Evidence for deficits in both encoding (83) and motor preparation/execution do exist for ADHD (84). We hypothesize that a lower encoding/motor function time may represents three distinct conditions: (1) an advantage in information processing that may further explain motivational deficits in activities that are not “fast enough” and therefore “not interesting enough for engaging effort”; (2) a faster but dysfunctional/inefficient encoding and/or motor function process (explaining a higher number of errors in all tasks in addition to the errors due to inefficient processing); (3) a compensatory mechanism secondary to the inefficient information accumulation.

It is important to note that our results were more consistent for the 2C-RT than for the CCT. Although differences between ADHD and TDC emerged for mean non-decision time and mean drift rates in both tasks; only in the 2C-RT deficits did drift rates differentiated ADHD from other psychopathological groups. Thus, we assessed task effects for these parameters (see supplemental material), exploring a potential role for cognitive load in determining these two deficits. No group by task effects were found for the main parameters, suggesting that a potential type II error is a suitable reason for our CCT negative findings in drift rates when other child mental disorders were compared to ADHD.

The results concerning the speed accuracy trade-off are of special interest, since response style in the 2C-RT task clearly differentiated ADHD subjects from ODD/CD patients, with ODD/CD group trading accuracy for speed, while ADHD subjects having a more cautious response style. Here, speed and accuracy were equally emphasized, suggesting that strategy rather than pure structural deficits in cognitive processing is contributing to attentional function in externalizing disorders (4, 85, 86). ODD/CD and not ADHD showed a more impulsive response style. ADHD, if anything, had a more cautious response style. However, none of these results were evident in the CCT and a task by group effect was found (see supplemental material), reflecting that this finding is highly dependent on task manipulations consistent with previous evidence (3).

The comorbid group with both ADHD and ODD/CD did not show any distinctive pattern to characterize them as a distinct entity from single diagnostic groups. This evidence supports the "*correlated risk factors model*", that predicts additive or synergistic effects of comorbidity, in contrast to the "*independent disorders model*" that predicts unique neuropsychological profiles (87, 88). Our findings are in agreement with studies that formally tested the interaction between these two clinical domains and failed to find any significant differences (37).

Regarding the implications of our study for theoretical models, the results fit well into the cognitive energetic model (14, 89). This model proposes that overall efficiency of information processing is determined by the interplay between computational mechanisms of attention, state factors (e.g., arousal, activation and effort) and management/executive control. Our findings are also consistent with state findings observed in a default mode network studies of ADHD (90).

This current study has some limitations. First, we were only able to investigate a restricted range of psychiatric disorders and important forms of psychopathology such as autism and reading disorders were not evaluated here. However, we used an empirically and theoretically derived taxonomy investigating differences between Fear, Distress, ADHD and ODD/CD as well as comorbid groups. Second, although our sample size is one of the biggest in this area of investigations, it might not have had enough power to confirm some of the findings on BIP in both tasks. Third, DM is not capable of detecting periodic oscillations in

performance that have been suggested to be characteristic of ADHD by some researchers (53, 91-93).

The current study has also some notable strengths. To our knowledge, this is the largest community-based study combining psychopathological and task-based data to study specificities and communalities in the neuropsychopathology of ADHD. All the groups came from the same community of subjects never medicated, providing a strong design against population stratification due to selection methods. All results were independent from age, site, gender and IQ effects. In addition, we used sophisticated analytic methods of performance, allowing us to decompose cognitive data into distinct processing components.

In conclusion, we were able to find that ADHD is distinctly affected in some BIP components that also explain deficits in IB-EF if measured with classical variables in the literature. Our results have important implications for research in pathophysiology of ADHD, since they point to both the involvement of lower order processing and strategy differences among clinical groups. Future studies are needed to reveal the neural networks underlying these BIP components and strategies and to advance our understanding of such deficits from a clinical and neurobiological perspective.

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Table 1 - Sample Description of Clinical Assessment, Age, IQ, SES, Gender and Site

	TDC (n=378)		Fear (n=90)		Distress (n=57)		ADHD (n=100)		ODD/CD (n=40)		ADHD+ODD/CD (n=39)	
	n	%	n	%	n	%	n	%	n	%	n	%
Site (POA)	150	0.4	60	0.7	43	0.8	53	0.5	34	0.9	22	0.6
Gender (male)	204	0.5	40	0.4	20	0.4	57	0.6	26	0.7	24	0.6
DSM-IV diagnosis												
Separation	-	-	31	0.3	-	-	-	-	-	-	-	-
Specific Phobia	-	-	50	0.6	-	-	-	-	-	-	-	-
Social Phobia	-	-	14	0.2	-	-	-	-	-	-	-	-
PTSD	-	-	-	-	7	0.1	-	-	-	-	-	-
GAD	-	-	-	-	19	0.3	-	-	-	-	-	-
Major dep	-	-	-	-	26	0.5	-	-	-	-	-	-
Other dep	-	-	-	-	4	0.1	-	-	-	-	-	-
Undiff anx/dep	-	-	-	-	2	0.0	-	-	-	-	-	-
ADHD-C	-	-	-	-	-	-	25	0.3	-	-	22	0.6
ADHD-I	-	-	-	-	-	-	41	0.4	-	-	10	0.3
ADHD -H	-	-	-	-	-	-	20	0.2	-	-	4	0.1
ADHD NOS	-	-	-	-	-	-	14	0.1	-	-	3	0.1
ODD	-	-	-	-	-	-	-	-	29	0.7	33	0.8
CD	-	-	-	-	-	-	-	-	9	0.2	7	0.2
Other disruptive	-	-	-	-	-	-	-	-	3	0.1	1	0.0
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Age (years)	9.7	2.0	9.8	1.9	10.5	2.0	9.6	1.8	10.0	2.0	9.4	2.0
IQ	105.7	15.6	100.9	16.8	100.3	16.5	99.6	16.6	101.6	13.3	100.8	18.4
SES (Score)	20.8	4.7	20.2	4.2	19.2	4.7	20.5	5.2	19.4	4.8	19.8	3.8

Note: M, Mean; SD, Standard Deviation; SES, Socioeconomic Status; IQ, Intelligence Quoeficient; TDC, Typically Developing Controls; PTSD, Post-traumatic stress disorder; GAD, Generalised Anxiety Disorder; Undiff, Undifferentiated; anx, anxiety; dep, depression; ADHD, Attention Deficit/Hyperactivity Disorder; -C, Combined; -I Innatentive; -H, Hyperactive; NOS, Not Otherwise Specified; ODD, Oppositional Defiant Disorder; CD, Conduct Disorder; TDC, Typically Developing Controls.

Table 2 – Post-hoc ANCOVAs showing between-group differences in Diffusion Model Parameters for Two Choice Reaction Time (2C-RT) task and Conflict Control Task (CCT)

	TDC		ADHD		Fear		Distress		ODD/CD		ANCOVAs			Significant contrasts	Hypothesis	
	M	SE	M	SE	M	SE	M	SE	M	SE	$F_{4,657}$	p	η_p^2		H1	H2
BIP (2C-RT)																
Q	-.074	.047	.102	.091	.013	.096	.187	.123	-.172	.146	1.773	0.132	0.011	-	**No**	**No**
Ter	.053	.049	-.286	.095	.108	.1	.125	.128	-.101	.151	3.212	**0.013**	0.019	**ADHD <** TDC, FEAR, DIST	**Yes**	**No**
a	-.048	.05	.216	.097	.043	.102	.101	.13	-.526	.154	4.635	**0.001**	0.028	**ADHD>**TDC, ODD/CD	**Yes**	**No**
e	-.13	.052	.24	.099	-.052	.105	.269	.134	.13	.158	4.131	**0.003**	0.025	**ADHD>**TDC, FEAR; DIST>**TDC**	**Yes**	**No**
v	.09	.048	-.313	.093	.019	.098	.022	.125	.059	.148	3.763	**0.005**	0.022	**ADHD<** all groups	**Yes**	**Yes**
BIP (CCT)																
Q	-.045	.046	.114	.087	-.014	.092	.064	.118	-.235	.14	1.406	0.23	0.009		**No**	**No**
*Ter (c)**	.089	.05	-.184	.095	.024	.101	-.055	.129	-.318	.152	2.784	**0.026**	0.017	**ADHD**<TDC	**Yes**	**No**
a	-.109	.052	.114	.1	.235	.105	.151	.135	.08	.159	2.87	**0.022**	0.017	FEAR>**TDC**	**No**	**No**
e	.007	.052	-.043	.101	-.018	.107	.007	.136	-.065	.161	0.085	0.987	0.001		**No**	**No**
*v (c)**	.117	.049	-.278	.095	.003	.1	-.214	.128	-.005	.152	4.135	**0.003**	0.025	**ADHD<**TDC, FEAR; DIST<**TDC**	**Yes**	**No**
IB-EF (CCT)																
v(i)-v(c)	-.015	.102	.118	.157	.235	.132	.073	.097	-.015	.102	1.356	0.248	0.008	-	**No**	**No**

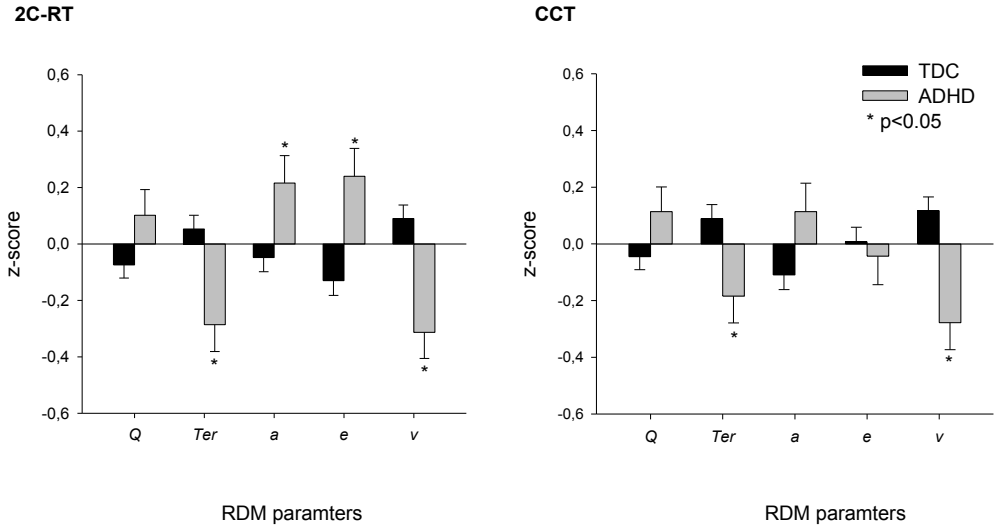
MANCOVAs: BIP (2C-RT), $F(20,2612)=2.69$, $p<0.001$, $\eta_p^2=0.02$; **BIP (CCT)**, $F(20,2612)=2.03$, $p=0.002$, $\eta_p^2=0.015$;
Note: Estimated Marginal Means for z-scores (corrected for age and IQ). IB-EF represented differences between raw scores of both trial conditions.
Abbreviations: IB-EF, Inhibitory-based Executive Function; M, Mean; SE, Standard Error; ANCOVA, Analysis of Covariance; TDC, Typical Developing Controls; DIST, Distress.
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency); v(i), Mean Drift Rates in incongruent trials; v (c), Mean Drift Rates in congruent trials. * Calculated only for congruent trials.
Contrasts: [a] Difference from controls; [b] Difference from ADHD subjects (gray areas mark the two comparison groups)..
Hypothesis testing: H1, Hypothesis 1 (Deficits in ADHD if compared to controls); H2, Hypothesis 2 (Deficits are specific to ADHD);
Yes, Not Rejected; No, Rejected

Table 3 – Partial correlations between Inhibitory-Based Executive Function classical indexes controlled for potential confounders and baseline basic information processing

			Partial correlations			
	Crude Analysis for Classical indexes		**Step 1** (Age, Gender, IQ, Site)		**Step 2** (+BIP)	
	% CI CCT	% CI GNG	% CI CCT	% CI GNG	% CI CCT	% CI GNG
% CI CCT	-	.421**	-	0.385**	-	0.256**
% CI GNG	.421**	-	0.385**	-	0.256**	-
Groups						
ADHD	-.094*	-.096*	-0.066	-0.082*	0.005	-0.021
ODD/CD	-.012	-.034	0.012	-0.022	0.029	0.006
Fear	-.019	-.004	-0.01	-0.011	-0.02	-0.038
Distress	.029	.004	-0.003	-0.028	0.007	-0.042
Potential Confounders						
Age	.293**	.209**				
IQ	.090*	-.04				
Site (POA)	.013	-.001				
Gender (male)	.061	.144**				
BIP (2C-RT)						
Q	-.259**	-.075*				
Ter	.156**	.335**				
a	-.155**	-.032				
e	-.162**	-.153**				
v	.460**	.447**				

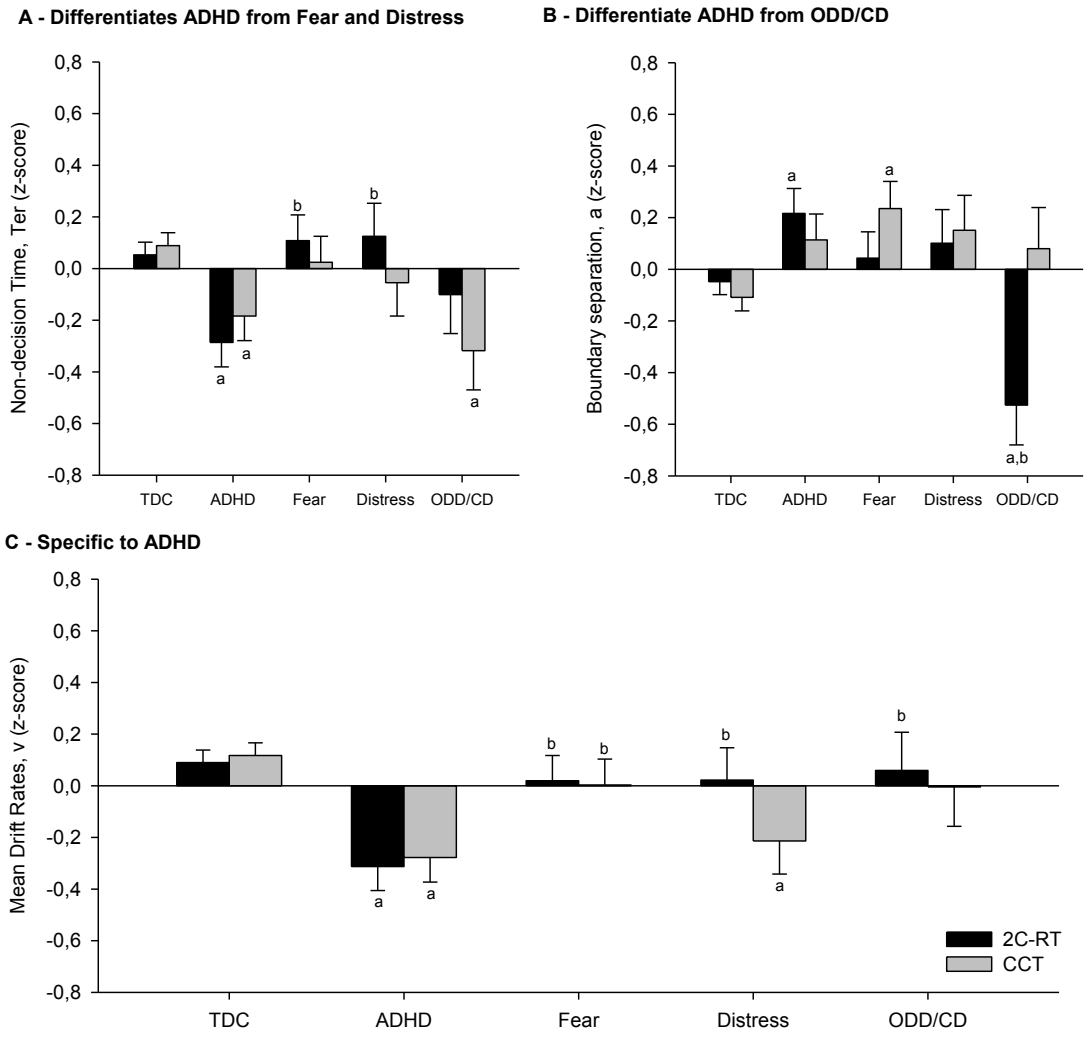
Note: IB-EF, Inhibitory Based Executive Function; GNG, Go/No-Go; CCT, Conflict Control Task; 2C-RT, 2 Choice Reaction Time Task; ADHD, Attention Deficit/Hyperactivity Disorder; ODD/CD, Oppositional Defiant / Conduct Disorder; IQ, Intelligence.
DM parameters: Q, Trial to Trial variability in Non-decision Time; Ter, Mean Non-decision Time; a, Boundary Separation; e, Trial to Trial variability in Drift Rates; v, Mean Drift Rates;
Classical indexes for GNG is % of correct inhibitions and for CCT is % of correct responses in the incongruent trials. Values represent Pearson and point-biserial correlation coefficients. Gray line represent correlations for ADHD; * p<0.05; **p<0.01.

Figure 1 – Primary differences between Attention Deficits/Hyperactivity Disorder (ADHD) subjects from Typically Developing Controls (TDC) in Basic Information Processing



Abbreviations: TDC, Typical Developing Controls; ADHD, Attention Deficit/Hyperactivity Disorder; 2C-RT, Two-choice Reaction Time task; CCT, Conflict Control Task; BIP, Basic Information Processing;
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency). *Ter* and *v* in CCT were generated only with congruent trials.

Figure 2 – Specific processing deficits in Attention Deficit/Hyperactivity Disorder compared to ODD/CD, Fear and Distress groups



Abbreviations: TDC, Typical Developing Controls; ADHD, Attention Deficit/Hyperactivity Disorder; ODD/CD, Oppositional Defiant/Conduct Disorder; 2C-RT, 2 Choice Reaction Time Task; CCT, Conflict Control Task;
Contrasts: [a] Different from controls; [b] Different from ADHD;
Panel A – ADHD is different from Fear and Distress disorders in the 2C-RT.
Panel B – ADHD is different from ODD/CD in the 2C-RT.
Panel C – ADHD is different from all groups in the 2C-RT, and from Fear in the CCT.

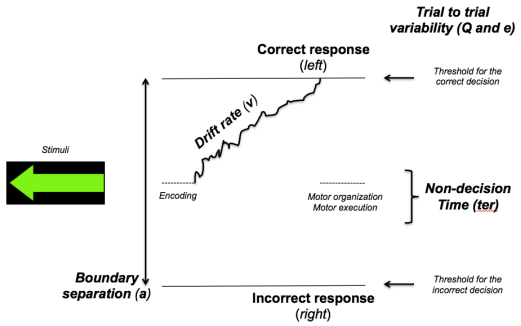
Supplemental material

Measuring IB-EF with Diffusion Model (Alternative method)

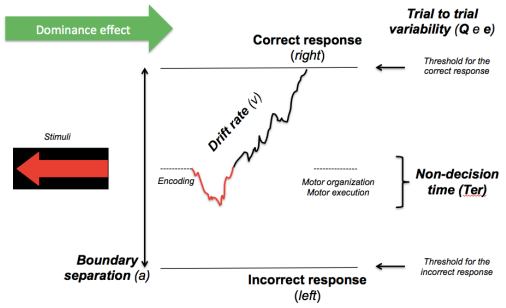
An advantage of using Diffusion Models is the opportunity to look for high order functions (such as inhibitory control) in an independent way of potential pre-existing deficits in BIP. We performed this analysis using the CCT, comparing congruent and incongruent trials and investigating the effect of “conflict” between groups in mean drift rates. This is based on the assumption that in incongruent trials the subject starts to accumulate information towards the wrong boundary. This happens because: (a) it is intuitive to press the right button when you see an arrow pointing to the right direction and (b) we introduce a dominance effect introducing a majority of congruent trials (75%) reinforcing this intuitive process. Therefore in Incongruent trials the brain starts accumulating information towards the wrong boundary based on the direction (both intuitively and reinforced by frequency) and has to change the accumulation of information towards the correct boundary when the instruction of pressing the opposite button based on the color of the arrow is integrated in the process of accumulation of evidence (a more "high" order interference in the decision making process). Subtracting from incongruent trials (that include conflict + BIP), the processing efficiency from congruent trials (only composed of BIP) provides a reliable and independent measure of the IB-EF (conflict effect), measured in the context of potential BIP deficits.

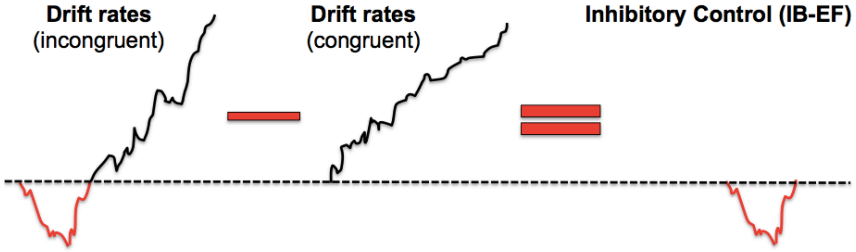
Schematic representation of the Diffusion Process in the Conflict Control Task

Congruent trials



Incongruent trials





Complementary analysis: "task" effects

Since results from the two tasks regarding BIP (2C-RT and CCT) are somewhat mixed, we conducted an additional analysis in order to investigate "task" effects and "task by group" effects, i.e., to investigate whether differences in the executive load of the task would affect DM parameters comparing the trials from the 2C-RT with the congruent trials from the CCT (that are exactly the same), using a mixed analysis of covariance. A main effect of task was found for all parameters and reflected that the more executive demanding the task implicates in a higher variability in non-decision time, slower encoding/motor-function, more cautiousness, more variability in deciding and lower processing efficiency, as expected.

Two task parameters produced a task by group interaction: boundary separation (*"a"*) ($F(4,654)=3.6$, $p=0.007$, $\eta_p^2=0.022$) and trial-to-trial variability in drift rates (*"e"*) ($F(4,654)=2.69$, $p=0.030$, $\eta_p^2=0.016$). In order to identify in which groups this effect occurred, stratified analysis were performed for each group.

This analysis revealed that subjects with ODD/CD were less cautious in the 2C-RT compared to the CCT and that TDC and Fear group was more cautious in the CCT compared to the 2C-RT; no differences were detected for ADHD and Distress groups. Differences in trial-to-trial variability in drift rates were only significantly associated with ADHD in the 2C-RT and not in the CCT. This analysis indicates that groups differ in the effects that variation in task parameters and task demands affect some BIP parameters. However, no groups by task interactions were found for the major parameters that are implicated in ADHD (processing efficiency and encoding/motor-function).

Supplementary Table 1 - Correlation Matrix for age, IQ and gender and Diffusion Model Parameters for Two-Choice Reaction Time (2C-RT) task and Conflict Control Task (CCT) in Typical Developing Controls (n=378)

	Q		**Ter**			**a**		**e**		**v**		
	2C-RT	CCT	2C-RT	CCT-I	CCT-C	2C-RT	CCT	2C-RT	CCT	2C-RT	CCT-I	CCT-C
Age	-.399**	-.509**	-.272**	-.266**	-.297**	-.195**	-.089	-.015	.155**	.341**	.181**	.262**
IQ	-.046	-.031	.045	-.008	-.023	-.02	.041	.047	-.059	.081	.099	.037
Males	-.011	.051	.135**	.139**	.110*	.025	.091	-.125*	-.026	.046	-.005	-.006
Q 2C-RT	-	.518**	.390**	.278**	.288**	.088	.053	.314**	-.034	-.202**	-.132*	-.205**
Q CCT		-	.252**	.521**	.587**	.312**	-.168**	-.033	.038	-.412**	-.056	-.166**
Ter 2C-RT			-	.580**	.560**	-.209**	-.008	-.053	-.305**	.508**	.106*	.158**
Ter CCT-I				-	.877**	.131*	-.198**	-.217**	-.281**	.169**	.412**	.305**
Ter CCT-C					-	.121*	-.344**	-.208**	-.181**	.125*	.296**	.297**
a 2C-RT						-	.216**	.001	-.110*	-.356**	-.002	-.126*
a CCT							-	-.01	-.169**	-.022	-.110*	-.155**
e 2C-RT								-	.124*	.001	-.133**	-.088
e CCT									-	-.216**	-.292**	-.179**
v 2C-RT										-	.358**	.486**
v CCT-I											-	.545**
v CCT-C												-

Note: Pearson product-moment correlation coefficients (r). For gender, point-biserial correlation coefficient (r_{pb}) is presented.
Abbreviations: Q, Trial to Trial variability in Non-decision Time; Ter, Mean Non-decision Time; a, Boundary Separation; e, Trial to Trial variability in Drift Rates; v, Mean Drift Rates. (c) congruent trials; (i) incongruent trials;
* p<0.05; ** p<0.01.

8. ARTIGO #4

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Neuropsychological Mechanisms Underpinning Inattention and Hyperactivity/Impulsivity: Neurocognitive Support for a Dimensional Model of ADHD

Running Title: Neurocognitive Support for ADHD Dimensionality

Giovanni Abrahão Salum, MD, PhD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; Federal University of Rio Grande do Sul, Brazil*

Edmund Sonuga-Barke, PhD - *Southamptom University, United Kingdon; Ghent University, Belgium*

Joseph Sergeant, PhD - *Vrije Universiteit, The Netherlands*

Joachim Vandekerckhove, PhD - University of California, United States

Ary Gadelha, MD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; Federal University of São Paulo, Brazil*

Tais Moriyama, MD- *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; São Paulo University, Brazil*

Ana Soledade Graeff-Martins, MD, PhD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; São Paulo University, Brazil*

Gisele Gus Manfro, MD, PhD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; Federal University of Rio Grande do Sul, Brazil*

Guilherme Polanczyk, MD, PhD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; São Paulo University, Brazil*

Luis Augusto Paim Rohde, MD, PhD - *National Institute of Developmental Psychiatry for Children and Adolescents, Brazil; Federal University of Rio Grande do Sul, Brazil*

Address correspondence and reprint requests

Giovanni Abrahão Salum

Hospital de Clínicas de Porto Alegre

Ramiro Barcelos, 2350 – room 2202; Porto Alegre, Brazil – 90035-003;

E-mail:gsalumjr@gmail.com - Phone/Fax: +55 51 3359 8094

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Tables: 2 / **Figures:** 1 **/ Supplemental material**: 2 (Table S1 and Table S2)

Abstract (word count 250)

Objective: Evidences from epidemiology, behavioral genetics and psychometrics suggest that Attention Deficit/Hyperactivity Disorder (ADHD) is a dimensional construct. Nevertheless, whether neuropsychological mechanisms operate at different levels of ADHD symptom severity remains to be studied. We investigated whether deficits in neuropsychological mechanisms previously associated with ADHD - Basic Information Processing (BIP) and Inhibitory-Based Executive Function (IB-EF), have a linear relationship with symptoms of Inattention and Hyperactivity/Impulsivity across the whole spectrum of ADHD.

Methods: A total of 1,547 children (6 to 12 years old) participated in the study. The Development and Well Being Behavior (DAWBA) was used to classify children into groups according to ADHD symptoms in inattention and hyperactivity assessed independently: (1) asymptomatic, (2) minimal, (3) moderate, (4) clinical ADHD. Neurocognitive performance was evaluated using: 2-choice reaction time task (2C-RT) and Conflict Control Task (CCT). BIP and IB-EF were derived from Diffusion Models.

Results: Deficient BIP was found in subjects with minimal, moderate and full ADHD for both Inattention (in both tasks) and Hyperactivity/Impulsivity (in 2C-RT) if compared to asymptomatic patients. In all significant results, a linear trend was detected (p linear trend <0.05). No significant findings emerged for IB-EF.

Conclusion: We were able to show that deficits in BIP operate at several levels of ADHD spectrum and that increase in symptom severity were related linearly to neuropsychological impairment and were not restricted to the clinical syndrome. This data provides neurocognitive support for a dimensional model of ADHD in which diagnostic thresholds reflect clinical and societal burden rather than pathophysiological states.

Key-words: ADHD, Inattention, Hyperactivity, neuropsychology, neurocognitive, dimensionality.

Introduction

The controversy of whether ADHD should be regarded as a category or as a dimension has been a key issue in the literature on ADHD for over a decade[1-5] and it was revisited recently in the context of the development of new classificatory systems in Psychiatry[6,7]. A categorical view of ADHD propose that the disorder differs from normality in both degree and kind; whereas a dimension view of ADHD suggest that the disorder is different from normality only in degree but not in kind[6]. In the former, diagnostic thresholds actually reflect "natural" boundaries linked to underlying causes; in the last one, they are somewhat arbitrary, reflecting clinical and societal burden rather than pathophysiological states.

Evidence from the dimensionality of ADHD comes from behavioral genetics[4,8,9], taxometric studies[10-13] as well as neuroimaging[14]. For instance, behavioral genetic have addressed the issue of whether different levels of ADHD symptoms have the same or different etiology[8]. Data from this line of investigation suggested that ADHD is best viewed as a quantitative extreme determined by genetic and environmental factors operating dimensionally throughout the distribution of ADHD symptoms[8]. Nevertheless, studies investigating this issue from a neurocognitive perspective are still lacking.

Deficits in both Inhibitory-Based Executive Function (IB-EF) [15-17] and Basic Information Processing (BIP) [18-22] are considered central to ADHD pathophysiology. More recently, a considerable body of evidence has shown that deficits in IB-EF tasks in children with ADHD may be totally or partially attributable to underlying BIP deficits[18-20]. Concurring with these findings, a previous study from our group showed that children with ADHD have deficits in encoding/motor-function and decision-making processes[23]. These findings emerged using a Diffusion Model (DM) for two-choice response times[24]. This model allows both disentangling decision-making from sensory and motor processing and investigating strategic response style. No significant IB-EF deficits were found in ADHD. Our results, along with previous DM analyses[25], suggest a major role for BIP in explaining differences between ADHD and typical developing children. These findings were also found for other authors using a similar DM approaches[25].

We therefore used the documented association between BIP deficits and ADHD subjects from our previous study to formally test competing models of ADHD (e.g., dimensional and categorical). In order to address this specific question we selected a large sample of typically developing children with different levels of ADHD symptoms classified into three groups (asymptomatic, minimal symptoms and moderate symptoms) and children with ADHD defined by DSM-IV (high number of symptoms). All symptomatic groups were defined as having the same symptom interval between each other. Using that approach we were able to test the shape of the link function between BIP and four groups with different levels of ADHD symptoms. A linear relationship between neurocognitive deficits and symptoms of ADHD would favor a dimensional model. In counterpart, a discontinuity in neuropsychological mechanisms related to the clinical end of the spectrum – (a) between-group differences would be evident only in the clinical group or (b) the clinical group would be disproportionally affected if compared to the other groups – can be seen as evidence for a categorical model.

Indeed studies either have assessed the associations between symptoms of ADHD in the general population[26] (without DSM-IV formal diagnostic criteria) or in extreme ends of the distribution –clinical cases of ADHD and selected typical controls with low number of symptoms[18,20] – but they rarely look at the shape of the function that links neurocognition and ADHD symptoms across the spectrum more directly. Therefore, previous studies are limited in investigating different models of ADHD because they either focus on one or in the other model of the disorder.

Since this paper aims to "*test a concept"* rather then explore differences between clinical groups*,* we constructed groups of children without any other psychiatric disorders (including Oppositional Defiant Disorder, ODD/CD). This provides a strong design for testing this specific hypothesis, since spurious associations driven by other clinical disorders (such as ODD/CD) are diminished. This methodological refinement would be unfeasible in clinical samples that have high rates of comorbidity[27,28]. Our main hypothesis is that deficits in BIP (specifically processing efficiency and encoding/motor function) will be observed at subclinical as well as full clinical levels of ADHD and that ADHD symptom severity (both Inattention and Hyperactivity/Impulsivity) will be related to deficits in a linear way. Based on our previous

results with the clinical syndrome, no associations are expected for other strategic BIP variables (speed-accuracy trade off) or for IB-EF measures.

Methods

Participants

The sample is part of a large community school-based study. The ethics committee of the University of São Paulo approved the study. We obtained written consent from parents of all participants, and verbal assent from all children. The screening phase of the study included children from public schools close to the research centers in Porto Alegre and São Paulo, Brazil. Eligible subjects were those: (1) registered for school by a biological parent capable of providing consent and information about the child's behavior; (2) between 6-12 years of age (at the screening phase); (3) who remained in the same school during the study period.

During the screening phase 9,937 informants were interviewed using the *Family History Survey* (FHS)[29]. From this pool, we recruited two subgroups: a random-selection stratum and high-risk stratum. For the random-selection sample, a simple randomization procedure from school directories was used and a total of 958 subjects provided data on psychopathology. Selection for the high-risk stratum involved a risk-prioritization procedure, focused on individuals with a family history of specific disorders and current psychiatric symptoms and a total of 1514 provided data on psychopathology (see [30] for further details), resulting in a total sample of 2512 subjects.

From these 2512 subjects, 2177 (86.7%) performed the 2-Choice Reaction Time Task (2C-RT) [31], 2166 (86.2%) performed the Conflict Control Task (CCT) [31], and 2243 (89.3%) performed the IQ evaluation. A total 2002 (74%) had data available for all three evaluations. Subjects that did not perform the tasks did not differ from those completing both instruments regarding psychopathology (all $p>0.05$; data not shown). An additional 36 (1.6%) were excluded based on poor task compliance for having more than 50% of missing/outlier responses and 108 (4.3%) were excluded for poor task compliance estimated by the DM. No differences were detected between the 144 subjects excluded due to task compliance issues and the remaining sample regarding psychopathology (all $p>0.05$; data not shown).

The goal of the current study was to select from the remaining sample (n=1858), a very specific group of patients lying at different levels of the spectrum of ADHD symptoms, but without comorbidity with other psychiatric disorders. The ADHD section of the DAWBA was used in this study without any skipping rule to allow the assessment of inattention and hyperactivity/impulsivity in the total sample. Each of the 18 ADHD symptoms have a 3-option response scale "No more than other", "A little more than others" and "A lot more than others"; representing a score of 0, 1 and 2, respectively. Groups were constructed based on similar symptom cut-offs suggested by previous studies[14,32]. Therefore, we selected overlapping hierarchically defined sets of groups as described below in order to test our hypotheses:

Inattention: (1) TDC (asymptomatic) – randomly selected subjects, scoring 0 in inattentive symptoms; (2) TDC (minimal) – subjects scoring from 1 to 5 in inattentive symptoms scale (maximum of 2 full ADHD symptoms); (3) TDC (moderate) – subjects scoring from 6 to 11 in inattentive symptoms (maximum of 5 full ADHD symptoms in typical developing children); (4) Predominantly Inattentive ADHD subtype (n=36) or a Combined ADHD subtype (n=17) (Full ADHD DSM-IV diagnoses).

Hyperactivity/Impulsive: (1) TDC (asymptomatic) – randomly selected subjects, scoring 0 in hyperactive/impulsive symptoms; (2) TDC (minimal) – subjects scoring from 1 to 5 in hyperactive/impulsive symptoms (maximum of 2 full ADHD symptoms); (3) TDC (moderate) –subjects scoring from 6 to 11 in hyperactive/impulsive symptoms (maximum of 5 full ADHD symptoms in typical developing children); (4) Predominantly Hyperactive/Impulsive ADHD subtype (n=17) or a Combined ADHD subtype (n=15) (Full ADHD DSM-IV diagnoses).

For all analyses, we excluded patients that ever received any psychiatric medication (n=74, 4%), those with an IQ bellow 70 (n=36; 1.8%), individuals with comorbid conduct/oppositional disorder (n=127; 6.8%), anxiety disorders (n=100; 5.4%), depressive disorders (n=64; 3.5%), mania (n=3; 0.2%), psychoses (n=1; 0.1%), pervasive developmental disorders (n=9; 0.6%), tic disorders (n=15; 0.8%), and eating disorders (n=8; 0.5%).

Psychiatric diagnosis

We used the Development and Well-Being Assessment (DAWBA)[33], a structured interview administered to biological parents by trained lay interviewers in order to perform

psychiatric diagnoses. The DAWBA was administered to biological parents who indicated they could provide accurate information in accordance with previously reported procedures[33]. Nine psychiatrists performed rating procedures. All were trained and supervised by a senior child psychiatrist.

Neurocognitive Tasks

Two tasks were used to assess BIP and IB-EF: a simple two-choice reaction time task (2C-RT) and a conflict-control task (CCT) [31].

2C-RT: this task measures the ability of the participant to perform extremely basic perceptual decisions about the direction an arrow on the screen is pointing. It has little or no executive component. A total of 100 arrow stimuli were presented, half requiring left and half requiring a right button press.

CCT: this task measures builds on the 2-CRT and includes a second inhibitory executive component requiring participants to occasionally suppress a dominant tendency to respond to the actual direction of an arrow and to initiate a response indicating the opposite direction. This requirement was indicated by a change in the color of the arrow (a "conflict" effect). There were 75 congruent trials with green arrows - participants had to press the button indicating the actual direction of the arrow and 25 incongruent trials (n=25), when red arrows were presented and participants had to respond in the opposite direction to that indicated by the arrows presented.

Inter-trial interval was 1500 msec and the stimulus duration was 100 msec for all three tasks in order to allow the investigation of our fourth hypothesis. These two tasks were used to derive BIP variables using Diffusion Models (2C-RT and CCT) and IB-EF measured in the context of BIP deficits (above and beyond deficits in BIP, i.e., IB-E deficits measured independently from potential BIP deficits).

Deriving Basic Information Processing (BIP) – Diffusion Model

BIP variables were derived from Diffusion Models [24,34] in both 2C-RT and CCT. We use the following parameters for analysis: boundary separation ("*a"*), non-decision time ("*Ter"*), drift rate (*"v"*) as well as two parameters for variability from trial to trial for both extra-

decisional processes ("Q") and decisional processes ("e"). The boundary separation indicates the relationship between speed and accuracy (i.e., speed-accuracy trade-off – a response caution or impulsive response style). The non-decision time is thought to encompass encoding and motor function (preparation and execution). The drift rate reflects the efficiency with which information is processed - the rate at which an individual is able to acquire information from an encoded stimulus to make a forced choice response [25]. Both non-decision time and drift rates fluctuate from trial to trial in the course of the experiment also providing parameters of BIP variability. Diffusion Model analysis used a sophisticated method [35] for dealing with outliers and other contaminants (random guesses and fast guesses) resulting in the exclusions of some subjects as described above. DM parameters were calculated only for 2C-RT and for CCT task.

Inhibitory-Based Executive Function (IB-EF; measured in the context of BIP)

IB-EF was measured in the context of BIP, i.e., above and beyond deficits in BIP. In the CCT, we assessed the difference in performance in congruent and incongruent trials in mean drift rates (v-incongruent – v-congruent)[34]. This model assumes that the "conflict effect" of CCT induces an initial accumulation of evidence towards the wrong boundary in incongruent trials (the conflict effect), which is followed for the basic accumulation of evidence towards the correct boundary (BIP that is also embedded in incongruent trials). Subtracting from incongruent trials (that include conflict + BIP), the processing efficiency from congruent trials (only composed of BIP) provides a reliable and independent measure of the IB-EF (conflict effect only), measured in the context of potential BIP deficits.

Statistical Analysis

In order to investigate our hypothesis, we performed a series of Multivariate Analysis of Covariance, using BIP variables from 2C-RT and from CCT. Results from MANCOVAS and IB-EF from CCT were further decomposed with ANCOVAS and between group differences were analyzed with two specific contrasts: (1) differences from TDC (asymptomatic) and differences from clinically defined ADHD subjects. All models (MANCOVAs and ANCOVAs) controlled for site, gender and used age and IQ as covariates. In addition, we tested linear,

quadratic and cubic trends among the four groups independently for inattention and hyperactivity/impulsivity using polynomial contrasts. Hierarchical linear models were used to investigate the role of ODD symptoms in driving our results.

All variables were z-transformed and normalized using Van den Waerden transformation[36]. Effect sizes were defined in terms of % of explained variance and 1, 9 and 25% were defined as small, medium, and large effects corresponding to 0.01, 0.06 and 0.14 partial eta square (η_p^2) values [37]. Diffusion Model Analysis was performed using computer codes from hierarchical diffusion models for two-choice response times [38]. All tests were 2-tailed, with an alpha value of 0.05.

Results

Sample description can be seen in table 1. Groups did not differ in terms of gender (all p-values>0.05) and age differences were minimal. Analyses for inattentive and hyperactive ADHD symptoms are depicted in Tables 2 and 3, respectively.

For Inattention, we found that poorer processing efficiency ("v") was present even in children with minimal inattentive symptoms in both tasks compared to asymptomatic TDC. Those with moderate level of inattention were also impaired in processing efficiency and had a higher trial-to-trial variability in extra-decisional processes ("Q") in both tasks. Full ADHD Inattentive/Combined had poorer processing efficiency in both tasks and higher variability in non-decision time in the CCT task if compared to asymptomatic TDC. The clinical group also had higher variability in decisional time ("e") and faster encoding/motor-function in the 2C-RT ("Ter"), but not in CCT. For all between group differences we found a significant linear trend supporting a dose-response relationship between Inattentive symptoms and BIP (Table 2, Figure 1). No between-group differences in IB-EF were found (Table 2).

For Hyperactivity/Impulsivity, TDC subjects with minimal hyperactivity symptoms already presented a poorer processing efficiency that was also shared with those with moderate hyperactivity symptoms and with ADHD Hyperactive/Combined subjects in the 2C-RT task. Those with moderate hyperactivity symptoms and those with ADHD also had a faster encoding/motor function only in the 2C-RT. The overall MANCOVA for CCT was not significant and therefore no further ANCOVAs for CCT were performed (Table 3, Figure 1).

Again, all between group differences had a significant linear trend and therefore support a dose-response relationship between hyperactive symptoms and BIP. No between group differences were detected for IB-EF (Table 3).

Additional analyses were also performed in order to investigate whether variations in subthreshould ODD symptoms according to Child Behavior Checklist (CBCL)[39] would have any role in driving our results (Supplemental material; Tables S1 and S2). For 2C-RT, both ODD and ADHD symptoms were associated with Variability in Non-decision Time ("Q") and Mean Drift rates ("v"). Hierarchical analysis reveal that for "Q" it is the common variance between ODD and ADHD that was driving the association; whereas for "v" it is the unique variance related to ADHD (since in the model including both ADHD and ODD, only ADHD is significantly associated with "v"). Only ADHD was associated with Mean Non-decision Time ("Ter") and effects are above and beyond variations in ODD symptoms. For CCT, only ADHD was associated with Mean Non-Decision Time ("Ter") and Mean Drift Rates ("v") and results were also robust against variations in ODD symptoms. No interactions between ADHD and ODD emerged from our analysis. No collinearity was detected (all Variance Inflation Factors lower than 2).

Discussion

In this study, we were able to demonstrate that deficits in information processing were found in TDC with minimal, moderate as well as in individuals with ADHD for Inattention (in both tasks) and Hyperactivity/Impulsivity (in the 2C-RT). No significant findings emerged for IB-EF. Crucially, these trends followed a linear function and there was no evidence for a categorical boundary between sub- and clinical levels of ADHD. Advancing our findings from a previous categorical analyses, the current study provides further evidence that processing efficiency is not only a key mechanism for ADHD as a syndrome, but it is also strongly implicated with both inattention and hyperactivity problems even at minimal levels. These results therefore show that neuropsychological mechanisms operate at several levels of the spectrum of ADHD symptoms and provide neurocognitive support for a dimensional model of ADHD.

A higher variability in non-decisional time for Inattention is an additional interesting research finding not previously reported using DM. Previous studies in ADHD have shown that higher intra-subject variability in reaction time is one of the most consistent neurocognitive markers of ADHD [40]. The hierarchical analysis has also shown that this parameter is also linked to symptoms of ODD and that is the common variance between ODD and ADHD that is likely to be associated with such variability. Using DM, we were able to understand that part of this variability may be linked to behavioral inconsistencies related to extra-decisional processes, such as encoding and motor preparation or execution. Further studies are needed in order to understand the clinical and biological nature of such association.

Our study adds to a growing literature supporting the notion that ADHD is best considered as a dimension, lying at the end of a continuum; rather than a category, with a distinct pattern of discontinuity within the spectrum of inattention and hyperactivity. Evidences for dimensionality arise from several sources. Those from psychometrics are particularly strong and have supported a dimensional rather than a categorical view of ADHD using several statistical techniques such as latent class analysis [41,42], factor mixture models [43,44] and different taxometric approaches[10-13]. Behavioral genetics studies also support dimensionality[4,8,9]. Studies suggest that the degree of heritability is similar between those with low levels of attention problems compared with those with moderate and high levels of attention problems[45].

Our results are also in agreement with other studies that investigated directly (and not with latent models) etiological and neuropsychological markers of ADHD. For example, evidence from epidemiological studies [46], structural neuroimaging studies [14], clinical trials [47,48] and personality traits [49,50], have suggest a similar patterns between sub-threshould and clinical cases. One study in adults also found evidence for dimensionality using neurosychological findings[51]. Regarding neuroimaging, Shaw et al[14] used a very similar approach to ours. The authors found that subjects with minimal and moderate hyperactivity symptoms presented patterns of cortical development similar to those with ADHD, also showing a linear relationship between cortical thickness in specific brain areas associated with ADHD and levels of hyperactivity symptoms.

This evidence that ADHD is best seen as a dimension rather than a category has several clinical implications. Of note, we underscore the implications to the etiology of ADHD. Dimensional phenotypes cannot arise from a single dichotomous causal factor and are most typically the result of an interaction of multiple etiological factors [52]. In addition, the extremely relevant clinical question of where to put the threshold designating the categorical diagnosis[53] is inherent to a dimensional approach. Pragmatically we will still need practical decision rules for clinical purposes and the "thresholds" decision will need to be addressed for ADHD as it has been for other continuous traits in medicine, such as hypertension and levels of cholesterol. Therefore focusing on defining these "threshoulds" will be a crucial step for us to better stratify risk and start doing rational stepped care for children suffering from attention problems.

Our study has limitations. First, other scales, such as "Strenghts and Weaknessess of ADHD Symptoms and Normal Behavior (SWAN)", that have a more appropriate normal distribution of its scores in the population could have been more sensitive to between group differences in inattention and hyperactivity [9]. Second, our analyses were limited to the evaluation of BIP and IB-EF and results don't necessarily imply that ADHD is a continuous disorder. Other neurocognitive domains, such as temporal processing and delay aversion, could cause a discontinuity within the ADHD spectrum. Despite that, our study shows that for those specific measures there is a clear linear relationship. Our study has also notable strengths. This study provides neurocognitive evidence that processing efficiency is implicated with both inattention and hyperactivity at different levels of symptom severity across the ADHD spectrum including mild non-clinical levels. Our study design is strong against results due to comorbid problems, medication profiles and referral bias. Furthermore, all the effects reported are above and beyond effects of age, gender, IQ and investigational site.

In conjunction with accumulating previous evidence, our findings suggest that research in neurobiology of ADHD may benefit to changing focus from extreme group comparisons to dimensional designs[12]. This approach may even facilitate scientific discoveries on the neurobiology of inattention and/or hyperactivity/impulsivity problems.

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Table 1 - Sample Description

	Symptoms of Inattention (n=1,184)											
	TDC (asymptomatic) n=229 (19.3%)			**TDC (minimal)** n=590 (49.8%)			**TDC (moderate)** n=312 (26.4%)			**ADHD Inattentive/Combined** n=53 (4.5%)		
	n	**%**		**n**	**%**		**n**	**%**		**n**	**%**	
Gender (male)	104	45.4		300	50.8		166	53.2		30	56.6	
	M	**p25**	**p75**	**M**	**p25**	**p75**	**M**	**p25**	**p75**	**M**	**p25**	**p75**
Age (years)	9	8	11	10	8	11	10	8	11	9	8	10
IQ (score)	106	97	115	103	91	112	100	91	112	97	91	109
SES (score)	21	18	24	20	17	23	19	17	23	20	16	23
DAWBA												
Inattentive	0	0	0	3	1	4	8	6	9	16	14	17
Hyp/Imp	0	0	1	2	0	4	5	2	8	11	8	14

	Symptoms of Hyperactivity/Impulsivity (n=1,142)											
	TDC (asymptomatic) n=227 (19.8%)			**TDC (minimal)** n=658 (57.6%)			**TDC (moderate)** n=225 (19.7%)			**ADHD Hyperactive/Combined** n=32 (2.8%)		
	n	**%**		**n**	**%**		**n**	**%**		**n**	**%**	
Gender (male)	104	46		327	49.8		126	56		17	53.1	
	M	**p25**	**p75**	**M**	**p25**	**p75**	**M**	**p25**	**p75**	**M**	**p25**	**p75**
Age (years)	10	8	11	10	8	11	9	8	11	9,5	8	11
IQ (score)	106	94	115	100	91	112	103	91	112	97	88	109
SES (score)	20	18	24	20	17	23	19	17	23	20	16	23
DAWBA												
Inattentive	0	0	1	3	1	5	6	3	9	13	11.5	16
Hyp/Imp	0	0	0	2	1	4	7	6	9	15	13.5	16

Note: M, Median; p25, 25th percentile; p75, 75th percentile; Hyp, Hyperactivity; Imp, Impulsivity; IQ, Intelligence Quotient; SES, Socioeconomic Status; DAWBA, Development and Well-Being Behavior; POA, Porto Alegre city (site).

Table 2 – Post-hoc ANCOVAs showing differences between groups of Inattention in Diffusion Model Parameters for Two Choice Reaction Time (2C-RT) task and Conflict Control Task (CCT)

	Symptoms of Inattention								ANCOVA						
	TDC (Asym)		TDC (Min)		TDC (Mod)		ADHD (Inatt/Comb)		$F_{3,1084}$	p-value	η_p^2	*Significant Contrasts*	*Trend*		
	M	SE	M	SE	M	SE	M	SE					L	Q	C
BIP (2C-RT)															
Q	-.116	.063	-.045	.039	.163	.053	.112	.126	4.883	**.002**	.013	**Asym**<Mod;	**.038**	.433	.106
Ter	.158	.067	.032	.042	.041	.057	-.334	.134	3.609	**.013**	.01	**Asym**>ADHD; **ADHD**<Min, Mod	**.001**	.133	.046
a	-.092	.067	.014	.041	-.016	.057	.285	.133	2.221	.084	.006	-	**-**	-	-
e	-.127	.069	.022	.042	.012	.058	.318	.137	3.031	**.029**	.008	**Asym**<ADHD; **ADHD**<Min, Mod	**.004**	.35	.073
v	.227	.063	.031	.039	-.056	.054	-.395	.127	7.739	**<0.001**	.021	**Asym**>Min, Mod, ADHD; **ADHD**<Min, Mod	**<0.001**	.354	.14
BIP (CCT)															
Q	-.138	.06	-.005	.037	.098	.051	.279	.12	4.705	**.003**	.013	**Asym**<Mod, ADHD	**.001**	.747	.635
Ter (c)	.113	.067	-.015	.041	.073	.057	-.06	.133	1.255	.288	.003	-	-	-	-
a	-.078	.069	.014	.042	.072	.058	.022	.137	.929	.426	.003	-	-	-	-
e	-.099	.069	.016	.042	.027	.058	.016	.137	.819	.483	.002	-	-	-	-
v (c)	.185	.067	.022	.042	-.071	.057	-.333	.134	5.089	**.002**	.014	**Asym**>Min, Mod,ADHD; **ADHD**<Min	**<0.001**	.551	.361
IB-EF (CCT)															
v(i)-v(c)	.009	.068	-.052	.042	-.015	.057	.241	.135	1.512	.21	.004	-	-	-	-

MANCOVAS: BIP(2C-RT), $F(15,3246)=3.07$, $p<0.001$, $\eta_p^2=0.014$; **BIP(CCT)**, $F(15,3246)=2.63$, $p=0.001$, $\eta_p^2=0.012$;
Polynomial Contrasts: Trend: L, Linear; Q, Quadratic; C, Cubic. Differences between asymptomatic and non-clinical groups are underscored in gray.
Note: Estimated Marginal Means for z-scores (corrected for age and IQ).
Abbreviations: Inat, Inattentive; Comb, Combined; IB-EF, Inhibitory-based Executive Function; M, Mean; SE, Standard Error; ANCOVA, Analysis of Covariance; TDC, Typical Developing Controls; asym, Asymptomatic; min, Minimal Symptoms; mod, Moderate Symptoms; ADHD, Attention Deficit/Hyperactivity Disorder (Predominantly Inattentive or Combined subtypes).
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency); T(c), Mean Non-decision Time in congruent trials; v(i), Mean Drift Rates in incongruent trials; v (c), Mean Drift Rates in congruent trials.

Table 3 – Post-hoc ANCOVAs showing differences between groups of Hyperactivity/Impulsivity in Diffusion Model Parameters for Two Choice Reaction Time (2C-RT) task and Conflict Control Task (CCT)

	Symptoms of Hyperactivity/Impulsivity								ANCOVA					
	TDC (Asym)		TDC (Min)		TDC (Mod)		ADHD (Hyp/Comb)		$F_{3,1044}$	p-value	η_p^2	*Significant Contrasts*	*Trend*	
	M	SE	M	SE	M	SE	M	SE					L	Q	C
BIP (2C-RT)														
Q	-.033	.064	-.029	.037	.06	.064	.255	.164	1.381	.247	.004	-	-	-	-
Ter	.175	.067	.04	.039	-.056	.068	-.372	.173	3.907	**.009**	.011	**Asym**>Mod,ADHD; **ADHD**<Min	**0.002**	0.369	0.385
a	-.064	.067	-.007	.039	.047	.068	.314	.173	1.57	.195	.004	-	-	-	-
e	-.084	.069	-.029	.04	-.066	.069	.38	.177	2.079	.101	.006	-	-	-	-
v	.189	.063	.027	.037	-.087	.064	-.521	.164	6.946	**<0.001**	.02	**Asym**>Min,Mod,ADHD; **ADHD**<Min,Mod	**<0.001**	0.154	0.194
BIP (CCT)															
Q	-.044	.061	.001	.035	.126	.061	.121	.157	-	**-**	-	-	-	**-**	-
*Ter**	.135	.067	.038	.039	.016	.067	-.147	.172	-	**-**	-	-	-	**-**	-
a	-.037	.068	.003	.04	.007	.068	.076	.175	-	**-**	-	-	-	**-**	-
e	-.001	.068	-.029	.04	.155	.069	.061	.176	-	**-**	-	-	-	**-**	-
*v**	.172	.068	.017	.04	-.035	.069	-.356	.175	-	**-**	-	-	-	**-**	-
IB-EF (CCT)															
v(c)-v(i)	-.044	.067	-.027	.039	-.076	.068	.286	.173	1.281	.279	.004	-	-	-	-

MANCOVAS: BIP (2C-RT), $F(15,3126)=2.07$, $p=0.009$, $\eta_p^2=0.010$; **BIP(CCT)**, $F(15,3126)=1.56$, $p=0.077$, $\eta_p^2=0.007$;
Polynomial Contrasts: Trend: L, Linear; Q, Quadratic; C, Cubic. Differences between asymptomatic and non-clinical groups are underscored in gray.
Note: Estimated Marginal Means for z-scores (corrected for age and IQ).
Abbreviations: Hyp, Hyperactivity; Comb, Combined; IB-EF, Inhibitory-based Executive Function; M, Mean; SE, Standard Error; ANCOVA, Analysis of Covariance; TDC, Typical Developing Controls; asym, Asymptomatic; min, Minimal Symptoms; mod, Moderate Symptoms; ADHD, Attention Deficit/Hyperactivity Disorder (Predominantly Hyperactive/Impulsive or Combined subtypes).
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed Accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency); T(c), Mean Non-decision Time in congruent trials; v(i), Mean Drift Rates in incongruent trials; v (c), Mean Drift Rates in congruent trials.

Table S1 - Hierarchical Linear Models for Attention Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder dimensions for 2-Choice Reaction Time task (2C-RT)

	Q		*Ter*		*a*		*e*		*v*	
	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2
Step 1		.166***		.041***		.052***		.01***		.151***
State (SP)	-.07***		-.059*		.009		-.009		-.027	
Age (years)	-.418***		-.132***		-.237***		-.024		.384***	
Gender (male)	.013		.158***		.024		-.11***		.062*	
IQ (score)	-.106***		.012		-.053*		-.007		.144***	
Step 2a		.169*		.046***		.054		.011		.165***
ADHD	.058*		-.079**		.049		.045		-.122***	
Step 2b		.168*		.043		.052		.009		.154*
ODD	.05*		-.048		.023		.016		-.062*	
Step 3 – Both										
ADHD	.043	.169	**-.074***	.046	.05	.053	.049	.01	**-.122*****	.164
ODD	.028	.169	-.01	**.046***	-.003	.053	-.009	.01	0	**.164*****
Step 4 - Interaction		.169		.045		.053		.011		.164
ADHD*ODD	-.053		-.016		-.05		.093		.023	

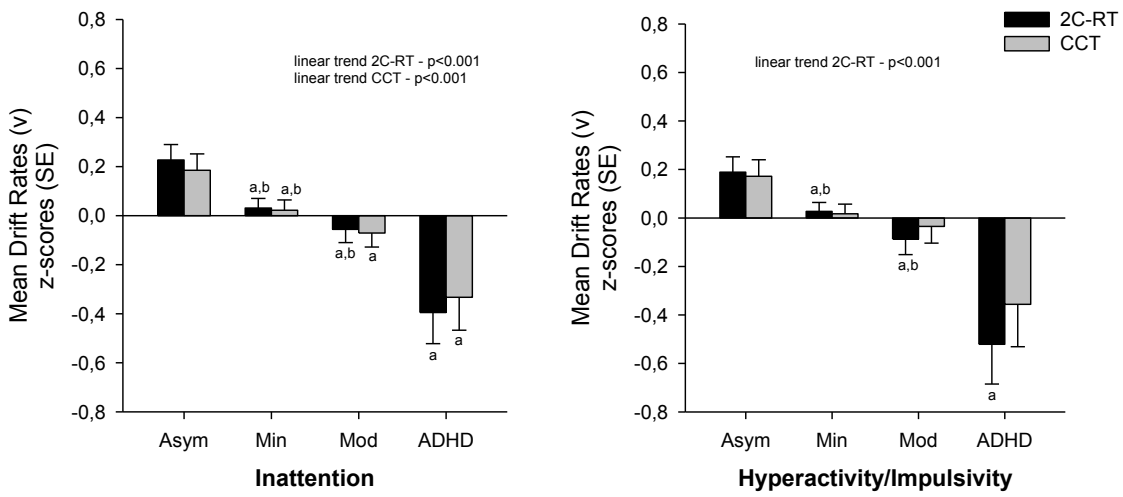
Note: IQ, Inteligence Quotient; ADHD, Attention Deficit/Hyperactivity symptoms (symptom count Development and Well-Being Behavior); ODD, Oppositional Defiant Disorder (according to Child Behavior Checklist).
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed Accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency).
*p<0.05; **p<0.01; ***p<0.001

Table S2 - Hierarchical Linear Models for Attention Deficit/Hyperactivity Disorder and Oppositional Defiant Disorder dimensions for Conflict Control Task (CCT)

	Q		*Ter (congruent)*		*a*		*e*		*v (congruent)*		*v(i)-v(c) (IB-EF)*	
	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2	Variable β	Model ΔR2
Step 1		0,241***		0,07***		0,008		0,003**		0,071***		<0.001
State (SP)	-0,038		-0,056*		0,038		-0,005		0,011		0,004	
Age (years)	-0,494***		-0,225***		-0,06*		0,047		0,273***		-0,015	
Gender (male)	0,094***		0,155***		0,064*		-0,054*		0,004		0,039	
IQ (score)	-0,134***		-0,037		0,034		-0,017		0,102***		0,036	
Step 2a		0,243*		0,071		0,008		0,003		0,08***		<0.001
ADHD	**0,054***		-1,454		0,021		0,027		**-0,1*****	0,014		
Step 2b		0,24		0,071		0,008		0,002		0,072		<0.001
ODD	0,023		-0,027		-0,013		0,022		-0,039		-0,015	
Step 3 – Both												
ADHD	**0,057***	0,242	-0,032	0,071	0,037	0,008	0,021	0,002	**-0,109*****	0,08	0,03	<0.001
ODD	-0,006	**0,242***	-0,011	0,071	-0,032	0,008	0,012	0,002	0,017	**0,08*****	-0,03	<0.001
Step 4 - Interaction		0,242		0,07		0,009		0,001		0,08		<0.001
ADHD*ODD	-0,028		-0,018		-0,095		0,018		0,044		0,053	

Note: IQ, Inteligence Quotient; ADHD, Attention Deficit/Hyperactivity symptoms (symptom count Development and Well-Being Behavior); ODD, Oppositional Defiant Disorder (according to Child Behavior Checklist).
DM parameters: Q, Trial-to-trial variability in Non-decision Time; Ter, Mean Non-decision time (Encoding/Motor function); a, Boundary Separation (Speed accuracy Trade-off); e, Trial-to-trial variability in Drift Rates; v, Mean Drift Rates (Processing Efficiency); v(i), Mean Drift Rates in incongruent trials; v (c), Mean Drift Rates in congruent trials; IB-EF, Inhibitory-based Executive Function
*p<0.05; **p<0.01; ***p<0.001

Figure 1 – Between group differences Mean Drift rates in Two Choice Reaction Time Task (2C-RT) and Conflict Control Task (CCT) for Inattention and Hyperactivity/Impulsivity symptoms.



Note: v, Mean Drift Rates; Asym, Asymptomatic; Min, Minimal; Mod, Moderate; ADHD, Attention Deficit/Hyperactivity Disorder; SE, Standard Error; 2C-RT, 2-Choice Reaction Time Task; CCT, Conflict Control Task. For CCT, mean drift rates from congruent trials were used.
Contrasts: [a] significant differences from TDC; [b] significant differences from ADHD.

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9. CONCLUSÕES E CONSIDERAÇÕES FINAIS

Nesta tese foram apresentados quatro artigos que têm em comum a intenção de buscar meios biológicos e psicológicos de entender os mecanismos envolvidos nos transtornos mentais comuns na infância.

O primeiro estudo dedica-se ao estudo de mecanismos genéticos relacionados aos sintomas de depressão na infância. Os achados demonstram a complexidade da perspectiva longitudinal do desenvolvimento nas variáveis de risco em psiquiatria. Enquanto que em sujeitos pré-púberes e em sujeitos que se encontram na puberdade as variações na região promotora do gene transportador da serotonina não tem influência nos sintomas de depressão na infância, após a puberdade (um período de alta incidência de depressão) as variações de alta expressividade (LgLg) passam a ser um fator protetor para o desenvolvimento de psicopatologia depressiva nesse grupo. Os achados, se replicados, tem implicações importantes para o entendimento dos mecanismos envolvidos com esse polimorfismo genético comum e sugerem que as regulações programadas da puberdade tenham implicações neste gene ou nos sistemas em que este gene está envolvido.

O segundo estudo dedica-se ao estudo de diferenças individuais nos mecanismos relacionados à orientação da atenção para estímulos ameaçadores (faces de raiva) e recompensadores (faces de felicidade) no ambiente. Neste estudo nós demonstramos que sintomas de internalização na infância (emocionais) estão relacionados à vigilância para estímulos ameaçadores no ambiente (como outros estudos no campo tinham demonstrado). A inovação deste estudo está no fato de que, esse efeito varia de acordo com o tipo da doença psiquiátrica. Em sujeitos com

transtornos do estresse (depressão, ansiedade generalizada e estresse pós-traumático) os sintomas de internalização também estão associados à vigilância para ameaças; no entanto, em sujeitos com transtornos fóbicos, esses sintomas estiveram associados com evitação de ameaças. Nenhum efeito foi encontrado em sujeitos com transtornos comportamentais. Esses resultados ajudam na discriminação de mecanismos distintos dentro do grupo de transtornos emocionais. Além disso, esses achados tem potencial valor terapêutico, tendo em vista que estratégias de re-treinamento atencional (baseadas principalmente em favorecer uma memória implícita com o objetivo de evitar ameaças) estão sendo utilizadas para sujeitos com transtornos de ansiedade. No entanto, segundo nossos achados sujeitos fóbicos, com elevados escores de sintomas internalizantes, podem ser um grupo específico que não se beneficia de ter sua atenção treinada para evitar ameaças.

O terceiro estudo dedica-se a estudar aspectos do processamento básico e de controle inibitório no TDAH. Nesse estudo fomos capazes de demonstrar que o TDAH possui diversas alterações no processamento básico, mesmo em tarefas sem componente executivo. Um dos achados é de especial importância: uma ineficiência no processamento de informações básicas especialmente em tarefas sem nenhum componente "executivo" (de ordem maior). Esse achado foi específico do TDAH, isto é, não esteve presente em nenhum outro grupo de psicopatologia e foi capaz de diferenciar o TDAH de todos os grupos de psicopatologia. Esse achado é inédito na literatura de TDAH e tem importantes implicações tanto para os modelos teóricos quanto para a base empírica corrente. Outro achado interessante deste estudo é que após controlar para os déficits em processamento básico, nenhum achado de controle inibitório foi encontrado. Isso corrobora achados em outras disciplinas da ciência em

demonstrar a primazia e a importância de processos básicos no TDAH. Além disso, a comorbidade entre TDAH e TOD/TC demonstrou ser apenas uma combinação de efeitos aditivos do TDAH e TOD/TC e não uma entidade clínica diferente, no que se refere a essas funções cognitivas.

O quarto estudo teve a intenção de prover evidências para a concepção dimensional do TDAH. Corroborando evidências das análises taxométricas, de genética comportamental e de neuroimagem estrutural, esse estudo demonstrou que os déficits em processamento básico de informações se associam de forma linear à desatenção e hiperatividade/impulsividade, mesmo em sujeitos com desenvolvimento típico. Neste estudo demonstrou-se que esse mecanismo está presente em todo o espectro de problemas com desatenção e hiperatividade/impulsividade e não está restrito aos casos clínicos de TDAH.

O estudos que compõe esta tese são inovadores no intuito de investigar tantos os mecanismos biológicos relacionados a ação de genes comuns nos sintomas depressivos, quanto em buscar os mecanismos psicológicos específicos para transtornos emocionais e do comportamento. Até onde os autores tem conhecimento, nenhum estudo da literatura investigou o papel da puberdade como moderador da ação de genes do sistema serotonérgico nos sintomas depressivos. Além disso, os estudos investigando fatores psicológicos associados aos transtornos mentais nunca investigaram a especificidades dos déficits neuropsicológicos em relação aos transtornos de interesse. No entanto, os achados vão ao encontro da literatura, demonstrando a importância da orientação da atenção para ameaças em sintomas de internalização, a importância do processamento básico para o TDAH e, fornecendo evidências neurocognitivas para a dimensionalidade do TDAH.

No que se refere às discussões nosológicas abordadas na tese, estes estudos se encontram no meio entre as abordagens fenomenológicas clássicas (DSM e CID) e abordagens transdiagnósticas como o RDoC. Isso porque eles comparam processos psicológicos previstos em estratégias como o RDoC entre grupos de transtornos psiquiátricos baseados nos critérios clássicos do DSM-IV. Os estudos estão de acordo com a visão que Kendler (Kendler, 2008, 2009, 2012, Kendler and First, 2010) propõe para o avanço das pesquisas nesta área, através de sucessivas desmontagens e re-montagens das evidências empíricas. Estudos que investigam este hiato entre as abordagens inovadoras e clássicas são fundamentais. Eles têm a intenção de prover sentido clínico aos processos mentais investigados direcionar as pesquisas relacionadas aos mecanismos, priorizando os que tem maior probabilidade de informar a psicopatologia das doenças.

Embora haja entusiasmo acerca da nova nosologia proposta pelo RDoC, há inúmeros motivos para ter cautela. Os estudos neste campo ainda são embrionários e ainda é cedo para dizer se de fato uma nova abordagem baseada em processos irá trazer progressos no que se refere a revelar "a biologia por trás dos transtornos psiquiátricos". Além disso, é de extrema importância que esses mecanismos sejam validados também do ponto de vista empírico. Muitas vezes assume-se, sem crítica, de que esses mecanismos são mais válidos e mais confiáveis do que sintomas e síndromes, apenas por diminuírem o componente "subjetivo" das avaliações. No entanto, os componentes ditos "objetivos" estão também sujeitos a diversas fontes de erro e variação.

Não há razão para se falar em substituição dos modelos classificatórios vigentes (DSM-5 e CID-11) pelo RDoC. Ao se conhecer as matrizes do RDoC, fica

clara a proposta de seu uso exclusivo para ambientes de pesquisa. Portanto, nossas "iterações epistêmicas" continuarão por muito tempo ainda trabalhando com as síndromes que estamos acostumados a conhecer. A posição longitudinal que a grande disciplina das "neurociências clínicas" está assumindo dentro do contexto de pesquisa em psiquiatria é inegável e, talvez, irreversível. A integração de conhecimentos de genética básica e, especialmente, de neuroimagem e neuropsicologia vão, provavelmente, fazer cada dia mais parte da vida do psiquiatra. A integração desses conhecimentos com a fenomenologia será fundamental para avançar o campo no principal objetivo de longo prazo dessas iniciativas que é o melhor interesse dos pacientes que sofrem de problemas de saúde mental.

10. ANEXOS

10.1. Outros artigos com foco específico em fisiopatologia dos transtornos mentais publicados durante o período doutorado

10.1.1. Artigo anexo #1 (resumo)

Publicado no periódico ***Current Opinion in Psychiatry***

Current Opinion in Psychiatry

November 2010 – Volume 23 – Issue 6 – p 498-503

doi: 10.1097/YCO.0b013e32833ead33

Effects of childhood development on late-life mental disorders

Giovanni Abrahão Salum, Guilherme Vanoni Polanczyk, Eurípedes Contantino Miguel, Luis Augusto Paim Rohde

Purpose of review: To explore recent findings bridging childhood development and common late-life mental disorders in the elderly.

Recent findings: We addressed aging as a part of the developmental process in central nervous system, typical and atypical neurodevelopment focusing on genetic and environmental risk factors and their interplay and links between psychopathology from childhood to the elderly, unifying theoretical perspectives and preventive intervention strategies.

Summary: Current findings suggest that childhood development is strictly connected to psychiatric phenotypes across the lifespan. Although we are far from a comprehensive understanding of mental health trajectories, some initial findings document both heterotypic and homotypic continuities from childhood to adulthood and from adulthood to the elderly. Our review also highlights the urgent need for investigations on preventive interventions in individuals at risk for mental disorders.

10.1.2. Artigo anexo #2 (resumo)

Publicado no periódico ***Journal of Psychiatric Research***

Journal of Psychiatric Research

February 2012 – Volume 46 – Issue 2 – p147-151

http://dx.doi.org/10.1016/j.jpsychires.2011.09.023

Anxiety disorders in adolescence are associated with impaired facial expression recognition to negative valence

Rafaela Behs Jarros, Giovanni Abrahão Salum, Cristiano Tschiedel Belém da Silva, Mariana de Abreu Costa, Jerusa Fumagalli de Salles, Gisele Gus Manfro

Objective: The aim of the present study was to test the ability of adolescents with a current anxiety diagnosis to recognize facial affective expressions, compared to those without an anxiety disorder.

Methods: Forty cases and 27 controls were selected from a larger cross sectional community sample of adolescents, aged from 10 to 17 years old. Adolescent's facial recognition of six human emotions (sadness, anger, disgust, happy, surprise and fear) and neutral faces was assessed through a facial labeling test using Ekman's Pictures of Facial Affect (POFA).

Results: Adolescents with anxiety disorders had a higher mean number of errors in angry faces as compared to controls: 3.1 (SD=1.13) vs. 2.5 (SD=2.5), OR=1.72 (CI95% 1.02 to 2.89; p=0.040). However, they named neutral faces more accurately than adolescents without anxiety diagnosis: 15% of cases vs. 37.1% of controls presented at least one error in neutral faces, OR=3.46 (CI95% 1.02 to 11.7; p=0.047). No differences were found considering other human emotions or on the distribution of errors in each emotional face between the groups.

Conclusion: Our findings support an anxiety-mediated influence on the recognition of facial expressions in adolescence. These difficulty in recognizing angry faces and more accuracy in naming neutral faces may lead to misinterpretation of social clues and can explain some aspects of the impairment in social interactions in adolescents with anxiety disorders.

10.1.3. Artigo anexo #3 (resumo)

Publicado no periódico ***Neuroscience Letters***

Neuroscience Letters

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Evidence of association between Val66Met polymorphism at BDNF gene and anxiety disorders in a community sample of children and adolescentes

Andrea Goya Tocchetto, Giovanni Abrahão Salum, Carolina Blaya, Stephania Teche, Luciano Isolan, Andressa Bortoluzzi, Rafael Rebelo e Silva, Juliana Becker, Marino Bianchin, Luis Augusto Rohde, Sandra Leistner-Segal, Gisele Gus Manfro

Different lines of evidence support BDNF as a candidate gene in mood and anxiety modulation. More recently, the Met allele of the BDNF Val66Met polymorphism has been implicated in anxiety in animal models and anxiety-traits in humans. The aim of this study is to evaluate the a priori hypothesis that the association between anxiety disorders and Val66Met polymorphism at the BDNF gene would be replicated in a community sample of children and adolescents. 240 subjects from a total sample of 2457 children and adolescents aged 10-17 years from the public schools in the catchment area of the primary care unit of a university hospital participated in this case-control study and were assessed for psychopathology using the K-SADS-PL. A sample of saliva was collected for DNA analysis of Val66Met polymorphism. BDNF was the single gene evaluated in this sample. We found a significant association between carrying one copy of the Met allele and higher chance of anxiety disorders in children and adolescents. The association remained positive even after the adjustment for potential confounders (228 subjects; OR=3.53 (CI95% 1.77-7.06; p<0.001)). Our results support the a priori hypothesis of an association between anxiety and the polymorphism Val66Met. To our knowledge, this is the first study documenting a potential role of this polymorphism in a community sample of anxious children and adolescentes.

10.1.4. Artigo anexo #4 (resumo)

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Preliminary evidence of association between EFHC2, a gene implicated in fear recognition, and harm avoidance.

Carolina Blaya, Priya Moorjani, Giovanni Abrahão Salum, Leonardo Gonçalves, Lauren Weiss, Sandra Leistner-Segal, Gisele Gus Manfro, Jordan Smoller

Genetic variation at the EF-hand domain containing 2 gene (EFHC2) locus has been associated with fear recognition in Turner syndrome. The aim of this study was to examine whether EFHC2 variants are associated with non-syndromic anxiety-related traits [harm avoidance (HA) and behavioral inhibition (BI)] and with panic disorder (PD). Our sample comprised 127 PD patients and 132 controls without psychiatric disorder. We genotyped nine SNPs within the EFHC2 locus and used PLINK to perform association analyses. An intronic SNP (rs1562875) was associated with HA (permuted p=0.031) accounting alone for over 3% of variance in this trait. This same SNP was nominally, but not empirically, associated with BI (r(2)=0.022; nominal p=0.022) and PD (OR=2.64; nominal p=0.009). The same association was found in a subsample of only females. In sum, we observed evidence of association between a variant in EFHC2, a gene previously associated with the processing of fear and social threat, and HA. Larger studies are warranted to confirm this association.

10.1.5. Artigo anexo #5

Publicado no periódico ***Revista Brasileira de Psiquiatria (acesso livre)***

The multidimensional evaluation and treatment of anxiety in children and adolescents: rationale, design, methods and preliminary findings

Avaliação multidimensional e tratamento da ansiedade em crianças e adolescentes: marco teórico, desenho, métodos e resultados preliminares

Giovanni Abrahão Salum,[1,2,3] Luciano Rassier Isolan,[1,3] Vera Lúcia Bosa,[4] Andrea Goya Tocchetto,[1] Stefania Pigatto Teche,[1] Ilaine Schuch,[4] Jandira Rahmeier Costa,[1] Marianna de Abreu Costa,[1] Rafaela Behs Jarros,[1,2,3,7] Maria Augusta Mansur,[1,3] Daniela Knijnik,[1] Estácio Amaro Silva,[1,3] Christian Kieling,[3] Maria Helena Oliveira,[1] Elza Medeiros,[1,3] Andressa Bortoluzzi,[1,5] Rudineia Toazza,[1,5,6] Carolina Blaya,[1,7] Sandra Leistner-Segal,[8] Jerusa Fumagalli de Salles,[6] Patrícia Pelufo Silveira,[4,5] Marcelo Zubaran Goldani,[4] Elizeth Heldt,[1,3] Gisele Gus Manfro[1,2,3,5]

[1] Anxiety Disorders Program for Child and Adolescent Psychiatry (PROTAIA), Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[2] National Science and Technology Institute for Child and Adolescent Psychiatry (INPD)
[3] Postgraduate Program in Medical Sciences: Psychiatry, Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[4] Center for Child and Adolescent Health Studies (NESCA), Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[5] Postgraduate Program in Neuroscience, Institute of Basic Sciences/Health (ICBS), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[6] Cognitive Neuropsychology Research Center (Neurocog), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[7] Universidade Federal de Ciências da Saúde de Porto Alegre (UFSCPA), Porto Alegre, RS, Brazil
[8] Medical Genetics Service, Hospital de Clinicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Abstract

Objective: This study aims to describe the design, methods and sample characteristics of the Multidimensional Evaluation and Treatment of Anxiety in Children and Adolescents – the PROTAIA Project. **Method:** Students between 10 and 17 years old from all six schools belonging to the catchment area of the Primary Care Unit of Hospital de Clínicas de Porto Alegre were included in the project. It comprises five phases: (1) a community screening phase; (2) a psychiatric diagnostic phase; (3) a multidimensional assessment phase evaluating environmental, neuropsychological, nutritional, and biological factors; (4) a treatment phase, and (5) a translational phase. **Results:** A total of 2,457 subjects from the community were screened for anxiety disorders. From those who attended the diagnostic interview, we identified 138 individuals with at least one anxiety disorder (apart from specific phobia) and 102 individuals without any anxiety disorder. Among the anxiety cases, generalized anxiety disorder (n = 95; 68.8%), social anxiety disorder (n = 57; 41.3%) and separation anxiety disorder (n = 49; 35.5%) were the most frequent disorders. **Conclusion:** The PROTAIA Project is a promising research project that can contribute to the knowledge of the relationship between anxiety disorders and anxiety-related phenotypes with several genetic and environmental risk factors.

Descriptors: *Anxiety; Phobic disorders; Panic; Epidemiological; Comorbidity*

Resumo

Objetivo: o objetivo deste estudo é descrever o desenho, os métodos e as características amostrais da Avaliação Multidimensional e Tratamento da Ansiedade em Crianças e Adolescentes – Projeto PROTAIA. **Método:** Escolares entre 10 e 17 anos de todas as escolas pertencentes à área de abrangência da unidade de atenção primária do Hospital de Clínicas de Porto Alegre foram incluídos no projeto. O projeto compreende cinco fases: 1) triagem comunitária; 2) diagnóstico psiquiátrico; 3) avaliação multidimensional, incluindo fatores ambientais, neuropsicológicos, nutricionais e marcadores biológicos; 4) tratamento; e 5) fase translacional. **Resultados:** Um total de 2.457 sujeitos foram triados para transtornos de ansiedade na comunidade. Dos indivíduos que compareceram à avaliação diagnóstica, 138 foram detectados com ao menos um transtorno de ansiedade (excluindo fobia específica) e 102 indivíduos sem nenhum transtorno de ansiedade. Dentre os casos de ansiedade, o transtorno de ansiedade generalizada (n = 95; 68,8%), transtorno de ansiedade social (n = 57; 41,3%) e o transtorno de ansiedade de separação (n = 49; 35,5%) foram os mais frequentes. **Conclusão:** O projeto PROTAIA é um projeto de pesquisa promissor que pode contribuir para o entendimento da relação entre transtornos de ansiedade e fenótipos relacionados à ansiedade com vários fatores de risco, tanto genéticos quanto ambientais.

Descritores: Ansiedade; Transtornos fóbicos; Pânico; Epidemiologia; Comorbidade

Correspondence
Gisele Gus Manfro
Hospital de Clínicas de Porto Alegre
R. Ramiro Barcelos, 2350 – room 2202
90035-003 Porto Alegre, RS, Brazil
Phone/Fax: (+55 51) 3359-8983
Email: gmanfro@gmail.com

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Introduction

Cross-sectional studies have shown that anxiety disorders are the most prevalent psychiatric disorders,[1,2] with lifetime inter-quartile range prevalence rates of 9.9 to 16.7% worldwide.[1] Childhood and adolescence are the principal risk phases for the development of anxiety symptoms[3] with 75% of all anxiety disorders having their onset before the age of 21 and about 50% before age 11. Prospective studies have also shown that 55% of those with a diagnosis of anxiety disorder in adulthood have already had a positive diagnostic assessment at 11 to 15 years of age.[4]

Different endophenotypes,[5] such as behavioral inhibition, neuroticism, anxiety sensitivity, introversion and harm avoidance have been associated with the complexity of anxiety-proneness. Although anxiety can be expressed as a continuum, the Diagnostic and Statistical Manual of Mental Disorders – fourth revised edition (DSM-IV-TR)[6] clinically categorises the following disorders: separation anxiety disorder (SeAD), specific phobias (SP), social anxiety disorder (SoAD), agoraphobia (AG), panic disorder (PD), generalized anxiety disorder (GAD). Obsessive-compulsive disorder and post-traumatic stress disorder are also classified in the anxiety disorders group, according to the current version of the DSM-IV-TR, however, their grouping with the other anxiety disorders is controversial.[7-9]

The continuous nature of anxiety impairs the longitudinal study of these disorders. Some authors have pointed out that a diagnosis of an anxiety disorder has low stability across the lifespan, with a considerable degree of fluctuation in diagnostic status and a strong tendency to naturally wax and wane over time, particularly among younger groups.[10] Despite this, longitudinal studies have demonstrated that a few anxious children and adolescents enter adulthood without any diagnosis. For instance, only 13% of baseline SoAD cases in the Early Developmental Stages of Psychopathology were free of any diagnosis during the 10-year follow-up; 35% reported the same disorder and 64% reported the presence of another anxiety disorder or depression.[11] It seems that there is a heterotypic continuity across time or a sequential comorbid pattern.[12,13]

These fluctuating patterns across the lifespan are best understood from a developmental perspective. Genes and environmental factors have several ways to interplay in order to change neural substrate, human behaviors and emotions. A variety of developmental progressions can arise from the same set of risk and protective factors which may result either in a particular disorder (equifinality), or differing outcomes (multifinality).[14] These influences can be observed even later in life.[15]

Taking this into consideration, a challenging task is to establish specific risk factors for anxiety disorders. Low socioeconomic status, poor parenting style, parental psychopathology, childhood maltreatment, and life events[3] have already been implicated in the development of anxiety disorders. However, the complex relationship between these risk factors, genetic factors and phenotypic presentations is poorly understood. In addition, few studies have evaluated other factors intimately related to anxiety, such as diet, food intake and their consequences[16] or investigated evidence-based cognitive behavioral manuals for treating anxiety disorders in low and middle income countries (LMIC).

The objective of this article is to briefly describe the multi-stage design, the methods and to present preliminary findings of the Multidimensional Evaluation and Treatment of Anxiety in Children and Adolescents – the PROTAIA Project.

Method

The PROTAIA (Anxiety Disorders Program for Child and Adolescent Psychiatry) is an emerging program at the *Hospital de Clínicas de Porto Alegre – Universidade Federal do Rio Grande do Sul* (HCPA-UFRGS) that aims to study anxiety disorders using a comprehensive, research-based perspective to conduct a multidisciplinary project. In this collaborative project there are many hypotheses established on an *a priori* basis being tested under several theoretical approaches. It has an exploratory nature in order to generate hypotheses to be confirmed in larger samples. This prolific new working group comprises psychiatrists, child and adolescent psychiatrists, pediatricians, speech therapists, nurses, therapists, psychologists, molecular biologists, experimental researchers and nutritionists.

1. Phases of the PROTAIA Project

The starting point of the PROTAIA Project is the **Community Screening Phase**, in which all children and adolescents between 10 and 17 years of age from the six schools belonging to the Primary Care Unit of HCPA catchment area were invited to participate. A screening scale for anxiety disorders (Screen for Child and Anxiety Related Emotional Disorders - SCARED) and other instruments were administered to all students that agreed to participate. The cross-sectional design as a starting point for this study has three main objectives: (1) to screen for anxiety disorders in the community; (2) to provide data for validation of clinical scales and normative scores; and (3) to identify subjects with high probability of having anxiety disorders and a community control group from the same population for subsequent projects.

The second step, directly related to the Community Screening Phase, is the **Diagnostic Phase**. In this phase all subjects above the 75th percentile in the screening scale (SCARED)[17,18] and their parents were invited to undergo a diagnostic clinical interview and a structured clinical interview (K-SADS-PL) with psychiatrists, based on a DSM-IV structured interview. Additionally, a random sample of controls equally distributed in the other three quartiles of the SCARED was invited to participate in the psychiatric evaluation. The two main objectives of this step are: (1) to estimate prevalence rates of anxiety disorders in the regional population and (2) to define a community sample of cases with anxiety and a control sample of subjects without anxiety from the same population.

The third step, also associated with the previous steps, is the **Multidimensional Evaluations Phase**. In this phase, nutritional, obstetric and pediatric history was assessed and metabolic

and neuropsychological tests were performed. Moreover, we evaluated genetics from family trios, environmental measures associated with stress (e.g., bullying, peer victimization, parental bonding, childhood trauma, family functioning, etc), parental psychopathology, endophenotypic measures from children, adolescents and their parents, as well as measures of quality of life. This assessment was performed in sub-samples in order to allow exploratory analysis and to study different hypotheses defined *a priori* based on the literature. The main objective of this phase is to provide a large dataset of measures in order to better understand the complexity of and the relationship between anxiety symptoms and disorders with genetic and environmental factors.

The fourth step is the **Treatment Phase**. Since there is no validated protocol to treat young patients with anxiety disorders in Brazil, a group of therapists with large experience in Cognitive Behavior Group Therapy (CBGT) developed a manual of CBGT based on the most used foreign manuals to date.[19-21] The main objective of this phase is to develop a new manual, based on the previous ones, in order to treat internalizing disorders in-group as an alternative approach to public health strategies in Psychiatry.

The fifth step is the **Translational Phase**. PROTAIA also serves as a base for the development of translational models in experimental animal research, aiming to clarify the possible mechanisms involved in the human findings.

2. Training

1) Community phase training

The community phase was carried out in three stages: (1) June 2008 (for the biggest school included); (2) November 2008 (for the second biggest school included) and (3) April 2009 for the remaining schools. The community study was performed in three different stages in order to provide an optimal time between screening evaluation and diagnostic assessment.

Twelve research assistants were trained over two days to administer the research protocol to 10 to 17 year old children and adolescents. Training involved instructions regarding "what to do" and "what to answer" during school-administered self-rated protocols and to assess accurate information about truancy, school transfer and school dropout with the teachers and directors. Training also involved a pilot study in a non-participant school with 85 students.

2) Diagnostic evaluation training and inter-rater reliability

Diagnostic assessment was performed between August 2008 and December 2009, by Psychiatry residents (n = 4), psychiatrists (n = 1) and child and adolescent psychiatrists (n = 4) under the supervision of a senior psychiatrist (GGM). All interviewers had undergone a K-SADS-PL training process for one month that consisted of four phases: (1) 4 seminars of 2 hours each about the structure and diagnostic criteria of the instrument, conducted by two child and adolescent psychiatrists (AGT and LRI) and a highly trained researcher with an experience of more than 100 K-SADS-PL interviews (CK); (2) observation of 5 K-SADS-PL interviews, *in vivo,* performed by a senior interviewer; (3) administration of the K-SADS-PL in 2 patients by the trainees under the supervision of a trained interviewer; (4) pair by pair factorial combination of each interviewer (i.e., at least two interviews with every interviewer). Decisions over final diagnoses were reached in a clinical committee (whenever necessary), conducted by child and adolescent psychiatrists with clinical experience (LRI and AGT) and a senior psychiatrist (GGM).

Inter-rater reliability was achieved by watching and rating 16 DVD K-SADS-PL interviews with child and adolescent patients and healthy controls. Inter-rater reliability resulted in a kappa-value of 0.932 for the anxiety disorders module. Regarding the presence of a specific anxiety disorder, the research assistants reached a kappa value of 1.00 for PD, GAD and SeAD; a kappa value of 0.917 for SoAD and 0.873 for SP.

The subjects were invited to undergo clinical evaluation by phone. A loss of contact was defined after 5 calls over 5 different days, at different times of day.

3) Nutritional and body composition evaluation

All researchers involved in the evaluation of nutritional and body composition were trained for 40 hours in the study of anthropometric techniques and bioelectrical impedance analysis (BIA), the study of the tools to collect and record data and the study of the ethical aspects of research. Afterwards, trainees were shown how to handle the calibration of the scale, stadiometer, calipers, BIA and software analysis of macro and micronutrients; they followed this by training the nutritional measurements and procedures to a pilot group of children and adolescents.

3. Clinical evaluations and rating scales in the PROTAIA Project

In order to elicit new research collaboration, we decided to publish the research protocol used in this project.

1) Psychiatric scales

Both validated and non-validated scales were used in the PROTAIA protocol. Since there are few validated instruments in child and adolescent Psychiatry, non-validated scales were subjected to a process of transcultural adaptation that consisted of two translations followed by the evaluation of the revised translated version by a group of experts and focus groups. One of the objectives of the PROTAIA project is to validate psychiatric scales. Tables 1 and 2 provide an overview of the psychiatric scales used in the community and diagnostic phases.

In the community phase, the self-rated instruments were administered in school classes with careful supervision of the research assistants. Random scales were administered using a systematically random process involving an "S" distribution of questionnaires (in order to avoid bias related to the seating places in the classroom), in a ratio of 1 questionnaire per 6 students in the June/2008 data collection and 1 questionnaire per 5 in the August/2008 and April/2009 data collections. In the multidimensional evaluation phase, the self-rated instruments were delivered in manila envelopes after the diagnostic assessment and were collected at the school.

a) The Screening Scale

The SCARED scale is a 41-item broad screening instrument

Table 1 – Self-rated Instruments used in the community phase by scholars

Community phase	Participants	Validated in Brazil	Construct
1. Sociodemographic questionnaire	All	Yes	Socio-demographic
2. Screen for Children and Adolescent Emotional Related Disorders – Child version (SCARED-C) [17,18]	All (n = 2457)	No*	Anxiety symptoms according to DSM-IV
3. Olweus questions to assess Bullying[45,46]	All (n = 2476)	No*	Bullying in general
4. Strengths and Difficulties Questionnaire (SDQ) [47,48]	Random (n = 475)	Yes[33]	Difficulties related to emotional problems, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behavior
5. Multidimensional Anxiety Scale for Children (MASC)[49]	Random (n = 459)	No**	Anxiety according with some constructs of DSM-IV and alternative phenotypes
6. Childhood Depression Inventory (CDI)[50]	Random (n = 454)	Yes[51-53]	Depression according to DSM-IV and Suicide Ideation
7. Youth Quality of Life (YQOL)[54,55]	Random (n = 419)	No**	Quality of life in children and adolescents
8. Childhood Anxiety Sensitivity Index (CASI)[56†]	Random (n = 158))	No*	Anxiety Sensitivity
9. Peer Interaction for the Primary School (PIPS)[57†]	Random (n = 157)	No*	School Bullying
10. Behavioral Inhibition Instrument (BII)[58††]	Random (n = 869)	No*	Behavioral Inhibition for children
11. Retrospective Self-report of Inhibition (RSRI)[59] – *adapted for children and adolescents*[†††]	Random (n = 454)	No***	Behavioral Inhibition for adults adapted in order to measure current inhibition in the scholars
12. Childhood Trauma Questionnaire (CTQ)[60†††]	Random (n = 307)	Yes[61]	Traumatic experiences in a retrospective basis (abuse and neglect – emotional, physical and sexual)
13. Resilience Scale (RES)[62‡]	Random (n = 244)	Yes[63]	Resilience construct
14. Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)[64‡‡]	Random (n = 235)	Yes[65]	Screening test for alcohol, smoking and substance use

***Validation information**: * Cross-cultural adaptation for the purposes of PROTAIA project; ** Scales with complete translational process, but without validation studies in the country; *** Scales adapted from adults to children and adolescents.*
***Note**: † Only used in April 2009 data collection (systematically random in 1 from each 5 students); †† Only used in April 2009 data collection (for all students); ††† Only used in August 2008 (systematically random in 1 from each 5 students) and April 2009 data collection (systematically random in 1 from each 5 students); ‡ Only used in June 2008 (systematically random in 1 from each 6 students) and August 2008 data collection (systematically random in 1 from each 5 students); ‡‡ Only used in June 2008 data collection (systematically random in 1 from each 6 students).*

which offers a self- and a parent-report version.[17,18] This instrument has four subscales that were developed on the basis of the DSM-IV classification of anxiety disorders (panic disorder, generalized anxiety disorder, separation anxiety disorder and social anxiety disorder) and a fifth subscale (school anxiety) that represents a common anxiety problem in children and adolescents. A recent meta-analysis evaluating the cross-cultural psychometrics of SCARED suggested that this scale has robust psychometric properties demonstrating good internal consistency, test-retest reliability, parent-child correlation, convergent and discriminant validity.[22]

2) Nutritional evaluation

Anthropometric measurements were performed in duplicate and taken by using standard techniques and calibrated equipment.[23] Body weight was measured with portable digital electronic balance scales (*Marte®),* (Marte, SR Sapucaí, MG, Brazil), and height with an extensible portable stadiometer (Alturexata, BH, MG, Brazil). Arm circumference and waist circumference were measured with a tape measure (Sanny, SBC, SP, Brazil).[24,25] The subscapular and triceps skinfolds were measured using a caliper (Cescorf, Porto Alegre, RS, Brazil).[26] The sexual maturation stage was determined by a self-assessment, according to Tanner's criteria.[27]

The assessment of the body composition was measured by bioelectrical impedance analysis (BIA) (Biodynamics-450, Seattle, WA, EUA).[28] Physical activity was assessed based on 3-day physical activity records (PAR24h).[29] The levels of regular physical activity were determined by means of a self-report instrument which provided an estimate of energy expenditure and time spent in different activities.

Food intake estimates were made using 24-h food records and by a food frequency questionnaire for adolescents (AFFQ),[30,31] with the aid of a food and utensils photo album. The quantitative analysis of macro- and micronutrients consumed was calculated with the use of NutriBase® software (Version NB7 Network) (Phoenix, AZ, USD).

3) Neuropsychological evaluation

In addition to the above assessments, a sub-sample of cases and controls were evaluated through neuropsychological tests. The neuropsychological battery is presented in Table 4 and was performed in three 40-minute weekly sessions at school. Sixty-eight children were assessed (41 with a current anxiety diagnosis and 27 controls without current anxiety diagnosis). Cases and controls did not differ regarding age or gender (data not shown).

Table 2 – Instruments used in the diagnostic phase and multidimensional evaluation phase

Community phase	Participants (Valid sample size)	Validated in Brazil	Construct
Clinical Interview with the primary caretaker and with the children or adolescents			
1. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL)[66]	All selected and that accepted participation (n = 240)	In process of validation	Diagnostic interview according to DSM-IV
Self-rated instruments rated by the mother and father about their child			
2. Screen for Children and Adolescent Emotional Related Disorders – Parent version (SCARED-P)[17,18]	All selected that return self-rated questionnaires (n = 132)	No*	Anxiety symptoms according to DSM-IV
Self-rated instruments rated by the mother and father about themselves			
3. Screen for Children and Adolescent Emotional Related Disorders (SCARED)[18] – *adapted to be retrospective version (called SCARED-R)*	All selected that return self-rated questionnaires (n = 132)	No*	Anxiety symptoms of parents when they were at the same age of the index child according to DSM-IV
4. Beck Anxiety Inventory (BAI)[67]	All selected that return self-rated questionnaires (n = 127)	Yes[68]	General anxious Symptoms
5. Beck Depression Inventory (BDI)[69]	All selected that return self-rated questionnaires* (n = 128)	Yes[70,71]	General depressive Symptoms
6. Harm Avoidance and Novelty Seeking scales of Temperament and Character Inventory (TCI)[72]	All selected that return self-rated questionnaires (n = 128)	No†	Temperament
7. Retrospective Self-report of Inhibition (RSRI)[59]	All selected that return self-rated questionnaires (n = 131)	No	Retrospective behavioral inhibition
8. Family Environment Scale (FES)[73,74]	All selected that return self-rated questionnaires (n = 131)	Yes[74]	Family Functioning
Self-rated Instruments rated by the child and his/her sibling (Full evaluation)			
9. Screen for Children and Adolescent Emotional Related Disorders – Child version (SCARED-C)[18]	All selected that return self-rated questionnaires (n = 57)	No*	Anxiety symptoms according to DSM-IV
10. Strengths and Difficulties Questionnaire (SDQ)[47,48]	All selected that return self-rated questionnaires (n = 57)	Yes[33]	Difficulties related to emotional problems, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behavior
11. Multidimensional Anxiety Scale for Children (MASC)[49]	All selected that return self-rated questionnaires (n = 57)	No**	Anxiety according with some constructs of DSM-IV and alternative phenotypes
12. Childhood Depression Inventory (CDI)[50]	All selected that return self-rated questionnaires (n = 57)	Yes[51-53]	Depressive symptoms and Suicide Ideation
13. Youth Quality of Life (YQOL)[54,55]	All selected that return self-rated questionnaires (n = 57)	No**	Quality of life in children and adolescents
14. Retrospective Self-report of Inhibition (RSRI)(59) – *adapted for children and adolescents*	All selected that return self-rated questionnaires and have less than 14 years old (n = 57)	No**	Behavioral Inhibition for adults adapted in order to measure current inhibition in the scholars
15. Harm Avoidance and Novelty Seeking scales of Temperament and Character Inventory (TCI)[72]	All selected that return self-rated questionnaires and have age higher than 14 years old (n = 57)	No†	Temperament evaluation
16. Childhood Trauma Questionnaire (CTQ)[60]	All selected that return self-rated questionnaires (n = 57)	Yes[60]	Traumatic experiences on a retrospective basis (abuse and neglect – emotional, physical and sexual)
17. Parental Bonding Instrument (PBI)	All selected that return self-rated questionnaires (n = 57)	Yes[75]	Bonding patterns of care, overprotection an authoritarianism

***Validation information**: * Cross-cultural adaptation for the purposes of PROTAIA project; ** Scales with complete translational process, bu without validation studies; *** Scales adapted from adults to children and adolescents; † Only the TCI-revised version is validated in Brazil,[76] bu this version was not available at the time of protocol elaboration and data collection in 2007/2008 and therefore another version with some previous investigation in anxiety disorders was used.[77] Note: The sibling with the least age difference related to the index child.*

Table 3 - Nutritional, body composition and metabolic evaluation

Instrument	Participants	Validated in Brazil	Construct
1. The 24-hour food records (FR24h)[3]	Participants of Nutritional and Body Composition evaluation	N/A	Food intake of the Macronutrients and micronutrients / feeding behavior
2. Food frequency questionnaire for adolescents (AFFQ)[1]		Yes[63]	Food intake of the Macronutrients and micronutrients / feeding behavior
3. 3-day physical activity record (PAR24h) [2]		Yes[63]	Physical activity
4. Self-assessment of sexual maturation stage[4]		Yes[63]	Sexual maturation
5. Anthropometric assessment and bioelectrical impedance analysis (BIA)[28]		N/A	Weight (W), stature (S), arm circumference (AC), waist circumference (WC), tricipital skinfolds (TSF), subscapular skinfolds (SSF) and bioelectrical impedance analysis (BIA)
6. Biochemical indicators[9]	All selected that accepted participation with collection of biological material through blood samples	N/A	Glycemia, total cholesterol, high-density lipoproteins (HDL¬c), low-density lipoprotein (LDLc), triglycerides (TG), thyroid stimulating hormone (TSH), hormones insulin, and Homeostasis Model Assessment (HOMA)

N/A, Not applicable

4) DNA extraction and genotyping

DNA was extracted from saliva using the Oragene® DNA Self-collection kit (DNA Genotek) according to the manufacturer's instructions. The biological sample was collected from the participants and their parents. When one of the parents was unavailable, the biological sibling with the least age difference available at the time was invited to participate in the study. The DNA samples were stored at -4ºC and the amplification of the region of interest was performed by Polymerase Chain Reaction (PCR), using reported primers, followed by digestion with specific restriction enzymes (RFLP). The digested products were submitted to 3% agarose gel electrophoresis and visualized with ethidium bromide staining under UV light.

5) Blood sample collection and storage

Blood collection was performed in the outpatient research clinic of the HCPA. The adolescents arrived at the center in the morning (between 7 and 10 am) accompanied by the legal guardian, having fasted for 10 to12 hours. Three tubes containing 4.5ml of blood samples were obtained by venipuncture and transported immediately in ice boxes to the Clinical Pathology laboratory for analysis of glucose, TSH, total cholesterol, HDL, triglycerides and insulin. Two other samples were stored for future molecular and hormonal studies: total blood in EDTA tubes, stored at -20C, and serum (separated from the other blood components after centrifugation for 5 minutes at 4.500 rpm) stored at -80C in the Protein and Molecular Analysis Laboratory.

4. Cognitive behavior therapy protocol development

Four therapists (two clinical psychologists and two psychiatrists) supervised by researchers with a minimum of 10 years' experience in CBT developed a treatment protocol for children and adolescents with anxiety disorders based on the Coping Cat – Workbook (19, 20), FRIENDS Programme[21] and personal experience, taking into consideration particular cultural issues.

Table 4 - Neuropsychological tests

Dimensions	Participants	Neuropsychologic tests
1. Intelligence	Selected case-controls	Wechsler Abbreviate Scale of Intelligence– WASI*[78]
2. Working memory	Selected case-controls	Digit Verbal Span[79]
3. Attention	Selected case-controls	Trail Making Test – A and B[80] D2[81] Go-No-Go test[82]
3. Mental flexibility	Selected case-controls	Wisconsin Card Sorting Task (WCST);[83]
4. Memory and planning	Selected case-controls	WMS-R – Wechsler Memory Scale – Revised – Logic memory[79] The Rey Complex Figure[84] RAVLT – Rey Auditory Verbal Learning Test[85]
5. Emotional processing	Selected case-controls	Labeling Pictures of Facial Affection (POFA) / Ekman[86]

** The Wechsler Abbreviate Scale of Intelligence– WASI is currently being validated. The version used in this project was the same used in the validation procedure provided by the validation team.*

Due to the different developmental characteristics of individuals between 10 to 17 years, the treatment was stratified into two age groups: children from 10 to 13 years, and adolescents from 14 to 17 years. The final CBT protocol was tested in a pilot group and was administered in group format (6 to 10 patients per group), limited to 14 90-minute sessions (10 to 13 years) and 12 90-minute sessions (14 to 17 years), over 4 months. In brief, the four main elements of CBT were: (1) the recognition and description of the physical symptoms of anxiety, (2) the recognition and modification of thoughts that contribute to their anxious experiences (negative self-talk), (3) the development of a plan (confrontation strategies) to deal with the situations which cause anxiety, and (4) performance evaluation and the choice of self-reward. Although the treatment was focused on the child or adolescent, two psychoeducational sessions (one in the middle and another at the end) with parents were included.

5. Data entry

Double entry of the data was performed item-by-item generating more than 3,000 core variables. Paper questionnaires were checked if discrepancies between the two entries were found. In general, replacement of missing values with the linear trend of a point were allowed if missing values item by item did not represent more than 20% of the whole scale.

6. Ethical considerations

This study was approved by the ethical committee of *Hospital de Clínicas de Porto Alegre* (number 08-017). In the initial community phase we used dissent forms. For the subsequent phases, separate written informed consents from primary caretakers and children and adolescents were collected.

Results

From the six public schools in the primary care system area, encompassing 2,754 students, 2,537 were covered by the survey (92.1%), 2,325 (91.6%) by the first visit at the school and 212 (8.4%) at rescue days for the initially missing students. From these 2,537 students, 80 (3.2%) refused to participate. From this sample, 842 subjects were selected for further clinical evaluation and 160 (26.6%) and 80 (33.3%) from the positive and negative screening groups respectively attended the diagnostic evaluation interview. A biological sample for DNA analysis was collected from 242 children. Figure 1 describes the flow diagram of subjects enrolled.

The sample that attended school screening was fairly similar to the one that refused to participate, with the exception of a higher proportion being female (OR = 1.6; p = 0.049) and younger [12.8 years (SD = 2.37) vs. 14.0 years (SD = 2.51); p < 0.001]. The sample that attended school screening but not diagnostic assessment was also similar, with no difference regarding gender (OR = 0.79; p = 0.151), but with a higher chance of being older [12.8 (SD = 2.38) vs. 13.9 (SD = 2.51); (p < 0.001)]. There were no other significant differences regarding symptoms or risk factors. Clinical characteristics of the sample that attended diagnostic assessment are depicted in Table 5.

The epidemiological design was intended to adjust for complex samples adjusting for oversampling in the upper quartile. However, unfortunately, males were less likely to attend the diagnostic evaluation than females. Out of those selected for diagnostic evaluation, 60%, 44%, 18% and 16% of males and 75%, 73%, 48%, and 20% of females, in each quartile respectively, attended the diagnostic evaluation. Therefore, the male:female ratio regarding selection in and attendance of the diagnostic phase became unbalanced in each of the quartiles not favoring the weighting in the cross-sectional oversampling design.

On the other hand, the selection based on the 75th percentile of the screening scale increased the number of anxious cases in our sample between 3 and 8 times as compared to the sample below the arbitrary threshold, allowing comparisons between cases and controls selected from this community sample. Between those with a positive lifetime diagnosis for anxiety disorders 95 (68.8%) had GAD, 57 (41.3%) had SoAD, 49 (35.5%) had SeAD and 9 (6.5%) had PD.

A sub-analysis undertaken only by the psychiatrists blinded to the screening results in randomly selected subjects equally distributed into the four quartiles of SCARED, revealed that SCARED has good predictive characteristics of lifetime anxiety diagnosis as a group as compared to psychiatric diagnosis using K-SADS-PL (area under the curve = 0.739; CI95% 0.651-0.826; p < 0.001; n = 119). However, the 75th percentile has demonstrated low sensitivity (50%) and high specificity (81%) for case detection and, therefore, it is possible that severe cases of anxiety disorder are over-represented in this sample.

Although we have demonstrated high rates of comorbidity between anxiety diagnoses, out of the 15 possible presence/absence combinations between SeAD, GAD, SoAD and PD in patients with at least one anxiety disorder, the diagnosis of GAD was the most frequent condition (30.4%; n = 42), followed by SoAD (14.5%; n = 20) and SeAD (12.3%; n = 17) without any other anxiety disorder comorbidity. PD was the only anxiety disorder diagnosis more common in comorbidity with other anxiety disorders (3.5%; n = 5) than without comorbidity (2.2%; n = 3) in our sample. Regarding comorbid combinations, GAD with SoAD had the highest rate (15.2%; n=21) followed by SeAD and GAD (10.9%; n = 15), and the comorbidity between these three conditions, SoAD, SeAD and GAD (8.7%; n = 12). Further combinations did not reach more than 2% of the total sample. These results can be seen in Figure 2.

There were no associations between having at least one anxiety disorder with non-anxious psychiatric comorbidities considering the negative screening sample (all p-value > 0.05), except for specific phobia (OR = 3.68; CI95% 1.37-9.92; p = 0.012). On the other hand, there was an association between having at least one anxiety disorder and major depression (OR = 3.23; CI95% 1.17-8.91; p = 0.022) and between having at least one anxiety disorder and specific phobia (OR = 7.45; CI95% 2.75-20.22; p <

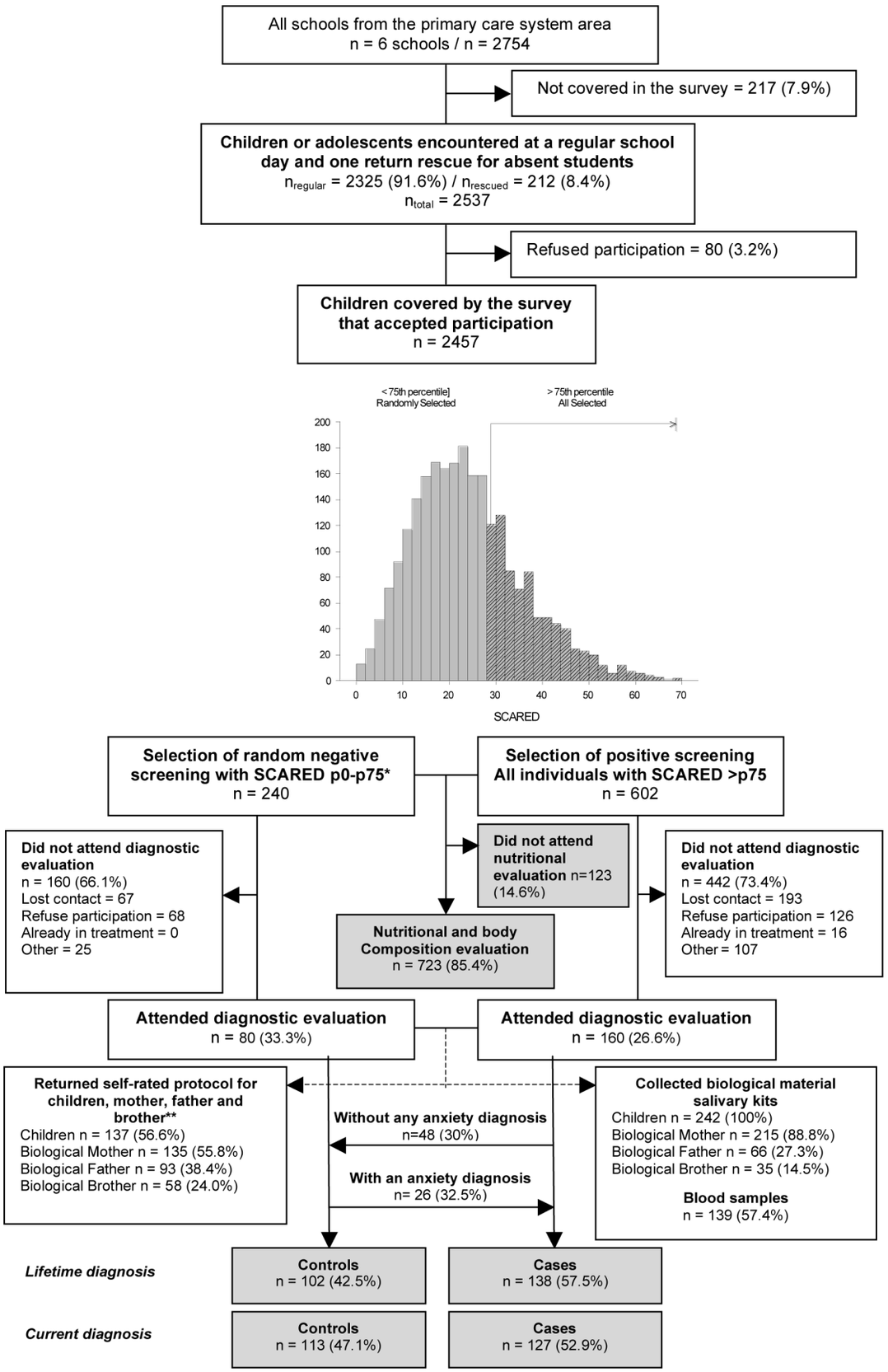

Figure 1 - Flow diagram of subjects enrolled.

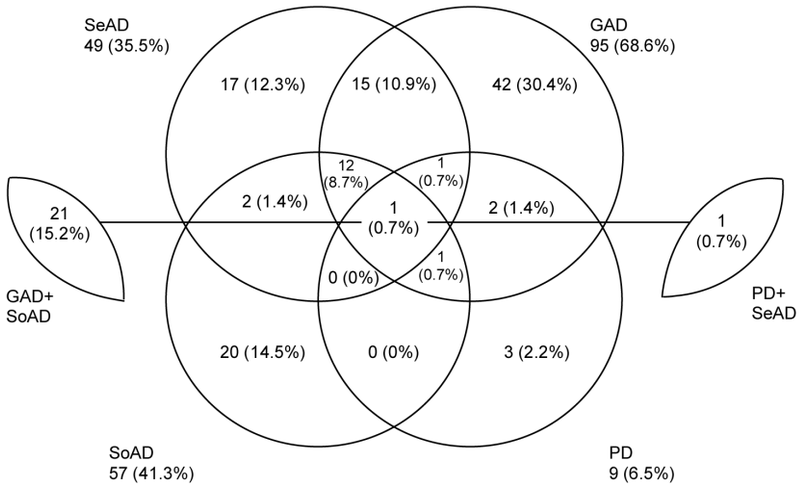

Figure 2 – Anxiety comorbidity between anxious cases.
Obs.: n (%) inside circles represent prevalence rates within anxious individuals. Number (%) inside intersections represent prevalence rates within anxious individuals with comorbid disorders between the conditions of the given circle. There are 15 combinations possible between SoAD, SeAD, PD and GAD.

0.001) among those from the positive screening sample. Gender, age and socio-economic status did not differ between anxious and non-anxious groups (all p-values > 0.05) in both positive and randomly negative screening samples. These results are depicted in Table 5.

Discussion

The PROTAIA Project is an example of a planned multidisciplinary project with different dimensional types of assessment. It involves several types of evaluation with a careful methodological approach, through which we were able to identify 138 cases of anxiety disorders. This report aims to describe our research protocol and the preliminary results.

We were able to successfully increase the number of anxious cases in our sample with the use of the 75th percentile of the SCARED oversampling procedure. However, since ROC analysis reveal a low sensitivity, it is possible that severe cases are over-represented. Another study that used a similar selection procedure selecting the top 15% most anxious (high anxious) on SCARED and ± 2 points on SCARED from the median score (median anxious) was also able to increase the number of anxious cases using this screening method.[32]

The most common anxiety disorder found in our sample was GAD, followed by SoAD and SeAD. In one epidemiological study restricted to school children between 7 and 14 years old in one southeast Brazilian city, not otherwise specified anxiety was the most prevalent disorder (2.1%) followed by SeAD (1.4%), SoAD (0.7%) and GAD (0.4%).[33] In addition, in another well-designed epidemiological study of adolescents (13 to 18 year old children), higher prevalences of SoAD (9.1%) and SeAD (7.6%) were found compared to GAD (2.2%).[34] Studies that used similar designs using SCARED as a screening method also find SoAD and SeAD (prevalence rates within high anxious individuals: 21% and 16%, respectively) to be more prevalent than GAD (15%).[32] We believe that differences in frequency rates between these diagnoses can be attributed to different diagnostic instruments, differences in attendance of diagnostic interviews (the lower rates of attendance in our study can decrease the prevalence of disorders with a higher phobic and avoidant component such as SoAD and SeAD). Additionally, we cannot rule out that these differences are not due to SCARED.

Like other studies,[33] our results demonstrated an association between anxiety disorders and major depression once these two conditions consistently are classified as internalizing disorders.[35] We observed neither an association between ODD and CD, as indicated by some studies[33] nor between anxiety and ADHD.[36] The comorbidity patterns regarding internalizing and externalizing disorders are still controversial in epidemiological studies. This may be due to differences between shared and non-shared genetic and environmental risk factors as well as differences in the diagnostic process used. Moreover, the oversampling procedure and differential sex attendance to the diagnostic evaluation in our study may be responsible for our findings.

Furthermore, our sample is composed of a high number of cases of ADHD (n = 63) and ODD (n = 38) in both positive and randomly selected negative screening. Assuming an independent

Table 5 – Descriptive characteristics of the sample who attended diagnostic assessment stratified by screening scale and anxiety diagnosis

	Randomly selected from negative screening				Positive screening			
	Any anxiety disorder (n = 80)				Any anxiety disorder (n = 160)			
	Absent (n = 54)		Present (n = 26)		Absent (n = 48)		Present (n = 112)	
	n	%	n	%	n	%	n	%
Socio-demographic variables								
Gender (female)	30	55.6%	19	73.1%	32	66.7%	83	74.1%
White skin color*	45	83.3%	20	76.9%	28	59.6%	67	62.6%
Low socio-economic status*	30	66.7%	9	40.9%	24	61.5%	57	64.8%
DSM-IV Anxiety Diagnosis (Lifetime)								
GAD	-	-	11	42.3%	-	-	84	75.0%
Panic Disorder	-	-	1	3.8%	-	-	8	7.1%
Separation anxiety	-	-	11	42.3%	-	-	38	33.9%
Social anxiety	-	-	11	42.3%	-	-	46	41.1%
Other DSM-IV KSADS-PL Diagnoses (Lifetime)								
ADHD	17	30.4%	6	23.1%	12	25.0%	28	25.0%
Depression	7	12.5%	4	16.0%	5	10.4%[a]	30	27.3%[b]
ODD	9	16.1%	3	11.5%	10	20.8%	16	14.3%
Enuresis	3	5.4%	0	0%	4	8.3%	14	12.5%
Encopresis	1	1.8%	0	0%	0	0%	2	1.8%
Tic disorder	3	5.4%	4	15.4%	2	4.2%	8	7.2%
OCD†	2	3.6%	0	0%	0	0%	3	2.7%
PTSD†	0	0%	0	0%	1	2.1%	9	8.1%
Specific phobia†	13	23.2%[a]	14	53.8%[a]	5	10.4%[a]	52	46.4%[b]
Conduct Disorder	5	8.9%	0	0%	1	2.1%	1	0.9%
Mental retardation***	0	0%	1	3.8%	3	6.3%	3	2.7%
Clinical scores	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SCARED	18.73[a]	7.15	22.59[b]	4.45	38.50[a]	7.11	41.38[b]	7.92
MASC*	35.22[a]	15.93	34.92[b]	11.91	46.72	14.20	58.83	13.92
CDI*	7.13	6.23	5.89	2.14	5.30[a]	7.54	11.04[b]	6.50

***Abbreviations**: ADHD, Attention Deficit/Hyperactivity Disorder; ODD, Oppositional Defiant Disorder; OCD, Obsessive Compulsive Disorder; GAD, Generalized Anxiety Disorder; PTSD, Post-traumatic Stress Disorder; SCARED, Screen for Children and Anxiety Related Emotional Disorders; MASC, Multidimensional Anxiety Scale for Children; CDI, Childood Depression Inventory.*
***Note**: Bipolar disorder, Psychotic disorders, Anorexia, Bulimia, Tobacco dependence, Alcohol abuse and dependence, substance abuse and dependence and pervasive developmental disorders were not shown since frequency in the sample was lower than 3%.*
† Specific phobia, OCD and PTSD diagnosis were not considered for the "any anxiety diagnosis" group.
**Missing data.*
**** Mental retardation was defined using a probability method based on clinical suspicion.*
Different letters (a,b) indicated statistical significant results (p-value < 0.05).

relation between ADHD and SCARED scores, the estimated prevalence of lifetime ADHD in our sample would be 23%, greatly exceeding the worldwide estimated prevalence of 5%.[37] Therefore, it seems that our sample has a larger number of individuals seeking treatment for ADHD (as well as ODD) unbalancing the case numbers that attended diagnostic assessment.

There were no differences between anxious and non-anxious groups in terms of age, gender and socioeconomic status. The association between anxiety disorders and socioeconomic characteristics is controversial:[3] although there is some evidence favoring a positive association,[38] there are studies suggesting more complex relationships between poverty and mental disorders.[39] Females are twice as likely as males to develop anxiety disorders,[38,40] however some studies have shown that this sex difference, with respect to prevalence, is small in childhood and increases with age.[41]

Small- to medium-sized research centers frequently delineate research projects that aim to address one specific research question. Although this design brings some advantages (e.g. a more specific control for confounders, for example), it generally results in a lonely

process of scientific exploration, is very expensive and does not provide data for testing further hypotheses of a complex phenomenon such as psychiatric disorders. Therefore, a collaborative work that considers different theoretical approaches is a notable advantage.

A randomized clinical trial (RCT) followed by evaluations of treated cases was planned in the PROTAIA project in order to evaluate treatment efficacy with previously tested medication.[42] However, due to the low participation rate of the subjects in the clinical evaluations, even after several attempts to make contact, this treatment research plan could not be implemented. This situation reflects one of the difficulties in carrying out research in community settings, especially concerning anxiety disorders. Although anxiety disorders are responsible for disability and suffering, few subjects agreed to participate in the study, in which CBGT was offered at no cost.

The development of validated and effective techniques of group CBT is needed, especially when looking from a public health perspective. Very few studies have been published in the country evaluating the effectiveness of psychotherapeutic approaches in childhood. If these protocols prove their effectiveness, CBT could have a major role in the treatment of anxious children and adolescents in the public health system. Research in this area is essential given that protocols from other parts of the world without any type of cultural adaptation are unlikely to be effective for the Brazilian population. It is known that strategies for coping with anxiety disorders are very dependent on the cultural environment.[43]

The whole design of our protocol has some limitations. First, study participation in the diagnostic phase was low compromising some of the clinical profile of our sample. It was thought that perhaps more phobic subjects were less likely to attend the diagnostic interview. Second, 75th percentile has shown a low sensitivity and therefore more severe cases of anxiety could be over-represented in our sample since prevalence rates could not be adjusted for complex samples. Third, the method of selection using the SCARED is intrinsically related to scale performance and this scale is under the process of validation. However, there are no reliable scales to measure this specific construct of anxiety disorders for the Brazilian population. Otherwise, this is the first study (to the authors' knowledge) to evaluate a sample specifically in order to investigate symptoms of anxiety disorders in a Brazilian population with a probabilistic care, and to include several other clinical, nutritional and biological measures.

Conclusion

Future perspectives for the PROTAIA group include a neuroimaging study and the inclusion of inflammatory and biological markers in the blood samples. In addition, this paper aims to describe the preliminary results as well as to allow research collaboration with other emerging groups[44] that share research interests and similar research protocols. The PROTAIA Project is a promising research project that can contribute to the knowledge of the relationship between anxiety disorders and anxiety-related phenotypes with several genetic and environmental risk factors.

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Disclosures

Writing group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Giovanni Abrahão Salum	-	CAPES CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Luciano Rassier Isolan	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Vera Lúcia Bosa	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Andrea Goya Tocchetto	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Stefania Pigatto Teche	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Ilaine Schuch	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Jandira Rahmeier Costa	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Marianna de Abreu Costa	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Rafaela Behs Jarros	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Maria Augusta Mansur	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Daniela Knijnik	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Estácio Amaro Silva	-	CAPES CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Christian Kieling	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Maria Helena Oliveira	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Elza Medeiros	-	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Andressa Bortoluzzi	-	CAPES CNPq FIPE-HCPA FAPERGS	-	-	-	-	-

(TO CONTINUE)

(CONTINUATION)

Writing group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Rudineia Toazza	-	CAPES CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Carolina Blaya	UFCSPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Sandra Leistner-Segal	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Jerusa Fumagalli de Salles	UFRGS	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Patrícia Pelufo Silveira	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Marcelo Zubaran Goldani	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Elizeth Heldt	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	-
Gisele Gus Manfro	UFRGS-HCPA	CNPq FIPE-HCPA FAPERGS	-	-	-	-	Roche*

** Modest*
*** Significant*
**** Significant: Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*
Note: UFRGS-HCPA = Universidade Federal do Rio Grande do Sul-Hospital de Clínicas de Porto Alegre; UFCSPA = Universidade Federal de Ciências da Saúde de Porto Alegre; CAPES = Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; CNPq = Conselho Nacional de Desenvolvimento Científico e Tecnológico; FIPE-HCPA = Fundo de Incentivo à Pesquisa e Eventos-Hospital de Clínicas de Porto Alegre; FAPERGS = Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul.
For more information, see Instructions for Authors.

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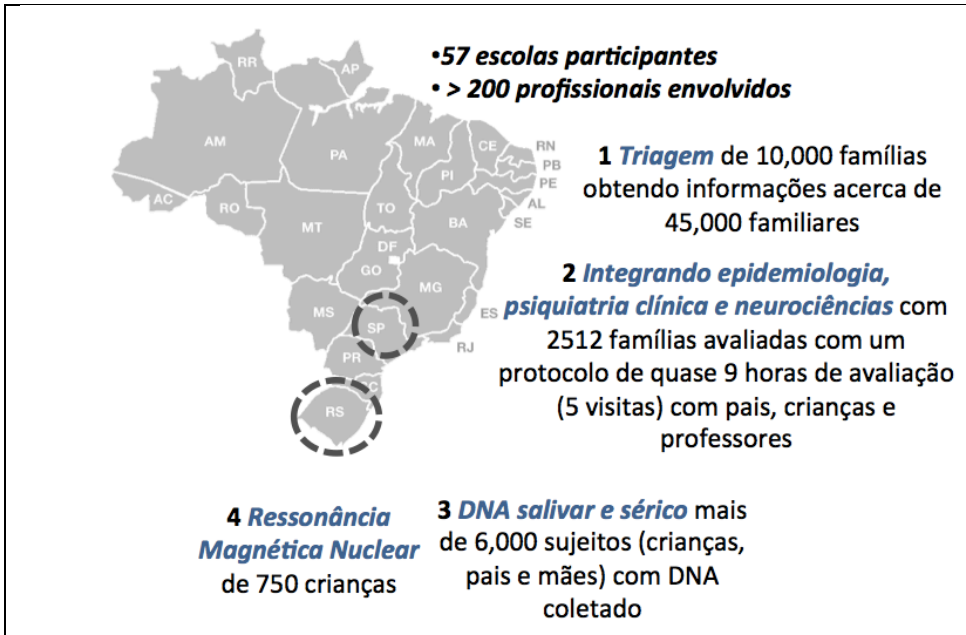
10.2. Resumo do Projeto "Coorte de Alto Risco para o Desenvolvimento de Transtornos Psiquiátricos na Infância e Adolescência"

RESUMO DO PROJETO 2

COORTE DE ALTO RISCO PARA O DESENVOLVIMENTO DE TRANSTORNOS PSIQUIÁTRICOS NA INFÂNCIA E ADOLESCÊNCIA

1. APRESENTAÇÃO

Até onde vai o conhecimento dos autores, este é o maior estudo de psiquiatria da infância e adolescência já realizado no país. O projeto envolveu a coordenação de mais de 200 profissionais, entre entrevistadores, psicólogos, fonoaudiólogos, geneticistas, físicos, enfermeiros, etc. Trata-se de um projeto colaborativo entre a Universidade de São Paulo, a Universidade Federal do Rio Grande do Sul e a Universidade Federal de São Paulo.



Esquema geral da Coorte de Alto Risco

O desenho do projeto é extremamente inovador. Os estudos de seguimento realizados em outros locais do mundo acabam por selecionar sujeitos com risco basal baixo e acabam sem poder estatístico para identificar riscos específicos. No desenho desse projeto, optou-se por seguir sujeitos (crianças) com risco elevado (com alto número de sintomas e com história familiar positiva), o que acreditamos que no seguimento nos dará vantagens importantes no que se refere ao número de casos detectados e aumentar o poder estatístico.

A possibilidade de detectar sujeitos de alto risco para desfechos negativos em psiquiatria é uma inovação em si. E, se possibilitada por nossa abordagem multidisciplinar, envolvendo avaliações clínicas, genética, neuropsicológica e de neuroimagem, possuem potencial para gerar uma mudança importante no entendimento dessas doenças e avançar nas alternativas de tratamento disponíveis até o momento.

2. METODOLOGIA

2.1. Delineamento

Coorte de escolares de alto risco e de risco basal para psicopatologia na infância e adolescência

2.2. Amostragem

O projeto conta com 4 etapas específicas (triagem, etapa domiciliar, etapa escolar e etapa de neuroimagem) em inicialmente 3 fases (linha de base, seguimento de 3 anos e seguimento de 6 anos).

(1) **etapa de triagem** dos casos de alto risco, baseados na psicopatologia familiar e sub-sindrômica das crianças;

(2) **etapa de avaliação domiciliar** dos diagnósticos psiquiátricos da criança, dos pais, incluindo coleta de fatores de risco gerais e coleta de saliva do trio (pai, mãe, criança);

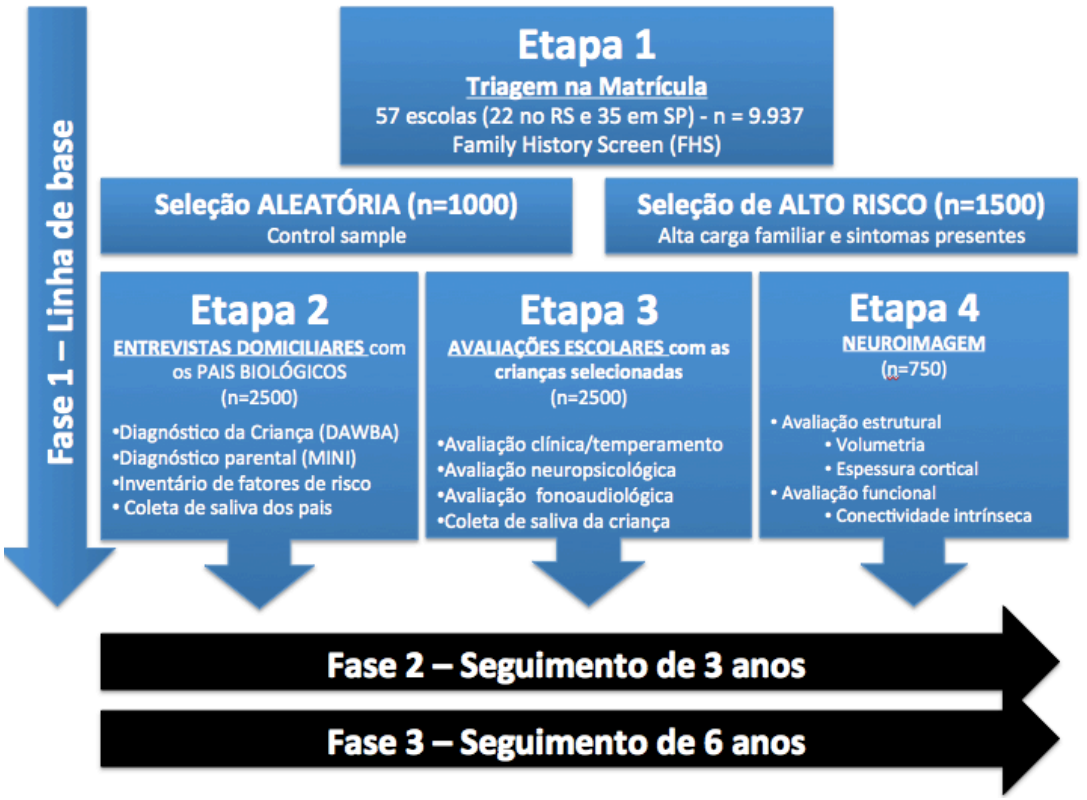
(3) **etapa de avaliação escolar** das características neuropsicológicas e detalhamento clínico de determinadas condições e onde será realizado o diagnóstico e coletados fatores de risco psiquiátricos gerais;

(4) **etapa de avaliação com neuroimagem** de uma sub-amostra das crianças de risco.

Todas as etapas de fase 1 (linha de base) já foram concluídas. O início da fase 2 está previsto para o primeiro semestre de 2013.

Nas fases 2 (seguimento de 3 ano) e 3 (seguimento de 6 anos), pretende-se reaplicar o mesmo protocolo das etapas já descritas.

O desenho geral do projeto com suas 4 etapas e 3 fases encontra-se na figura abaixo.



Etapas e Fases do Projeto

2.3. Descrição das Etapas do projeto

2.3.1. Etapa de triagem

A fase de triagem ocorreu durante o período de matrícula e re-matrícula em 57 escolas da rede estadual de ensino (22 em Porto Alegre e 35 em São Paulo) em 2010. Durante esse período entrevistadores treinados convidaram os pais e mães biológicos de crianças de 6 a 12 anos que estavam em processo de matrícula ou re-matrícula para participarem da pesquisa. Os sujeitos que aceitaram participar foram avaliados pelos entrevistadores com um questionário sócio-demográfico preparado especialmente para os objetivos do estudo e com um questionário de Rastreamento

de História Familiar de Transtornos Psiquiátricos (Family History Screen –FHS (Weissman *et al*., 2000). O FHS foi adaptado para prover informações acerca da criança índice, dos irmãos biológicos dessa criança, dos meio irmãos e acerca dos dois pais biológicos, através de informações *by proxy*. Este instrumento permite avaliação preliminar de sintomas depressivos, de mania/hipomania, de transtorno do pânico, ansiedade generalizada, fobia social, agorafobia, fobia específica, transtorno obsessivo compulsivo, sintomas psicóticos, álcool e drogas.

Foram considerados sujeitos elegíveis para participação dessa fase da pesquisa:

- Indivíduos na faixa etária de 6 a 12 anos completos;
- Estarem em processo regular de matrícula ou re-matrícula na escola participante do projeto;
- Comparecerem à matrícula com pai biológico ou mãe biológica;

Um total de 9.937 crianças foram incluídas nessa primeira fase. As entrevistas foram realizadas predominantemente com a mãe biológica (87.5%), levando em consideração sintomas de 45.394 familiares. A média de idade foi de 9 anos (DP=1,9), com uma leve predominância de sujeitos do sexo masculino 52,1% (n=5179).

Nome do Instrumento ou Procedimento	Objetivo
***Family History Screen* (FHS)**	Rastreamento de sintomas no menor e familiares de primeiro grau
Questionário sócio-demográfico	Identificação de fatores sócio-demográficos

2.3.2. Seleção dos indivíduos de risco para a coorte

Dentro do universo de 9.937 sujeitos de pesquisa avaliados quanto à história familiar de transtornos psiquiátrico e quanto à apresentação sintomática da criança, os 1500 sujeitos de mais alto risco para os cinco principais transtornos de interesse para este projeto (TDAH, Ansiedade, TOC, Psicose e Aprendizagem) e uma amostragem aleatória de 1000 sujeitos foram convidados para participarem das etapas domiciliar e escolar.

No intuito de permitir o estabelecimento de parâmetros para as medidas clínicas dentro dessa população estudada e ter um controle da incidência em uma amostra não selecionada pelo risco, 1.000 indivíduos foram selecionados aleatoriamente para compor a amostra com risco basal para o estudo, de onde sairão os sujeitos para comparações transversais.

Os 1500 indivíduos de maior alto risco para os 5 transtornos de interesse (Transtornos de Ansiedade, Transtorno de Déficit de Atenção e Hiperatividade, Transtorno Obsessivo Compulsivo, Transtornos Psicóticos e Transtornos de Aprendizagem) foram selecionados através de uma estratégia de priorização descrita a seguir. Definiu-se que os indivíduos de maior risco para os 5 transtornos seriam as crianças que positivarem a avaliação de sintomas no FHS para cada um dos transtornos e que tivessem a maior densidade de sintomas da mesma ordem na família, através do mesmo instrumento. Dentro de cada família, no intuito de preservar a independência nas futuras comparações estatísticas, apenas 1 indivíduo por família foi selecionado.

2.3.3. Etapa domiciliar

Os 2.500 indivíduos selecionados foram avaliados no domicílio com uma entrevista domiciliar diagnóstica. Esta entrevista também foi realizada por entrevistadores leigos, intensivamente treinados pela equipe de pesquisa e com supervisão contínua. O protocolo de avaliação domiciliar é composto por:

- Uma entrevista diagnóstica estruturada com o DAWBA (*Development and Well-Being Assessment - DAWBA*), realizada com o cuidador principal acerca da criança índice, que provê diagnósticos psiquiátricos de acordo com o DSM-IV e CID-10. Tendo em vista a importância do diagnóstico psiquiátrico, essa entrevista possui uma avaliação aberta (semiestruturada), que recebe supervisão de um psiquiatra treinado para aumentar a validade dos diagnósticos clínicos.
- Uma avaliação dimensional da psicopatologia infantil através do *Child Behavior Checklist (CBCL)*, realizada com o cuidador principal acerca da criança.
- Uma entrevista diagnóstica estruturada com o *Mini International Neuropsychiatric Interview (M.I.N.I)*, realizada com pai e mãe biológicos da criança. A entrevista com o pai biológico que não foi o respondedor do protocolo foi realizada pelo telefone.
- Uma avaliação de fatores de risco psiquiátricos gerais que incluem: (a) fatores gestacionais e perinatais (como tabagismo e álcool na gestação, etc.), (b) estressores na infância (abuso, maus tratos, *Bullying*, rede de apoio, etc.); (c) doenças clínicas; (d) qualidade de vida geral; (e) desfechos escolares (faltas, suspensões, expulsões).

- Uma avaliação de tratamentos e uso de serviços através de uma avaliação semiestruturada,
- Avaliação de funcionamento familiar através da *Family Environmental Scale (FES)*.
- Coleta de material genético dos pais biológicos ou irmão biológico mais velho disponível (na ausência de um dos pais biológicos).

DAWBA (*Development and Well-Being Assessment* – DAWBA)	Entrevista diagnóstica estruturada
***Child Behavior Checklist* (CBCL),**	Avaliação dimensional da psicopatologia
Mini International Neuropsychiatric Interview	Avaliação diagnóstica estruturada dos pais
Avaliação de fatores de risco psiquiátricos gerais	Avaliação de fatores gestacionais e perinatais; estressores na infância; doenças clínicas; qualidade de vida; desfechos escolares
Avaliação de uso de serviços	Uso de medicações, terapia, tipo de serviço utilizado e satisfação com o serviço
***Family Environmental Scale* (FES).**	Avaliação de funcionamento familiar

2.3.4. Etapa escolar

No mesmo período de tempo em que os entrevistadores treinados realizaram as entrevistas domiciliares, psicólogos e fonoaudiólogos contratados pela equipe do projeto realizaram avaliações clínicas e neuropsicológicas com criança na escola.

O protocolo de avaliação na escola encontra-se na tabela abaixo. Um total de 26 *notebooks* foram adquiridos para realização dos testes que necessitam avaliações eletrônicas de tempo. O *Software e-prime* 2.0 foi adquirido para possibilitar testagens neuropsicológicas de ponta dentro do meio científico, com medidas de tempo de resposta e paradigmas complexos.

Strengts and Difficulties Questionnaire (SDQ)	Questionário de vulnerabilidades e capacidades das crianças
Escalas de Comportamento inibido e Sensibilidade à ansiedade	Escalas de fenótipos intermediários Ansiedade
Community Assessment of Psychic Experiences (CAPE)	Avaliação de sintomas psicóticos
Avaliação Neuropsicológica	Avaliação de funções cognitivas da criança, tais quais: QI estimado, atenção, memória, funções executivas, habilidades motoras e viés atencional relacionado às emoções
Teste de Desempenho Escolar (TDE)	Avaliação fonológica e do desempenho
Triagem processamento auditivo central	

Consciência fonológica (CONFIAS)	escolar
Teste de linguagem infantil (ABFW)	

Além disso, a coleta de saliva do trio (pai, mãe e criança) foi realizada de todos os sujeitos do projeto.

Coleta de Saliva	**Coleta de amostras de saliva do menor, pai e/ou mãe biológica para estudos genéticos futuros**

2.3.4.1. Avaliação neuropsicológica

Um dos principais focos deste projeto, em específico, é poder estudar os processos mentais associados aos transtornos psiquiátricos e, em especial para os Transtornos de Ansiedade e para o Transtorno de Déficit de Atenção e Hiperatividade. Os paradigmas de interesse para esses dois transtornos estão incluídos dentro de uma bateria neuropsicológica ampla, envolvendo 4 sessões de 1 hora cada um.

Tarefas de interesse para os Transtornos de Ansiedade

Uma tarefa é de especial interesse para os transtornos de ansiedade que é a tarefa relacionadas a orientação da atenção para estímulos ameaçadores e para estímulos sociais recompensadores – o *dot-probe*.

Tarefas de interesse para o Transtorno de Déficit de Atenção Hiperatividade

Em virtude de as hipóteses atuais para o TDAH contemplarem a ideia de múltiplos déficits, no intuito de explicar a heterogeneidade clínica, várias tarefas são de interesse para as hipóteses relacionadas ao TDAH, com relação com os seguintes domínios putativos:

Controle inibitório: *Go/No-Go Task* e *Conflict Control Task*

Aversão à espera (*delay aversion)*: *Delay Reaction Task* e *Choice-Delay Task*.

Processamento Temporal: *Duration Discrimination Task* e *Time Anticipation Task*

Déficits de Processamento básico: *2-Choice Reaction Time Task*

Memória de Trabalho: Span de dígitos (direto e inverso) e Blocos de Corsi (direto e inverso)

Quoeficiente de Inteligência: WISC-III

2.3.5. Avaliação genética

O DNA foi extraído da saliva utilizando o kit saliva *oragene* e sua extração está sendo realizada com o kit extração *oragene*. Em virtude das novas descobertas em genética dos transtornos psiquiátricos, as hipóteses específicas do projeto estão sendo re-discutidas. Varreduras no genoma inteiro serão idealizadas para identificação de variações comuns relacionadas aos transtornos de interesse e especialmente com a ideia de quantificar o número de variações em vias metabólicas específicas através de análises envolvendo a teoria dos grafos, como determinante dos desfechos em saúde mental e não continuar procurando variantes específicas no genoma capazes de explicar a variabilidade dos fenômenos.

4.3.6. Etapa de avaliação com Neuroimagem

Dos 2.500 sujeitos selecionados, uma sub-amostra de 750 crianças foram avaliadas para realização de exames por Ressonância Magnética estrutural (RM), por Tensores de Difusão (DTI) e de Conectividade Funcional (FC) em todo o projeto.

2.4. Fases 2 e 3 – Reavaliações de 3 e 6 anos

Os protocolos de reavaliação de 3 e 6 anos estão em presente discussão entre os pesquisadores participantes do projeto. Em princípio, todas as avaliações realizadas na linha de base serão repetidas, com exceção da etapa de triagem e coleta de saliva.

2.5. Aspectos Éticos

Os projetos que constituem este projeto estão aprovados no comitê de ética em pesquisa da Universidade de São Paulo, com parecer juntamente à Comissão Nacional de Ética em Pesquisa (CONEP). O estudo está de acordo com as Diretrizes e Normas Regulamentadoras de Pesquisas Envolvendo Seres Humanos (Resolução 196 / 96).