PONTÍFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

AVALIAÇÃO DAS BASES BIOLÓGICAS E SOCIAIS DO TEMPERAMENTO

MATIAS NUNES FRIZZO

Porto Alegre,
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Tese apresentada como requisito para a obtenção do Grau de Doutor ao Programa de Pós-graduação em Biologia Celular e Molecular da Pontifícia Universidade Católica do RS.

Orientador: Dr. Diogo Rizzato Lara

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RESUMO

O temperamento pode ser considerado como a base do humor, do comportamento e da personalidade, tem uma base biológica forte, manifesta-se cedo no desenvolvimento do indivíduo, norteia a formação dos hábitos sendo relativamente estável no decorrer do tempo. Apesar de se saber que há influências do meio, não está bem definida a relação entre temperamento e o meio social. Evidências sugerem que o temperamento e os traços de personalidade predispõem aos transtornos psiquiátricos e que a maioria deles é recorrente e crônico. Dessa forma, o presente estudo tem como objetivo investigar as bases biológicas e sociais do temperamento em modelos animais de temperamento e avaliar a relação do temperamento com hiperuricemia (elevação dos níveis séricos de ácido úrico) e com o autorrelato de ter sido vítima de *bullying* em humanos. Na avaliação das bases neurobiológicas do temperamento foram usados modelos animais, no qual foram selecionados camundongos com alta e baixa exploração em um teste campo aberto. Foram testados cem camundongos, e posteriormente selecionados os dez camundongos mais exploradores (HE) e os dez menos exploradores (LE), cujo mRNA de córtex frontal e estriado foi coletado para posteriormente ser avaliado através de chips para avaliação da expressão gênica (Genechip Mouse Gene 1.0 ST Array - Affymetrix). Os resultados mostraram 86 e 118 genes expressos diferencialmente (DEGS) no estriado e no córtex frontal, respectivamente. Através da análise dos DEGs os processos biológicos mais significativamente enriquecidos foram o do desenvolvimento do sistema nervoso e da função e sinalização celular, especialmente no estriado, numa comparação entre animas HE com LE. Estes resultados sugerem o envolvimento de processos de translação e pós-tradução, assim como os elementos sinápticos do estriado nas diferenças de características de comportamento exploratório. Nos estudos em humanos, os dados foram coletados em um grande levantamento via Web através do Brazilian Internet Study on Temperament and Psychopathology (BRAINSTEP). No estudo de bases biológicas do temperamento em humanos, analisamos o temperamento em 7.155 homens (5,1% hiperuricêmicos) e 22.225 mulheres (1,8% hiperuricêmicas). Indivíduos hiperuricêmicos apresentaram escores mais elevados em raiva e inferiores na inibição e controle, já as mulheres hiperuricêmicas também mostram uma maior sensibilidade emocional e um menor grau de vontade e de enfrentamento. Os resultados demonstraram que indivíduos com hiperuricemia têm mais traços emocionais e temperamentos afetivos externalizados e instáveis. No estudo sobre as bases sociais do temperamento avaliamos o bullying durante a infância e adolescência, através de uma pergunta sobre tempo de exposição ao *bullying* (nenhum, <1 ano, 1 a 3 anos e > 3 anos). Traços emocionais e temperamentos afetivos foram avaliados com a Escala de Temperamento Afetivo e Emocional (AFECTS). Cerca de metade da amostra relatou exposição ao *bullying* e 10% relataram ter sido vítimas por mais de 3 anos. Vitimas de *bullying* também apresentam uma proporção muito menor de temperamentos eutímicos e hipertímicos em ambos os sexos, o que foi compensado por um aumento, principalmente, na proporção de traços depressivos, ciclotímicos e volátil. Sofrer *bullying* foi associado com um impacto amplo e profundo sobre os domínios cognitivos e emocionais em todas as dimensões de traços emocionais, e com temperamento afetivo internalizado e instável. Esses resultados, em conjunto, mostram a importância de possíveis marcadores séricos (ácido úrico) e fatores genéticos e sociais sobre os traços de temperamento.

ABSTRACT

Temperament can be regarded as the basis of mood, behavior and personality. It has a strong biological basis, manifested early in the development of the individual, guiding the formation of habits and is relatively stable over time. Although it is known that there are environmental influences, the relationship of temperament with biological markers and social environment is not well defined. Evidence suggests that the temperament and personality traits predict psychiatric disorders and that most of them are recurrent and chronic. Thus, this study aims to investigate the biological and social basis of behavior and in animal models of traits to assess the relationship of temperament with hyperucemia and with self-report of having been bullied in humans. To study the neurobiological basis of this trait, we selected mice with high and low exploration of a central object in an open field. Out of one hundred mice tested, the ten mice with higher (HE) and lower exploratory (LE) activity were evaluated with gene expression (Genechip Mouse Gene 1.0 ST Array – Affymetrix) in the striatum and frontal cortex. The results showed 118 and 86 differentially expressed genes (DEGs) in the striatum and frontal cortex, respectively. Through analysis of DEGs biological processes were significantly more enriched in nervous system were development and function and cell-to-cell signaling, particularly in the striatum. These results suggest the involvement of translational and post-translational processes as well as striatal synaptic elements in the trait differences of exploratory behavior. Human studies were conducted with the data collected in a large web-survey on psychological and psychiatric measures (BRAINSTEP). In the study of biological basis of behavior we analyzed temperament in 7.155 males (5.1% hyperuricemic) and 25.225 women (1.8% hyperuricemic). Hyperuricemic subjects scored higher in anger and lower in inhibition and control, but hyperuricemic women also showed a higher emotional sensitivity and a lower degree of volition and coping. Subjects with hyperuricemia present more more externalizing and unstable emotional traits and affective temperaments In the study of the social bases of temperament assessed bullying during childhood and adolescence, through a question on time of exposure to bullying (none, <1 year, 1-3 years and> 3 years). Emotional traits and affective temperaments were evaluated with The Affective and Emotional Composite Temperament Scale (AFECTS). About half of the sample reported exposure to bullying and 10% reported being victimized by peers for longer than 3 years. Longer exposure to bullying was associated with lower Volition, Coping and Control. Bullying victimization was also associated with a much lower proportion of euthymic and hyperthymic types in both genders, which was compensated by an increase mainly in the proportion of depressive, cyclothymic and volatile types. Being bullied was associated with a broad and profound impact on emotional and cognitive domains in all dimensions of emotional traits, and with internalized and unstable affective temperaments. These results, taken together, show the importance of social factors and serum markers, as well as genetic markers of temperament.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AFECT - Escala Composta de Temperamento Emocional e Afetivo

AIS2C - Ativação-Inibição-Sensibilidade-*Coping*-Controle

ASRI - Adult Self-Report Inventory

BRAINSTEP - Brazilian Internet Study on Temperament and Psychopathology

CID-10 - Classificação Internacional das Doenças, 10ª edição

DSM-IV - Manual Diagnóstico e Estatístico de Transtornos Mentais 4ª edição

TCI - Inventário de Temperamento e Caráter

DNA - Ácido Desoxiribonucleico

MAOB - Monoamina oxidase B

TUBB3 - Tubulina classe III beta

GABBA - Ácido gama-aminobutírico

HRB - Animais alto respondedores

BLR - Animais baixos respondedores

mGLU2 - Receptor de glutamato metabotrópicos 2

VGLUT2 - Transportador Vesicular de Glutamato 2

CCK - Colecistoquinina

eIF2 - Fator de Iniciação eucariótico 2

H2B - Histona 2B

CBX3 - Chromobox homólogo da proteína 3

Cox7c - Citocromo c oxidase subunidade 7C

HIST2H2BE - Histona H2B

DEGS – Genes diferencialmente expressos

PMCH - Hormônio Concentrador de Melanina

CALB2 - Calbindina 2

Rpl21 - Proteína ribossomal 60S L21

FRMD7 - Domínio contendo a proteína 7

SCGN - Secretagogina

IFN-Z - Interferon zeta

IL-22 - Interleucina-22

Cma2/Mcpt9 - Quimase 2

SUMÁRIO

1 INTRODUÇÃO

1.1 TEMPERAMENTO

O temperamento pode ser considerado como a base do humor, do comportamento e da personalidade, e popularmente se refere ao jeito de ser de cada indivíduo (Lara et al., 2006). Está relacionado à natureza emocional, perceptual e cognitiva, tem uma base biológica forte, é relativamente estável no decorrer do tempo, mas também sofre influências do meio (Cloninger et al., 1993; Rothbart et al., 2000). Para Cloninger et al. (1993) as dimensões do temperamento são independentemente hereditárias, manifestam-se cedo no desenvolvimento do indivíduo e norteiam a formação dos hábitos e funções cognitivas futuras, pois estão ligadas às sensações e motivações básicas e automáticas do indivíduo. Evidências sugerem que o temperamento e os traços de personalidade predispõem aos transtornos psiquiátricos (Cloninger et al., 1993; Lara e Akiskal, 2006) e que a maioria deles é recorrente e crônico (Insel, 2005).

O conceito de temperamento surgiu cerca de 400 anos a.C.. Galeno e Hipócrates propuseram os temperamentos colérico, melancólico, sanguíneo e fleumático, baseando-se nos quatro elementos do filósofo Empédocles: água, ar, terra e fogo (Akiskal, 2005). No início do século XX, Kraepelin propôs os estados fundamentais depressivo, ciclotímico, irritável e hipertímico, que correspondem ao que hoje chamamos de temperamentos afetivos (Kraepelin,1921). Desde então, várias autores como Eysenck (1987), Cloninger (Cloninger et al.,1993), Akiskal (Akiskal et al.,1989) e outros apresentaram propostas de classificação e distinção

dos temperamentos, sendo que na psiquiatria os mais estudados são o modelo psicobiológico de Cloninger e o modelo de temperamentos afetivos de Akiskal.

Os modelos atuais da psiquiatria baseiam-se no diagnóstico dos transtornos de humor sem levar em conta como a personalidade. Os transtornos de humor, comportamento, cognição e personalidade classificados na psiquiatria pelos presentes nos manuais diagnósticos DSM-IV (*Diagnostic Statistical Manual*, 4ª edição) e CID-10 (Classificação Internacional das Doenças, 10ª edição) são concebidos como entidades distintas (Widiger e Samuel, 2005; Lara et al., 2006; Parker, 2008). Cada transtorno pode somente ser considerado presente ou ausente ("preenchem critérios") nesse modelo categórico, mesmo que muitos quadros subliminares ou subsindrômicos sejam clinicamente importantes (Judd et al., 2002). Apesar dos avanços da neurociência, da psicologia e da psicofarmacologia a classificação nosológica atual ainda não incorporou novos aspectos, como a fenomenologia descritiva nos sistemas diagnósticos (Insel e Quirion, 2005; Parker, 2008; Möller, 2008). Além da alta comorbidade de transtornos (Kendell e Jablensky, 2003) tem-se a validade questionável do modelo categórico fragmentado, também pelo fato de que uma mesma classe de medicações ser efetiva para muitos transtornos distintos (Lara e Souza, 2001; Insel, 2005).

Além disso, o alto grau de comorbidades e a utilização de um mesmo tratamento farmacológico para transtornos classificados distintamente (por exemplo, "antidepressivos" tratando vários transtornos de humor e de ansiedade, de personalidade e de comportamento) são alguns dos fatos que demonstram o equívoco conceitual das classificações atuais. Entre outras limitações, esses sistemas de classificação não consideram componentes do temperamento, um fator

que parece ser determinante para o desenvolvimento e/ou manifestação dos transtornos psiquiátricos (Lara et al., 2006).

Segundo Strelau e Angleitner (1991), o componente biológico do temperamento pode ser sustentado pelo fato de que as características e diferenças individuais do temperamento podem ser observadas desde as primeiras semanas de vida (Ito e Guzzo, 2002). Além disso, a semelhança de temperamento é bem maior em gêmeos homozigóticos, que compartilham 100% dos seus genes, do que em heterozigóticos, que ompartilham em média 50% de seus genes. Em outras palavras, quanto mais genes são compartilhados pelos indivíduos, mais similares eles são com respeito ao traço ou comportamento que tem origem genética (Strelau e Angleitner, 1991)

As dimensões do temperamento são herdadas independentemente, manifestam-se cedo no desenvolvimento do indivíduo e norteiam a formação dos hábitos e funções cognitivas futuras, pois estão ligadas às sensações e motivações básicas e automáticas do indivíduo (Cloninger et al., 1993). Com base nessa idéia, Cloninger e colaboradores (1993) descreveram um modelo psicobiológico dimensional de temperamento e caráter, na tentativa de sugerir uma nova forma para a classificação dos transtornos mentais. Nesse modelo, apesar de contemplar a personalidade normal e patológica, apresenta limitações para a aplicação clínica de rotina pela sua complexidade e por não ter sido criado para identificar indivíduos com risco para transtornos de humor, de déficit de cognição e de desvios de comportamento. O instrumento auto-aplicável relacionado a esse modelo, o Temperament and Character Inventory (TCI), é muito extenso (240 questões) para se tornar uma ferramenta útil na rotina clínica. Neste modelo, cada dimensão do temperamento é caracterizada por um traço herdado relacionado evitação de dano,

busca de novidades, apego e persistência, contemplando personalidades normais e patológicas. Cada uma dessas dimensões parece estar associada a sistemas neurais e emocionais distintos. Assim, o medo se relacionaria à evitação de dano; o desejo e o prazer a um aumento de busca de novidades; o apego à recompensa afetiva; e a ambição à persistência (Cloninger, 1999). Esse modelo tem sido utilizado em diferentes estudos que correlacionam temperamentos, transtornos mentais, bases genéticas (Cloninger, 1999; Lacht et al., 2007; Must et al., 2007).

O modelo de Akiskal tem como base os temperamentos afetivos ciclotímico, hipertímico, irritável, ansioso e depressivo. Apesar de ser um esquema prático, é limitado conceitualmente aos transtornos de humor. O instrumento usado para acessar esse construto é breve e de uso gratuito (Akiskal et al., 2005), mas se restringe a essencialmente dois fatores segundo as análises psicométricas, não possibilita uma orientação terapêutica clara e não apresenta referenciais de saúde mental.

Outros modelos psicológicos de personalidade, como o dos Cinco Grandes Fatores, surgiram a partir de análises psicométricas de diversas características psicológicas e comportamentais, sem um construto teórico consistente (McAdams, 1992). Os instrumentos que avaliam personalidade por esse modelo são relativamente longos e não são disponíveis para uso gratuito.

Desde 2005, nosso grupo tem buscado integrar as abordagens dimensionais e categóricas do temperamento e o uso combinado de traços e estados, a fim de manter as vantagens e minimizar as limitações de usá-las separadamente, como nos modelos anteriormente citados. Essa abordagem gerou o modelo AFECT (*Affective and Emotional Composite Temperament*), que é uma tentativa de integrar emoções

e afeto com transtornos psiquiátricos de maneira mais abrangente e eficaz. Para explicar este modelo, que serviu de base ao nosso trabalho, apresentamos a seguir um resumo do modelo AFECT conforme publicado em Lara et al., 2012.

1.1.1 O modelo AFECT

O modelo AFECT está calcado na premissa de que o temperamento é um elemento chave para o entendimento da saúde e doença no âmbito da mente, em concordância com diversos autores (Cloninger et al., 1993; Akiskal et al., 2005). A configuração de temperamento influencia a apreciação de eventos, gerando determinados vieses na qualidade e quantidade da percepção inicial e avaliação imediata dos estímulos, e depois na forma de lidar com eles. Assim, o temperamento está em uma posição central para influenciar e ser influenciado por outros domínios, como comportamento, cognição, percepção, atenção, relações, intenções, humor e afeto, trabalhando como uma força de ligação entre esses módulos e funções (Figura 1.1).

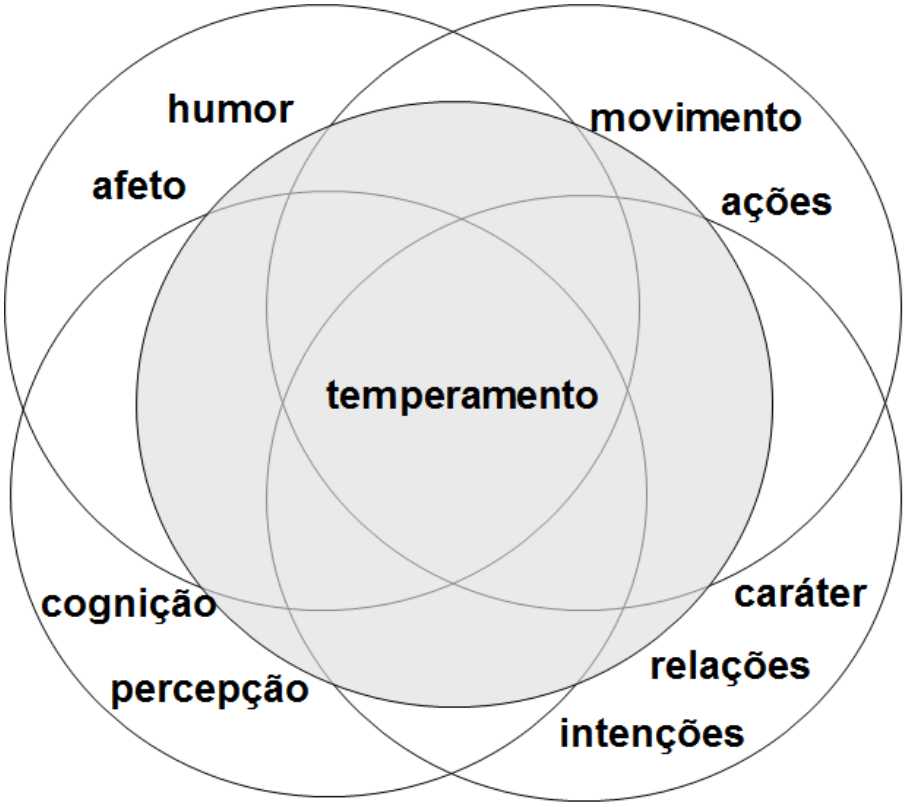

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Figura 1.1. O temperamento como função central e integradora de diversos elementos de humor, comportamento, cognição e valores.

O desenvolvimento teórico desse modelo teve como âncora a aplicação de princípios universais, que são:

1. **A mente funciona como um sistema.** Um sistema é um todo que envolve relações entre seus elementos. A mente é um sistema *aberto, autorregulado, complexo* e com elementos interconectados e *adaptativos*.

2. **O sistema mental tende a funcionar de forma coerente entre os seus diferentes módulos e planos.** Entender o funcionamento da mente como as relações entre as suas partes é fundamental para a compreensão do fenótipo resultante, com importantes implicações para saúde e transtornos mentais.

3. **Visões categóricas e dimensionais são complementares em seus pontos fortes e fracos e nenhuma das duas abordagens é suficiente para descrever todo o fenômeno mental.** Traços e estados, assim como categorias e dimensões oferecem diferentes visões dos fenômenos e podem ser integrados, por exemplo, muitos comportamentos específicos têm maior probabilidade de surgir quanto mais extremo é o indivíduo em uma dada dimensão ou grupo de dimensões de temperamento (por exemplo, ataques de pânico são mais comuns em pacientes com traços de medo e vulnerabilidade).

4. **O perfil de temperamento influencia quais transtornos podem se desenvolver de forma determinista e probabilística.** Os traços de temperamento formam um cenário sobre o qual os fenômenos mentais acontecem, independente de serem adaptativos ou desadaptativos, deliberados ou reativos. Tais traços podem, portanto, ser avaliados como fatores de risco ou de proteção para o desenvolvimento de transtornos mentais, ou até mesmo pelo uso de drogas.

5. **Níveis "ótimos" de traços de temperamento protegem de transtornos psiquiátricos.** A capacidade de autorregulação, contida no princípio de Ativação-Inibição-Sensibilidade-Coping-Controle (AIS2C), explicado a seguir, é crucial para adaptação e proteção contra transtornos do comportamento como o uso de drogas.

O princípio AIS2C é formado pelas relações entre Ativação-Inibição-Sensibilidade-*Coping*-Controle.

Segundo este princípio, a estrutura funcional de um sistema conta com duas forças independentes de ativação e de inibição, representadas ortogonalmente na forma de “X” na figura 1.2. Conceitualmente é importante considerar que a falta de ativação difere do excesso de inibição, e que o excesso de ativação é distinto de déficit de inibição. A interação entre essas duas forças principais gera tipicamente 5 tipos de resultante :

- alta ativação e baixa inibição = expansão

- baixa ativação e alta inibição = estagnação

- alta ativação e alta inibição = ambivalência, turbulência ou tensão

- baixa ativação e baixa inibição = indiferença, ou um estado “à deriva”

- ativação e inibição balanceadas = moderação

A resultante pode ser caracterizada medindo seu nível (alto-baixo) e estabilidade (estável-instável) ao longo do tempo. Essa interação de duas forças opostas gerando uma resultante é facilmente observável em seres vivos: os sistemas simpático e parassimpático geram as resultantes de pressão e tônus; o glucagon e a insulina modulam os níveis glicêmicos; e a entrada de cátions (sódio) e ânions (cloreto) resulta no grau de excitabilidade neuronal. Esse conceito também pode ser traduzido para o sistema mental.

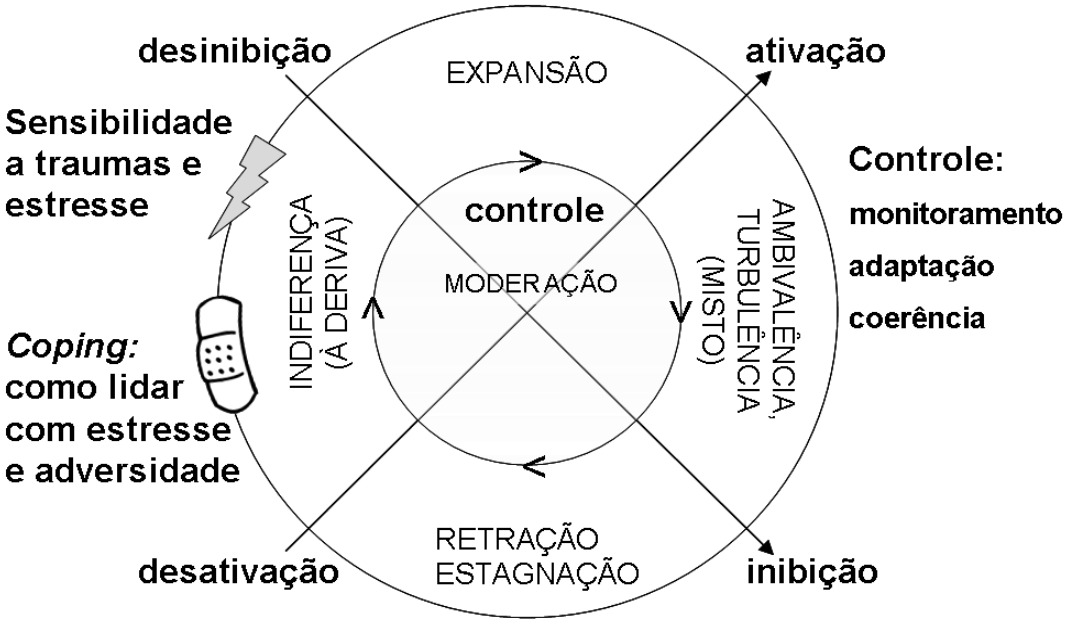


Figura 1.2 O modelo de sistema Ativação-Inibição-Sensibilidade-*Coping*-Controle. Ativação e inibição formam dois vetores independentes cuja interação produz as resultantes (sínteses) de expansão, retração, ambivalência, indiferença e moderação. O sistema conta com o Controle para monitorar e adaptar o sistema ao ambiente, um grau de sensibilidade (suscetibilidade) a estresse e uma capacidade de lidar com a adversidade (*Coping*).

O sistema conta com a capacidade de exercer sua autorregulação frente ao ambiente, monitorando e interpretando o contexto para sinalizar as mudanças necessárias na ativação e inibição adequadas. A essa função chamamos de Controle, representado como o círculo central com setas, para representar seu caráter dinâmico. O Controle tem a capacidade de coletar e gerenciar a informação do ambiente, para depois comunicar o que deve ser feito a partir de um *feedback*.

Na interação com o ambiente o sistema tem um determinado grau de Sensibilidade, que diz respeito a como o ele é abalado pela adversidade. Os recursos para resolver problemas enfrentados pelo sistema estão representados no *Coping* (palavra usada no inglês por não haver no português um substantivo com

essa exata conotação, relacionada psiquicamente à maturidade). O *Coping* é responsável por *lidar* com as adversidades, que envolve abordar o problema, ser capaz de resolvê-lo e, de preferência, fazer o sistema evoluir de modo que se torne mais apto e forte para lidar com futuros problemas de natureza semelhante. Portanto, de uma maneira diferente do Controle, a Sensibilidade e o *Coping* também processam informações advindas da interação com o ambiente.

Além da aplicação dos princípios universais, o modelo AFECT seguiu uma abordagem tanto analítica (partes) quanto sintética (resultante da interação entre as partes). Essa visão torna o desafio mais complexo, mas gera ganhos em poder explicativo. A abordagem analítica foi concebida com o temperamento emocional (traços emocionais específicos) e a sintética com temperamento afetivo.

1.1.2 Traços emocionais do temperamento

Traduzindo o modelo AIS2C para a mente, o temperamento emocional tem como base dois eixos ortogonais dos opostos complementares de *ativação* (vontade, desejo e raiva) e *inibição* (medo e cautela), que são modulados pelo *controle*. O sistema tem algum grau de *sensibilidade* a eventos ambientais e habilidades de *coping* (encarar e resolver problemas, aprendendo com isso).

Cada dimensão emocional é composta de 8 itens, com duas facetas de 4 itens para cada dimensão, com exceção do desejo, que é uma dimensão com uma só faceta de 4 itens. A composição final dos itens, gerados a partir de análises psicométricas, está mostrada na Tabela 1.1.

Tabela 1.1 Características dos temperamentos emocionais.

VONTADE DE DESEJO	Positividade	Pessimista	Otimista
		É difícil eu sentir prazer	É fácil eu sentir prazer
		Triste e desanimado	Alegre e animado
		Minha auto-estima é baixa	Minha auto-estima é alta
	Energia	Fico indiferente a novas atividades	Fico entusiasmado com novas atividades
		Desmotivado e desinteressado	Motivado e interessado
		Faltam-me objetivos e força de vontade	Tenho objetivos e força de vontade
		Parado e sem energia	Ativo e energético
	Impulsos	Meus impulsos do desejo são leves	Meus impulsos do desejo são fortes
		Sou moderado no que eu gosto	Exagero no que eu gosto
		Sei me conter na busca de prazer	Facilmente me rendo às tentações do prazer
		Mantenho o juízo quando quero algo	Faço loucuras quando quero algo
RAIVA	Intensidade	Tranquilo	Apressado e imediatista
		Ponderado	Sou de extremos, do tipo 8 ou 80
		Flexível	Teimoso
	Agressividade	Paciente	Impaciente
		Calmo	Irritado
		Pacífico	Agressivo
		Controlado	Explosivo
		Confio nas pessoas	Desconfiado
INIBIÇÃO	Medo	Medroso	Ousado
		Inibido e contido	Desinibido e espontâneo
		Preocupado	Despreocupado
		Fico paralisado frente ao perigo	Reajo rapidamente frente ao perigo
	Cautela	Cauteloso	Descuidado
		Penso antes de agir	Impulsivo, ajo sem pensar
		Prudente	Imprudente
		Evito correr riscos	Gosto de correr riscos

SENSIBILIDADE	Interpessoal	Eu me culpo facilmente	É raro eu me sentir culpado
		Lido mal com a rejeição	Lido bem com a rejeição
		Sou sensível a críticas	Suporto bem críticas
	A eventos	Eu fico magoado facilmente	Dificilmente fico magoado
		Tenho dificuldade em superar traumas	Tenho facilidade em superar traumas
		Sou sensível ao estresse	Resisto bem ao estresse
		Lido mal com situações de pressão	Lido bem com situações de pressão
		Tenho baixa tolerância à frustração	Tenho alta tolerância à frustração
COPING	Encarar	Jogo a culpa dos meus erros para os outros	Assumo a culpa pelos meus erros
		Tento me esquivar dos meus problemas	Enfrento meus problemas de frente
		Espero que meus problemas se resolvam sozinhos	Procuro resolver meus problemas
	Resolver	Deixo meus problemas pessoais acumularem	Resolvo meus problemas pessoais assim que posso
		Tenho dificuldade em resolver meus conflitos com pessoas	Tenho facilidade em resolver meus conflitos com pessoas
		Tenho dificuldade em encontrar soluções	Tenho facilidade em encontrar soluções
		Tendo a repetir meus erros	Aprendo com meus erros
		Sofrer me tornou mais frágil	Sofrer me tornou mais forte
CONTROLE	Foco	Desatento	Atento
		Dispersivo	Focado
		Planejo mal minhas atividades	Planejo bem minhas atividades
		Não concluo as tarefas que eu começo	Concluo as tarefas, mesmo as longas e difíceis
	Ordem	Desorganizado	Organizado
		Indisciplinado	Disciplinado
		Irresponsável	Responsável
		Displicente	Perfeccionista

1.1.3 Temperamento afetivo

O temperamento afetivo é um conceito sintético e está intimamente relacionado ao humor ou padrão energético. Assim, o desenvolvimento dos temperamentos afetivos segue a lógica de caracterizar as principais combinações entre as dimensões emocionais.

Estas dimensões podem variar em intensidade (alta, moderada e baixa) de tal forma que suas diversas e mais comuns combinações geram 12 temperamentos afetivos, sendo que 5 já haviam sido propostos por Kraepelin e Akiskal (ciclotímico, hipertímico, irritável, ansioso ou evitativo e depressivo) e outros 7 foram propostos por Lara et al. (2008) (volátil, apático, eutímico, disfórico, desinibido, eufórico e obsessivo).

Um objetivo importante do construto de temperamento afetivo é refletir os principais padrões de saúde e disfunção mental de forma sucinta e global. As definições dos temperamentos afetivos são as seguintes:

DEPRESSIVO: Tenho tendência à tristeza e à melancolia; vejo pouca graça nas coisas; tendo a me desvalorizar; não gosto muito de mudanças; prefiro ouvir a falar.

EVITATIVO: Sou muito preocupado e cuidadoso; frequentemente me sinto inseguro e apreensivo; tenho medo de que coisas ruins aconteçam; tento evitar situações de risco; estou sempre alerta e vigilante.

APÁTICO: Tenho pouca iniciativa; com frequência me desligo do que os outros estão dizendo ou fazendo; muitas vezes não concluo o que comecei; tendo à passividade e sou um pouco lento.

CICLOTÍMICO: Meu humor é imprevisível e instável (altos e baixos), muda rapidamente ou de maneira desproporcional aos fatos; tenho fases de grande energia, entusiasmo e agilidade que se alternam com outras fases de lentidão, perda de interesse e desânimo.

DISFÓRICO: Tenho uma forte tendência a me sentir agitado, tenso, ansioso e irritado ao mesmo tempo.

VOLÁTIL: Sou dispersivo, inquieto, desligado e desorganizado; às vezes sou precipitado ou inconveniente e só me dou conta mais tarde; mudo de interesse rapidamente; tenho dificuldade em concluir tarefas e fazer o que deveria.

OBSESSIVO: Sou exigente, dedicado, perfeccionista, detalhista e rígido; preciso ter o controle das coisas; não lido bem com incertezas e erros.

EUTÍMICO: Meu humor é equilibrado e previsível, costuma mudar só quando há um motivo claro; tenho boa disposição e, em geral, me sinto bem comigo mesmo.

HIPERTÍMICO: Estou sempre de bom humor, sou muito confiante e me divirto facilmente; adoro novidades; faço várias coisas sem me cansar; vou atrás do que quero até conquistar; tenho forte tendência à liderança.

IRRITÁVEL: Sou muito sincero, direto e determinado, mas também irritado, explosivo e desconfiado.

DESINIBIDO: Sou inquieto, ativo, espontâneo e distraído; muitas vezes ajo de maneira precipitada e inconsequente; é muito comum eu deixar para fazer as coisas na última hora; quando me irrito, logo fico bem de novo.

EUFÓRICO: Sou expansivo, rápido, falante e intenso; tenho muitas ideias e me distraio facilmente; sou imediatista, explosivo e impaciente; me exponho a riscos por excesso de confiança ou empolgação; exagero no que me dá prazer; não gosto de rotina e de regras.

O estudo desse modelo foi operacionalizado com o desenvolvimento da escala AFECTS. De acordo com os resultados dessa escala, 99% dos indivíduos se identificam com no mínimo um dos temperamentos afetivos propostos. Tais dados sugerem que esses 12 tipos afetivos abrangem o estilo afetivo e de padrão de humor da maioria das pessoas de forma prática e simples.

Para avaliar melhor essas tendências, índices de externalização-internalização e de instabilidade-estabilidade foram desenvolvidos, tornando evidente que os 12 temperamentos afetivos podem ser divididos em 4 grupos com 3 integrantes, com as seguintes definições gerais:

- intenalizados (depressivo, evitativo e apático): têm problemas por inibição e déficit de ativação, se prejudicam por deixar de fazer, por faltar atitudes e atributos adaptativos; tendem a evitar estímulos e são vulneráveis;

- instáveis (ciclotímicos, disfóricos e voláteis): erram por excesso e por déficit, umas vezes demais outras de menos; inconstância de relações e dificuldades no longo prazo pela falta de regularidade; alternam busca e evitação de estímulos e são reativos;

- estáveis (obsessivos, eutímicos e hipertímicos): a regularidade e moderação ajudam muito na adaptação; erram mais por acharem que estão sempre certos, porque comparados aos outros tipos, frequentemente estão bem e têm êxito, mas podem ter excesso de confiança e pecar por excesso de controle; busca moderada de estímulos ou alta busca de estímulos de média intensidade;

- externalizados (eufóricos, desinibidos e irritáveis): erram mais por excesso, fazem primeiro para depois pensar nas consequências e, muitas vezes, pagam um preço caro por isso; alta busca de estímulos e alta reatividade.

1.1.4 Relações entre os temperamentos emocionais e afetivos

De acordo com os resultados da escala AFECTS, cada um dos temperamentos afetivos tem uma configuração emocional específica, mas os tipos do mesmo grupo compartilham mais semelhanças. As configurações emocionais dos doze temperamentos afetivos estão representadas na Tabela 1.2.

Tabela 1.2 Configurações emocionais dos 12 temperamentos afetivos.

Temperamentos Afetivos	Vontade	Raiva	Desejo	Inibição	Sensibi-lidade	*Coping*	Controle
Depressivo	↓↓	↔	↓	↑↑	↑↑	↑↑	↓
Evitativo	↔	↔	↓	↑↑	↑	↓	↑
Apático	↓	↓	↓	↑	↑	↓↓	↓↓

Ciclotímico	↔	↑↑	↑↑	↔	↑↑	↓	↔
Disfórico	↔	↑↑	↑	↔	↑	↓	↔
Volátil	↓	↑	↑	↓	↑	↓↓	↓↓
Obsessivo	↑	↑	↔	↑	↔	↑	↑↑
Eutímico	↑↑	↓↓	↓↓	↔	↓↓	↑↑	↑↑
Hipertímico	↑↑	↓	↔	↓	↓↓	↑↑	↑↑
Irritável	↑	↑↑	↑	↔	↔	↑	↑
Desinibido	↑	↔	↑↑	↓↓	↔	↔	↓
Eufórico	↑	↑↑	↑↑	↓↓	↔	↑	↔

↓↓ = muito baixo, ↓ = baixo, ↔ = médio, ↑ = alto, ↑↑ = muito alto

Esse modelo de temperamento também fornece uma matriz sobre a qual os transtornos de humor, comportamento e personalidade podem se desenvolver. Esses transtornos podem ser concebidos a partir da natureza emocional e afetiva subjacente e em função dos seus pontos comuns, assim como pelas suas diferenças.

1.2 UTILIZAÇÃO DE MICROARRAYS PARA A AVALIAÇÃO DE EXPRESSÃO GÊNICA

Dentre as novas tecnologias desenvolvidas como um dos desdobramentos do seqüenciamento dos genomas, destaca-se a técnica de *microarrays*, ou chips de DNA. Esta técnica permite a investigação de milhares de genes de maneira simultânea, com grandes aplicações para a medicina preditiva, diagnóstica e farmacológica por meio do aumento substancial da capacidade analítica dos processos moleculares (Mocelin e Rossi, 2007; Guindalini e Tufik, 2007).

Através dos Chips de DNA pode-se examinar comparativamente a expressão gênica global que ocorre em diferentes tipos celulares ou em um tecido específico, quando submetidos ou expostos a uma determinada condição patológica ou experimental (Lockhart et al., 1996; Sharp et al., 2006). É possível, ainda, buscar variações estruturais na seqüência de DNA que possam contribuir para o aumento de susceptibilidade a doenças de uma maneira rápida, econômica e sistemática (Guindalini e Tufik, 2007).

No estudo de Choi et al. (2012) foram utilizados ratos selecionados para alto e baixo medo. Na avaliação da expressão gênica do DNA mitocondrial observou-se mudanças dependentes da idade na expressão dos genes monoamino axidase (MAOB) e tubulina 3 (TUBB3) no córtex pré-frontal dos animais com medo de altura. Com base nas alterações da expressão dos genes dos animais durante seu desenvolvimento observou-se uma maior predisposição ao desenvolvimento de perturbações do humor e ansiedade.

No estudo de Alttoa et al. (2010) foram avaliados os padrões de expressão gênica no hipocampo, cortex frontal e nos núcleos da rafe de ratos alto (HE) e baixo exploradores (LE). Na análise dos genes diferencialmente expressos (DEGs) e funções celulares encontrou-se uma superexpressão de genes envolvidos no desenvolvimento neuronal, morfogênese e diferenciação. Ainda, no estudo dos genes diferencialmente expressos, encontrou-se significativa diferença na expressão nos genes sinápticos, especialmente os serotoninérgicos e glutamatérgicos. Além disso, com base diferença de expressão gênicas nos animais, foi desenvolvida a análise das vias celulares, nas quais, identificou-se que a via de processos depressivos esta altamente enriquecida nos animais com baixa exploração.

No estudo de Clinton et al. (2011) avaliou-se o desenvolvimento cérebral em um modelo animal de diferenças de temperamento. Foram utilizados animais selecionados como *High Behavior Responses* (HBR) e *Low Behavior Responses* (LBR) que exibem diferenças de comportamento emocional. Animas HBRs apresentavam mais exploração, impulsividade, agressividade e animais LBRs demonstraram mais inibição, mais ansiedade e comportamento depressivo. Nos estudos de microarrays, avaliou-se a expressão gênica de LBR/HBR no hipocampo e no núcleo accumbens dos animais no período pós-natal. Na análise da expressão gênica encontrou-se uma diferença significativa de genes diferencialmente expressos no hipocampo. Além disso, os estudos comportamentais mostraram que a característica HBR/LBR de fenótipos de comportamento surgem no início da vida dos animais, ou seja, já nas primeiras semanas de vida observa-se os traços de temperamento destes animais e estes permaneciam a medida em que os animais se desenvolviam.

1.3 O USO DE ROEDORES PARA ESTUDO DOS TRANSTORNOS DE HUMOR

Há uma extensa gama de fatores genéticos e neurobiológicos homólogos entre roedores e humanos, responsáveis pela variedade de comportamentos bem conservados entre as espécies (Landgraf et al., 1999). Segundo Landgraf et al. (2007), o comportamento, a anatomia e as características fisiológicas entre roedores e humanos são semelhantes, permitindo uma extrapolação cuidadosa das emoções nos animais. Neste contexto, a aplicação de procedimentos para analisar o comportamento de ratos e camundongos são críticos para traduzir os rápidos avanços na genômica de mamíferos em avanços relevantes para o diagnóstico e tratamento de doenças psiquiátricas. Humanos e roedores têm uma origem evolutiva próxima (Murphy et al., 2001), o que sugere que o temperamento é uma característica genética estável que controla as motivações básicas e automáticas, organizado de modo semelhante em mamíferos (Cloninger, 1999).

Alguns estudos se valem da seleção de populações de roedores segundo características comportamentais para avaliar a participação de determinados genes. Por exemplo, Hovatta e colaboradores (2005) verificaram que linhagens de camundongos que se mostravam mais ou menos ansiosos em testes comportamentais como o campo aberto e a caixa de claro/escuro apresentavam aumento da expressão do gene que codificam para a enzima glioxilase. Essa enzima foi mais expressa em regiões cerebrais que modulam a ansiedade, correlacionando assim uma característica do temperamento a uma maior expressão gênica desta enzima específica. Além disso, a ansiedade foi revertida após a deleção desse gene, confirmando esses achados.

Modelos animais são ferramentas importantes para o estudo e compreensão dos transtornos psiquiátricos, principalmente na busca de novos e melhores tratamentos. Os modelos animais tem sido delineados utilizando uma variedade de parâmetros farmacológicos, comportamentais e genéticos. Kazlauckas et al. (2005) avaliaram características comportamentais em camundongos para selecionar fenótipos distintos com extremos de temperamento. O teste utilizado foi o teste de campo aberto com objeto central. Os resultados do estudo mostram que as diferenças individuais em temperamento podem influenciar uma variedade de comportamentos nos camundongos. O perfil comportamental de baixa e alta exploração pelos camundongos está associado ao temperamento depressivo e hipertímicos, semelhante aos temperamentos dos pacientes com depressão unipolar e transtorno bipolar, respectivamente, o que demonstra a importância da utilização de modelos animais nos estudos para transtornos de humor.

Muitos estudos baseiam-se na ideia de que diferenças individuais na resposta neural e hormonal a uma novidade contribuem para que se observem diferenças quanto ao comportamento explorador e susceptibilidades a psicopatologias (Zuckerman, 1990). Piazza et al. (1990) classificaram ratos com alta e baixa resposta locomotora. Animais com alta locomoção são mais susceptíveis às ações de pscicoestimulantes e apresentam diferentes perfis de susceptibilidades a drogas de abuso. Além disso, Thiel e colaboradores (1999) observaram que ratos com alta locomoção exploravam mais objetos novos (respondiam mais a novidades), no entanto respondiam de maneira semelhante aos ratos de baixa atividade exploratória em relação aos psicoestimulantes.

Landgraf e colaboradores (1999) utilizaram o teste de labirinto em cruz elevado para avaliar a alta e baixa ansiedade. Os pesquisadores observaram que

animais com baixa ansiedade são mais ativos, expressam maior agressividade e exploram mais a área central do campo aberto. No estudo de Piras et al. (2010), a partir da observação de animais selecionados com alto e baixo desempenho na tarefa de evitação na esquiva ativa, identificou-se um padrão de diferenças de comportamento que servem como modelo animal para analisar variações genéticas que predispõem a ansiedade. Dessa forma, bons modelos animais nos possibilitam a oportunidade única de examinar profundamente os mecanismos neurobiológicos, genéticos e ambientais que predispõem aos transtornos de humor (Ray e Hansen, 2004).

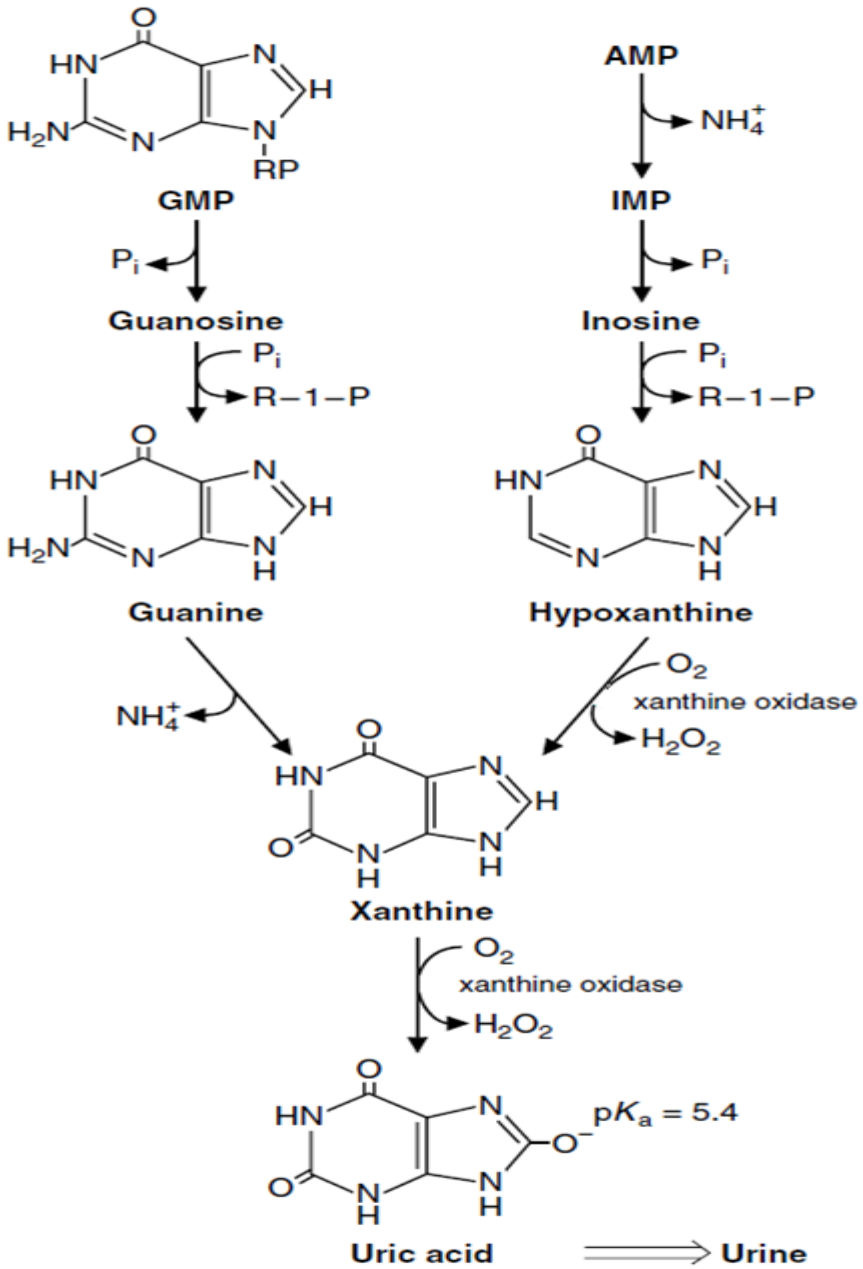
1.4 ÁCIDO ÚRICO E TEMPERAMENTO

O ácido úrico (2,6,8 trioxypurine-C5H4N4O3) é um composto orgânico, do metabolismo das purinas, encontrado nos animais. É produzido pelo fígado e excretado principalmente pelos rins (65-75%) e pelo o intestino (25 -35%). O ácido úrico é o produto final do metabolismo das purinas nos seres humanos, que apresentam níveis mais elevados do que os outros mamíferos em função da perda na atividade da uricase (Roch-Ramel e Guisan, 1999; Alvarez-Lario e Macarron-Vicente, 2010; de Oliveira e Burini, 2012).

Nos seres humanos o ácido úrico é sintetizado a partir da hipoxantina por meio da ação de xantina oxidase (Watts, 1966), como ilustra a figura . Doenças acompanhadas por destruição celular elevada, tais como leucocitoses, leucemias e distrofias, podem aumentar o fornecimento de ácidos nucleicos para o fígado e

resultar em uma maior produção de ácido úrico. Dessa forma, doenças resultantes de erros inatos do metabolismo da purina também podem provocar hiperuricemia (Richette e Bardin, 2010; Roddy e Doherty, 2010). Além disso, dietas ricas em carnes e o consumo de cerveja também elevam as concentrações de ácido úrico no sangue.

Figura 1.3 – Metabolismo das purinas: formação do ácido úrico



Em relação à patogênese o ácido úrico é geralmente associado a artrite gotosa e a nefrolitíase (Alvarez-Lario e Macarron-Vicente, 2010). A hiperuricemia provoca a formação de cristais, uma vez que o ácido úrico apresenta baixa solubilidade no meio extracelular. Dessa forma os cristais depositam-se em diversos tecidos induzindo a fagocitose e inflamação (de Oliveira e Burini, 2012). No contexto clínico a hiperuricemia, é considerada um indicador de prognóstico de doença renal, diabetes mellitus, doença cardiovascular e inflamação (Gagliardi et al., 2009; de Oliveira e Burini, 2012). Para Gao et al. (2008) hiperuricemia é um fator de risco independente para síndrome metabólica, doenças cardiovasculares, mas dietas ricas em urato são consideradas protetoras contras a doença de Parkinson. A hiperuricemia eleva em 16% todas as causas de mortalidade e em 39% os casos de mortalidade cardiovascular (Chen et al., 2009). No entanto, diversos estudos tem apontado que o ácido úrico, devido às suas ligações duplas, tem excelente capacidade antioxidante e pode ser responsável por 2/3 da capacidade antioxidante total do plasma (Sautin e Johnson, 2008).

El-Malakh e Jefferson (1999) sugeriram que desequilíbrios nas concentrações de ácido úrico podem estar associados à inúmeros transtornos psiquiátricos. Oslon e Houlihan (2000) também descrevem que reduções nas concentrações de purinas estão associadas a quadros hiperuricêmicos de pacientes com Síndrome de Lesch-Nyhan, à automutilação, retardo mental e coreoatetose. Além disso, o inibidor da xantina oxidase, alopurinol, reduz a produção de ácido úrico, e tem efeito antimaníaco (Machado-Vieira et al., 2001, 2008; Akhondzadeh et al., 2006), antiagressivo (Lara et al., 2000, 2003) e antipsicótico (Lara et al., 2001; Akhondzadeh et al., 2006;. Brunstein et al., 2005) quando usado como terapia adjuvante.

Com relação ao comportamento, o sistema purinérgico afeta o sono, atividade motora, cognição, atenção, agressividade e humor (Lara et al., 2001). Estudos dos anos 60 mostraram uma associação de ácido úrico plasmático com traços comportamentais e psicológicos, tais como alta energia, unidade de afeto, realização, bom desempenho, maior status social e liderança (revisto por Katz e Weiner, 1972). Recentes evidências de estudos genéticos e clínicos sugerem que a disfunção do sistema purinérgico pode desempenhar um papel importante na fisiopatologia e terapêutica de distúrbios bipolares (Machado-Vieira et al., 2008). De Berardis et al. (2008) relataram que os níveis de ácido úrico no plasma foram superiores apenas durante a fase maníaca dos distúrbios bipolares, mas não durante episódios depressivos ou fases eutímicas. Já Wen et al. (2011) evidenciou baixos índices de ácido úrico em pacientes depressivos.

Brooks e Mueller (1966) observaram um coeficiente de correlação de 0,66 entre os níveis de ácido úrico e os escores de motivação e vontade ($P < 0,001$) em um estudo com professores universitários. Da mesma forma relataram maior motivação, vontade e liderança em homens com hiperuricemia (> 7,0 mg / ml) em comparação com homens normouricêmicos. Rahe et al. (1976) também encontrou uma correlação positiva entre escores de motivação e níveis de ácido úrico no soro. No estudo de Lorenzi et al. (2010) os níveis de ácido úrico foram associados com desinibição (particularmente nas mulheres) e vontade (mais nos homens), bem como temperamentos irritáveis e hipertímicos.

1.5 BULLYING COMO ESTRESSOR SOCIAL

O *Bullying* envolve repetidas ações perniciosas entre pares, em que existe um desequilíbrio de poder (Olweus, 1993). O *bullying* é distinto de outras formas de comportamentos agressivos, abrangendo três elementos. Primeiro, o *bullying* ocorre entre indivíduos da mesma faixa etária, tendo lugar entre jovens ou entre adultos. As ações ofensivas que são perpetradas por adultos contra crianças ou adolescentes são considerados maus tratos e não *bullying*. Em segundo lugar, as ações ofensivas são repetidas ao longo do tempo, formando um padrão de interações entre os indivíduos que praticam *bullying* e a vítima. Em terceiro lugar, a relação entre os provocadores e a vítima é caracterizada por um desequilíbrio de poder através do qual é difícil para a vítima se defender. A força física, a popularidade e a idade são fatores que caracterizam o desequilíbrio de poder entre os *bulliers* e sua vítima (Arseneault et al., 2010). Presente nos estudos de Olweus, há mais de duas décadas atrás, o interesse nesse subconjunto de comportamento anti-social tem aumentado substancialmente, devido, em grande parte, à forte evidência de que ocorre em uma proporção notável de crianças e jovens (Boulton e Underwood, 1992; Nansel et al., 2001; 2004) e está associado simultaneamente (Hawker e Boulton, 2000) e ao longo do tempo (Reijntjes et al., 2010) com várias formas de desajustamento (Boulton, 2012).

O *bullying* pode ocorrer tanto pela forma aberta (confrontos físicos) como, secreta (toxicidade social), ou ocorrer eletronicamente via Web, fotos de celular, ou por meio de mensagens de texto desagradáveis. O *bullying* não é mais considerado um rito de passagem da infância. Alguns estudos determinaram que o *bullying* e

suas ramificações provocam sequelas psicológicas e físicas tanto a curto quanto a longo prazo. Problemas como dificuldade escolar, aumento de absenteísmo, distúrbios do sono, enurese, dor abdominal e dores de cabeça e até mesmo diminuição da função imunológica têm sido associados as vítimas de *bullying* (Williams et al., 1996;. Rigby e Peer, 1999; Hawker e Boulton, 2000; Vessey, 2012). Ser vítima de *bullying* está relacionado também com sintomas graves de saúde mental, incluindo sintomas depressivos e de ansiedade, ideação suicida, auto-agressividade, transtorno de compulsão alimentar, comportamento violento e sintomas psicóticos (Salmon et al., 1998; Kaltiala -Heino et al., 1999; Kaltiala-Heino et al., 2000; Striegel-Moore et al., 2002; Dake et al., 2003; Arseneault et al., 2010).

Em um estudo epidemiológico realizado em 25 países, em média, 11% das crianças relataram ser vítimas de *bullying* (Nansel et al. 2004). As vítimas tendem a mostrar sintomas crescentes de ansiedade e depressão (Hodges & Perry, 1999), de baixa auto-estima e as habilidades sociais pobres (Egan e Perry, 1998). As vítimas de *bullying* mostram sintomas de internalização, mas também problemas de externalização (Nansel et al., 2001; Juvonen et al., 2003; Veenstra et ai., 2005; Arseneault et al., 2010).

1.6 INTERNET COMO MEIO DE PESQUISA

Estudos face a face sobre questões delicadas, tais como maus tratos sofridos ao longo da vida, são propensos a subregistros. No entanto, dados de autorrelato coletados por computador podem aumentar a validade para questões de ordem moral e pessoal, em comparação aos métodos anônimos de papel e

caneta (Turner et al., 1998), face a face (Gosling et al,. 2004) e entrevistas telefônicas (Cuijpers et al., 2008). Especialmente quando os websites de pesquisa são acessados a partir de computadores pessoais remotos, os entrevistados podem se sentir menos preocupados com a forma como eles aparecem para os outros. Dados "*on-line"* sobre outras medidas são notadamente consistentes com dados "*off-line*" (Buchanan e Smith, 1999; Hewson e Charlton, 2005) e os usuários de Internet são similares aos não usuários nas medidas de ajustamento, interação social e traços de personalidade (Gosling et al., 2004). Além disso, a Internet fornece meios para aumentar a motivação dos participantes (por exemplo, *feedback* imediato personalizado) e possibilita inserir controles de validação, que aumentam significativamente a qualidade dos dados (Edwards et al., 2009). Em alguns estudos que avaliaram os questionários em diferentes versões, quase todos os entrevistados preferiram questionários em versões Web do que enviados pelo correio e entrevistas por telefone, ou não tinham preferência (Rankin et al., 2008; Touvier et al., 2010). Com base nessas evidências, questionários respondidos pela internet podem até mesmo ser considerados o padrão ouro para as questões sujeitas ao viés de desejabilidade social, especialmente em estudos populacionais.

2 JUSTIFICATIVA

O temperamento é um fator determinante para o desenvolvimento e/ou manifestação dos transtornos psiquiátricos (Cloninger et al., 1994; Lara et al., 2006). Além disso, várias evidências sugerem que parte do componente biológico da maioria dos transtornos mentais parece estar relacionado aos traços de temperamento ou padrão emocional básico (Cloninger et al., 1998; Must et al., 2007, Laucht et al., 2007; Benjamin et al., 1996; Ebstein et al.,1996; Lara et al, 2012). Em função disso, é importante identificar fatores biológicos associados as distintas características do temperamento, como diferenças na expressão de gênica e marcadores bioquímicos periféricos para ajudar no diagnóstico clínico e acompanhamento de pacientes. Para tais fins, avaliaremos a expressão gênica no corpo estriado e córtex frontal de camundongos com traços de alta e baixa atividade exploratória e o perfil temperamental de pessoas com hiperuricemia comparado a controles.

Além disso, há evidências de que o ambiente também influencia o temperamento, mas poucos estudos enfatizaram o impacto do ambiente social sobre traços psicológicos. Problemas como sintomas depressivos e ansiosos, dificuldade escolar, aumento de absenteísmo, distúrbios do sono, dores físicas, diminuição da função imunológica têm sido associados ao *bullying* (Williams et al., 1996;. Rigby e Peer, 1999; Hawker e Boulton, 2000; Vessey, 2012; Salmon et al., 1998; Kaltiala - Heino et al., 1999; Kaltiala-Heino et al., 2000; Striegel-Moore et al., 2002; Dake et al., 2003; Arseneault et al., 2010). Nesse sentido, avaliamos as relações do temperamento emocional e afetivo com a história de ter sido vítima de bullying na

infância e adolescência. Ambos os estudos em humanos se valeram de uma grande base de dados coletada pela internet no projeto BRAINSTEP.

Assim, o entendimento das bases neurobiológicas e sociais do temperamento pode contribuir para o entendimento da fisiopatologia de vários transtornos psiquiátricos e, consequentemente, para o desenvolvimento ou aprimoramento de estratégias preventivas, terapêuticas e diagnósticas.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Investigar as bases biológicas do temperamento em modelos animais e avaliar a relação do temperamento com o autorrelato de ter hiperuricemia e de ter sido vítima de *bullying* em humanos.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar a expressão gênica no corpo estriado de camundongos estratificados de acordo com os comportamentos de evitação de dano e busca de novidades.
- Avaliar a expressão gênica no córtex frontal de camundongos estratificados de acordo com comportamentos de evitação de dano e busca de novidades.
- Analisar as diferenças na expressão gênica do corpo estriado e do córtex frontal.
- Avaliar o temperamento afetivo e emocional em uma amostra de pessoas com autorrelato de hiperuricemia e controles coletada pela internet.
- Avaliar a freqüência do autorrelato de transtorno de humor e ansiedade em uma amostra de pessoas com autorrelato de hiperuricemia e controles coletada pela internet.
- Avaliar o temperamento afetivo e emocional em relação ao histórico de ter sofrido *bullying* em uma grande amostra de pessoas coletada pela internet.

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Differential Gene Expression in the Striatum and Frontal Cortex of High and Low Exploratory Mice

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Abstract

Exploration of environmental stimuli is an essential animal behavior expressed in various degrees. Novelty-seeking traits are associated with a higher intake of drug of abuse, among other externalizing disorders. To study the neurobiological basis of this trait, we selected mice with high and low exploration of a central object in an open field. Out of one hundred mice tested, the ten mice with higher (HE) and lower exploratory (LE) activity were evaluated with microarray gene expression in the striatum and frontal cortex. The results showed 118 and 86 differentially expressed genes (DEGs) in the striatum and frontal cortex, respectively. The biological processes most significantly enriched in differentially expressed genes (DEGs) were nervous system development and function and cell-to-cell signaling and interaction, particularly in the striatum. The top canonical pathways involved were eIF2 signaling in both the striatum and the cortex, and glutamate receptor signaling in the striatum. In the striatum in particular, there were downregulated genes related to the synapse such as the metabotropic glutamate receptor type 2 (mGLU2), vesicular glutamate transporter (VGLUT2), cholecystokinin and pro-melanocortin hormone (PMCH), all of which have been previously implicated in the regulation of exploratory behavior. Other significant DEGS in the striatum were Chromobox Protein Homolog 3 (CBX3), Cytochrome c oxidase subunit 7C (Cox7c) and Histone H2b type 2-E (HIST2H2BE), all upregulated, and calretinin (CALB2), which was downregulated. These results suggest the involvement of translational and post-translational processes as well as striatal synaptic elements in the trait differences of exploratory behavior.

Keywords – exploratory behavior; novelty-seeking; temperament; microarray; gene expression; striatum.

Introduction

Exploration of environmental stimuli is an essential animal behavior. Exploratory behavior is expressed in various degrees and individual differences can be studied in animal models. In rodents, exploratory activity has been classically evaluated with the open field (OF) using the parameters of locomotor activity in response to the novel environment or time in the center of the arena versus near the walls (thigmotaxis) (Redolat et al. 2009). Other uses measure the exploration of new versus a familiar environment, latency to approach and time spent with a novel object in an open field or using a hole board (Hughes, 2007).

Animal selection based on exploratory behavior is a useful tool to study the biological bases of temperament or personality, since this is a basic behavioral trait. Rodents expressing high exploratory activity have been shown to consume more drugs of abuse such as nicotine (Redolat et al. 2009), cocaine (Belin et al. 2011) and morphine (Pelloux et al. 2006). Of note, these findings parallel the evidence of high sensation seeking and novelty seeking in drug addicts (Blanchard et al. 2009). High exploration is also a trait observed in individuals with bipolar disorder and has been used to model this disorder in animals (Henry et al. 2010).

In a previous study, our group has characterized two behavioral extremes of mice, called low exploratory (LE) and high exploratory (HE), according to their exploratory behavior of an object placed in the center of an open field task (Kazlauckas et al. 2005). HE mice show less anxiety, more aggressive behavior against intruders, higher avoidance to conditioned punishment (electric foot-shock) and better performance in a maze with positive reinforcement (food) when compared to their LE counterparts. Exposure to chronic mild stress reduced central object exploration in both groups, increasing cortisol and reducing hippocampal BDNF only in HE mice (Kazlauckas et al. 2011a). In contrast, exposure to environmental enrichment increased central object exploration and hippocampal BDNF in both groups, with memory improvement particularly in LE mice (Kazlauckas et al. 2011b). These results suggest that HE and LE mice have distinct affective, cognitive and biological reactions to the environment.

Other studies have investigated the neurobiological substrates of behavioral profiles of HE and LE rodents. HE animals have higher basal and stimulated extracellular dopamine levels in the striatum but not in the nucleus accumbens (Mällo et al. 2007), and a higher proportion of dopamine-D2 receptors in the functional high-affinity state (Alttoa et al. 2009). LE animals also have significantly higher levels of 5-HT transporters in the frontal cortex (FC) and a larger increase in extracellular 5-HT levels after administration of the serotonin reuptake inhibitor citalopram (Mällo et al. 2008). Using microarray analysis in the raphe, hippocampus and FC, Alttoa et al. (2010) found several serotonin, GABA, and glutamate genes differentially expressed in LE- and HE-rats and overrepresentation of genes involved in neuron development, morphogenesis and differentiation. The most enriched pathways involved Wnt signalling, MAPK signalling, long-term potentiation, and long-term depression pathways. In another study with gene expression profiling, Clinton et al.(2009) found robust differences between high and low explorer rats during development particularly in the hippocampus, and especially regarding cell function and maintenance, development and intracellular signaling.

In this study, we evaluated the gene expression profiles in the striatum and frontal cortex of HE and LE mice selected according to central object exploration in the open field. The striatum was chosen due to its role in locomotion and emotional salience, whereas the frontal cortex exerts top-down control of limbic areas. Also, these regions have shown differences in previous studies with LE and HE animals using other selection protocols (Mällo et al. 2007, Mällo et al. 2008, Alttoa et al. 2009, Alttoa et al. 2010).

Materials and methods

Animals

One hundred male albino CF1 mice (2 months old), weighing approximately 35–40 g, were obtained from the State Foundation for Health Science Research (Porto Alegre, Brazil). They were housed in groups of six to eight in standard conditions of temperature and humidity, in a 12 h light/dark cycle (lights on at 7:00 am), with access to food and water ad libitum. All experimental procedures were

performed according to the NIH Guide for Care and Use of Laboratory Animals and Brazilian Society for Neuroscience and Behavior (SBNeC). Recommendations for animal care were followed throughout all the experiments in accordance with the project approved by the ethical committee from Universidade Federal do Rio Grande do Sul.

Behavioral separation of high and low exploratory mice

Mice were selected according to their exploratory behavior in the central area of the open field (OF) with a central object, as previously described (Kazlauckas et al. 2005). Briefly, the animal was placed in an open field (50cm×50cm×50 cm) with an object (a white cylinder of 1.5 cm radius and 5 cm high) placed in the center of the arena to stimulate exploration. Exploratory behavior was video recorded for 5 minutes and the time spent by the animal in and out of an imaginary center square of 30cm×30cm was analyzed with ANYmaze software (Stoelting, Woods Dale) for four animals simultaneously. This behavioral screening took place between 10:00 am and 01:00 pm. From the 100 mice screened in the OF, the bottom and top 10 explorers were selected to compose the low exploratory (LE) and high exploratory (HE) groups, respectively. All mice were maintained in their home cages appropriately identified without changing housemates for one week until sacrificed by decapitation. The difference between LE and HE groups were analyzed with Student's *t*-test.

Gene expression microarray analysis

Tissue dissection: Mice were decapitated; their brains were removed and immediately frozen in Trizol®. The right frontal cortex and striatum were dissected and separated in two eppendorf tubes with Trizol®.

RNA extraction: RNA extraction was performed with Trizol (Life – CA - USA) and purified with the silica-based method entitled RNeasy mini kit (Qiagen - CA) according to the manufacturer's recommendations. RNA quality was evaluated in an analytical 1% agarosis gel followed by spectrophotometry (Nanodrop ND-8000 spectrophotometer, Thermo Scientific) to evaluate the RNA concentrations and the 280/260 ratio.

Microarray experiments: The total RNA from each animal sample was converted to cDNA, biotin terminal labeled and hybridized into the Genechip Mouse Gene 1.0 ST Array (Affymetrix – Santa Clara - CA). The array covers 764,885 probes sets (on average 27 probes per gene) and interrogates 28,869 transcripts along the genome.

Quality control criteria. The arrays were corrected and normalized using RMA Robust Multiarray Averaging and no outliers were observed among the groups.

Data analysis. The Gene Chip Operating Software (Affymetrix) was used to scan the chips, determine cell intensities, and examine sample quality. Data was normalized with RMA (Robust Multi-array Average) (Irizarry et al. 2003) available in the R/Bioconductor software (www.bioconductor.org). Array quality was verified again with Affymetrix Expression Console (http://www.affymetrix.com/browse/level_seven_software_products_only.jsp?productId=131414&categoryId=35623#1_1). All arrays met quality requirements.

The ComBat method (Johnson et al. 2007) available in the R/Bioconductor software was used to remove a batch identified before data processing. The Inter-Quartile-Range (IQR) filter from R/Bioconductor was used to exclude all genes with IQR<0.2, which indicates low variability. The RankProd (Breitling et al. 2004; Hong et al. 2006) method was used to select differentially-expressed-genes (DEGs) with p-value < 0.05 corrected by False Discovery Rate (FDR) (Benjamini and Hochberg, 1995).

Cluster analysis was performed with the CLICK method available in the Expander software (http://acgt.cs.tau.ac.il/expander/index.html) (Sharan et al. 2003; Shamir et al. 2005)

Functional analysis (*in silico*) was performed with Ingenuity Pathway Analysis (IPA – www.ingenuity.com) and DAVID (http://david.abcc.ncifcrf.gov/summary.jsp), but as the results were the same, only IPA data is shown. The genes were organized according to their function using all databanks available. All MAPPs were established before data analysis and were not influenced by the results.

Results

Group selection according to Open Field exploration

Mice were selected in the open field task according to their exploratory behavior. The 10 mice spending less time in the central area of the arena (5.96 ± 1.24%) were denominated LE group and the top 10 explorers (43.35 ± 1.16%, $P < 0.001$ compared to LE mice) formed the HE group (Fig. 1A). Locomotor activity did not differ between LE and HE groups (Fig. 1B).

Gene expression analysis

The number of up and downregulated genes in the striatum and frontal cortex are shown in Table 1. HE mice had more genes downregulated than upregulated in the striatum and the reverse pattern was observed in the frontal cortex. Of the DEGs found in both regions, 25 were changed in the same direction (10 up and 15 downregulated) in HE mice, as listed in Table 2. The identified genes were mostly involved in intracellular processes, such as Rpl7 and Rpl15 of eIF2, but none were of obvious specific relevance to the nervous system.

Cluster analysis

In the frontal cortex, DEGs formed two clusters with higher gene expression in HE mice (Figure 2 A and B) and in the striatum only one cluster was found (Figure 2 C), with higher gene expression in LE mice.

Functional analysis with Ingenuity Pathway Analysis (IPA)

The biological processes most significantly enriched in DEGs are shown in Figure 3. The most robust differences were in nervous system development and function and cell-to-cell signaling and interaction, particularly in the striatum. Other interesting functions were cell function and maintenance, behavior, tissue development, cellular development, and cellular growth and proliferation.

The top canonical pathways involved were Eukaryotic Initiation Factor 2 (eIF2) signaling in both striatum and cortex, and glutamate receptor signaling in the striatum (Figure 4). Except for IL-22 signaling in the cortex, the other significant pathways were all in the striatum. Figure 5 shows the most significant hypothetical network in the striatum. As can be seen, the main genes involved in this network were eIF2 (most upregulated), cytochrome c oxidase subunit 7C (Cox7C/Gm10012, upregulated), glutamate signaling (mGlu2 and SCL17A6/VGLUT2 downregulated), cholecystokinin (CCK), calbindin 2 or calretinin (CALB2), triadin (TRDN), G protein-coupled-receptors (Gpcr) and Receptor activity modifying protein 3 (RAMP3), all downregulated in HE mice.

Most significant DEGs

In HE mice, the DEGs most significantly upregulated in the striatum were Chromobox Protein Homolog 3 (CBX3), Cytochrome c oxidase subunit 7C (Cox7c) and Histone H2b type 2-E (HIST2H2BE) and in the Frontal Cortex were 60S Ribossomal protein L21 (Rpl21), Domain-containing-protein 7 (FRMD7), Secretagogin (SCGN) and Tirosine Hydroxilase. The DEGs most significantly downregulated in the striatum were Pro-melanin-concentrating-hormone (PMCH), SLC17A6 (the vesicular glutamate transporter VGLUT2), Calbindin 2 or calretinin (CALB2) and in the Frontal Cortex were Interferon zeta (IFN-Z), Interleukin-22 (IL-22) and Chymase 2 (Cma2/Mcpt9).

Discussion

There was a reverse pattern of gene expression in the striatum and the frontal cortex in high versus low explorers as seen in the total number of DEGs (Table 1) and the cluster analysis (Figure 2). This can be interpreted as being in line with the regulatory role of the frontal cortex on the basal ganglia (Durston et al. 2011). Overall, there were few DEGs directly associated with synaptic function and many were involved in intracellular processes. However, the most significant functions were related to nervous system development and function and cell-to-cell signaling and interaction, particularly in the striatum. Thus, these results suggest that behavioral

differences in exploratory traits implicate intracellular processes in the striatum in particular, with some interesting elements related to the synapse, such as mGLU2, VGLUT2, cholecystokinin and PMCH.

Regarding intracellular processes, eIF2 is a heterotrimer (α, β, γ) required in the initiation of translation during protein synthesis (Hinnebusch et al. 2011). eIF2 activity is regulated by a mechanism involving both guanine nucleotide exchange and phosphorylation at the α-subunit, which is a target for a number of kinases derived from the pathways activated by stimuli such as amino acid deprivation, certain stresses and infection (Kimball, 1999). The convergence of these signaling networks on a translation initiation factor (eIF2B) plays an important role in oligodendrocyte viability and white matter disease (Carter, 2007). Also, alterations in the expression of the eIFs were associated with memory deficit (Naidoo, 2009). Jiang et al. (2010) suggested that increased eIF2α phosphorylation-dependent translations in CA1 pyramidal cells cause memory consolidation deficits of hippocampal memories without suppressing general translation. Furthermore, gene-specific transcription/translation following a slight increase in eIF2α phosphorylation is sufficient to elicit behavioral consolidation impairments (Jiang et al. 2010). Together, this data shows a highly specific relevance of eIF2 for the brain for such a universal process as translation initiation. Interestingly, HE show better long-term memory performance than LE mice, but not short-term memory (Kazlauckas et al. 2005; Kazlauckas et al. 2011a), which may be the result of differences in protein translation.

Other DEGs related to intracellular processes in the striatum were Histone H2B (HIST2H2BE) and Chromobox protein homolog (CBX3), which were upregulated. Post-translational modifications (PTMs) of histones are crucial for transcriptional control, since they define positive and negative chromatin territories, being an essential mechanism of neuronal plasticity in response to the environment (Keverne and Curley, 2009). Also, Cbx proteins (1, 3 and 5) are important for gene repression in heterochromatin. The chromodomains of Cbx proteins are thought to, at least in part, localize the proteins and their respective complexes to appropriately marked sites of the epigenome via recognition of histone H3 (Kaustov et al. 2011), playing important roles in human development and disease. Thus, H2N and CBX3 take part in epigenetic processes, which have been increasingly implicated in the

modulation of behavior, including in rodents, e.g. according to the pattern of maternal care of the pups (Lester et al. 2011). The other gene that was much upregulated in the striatum of HE mice was the mitochondrial enzyme of the respiratory chain Cytochrome c oxidase subunit 7C (Cox7c), which interestingly is expressed twice as much in the striatum of male mice after acute treatment with the psychostimulant caffeine (Jones et al. 2008)

The Calcium-Binding Proteins (CBPs) are a family of some 240 proteins and include parvalbumin, calbindin and calretinin (CALB2). Each of these CBPs exerts specific effects during different stages of calcium signaling, and is expressed differentially within sub-populations of cells (even between subcellular compartments of the same cell) (Mojumder et al. 2008). Calretinin (CALB2) is heavily expressed in peripheral sensory neurons of the visual, vestibular and auditory systems, and is selectively expressed in granule cells, the most abundant neuron type in the CNS (Cheron et al. 2008). The expression of calretinin as well as other calcium-binding proteins can also be down-regulated in functional systems (e.g. the visual system) following the disruption of neuronal connections. This refinement of localization and expression may be a cellular adaptation that confers spatial control over Ca^{2+} fluxes (Schwaller, 2009). Putative biological functions include a role of calretinin in the modulation of neuronal excitability (Schurmans et al. 1997, Gall et al.2003), long-term potentiation (Gurden et al. 1998) and regulation of calcium pools critical for synaptic plasticity (Schwaller et al.2002). Maternal separation lasting 6 hours increases hippocampal content of calretinin and calbindin, while it reduces their expression in the hypothalamus immediately after the separation procedure, suggesting possible modified feedback mechanisms in the HPA axis function during the stress hyporesponsive period (Lephart and Watson, 1999). Secretagogin (scgn) is another developmentally-regulated, neuron-specific CBP whose phylogenetic preservation and selective association with neurochemically distinct subsets of neurons suggest novel functional dimensions within the extended amygdala circuitry (Rogstam *et al.* 2007, Mulder et al. 2010).

FRMD7 has been shown to regulate the adhesion and morphogenesis of cells by modulating changes in the cytoskeleton (Chishti et al. 1998; Kubo et al. 2002). FRMD7 mRNA is mainly expressed in the cortex plate at the early fetal cortex in humans, and at the brain cortex in mice (Self et al. 2010; Jiali et al. 2011). Mutations

in the gene encoding FRMD7 are an important cause of idiopathic infantile nystagmus and a recent study showed that it plays a role in neurite development (Betts-Henderson et al. 2010).

Among the genes more closely related to the synapse, the glutamatergic system had two genes standing out, namely mGlu2 and VGLUT2. Both were downregulated in the striatum, which is compatible with a higher glutamatergic tone. Metabotropic glutamate receptors of types 2 and 3 have lately gained considerable relevance in the regulation of behavior. mGLU2 are distributed in the cortical and limbic regions (Ohishi et al. 1993) and are located in preterminal portions of neurons acting as autoreceptors and heteroreceptors (Shigemoto et al. 1997). Activation of mGLU2/3 receptors in the nucleus accubens mediates the effects of N-acetylcysteine on reducing cocaine relapse (Kupchik et al.2011) and attenuates methamphetamine-induced hyperlomocotion and increase in prefrontal serotonergic transmission (Ago et al. 2011). Also, reducing mGLU2/3 mediated transmission genetically or pharmacologically has antidepressant effects (Karasawa et al. 2005; Campo et al. 2011; Dwyer et al. 2011). In a similar vein, VGLUT2, which transports glutamate into synaptic vesicles, influences psychomotor and exploratory activity. Deletion of this transporter in the hippocampus, amigdala and the cortex of preadolescent mice leads to increased exploration of the open field central area and reduced anxiety (Wallén-Mackenzie et al. 2009). Also, a reduction of VGLUT2 was associated with enhanced locomotor response to amphetamine and the NMDA receptor antagonist MK-801 (Naert et al. 2011). Interestingly, glutamate is correleased in dopaminergic terminals, particularly in the ventral tegmental area-nucleus accumbens pathway (Stuber et al. 2010). Additionally, the loss of VGLUT2 in dopaminergic neurons is associated with an increase in sucrose and cocaine self-administration, less dopamine release (Alsio et al. 2011) and blunted response to amphetamine-induced locomotion (Birgner et al. 2010).

Cholecystokinin (CCK) is one of the most abundant neuropeptides in the brain, being highly expressed in the hippocampus, amygdala, septum, caudate nucleus, and hypothalamus (Lee and Soltesz, 2011). CCK is involved in several behaviors such as feeding and satiety through CCK1R, and nociception, anxiety, panic attacks, and learning and memory through CCK2R. CCK may mediate its effects through the dopaminergic mesocorticolimbic system, as CCK coexists with dopamine in the

ventral tegmental area projection onto the nucleus accumbens (Crawley, 1994). CCK2R deficient mice have increased dopaminergic tone as shown by D2 receptor affinity and behavioral responses (Koks et al. 2003). Our results showed an upregulation of CCK in low Explorer mice, which agrees with its behaviorally inhibiting effects (Lee and Soltesz, 2011). Accordingly, CCK gene expression was lower in the ventral tegmental area of rats with high locomotor response to a novelty environment when compared to low responding rats (Ballaz et al. 2008). Also, high responding animals to a new environment present higher object exploration, but the difference from low responding rats is abolished by the administration of a CCK2 antagonist (Ballaz et al. 2008). Thus, CCK system is a strong candidate for individual trait differences in exploratory behavior.

Pro-melanin-concentrating hormone (PMCH) is the precursor of the melanin-concentrating hormone (MCH), which is a cyclic neuropeptide confined largely to the lateral hypothalamus and zona incerta area with extensive neuronal projections throughout the brain, including the neurohypophysis (Chung et al. 2011a). The anatomic distribution suggests a neurotransmitter or neuromodulator role for MCH in a broad array of neuronal functions directed toward the regulation of goal-directed behavior, such as food intake, and general arousal. The MCH receptor (MCH1R) is expressed at high levels in the mesocorticolimbic, but not in the nigrostriatal dopaminergic pathway, and MCH potentiates dopamine-related responses, such as cocaine reward (Chung et al. 2009) and prepulse inhibition (PPI) (Chung et al. 2011b). Also, rats more susceptible to PPI deficit have high MCH expression in the hypothalamus and blocking these receptors reverses their PPI deficits (Chung et al. 2011). Also, low exploratory rats have higher MCH mRNA levels and reduced PMCH mRNA levels in the hypothalamus (Garcia-Fuster et al. 2011), which is in line with our finding of PMCH expression in the striatum, which was the most significantly downregulated gene in this region. Thus, exploratory behavioral traits may be modulated indirectly by the MCH system, since the only gene directly involved with the dopamine system was the upregulation of tirosine hydroxilase in the frontal cortex.

Novelty-seeking reflects curiosity, impulsivity, appetitive approach in response to novelty and reward, increased salience attribution and active avoidance of conditioned signals of punishment (Beckmann et al. 2011). High expression of these

traits has been demonstrated in patients with drug abuse and bipolar disorders, even when in periods of euthymia (Lara et al. 2006; Henry et al. 2010). Modeling these traits in mice, our results corroborate previous studies and point out synaptic candidates for these differences, such as the mGLU2 receptor, VGLUT2, CCK and the MCH system. These may be important targets for pharmacological intervention of trait related disorders, such as drug addiction, mood and personality disorders. Moreover, other molecules involved in intracellular processes and epigenetic phenomena (translation and post-translational), such as eIF2, H2N and CBX3, should be further studied.

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Table 1. Differentially expressed transcripts in the striatum and frontal cortex of HE mice in relation to LE mice (p<0.05 corrected by FDR).

	Striatum	Frontal cortex
Upregulated	39	54
Downregulated	79	32
Total number	118	86

Table 2. Differentially expressed transcripts expressed in both striatum and frontal cortex in the same direction (HE mice in relation to LE mice).

Probe set ID	Gene Symbol	Gene Title	Direction
10418193	Plac9	placenta specific 9	Down
10559818	-	-	Down
10559883	Vmn2r42	vomeronasal 2, receptor 42	Down
10563949	-	-	Down
10563959	-	-	Down
10568731	-	-	Down
10588026	Rpl7a	ribosomal protein L7A	Down
10598220	Gm2799	predicted gene 2799	Down
10602307	Ott	ovary testis transcribed	Down
10608260	Srsy	serine-rich, secreted, Y-linked	Down
10362428	Trdn	triadin	Up
10373702	Pisd-ps1	phosphatidylserine decarboxylase, pseudogene 1	Up
10399588	Zfp125	zinc finger protein 125	Up
10403978	Hist1h2bk	histone cluster 1, H2bk	Up
10417415	Gm1973	predicted gene 1973	Up
10428576	Rpl15	ribosomal protein L15	Up
10526718	Smok3a	sperm motility kinase 3A	Up
10536147	E330014E10Rik	RIKEN cDNA E330014E10 gene	Up
10563901	-	-	Up
10563925	-	-	Up
10582896	-	-	Up
10582916	-	-	Up
10599064	Gm10058	predicted gene 10058	Up
10602341	Ott	ovary testis transcribed	Up
10608368	LOC100041256	hypothetical protein LOC100041256	Up

Figure 1

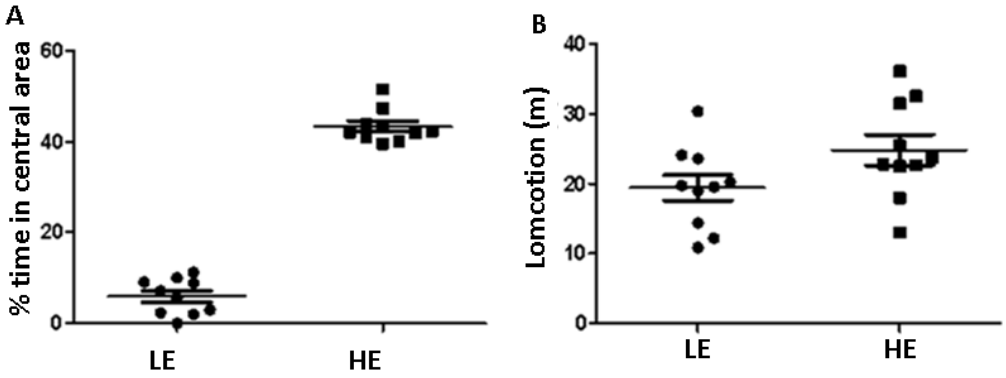

Figure 1. Selection of low (LE) and high (HE) exploratory mice based on open field behavior. Mice (n = 100) were subjected to the open field task with a central object, and the time spent in the central area together with locomotor activity was recorded for 5 minutes. LE (n = 10) and HE (n = 10) mice were evaluated for (A) time spent in the central area and (B) locomotion (cm). Results are presented as dot plot with mean ± S.E.M. Statistical analysis was performed using Student's t test. ***P < 0.001.

Figure 2

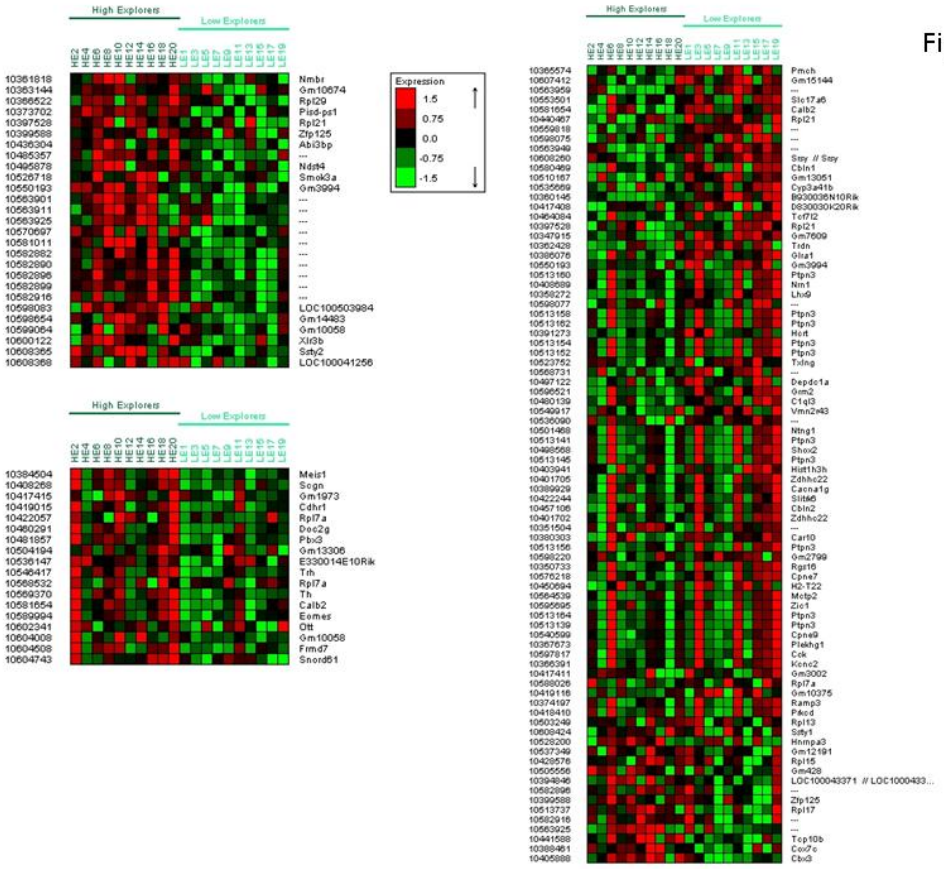

Figure 2. Gene expression profiles of brain samples. Hierarchical cluster analysis (HCA) of gene expression data from the frontal cortex (2 clusters - A and B) and striatum (C) of the high vs. low explorers using $p<0.05$.

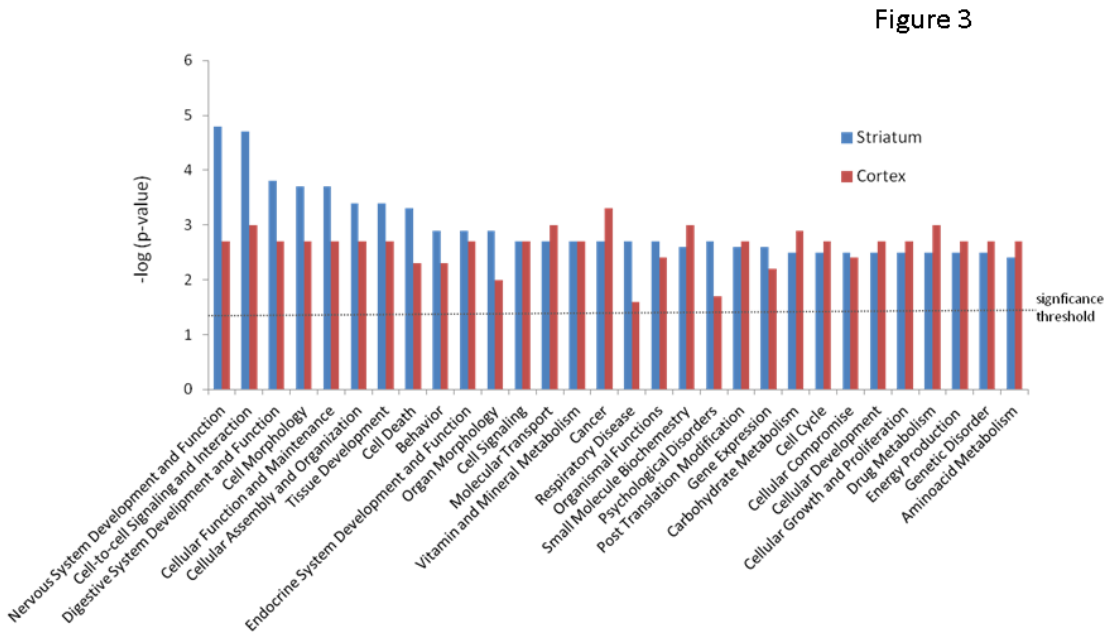

Figure 3. Significantly enriched Ingenuity Pathways Analysis (IPA) biological process terms in the lists of differentially expressed genes in the striatum and frontal cortex of the high vs. low explorers. The dotted line shows significance level of -log (p-value) 1.3 or $p<0.05$.

Figure 4

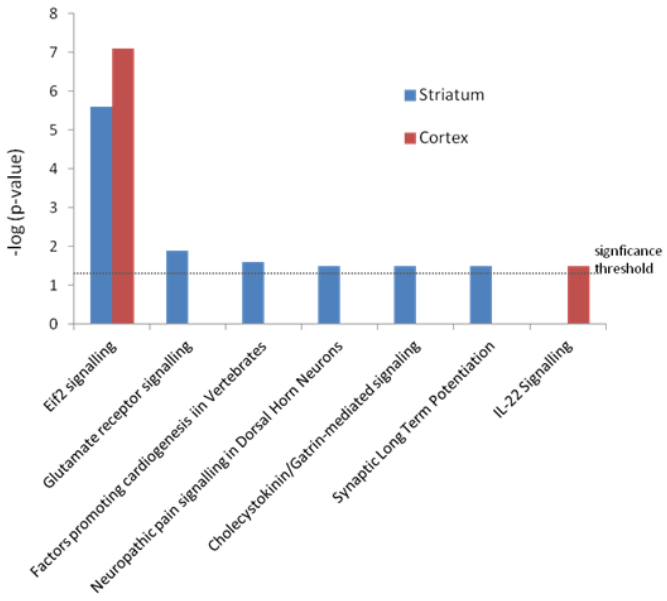

Figure 4. Significantly enriched Ingenuity Pathways Analysis (IPA) pathways in the lists of differentially expressed genes in the striatum and frontal cortex of high vs. low explorers. The dotted line shows significance level of -log (p-value) 1.3 or $p<0.05$.

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Figure 5

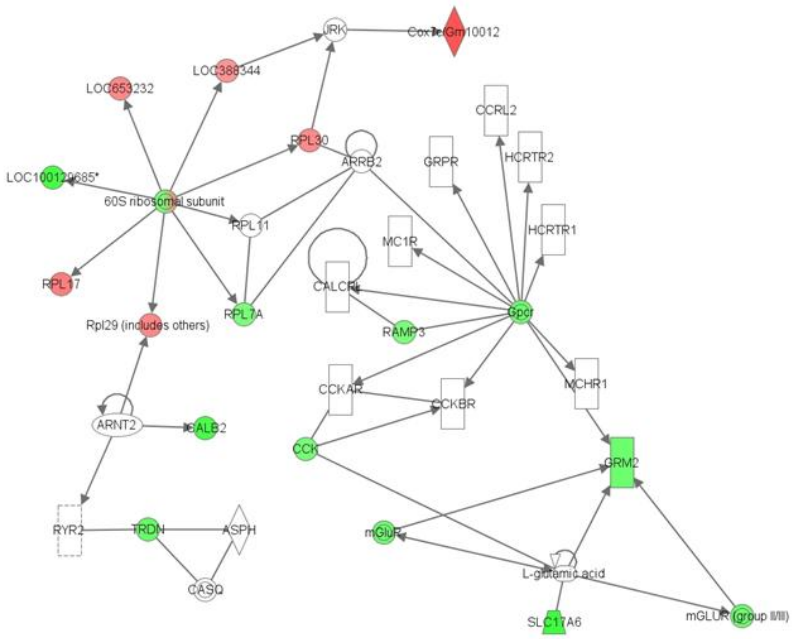

Figure 5. Ingenuity Pathways Analysis (IPA) showing differentially expressed genes mapped to pathway for Cell-To-Cell Signaling and Interaction, Nervous System Development and Function and Behavior. Each gene mapped to this pathway (marked in red or green) showed significantly altered expression in this pathway (p-value<0.05). Red=upregulated, green=downregulated

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Emotional Traits, Affective Temperaments and Mood Symptoms in Subjects with Hyperuricemia

Running head: Temperament and Mood in Hyperuricemia

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Abstract

Objective: Some externalized emotional traits and mood symptoms have been associated with uricemia. We aimed to evaluate temperament traits and mood symptoms (mania and depression) in subjects with self-reported hyperuricemia compared to controls in a large sample.

Methods: We analyzed temperament and mood symptoms in 7,155 males (5.1% hyperuricemic) and 22,225 women (1.8% hyperuricemic) from an Internet study in Brazil. Beer intake and age were included as covariates in the multivariate analyses.

Results: Hyperuricemic subjects scored higher in anger and lower in inhibition and control, but hyperuricemic women also showed a higher emotional sensitivity and a lower degree of volition and coping. Among hyperuricemic males, there were fewer apathetic and obsessive subjects and more dysphoric types, whereas in females, there were fewer anxious and more euphoric types. Both hyperuricemic males and females reported higher manic and depressive symptoms.

Conclusion: subjects with hyperuricemia present more mood symptoms and more externalizing emotional traits and affective temperaments.

Keywords: temperament; uric acid; gout; mania; depression; mood

1. Introduction

Uric acid is the end product of purine catabolism in humans and is produced by the enzyme xanthine oxidoreductase from xanthine[1]. High uric acid levels may result from increased purinergic turnover and/or reduced uric acid excretion and can cause gout, urolithiasis and nephropathy due to the deposit of urate crystals. Hyperuricemia has also been linked with hypertension, metabolic syndrome and cardiovascular disease, with a probable causal relationship of uric acid in at least some populations, such as young adults [2,3]. Uric acid is also considered a marker of oxidative stress, since reactive-oxygen species (ROS) are produced by xanthine oxidase [4]. Also, uric acid is a “danger” signaling molecule released from dying cells leading to an inflammatory response [5].

On the other hand, uric acid is a known scavenger of peroxynitrite [6] and accounts for up to 60% of the free radical scavenging activity in human blood [7]. Moreover, uric acid is able to suppress the inflammatory cascade, decrease blood–brain barrier permeability, and diminish central nervous tissue damage and neuronal death [8]. Another line of evidence shows that a higher prevalence and progression of some neurological diseases, such as Parkinson’s and Alzheimer’s disease, have been associated with low uric acid [9,10], and it is possible that they may predispose some other disorders, mainly due to the decrement of its antioxidant activity[2]. Thus, both beneficial and detrimental roles for uric acid have been postulated.

Individual differences in hyperuricemia are substantially influenced by genetic factors, but the phenotype of gout is mostly determined by environmental factors [11], such as the consumption of alcoholic beverages, meat, and fructose-rich industrialized food. However, a purine-rich diet would be responsible for an increment of only 1 to 2 mg/dL of uric acid [13]. Comparing the diet of gouty patients with

controls, the average daily intake of most purine-containing foods was similar, except for a significantly higher consumption of alcohol, mostly from beer[14]. Beer intake has been shown to be an independent factor for uric acid increments [15] due to both its alcoholic and purine content [15], and it has a stronger power to increase uric acid than liqueur and wine intakes [16,17].

In the 19th-century, the concept of “uric acid diathesis” suggested that mental disorders were the result of an imbalance of uric acid, and lithium was used to reduced uricemia [18]. Actually, the proposal that the purinergic system might be involved in bipolar disorders dates back to Kraepelin, who was the first to describe an association between manic symptoms, uric acid excretion, hyperuricemia, and gout [19]. Recent studies have strengthened this hypothesis. De Berardis et al. (2008) [20] showed that plasma uric acid levels were higher only during the manic phase, but not during the depressive or euthymic phases, whereas Salvadore et al. (2010) [21] confirmed that uric acid is increased during the first manic episode in drug naïve patients. Moreover, two randomized, placebo-controlled trials showed that the uric acid lowering drug allopurinol, a xanthine oxidase inhibitor, was effective in treating acute mania, when used adjunctively with lithium [22,23].

Temperament relates to the emotional nature and the quality of the prevailing mood, being mostly inherited and relatively stable over time [24,25]. Two influential temperament constructs in psychiatry are the psychobiological model by Cloninger [25], with a focus on behavior and basic emotions, and the model of affective temperaments by Akiskal [26], based on Kraepelin’s fundamental states [19]. Recently, we have proposed an integration of emotional traits and affective temperaments [27]. This model is based on the principle that activation (volition and anger) and inhibition (fear/caution) are the two main emotional forces, which are

integrated by the control system (attention and duty). Other regulatory dimensions are emotional sensitivity and coping. Their interaction would result in the prevailing mood or affective temperaments, which are divided into four major groups: internalized (depressive, anxious, apathetic), unstable (cyclothymic, dysphoric, volatile), stable (obsessive, euthymic, hyperthymic) and externalized (irritable, disinhibited and euphoric) [27].

Lorenzi et al. (2010) [28] recently reported an association of serum uric acid levels with the emotional traits of disinhibition and drive, as well as with hyperthymic and irritable affective temperaments. In the present study, our objective was to evaluate how hyperuricemia and gout were associated with temperament traits and mood symptoms (mania and depression). To this end, we analyzed the respective data from the Brazilian Internet Study on Temperament and Psychopathology (BRAINSTEP)[29] comparing the profile of individuals who reported having hyperuricemia or gout with controls (i.e. those who denied having hyperuricemia/gout).

2. Method

2.1 Participants

All participants gave their electronic informed consent before completing the scale. This form was elaborated to fulfill the requirements of the National Health Council of Brazil (Resolution 196/1996) and the Code of Ethics of the World Medical Association (Declaration of Helsinki). Their participation was voluntary and they could cancel their participation at any moment without justification. The study was approved by the Institutional Review Board of Hospital São Lucas from Pontifícia Universidade Católica do Rio Grande do Sul.

The data was collected by the BRAINSTEP project, a large web-based survey in Brazil (Lara et al., 2012b). Volunteers answered the Affective and Emotional Composite Temperament Scale (AFECTS) by Internet (www.temperamento.com.br), together with questions on having a diagnosis of gout or excessive uric acid (yes or no), as well as demographic variables, among various other scales and questionnaires. Since beer consumption is an important environmental factor associated with hyperuricemia, the data on beer consumption (transformed to alcohol units) within the last week was included.

To ensure the reliability of the data, questions checking attention were inserted within the instruments and throughout the system. Also, at the end of the system, there were two specific questions relating to the degree of attention and sincerity of the volunteer while completing the instruments. Only those who stated being attentive and sincere throughout the study and had correct answers in all attention validity checks were included. The initial sample was 46,685 volunteers, but only 29,380 passed all these validity checks.

Table 1 shows the demographic characteristics of the final sample, which was composed of 75.6% females (n=22,225). Proportionally, there were more hyperuricemic males (5.1%) compared to females (1.8%), as expected. Also, hyperuricemic subjects were older and had consumed more beer units within the last 7 days, so these variables were included as covariates in the analyses.

2.2 Instruments

Affective and Emotional Composite Temperament Scale (AFECTS)

The AFECTS (see [27] for the complete scale) consists of the following sections:

1) Emotional section: 52 seven-item multiple choice questions for the emotional dimensions of Volition, Anger, Inhibition, Sensitivity, Coping, and Control (8 items each). The questions are scored from 1 to 7 and the total score of each dimension is the sum of the scores of their respective questions. Each emotional dimension is composed by two facets of four questions as follows: Volition (positivity and energy), Anger (intensity and irritability), Inhibition (fear and caution), Emotional Sensitivity (interpersonal and to events), Coping (facing and solving), and Control (focus and order);

2) Affective section: from twelve short descriptions of the affective temperaments (depressive, anxious, apathetic, cyclothymic, dysphoric, volatile, obsessive, euthymic, hyperthymic, irritable, disinhibited, and euphoric) the subject was asked to select which of these profiles was the most suitable to represent his/her temperament. This allowed for a categorical evaluation of affective temperament.

Adult Self-Report Inventory (ASRI)

For the assessment of past depressive and manic symptoms, the ASRI[30] was used, since it also provides a quantitative evaluation (scores from 0 – never to 3 – very often to each item).

2.3 Statistical Analysis

Age differences between groups were analyzed with a t-test and the proportion of males and females in relation to the presence of hyperuricemia/gout was analyzed with a chi-square test. The mean scores of AFECTS emotional dimensions and mood symptoms for the control and hyperuricemia/gout groups were analyzed in males and females separately with multivariate analysis of covariance

(MANCOVA) and Bonferroni confidence interval adjustment, considering age and beer consumption as covariates. Differences in frequencies of affective temperaments according to the presence of gout/hyperuricemia were analyzed with a chi-square test for males and females separately. SPSS 18.0 software was used for all analyses, and a statistical significance was considered if $p<0.05$.

3. Results

The emotional profile of males showed that hyperuricemic subjects scored higher in anger traits ($F=9.054$, $p=0.003$), lower in inhibition ($F=4.071$, $p=0.04$) and lower at trend level for control ($F=3.815$, $p=0.051$) (Figure 1A). In contrast, hyperuricemic women had distinct profiles compared to controls in all dimensions: higher anger ($F= 6.394$, $p=0.011$) and emotional sensitivity ($F=9.185$, $p=0.002$), and lower volition ($F=7.895$, $p=0.005$), inhibition ($F=5.275$, $p=0.022$), control ($F=6.955$, $p=0.008$), and coping ($F=9.578$, $p=0.002$) (Figure 1B).

The analysis of affective types showed a lower frequency of apathetic and obsessive subjects and a higher frequency of dysphoric individuals in hyperuricemic males (Chi-square=26.052, $p=0.006$), whereas in females there were fewer anxious and more euphoric types (Chi-square=18.205, $p= 0.07$) (Figure 2).

As shown in Table 2, regarding mood symptoms, hyperuricemic males showed higher scores of mania ($F=16.535$, $p<0.001$) and depression ($F=5.405$, $p<0.05$) in the ASRI scale. Hyperuricemic females also showed more symptoms of mania ($F=44.554$, $p<0.001$) and depression ($F=38.624$, $p<0.001$), with more pronounced differences from controls compared to males. As expected, females reported more pronounced depressive symptoms than males (Table 2).

4. Discussion

This large Internet sample has an overrepresentation of young adults, who are less likely to have had an episode of gout, but it is precisely in the young that a causal role of uric acid in clinical diseases has been more clearly established [3]. This demographic profile also minimizes the proportion of subjects with hyperuricemia as part of metabolic syndrome, particularly in women, who show an increment in uric acid levels with age, particularly over 50 years of age [31]. Hyperuricemia usually precedes gout by many years and can be detected in routine lab examinations [12], allowing its identification even when asymptomatic. As expected, there were more hyperuricemic males (5.1%) compared to females (1.8%). Because is similar to the recently reported prevalence of gout, presently affecting 5.9% of males and 2.0% females in the US[32], and the male to female ratio of 2.4 in Italy, in patients who had their first gout attack between 2002-2009 [33]. Thus, the anticipated results regarding the relative prevalence of hyperuricemia and depressive symptoms in males and females, respectively, also point towards an adequate representation of the population and the quality of the data. Also, the finding that patients with gout drink more beer [34] was replicated in our sample of hyperuricemic subjects.

Regarding emotional traits, in both genders, hyperuricemia was associated with more externalizing traits, particularly anger, disinhibition and low control (the latter at trend level in males). However, compared to controls, hyperuricemic women also had lower volition and coping, and a higher emotional sensitivity. Lorenzi et al. (2010) [28], using the preliminary version of the AFECTS (called Combined Emotional and Affective Temperament Scale - CEATS), found that uric acid levels were correlated with disinhibition (particularly in women) and drive/volition (more in men). However, that study evaluated the association of these traits with the whole

range of serum uric acid levels. The present results suggest that, in the case of more extreme hyperuricemic women, other emotional dimensions also differ. Previous studies in males reported that serum uric acid levels were correlated with motivation, drive and leadership [35-37]. Although these are also more externalized traits, the present data indicates that those at the extreme high end of the uric acid range, have higher anger and disinhibition. Finally, although hyperuricemia is more prevalent in males, its associations with dysfunctional emotional traits were more robust in women. Accordingly, gout and hyperuricemia tend to have stronger associations with clinical comorbidities among women than among men [38-4].

The affective temperament profile also showed that males were less often self-ascribed as obsessive and apathetic, with a higher representation of dysphorics. Lorenzi et al. (2010) [28] found fewer euthymics and 20% were dysphorics among hyperuricemic male subjects, but the sample was small (n=44). Of note, the CEATS also did not have the obsessive temperament, which is part of the stable group, along with the euthymic temperament. Thus, there is partial agreement between these studies, pointing towards a shift from a more stable to a more dysphoric profile among hyperuricemic males. Regarding women, we found fewer anxious and more euphoric types. In Lorenzi et al. (2010) [28], when the top tertile female subjects were compared to the other two tertiles, hyperthymics and irritables were overrepresented, and there were no subjects with an anxious temperament among the hyperuricemic subjects. However, that version of the scale did not have the euphoric temperament, which is a more irritable and impulsive version of the hyperthymic temperament. Thus, the data of both studies is mostly in agreement and points to a shift from a more internalized (anxious, avoidant types) to a more externalized profile in women (euphoric, irritable types).

Using a quantitative self-report scale for the screening of depression and mania (ASRI), we observed a history of higher manic and depressive symptoms in hyperuricemic individuals, which is compatible with the temperament results. Accordingly, uric acid levels are higher in acute mania and directly correlate with manic symptom scores [20]. Salvadore et al. (2010) [21] also found increased serum uric acid levels in first episode and drug-naïve patients (80% females), although the correlation with symptoms was not replicated. Uric acid excretion also increased during the remission of manic episodes [42] and Mueller et al. (1970) [43] observed that those with marked mood lability also showed more daily variations of serum uric acid levels [44]. Conversely, serum uric acid levels of patients with major depression were significantly lower than those in a healthy control group, but also compared to patients with delirium, dementia, amnesia and other cognitive disorders, substances related disorders, schizophrenia, schizoaffective disorder and bipolar disorder [45]. Interestingly, those with anxiety disorders had lower uric acid levels [45]. Overall, these results indicate that higher uric acid levels are associated with externalizing disorders (e.g. mania) and traits (e.g. anger, drive, disinhibition) and lower levels are related to internalizing disorders (e.g. major depression and anxiety disorders). However, those with hyperuricemia may also experience more often from a general mood dysregulation, as shown by their increased depressive symptoms, as well as destabilizing emotional traits (low control and coping, high emotional sensitivity, especially in females). Along with clinical data [2,3], these results also raise the hypothesis that hyperuricemia may contribute to the increased morbid-mortality in patients with bipolar disorders [46].

Uric acid may have psychostimulant properties per se, similar to those of other xanthines, such as caffeine and theophylline, since the inhibition of uricase in rats

leads to increased uric acid levels and locomotion in rats [47]. Alternatively, uric acid may just reflect the activity of the purinergic system, or may be an index of metabolic activation, as the end-product of ATP catabolism. Behaviorally, the purinergic system, mainly through the effects of ATP and adenosine, modulates sleep, motor activity, cognition, attention, aggressive behavior and mood [48]. Importantly, the xanthine inhibitor allopurinol can exert antimanic [22,23,49], antiaggressive [50,51] and antipsychotic effects [22,52], suggesting a therapeutic role for the manipulation of the purinergic system.

This study has limitations to be considered. First, gout and hyperuricemia were assessed by self-reporting and uric acid levels were not determined. Thus, the comparison includes only those at the higher extreme of uric acid levels at some time point in their lives, compared to a mostly normouricemic population. Second, the majority of individuals with hyperuricemia should be hyperuricemic indeed (based on gout or a lab exam), but a small proportion of unrecognized hyperuricemic subjects may exist in the control group, given its normal distribution in the population. For reference, a recent population-based study in Brazil showed that 16% of males and 10% of females between 25 and 64 years of age had hyperuricemia (>6.8 mg/dL for men and >5.4 mg/dL for women) [53]. Third, the control group was not selected to represent a healthy population, which could lead to different results. Fourth, the sample consisted mostly of younger subjects (for age distribution of the sample)[27]. Lastly, beer intake within the last week was included as a covariate, but not other possible factors influencing uric acid levels, such as meat consumption, although this information did not affect the results of Lorenzi et al. (2010)[28] and intake of these food items was not clearly different in gouty patients [34]. In contrast, the major strength of our method was to be able to evaluate a large sample from the general

population, with a sufficient socioeconomic and educational level, to spontaneously and correctly complete the survey [27].

In conclusion, hyperuricemic subjects present more externalizing temperaments and mood symptoms both in males and females. The role of uric acid as a cause, consequence or just an indirect correlate of these traits, remains to be established, although the therapeutic effect of allopurinol in mania suggests at least a contributing role of purines in some patients. Further studies should investigate if hyperuricemia in subjects with externalizing traits derives from the higher production or lower excretion of uric acid.

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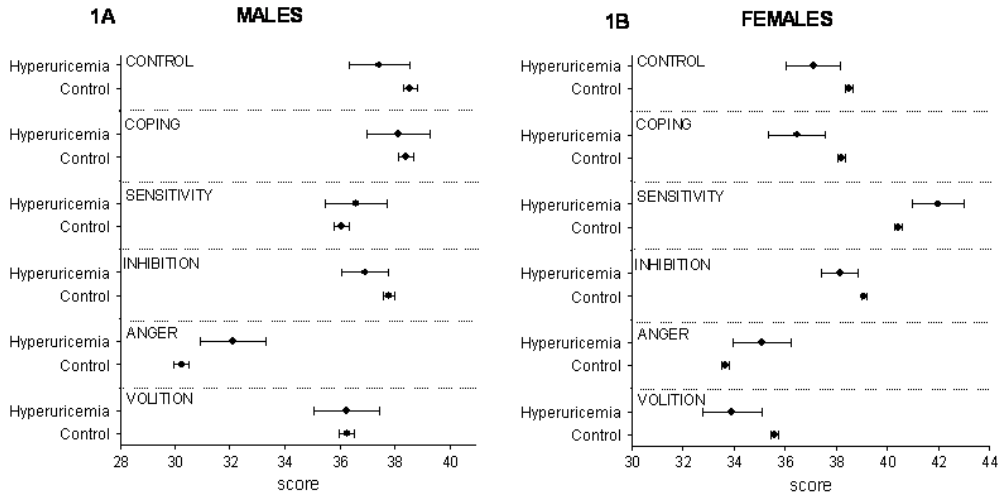

Figure 1. Emotional trait scores in hyperuricemic subjects and controls. Data is shown as mean ± 95% confidence interval in males (A) and females (B).

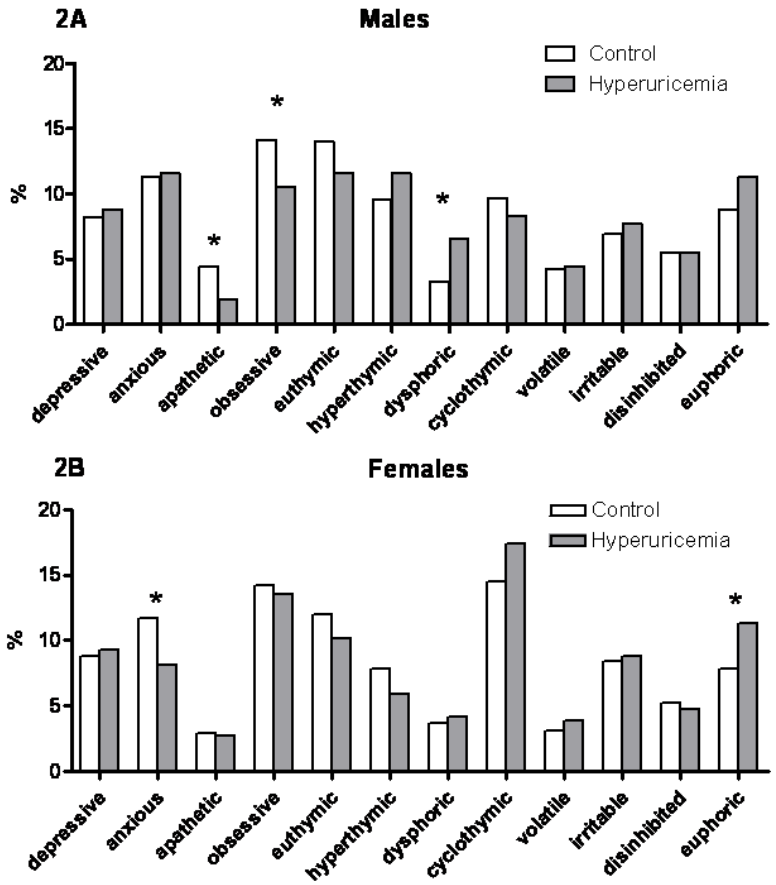

Figure 2. Distribution of affective temperament types in hyperuricemic subjects and controls. Data is shown as a percentage and * denotes a statistical difference ($p<0.05$, chi-square test).

Table 1 – Description of hyperuricemic subjects and controls (n=29,380).

	MALES		FEMALES	
	Control	**Hyperuricemia**	**Control**	**Hyperuricemia**
N (%)	6,793 (94.9)	362 (5.1)	21,818 (98.2)	407 (1.8)*
Mean age ± SD	32.1 ± 10.6	39.3 ± 11.4#	31.7 ± 10.4	33.6 ± 11.6#
Beer intake (units last 7 days)	5.0 ± 9.6	6.2 ± 10.4#	2.4 ± 3.5	3.8 ± 9.1#

* = lower proportion of hyperuricemic women compared to men ($p<0.05$)

= significantly higher compared to controls ($p<0.05$)

Table 2 – History of manic and depressive symptoms in hyperuricemic subjects and controls.

SYMPTOMS	**MALES**		**FEMALES**	
(ASRI scale)	**Control**	**Hyperurice mia**	**Control**	**Hyperurice mia**
Manic	16.1	17.1	16.4	17.9
mean (95%CI)	(16.0-16.2)	(16.6-17.5)	(16.3-16.5)	(17.5-18.3)
Depressive	23.2	24.0	25.2	27.3
mean (95%CI)	(23.0-23.3)	(23.4-24.6)	(25.1-25.3)	(26.6-27.9)

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Research report

Bullying victimization is associated with dysfunctional emotional traits and affective temperaments

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ABSTRACT

Background: Being bullied has been increasingly recognized as a risk factor for the development of psychiatric disorders, but there is very limited evidence on the association of bullying with temperament.
Methods: The data was collected in a large web-survey on psychological and psychiatric measures (BRAIN-STEP). Bullying was assessed with a question on time exposed to bullying (none, <1 year, 1–3 years and >3 years) during childhood and adolescence. Emotional traits and affective temperaments were evaluated with the Affective and Emotional Composite Temperament Scale (AFECTS). The final sample consisted of 50,882 subjects (mean age 30.8 ± 10.4 years, 73.4% females) with valid answers.
Results: About half of the sample reported exposure to bullying and ~10% reported being victimized by peers for longer than 3 years. Longer exposure to bullying was associated with lower Volition, Coping and Control scores, and more Emotional Sensitivity, Anger and Fear, with statistical significance between all groups. To a lower degree, exposure to bullying was associated with lower Caution and higher Desire scores. Bullying victimization was also associated with a much lower proportion of euthymic and hyperthymic types in both genders, which was compensated by an increase mainly in the proportion of depressive, cyclothymic and volatile types.
Limitations: Retrospective assessment of bullying with a single question on time exposed to bullying and use of self-report instruments only.
Conclusions: Being bullied was associated with a broad and profound impact on emotional and cognitive domains in all dimensions of emotional traits, and with internalized and unstable affective temperaments.

1. Introduction

Bullying refers to persistent and repetitive verbal and physical actions deliberately performed by one or more children towards another child (Arseneault et al., 2010). Although bullying, harassment, and victimization can take many forms (e.g., threatening, spreading rumours, pushing), the core elements of this behavior are repetition, aggression, and a relationship with an imbalance of power in which victims perceive themselves as unable to retaliate (Olweus, 1994; Smith et al., 1999).

Being a victim of bullying has been associated with longlasting effects and severe symptoms of mental health problems, including depression, anxiety, suicidal ideation, self-harm, binge eating disorder, violent behavior and psychotic symptoms (Salmon et al., 1998; Kaltiala-Heino et al., 1999b; Kaltiala-Heino et al., 2000; Striegel-Moore et al., 2002; Dake et al., 2003; Arseneault et al., 2010). They also more often complain from sleep disturbances, enuresis, abdominal pain and headaches than children who are not bullied (Williams et al., 1996; Rigby, 1999). A history of being bullied has also been associated with symptoms of borderline personality disorder (Sansone et al., 2012; Wolke et al., 2010). Despite these findings, to our knowledge there is little evidence on the association of being bullied during childhood and adolescence with temperament and personality traits in adulthood.

We have recently developed an integrative temperament model combining specific emotional traits and global affective temperaments, named the Affective and Emotional Composite Temperament (AFECT) model (Lara et al., 2012a), which is the evolution of the previous "fear and anger" model (Lara and Akiskal, 2006; Lara et al., 2006). The emotional traits are represented by Volition (positive affect, motivation, energy), Desire (impulses, indulgence), Anger (emotional intensity and aggressive behavior), Fear (worry, shyness, fearfulness), Caution (prudence, carefulness, risk-avoidance), Emotional Sensitivity (to interpersonal attrition such criticism, rejection and to events), Coping (maturity to face and solve problematic situations) and Control (attention, focus, sense of duty, discipline, planning). These are independent but interactive emotional traits

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which produce the general affective temperament, which can be classified into twelve types: depressive, anxious, apathetic (internalized types), cyclothymic, dysphoric, volatile (unstable types), obsessive, euthymic, hyperthymic (stable types), irritable, disinhibited, and euphoric (externalized types) (for further details see (Lara et al., 2012a). Such concepts of affective temperaments derived from the seminal work on underlying traits related to mood disorders proposed by Akiskal et al. (2005), but extended to represent other possible variations. To study our model, we have recently developed and validated a brief self-reporting scale for the simultaneous assessment of these emotional and affective temperaments, the Affective and Emotional Composite Temperament Scale (AFECTS) (Lara et al., 2012a).

We have designed a large web-survey to collect data on psychological and psychiatric measures called the Brazilian Internet Study on Temperament and Psychopathology (BRAINSTEP) (Lara et al., 2012b). This web research system contains several scales, questionnaires and behavioral measures, starting with demographic characteristics and the AFECTS. For the present study, our aim was to evaluate how emotional traits and affective temperaments, evaluated with the AFECTS, were associated with being exposed to bullying during childhood and adolescence in a large sample from the general population.

2. Methods

2.1. Participants

All participants gave their electronic informed consent before completing the scale. This form was elaborated to fulfill the requirements of the National Health Council of Brazil (Resolution 196/1996) and the Code of Ethics of the World Medical Association (Declaration of Helsinki). Their participation was voluntary and they could cancel their participation at any moment without justification. The study was approved by the Institutional Review Board of Hospital São Lucas from Pontifícia Universidade Católica do Rio Grande do Sul.

The data presented is part of a large web-based survey BRAINSTEP. Volunteers answered by Internet (www.temperamento.com.br) the AFECTS, and questions on demographic variables, among various other scales and questionnaires. To ensure the reliability of the data, questions checking for attention were inserted within the instruments and throughout the system. Also, at the end of the system, there were two specific questions on the degree of attention and sincerity of the volunteer while completing the instruments. Only those who stated being attentive and sincere throughout the study and had correct answers in the attention validity items were included. The initial sample was 63,345 volunteers who completed all the measures, but only 50,882 passed all of these validity checks.

2.2. Instruments

2.2.1. Affective and Emotional Composite Temperament Scale (AFECTS)

The AFECTS (see Lara et al., 2012a, for the complete scale) consists of the following sections:

(1) Emotional section: 52 seven-item multiple choice questions for the emotional traits of Volition, Anger, Emotional Sensitivity, Coping, and Control (8 items each) and Fear, Caution and Desire (4 items). The questions are scored from 1 to 7 and the total score of each dimension is the sum of the scores of their respective questions. Except for the Desire dimension, each emotional dimension is composed by two facets of four questions as follows: Volition (positivity and energy), Anger (intensity and irritability), Inhibition (fear and caution), Sensitivity (interpersonal and to events), Coping (facing and solving), and Control (focus and order).

(2) Affective Section: short descriptions of the twelve affective temperaments (depressive, anxious, apathetic, cyclothymic, dysphoric, volatile, obsessive, euthymic, hyperthymic, irritable, disinhibited, and euphoric) are presented with a 5-item Likert scale, from 'nothing like me' (rated as 1) to 'exactly like me' (rated as 5). This is the quantitative assessment of affective temperament. After these twelve descriptions, the subject has to select which of these profiles is the most suitable to represent his/her temperament. This allows for a categorical evaluation of affective temperament.

2.2.2. Assessment of being a victim of bullying

Given the lack of very short scales for assessment of bullying, we included the following question in our assessment: "During your childhood or adolescence, have you been a victim of intentional harmful acts (insults, humiliation, isolation, discrimination or physical aggression) in a repeated fashion by peers, with no obvious reason?" The following four possible answers were offered: no; yes, for less than 1 year; yes, between 1 and 3 years; and yes, for more than 3 years.

2.3. Statistical analysis

Age differences between bullying groups were analyzed with ANOVA. The proportion of males and females in relation to being exposed to bullying was analyzed with a chi-square test. Since preliminary analysis of emotional dimensions showed overall similar results for males and females, the mean scores of AFECTS emotional dimensions were analyzed with a multivariate analysis of variance with Bonferroni confidence interval adjustment, considering age and gender as covariates. Differences in proportions of affective temperaments according to involvement in bullying were analyzed with the chi-square test for males and females separately. SPSS 18.0 software was used for all analyses, and a statistical significance was considered if $p < 0.05$.

3. Results

The final sample consisted of 13,520 males (26.6%, mean age$=30.8 \pm 10.8$ years) and 37,362 females (73.4%, mean age$=30.8 \pm 10.1$ years). The distribution according to bullying groups and gender is shown in Table 1. There were significantly more females with no bullying than males and significantly more males in all bullying groups. Age was significantly higher in the no bullying group when compared to all bullying groups ($F=160.162$, $p < 0.05$, ANOVA) and significantly higher in bullying $1 < 3$ years when compared to another's bullying groups.

Regarding emotional traits, the most robust differences were found for the dimensions of Emotional Sensitivity ($F=715.9$, $p < 0.001$), Volition ($F=587.4$, $p < 0.001$), Coping ($F=423.5$, $p < 0.001$), Control ($F=381.9$, $p < 0.001$) and Anger ($F=181.3$, $p < 0.001$), with smaller differences for Desire ($F=65.2$, $p < 0.001$), Caution ($F=56.9$, $p < 0.001$) and Fear ($F=107.4$, $p < 0.001$).

As shown in Fig. 1, the longer the exposure to bullying the lower the Volition, Coping and Control scores, with statistical significance between all groups. Also, longer exposure to bullying was associated with more Emotional Sensitivity, Anger and Fear, also with statistical significance between all groups. To a lower degree, more exposure to bullying was associated with lower Caution and higher Desire scores.

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Analyzing individual items of the emotional section of AFECTS, the most significant differences ($p < 0.001$ for all) were found for the items referring to low/high self-esteem (Volition, $F=915.6$), sadness/joy (Volition, $F=715.7$), low/high difficulty to overcome traumas (Emotional Sensitivity, $F=600.2$), low/high difficulty to handle conflicts with people (Coping, $F=482.2$), and low/high rejection sensitivity (Emotional Sensitivity, $F=438.5$).

Regarding affective temperaments (Fig. 2), increased exposure to bullying was associated with a lower proportion of euthymic and hyperthymic types in both genders. The frequency of these temperament types in the group with ≥ 3 years of bullying was around 30–45% of the no bullying group. This decrement was compensated by an increase mainly in the proportion of depressive, cyclothymic and volatile types. In females, the frequency of apathetic types was also higher in subjects exposed to ≥ 1 year of bullying. The proportion of other temperament types (anxious, dysphoric, obsessive, irritable, disinhibited and euphoric) was mostly unrelated to the degree of exposure to bullying.

4. Discussion

The prevalence of being bullied in childhood and adolescence was around ~54% in males and ~44% in females in our sample, being more long-lasting in males. Similarly, in a UK sample of subjects 18–24 years from the general population, ~60% of males and ~39% of females retrospectively reported being a victim of bullying (Radford et al., 2011). Also a large national survey conducted in the US youth reported that 53% of males and 37% of females are exposed to bullying during their current school term (Nansel et al., 2001), and the most pronounced gender differences were in those exposed regularly (26% in males and 14% in females). In a large study conducted in Brazilian schools, the prevalence of being bullied over the previous 30 days in the 9th grade was 32% in boys and 29% in girls (Malta et al., 2009). Thus, despite differences in methodologies among studies and populations, the prevalence of bullying in our sample was in general agreement with the literature.

The main finding of our study was that a history of being bullied was associated with clear and time-dependent differences in temperament towards a dysfunctional profile. For emotional traits, being a victim of bullying was particularly associated with more Emotional Sensitivity (trauma and rejection sensitivity) and less Volition (low self-esteem and sadness), but all dimensions were affected, including Control, which relates to attention and executive functions. Thus, being bullied seems to be linked with a broad and profound impact on emotional and cognitive domains. Accordingly, being bullied was related to a shift from stable and healthy types (euthymic and hyperthymic) to internalized (depressive, apathetic) and unstable types (cyclothymic and volatile). The associations between being bullied and affective temperaments were quite similar between genders, except for the slight increase of apathetic types in females.

The cross-sectional design of our study does not allow to differentiate if such temperament traits make subjects more likely to be exposed to bullying or if bullying per se disrupts their temperaments. Previous research suggests that the interaction between emotional traits and being bullied works both ways. Fekkes et al. (2006) have shown that children with anxiety and depressive symptoms are at increased risk of being victimized, but also many psychosomatic and psychosocial health problems follow an episode of bullying victimization. The temperamental features more likely to render an individual attractive to bullying perpetrators is an inhibited temperament (Gladstone et al., 2006), which is prone to high Fear and Emotional Sensitivity, and low Volition (i.e., depressive) and Anger traits. However, our results showed that Anger increased proportionally to the length of peer

Table 1
Demographic description of the sample.

Groups	Mean age (years) ± S.D.	Males ($n=13,520$)	Females ($n=37,362$)
No bullying	31.6 ± 10.7[a]	6240 (46.2%)	21,096 (56.5%)[c]
Bullying < 1 year	30.5 ± 9.7[b]	3600 (26.6%)	9,326 (25.0%)[d]
Bullying 1 < 3 years	29.0 ± 9.0	1936 (14.3%)	3,894 (10.4%)[d]
Bullying ≥ 3 years	29.2 ± 9.0	1744 (12.9%)	3,046 (8.2%)[d]

[a] Significantly higher than other bullying groups.
[b] Significantly higher than bullying 1 < 3 and bullying ≥ 3 years groups. (ANOVA, $F=160.162$, $p < 0.05$).
[c] Higher and
[d] Lower proportion compared to males (Chi-square = 576.469, $p < 0.001$).

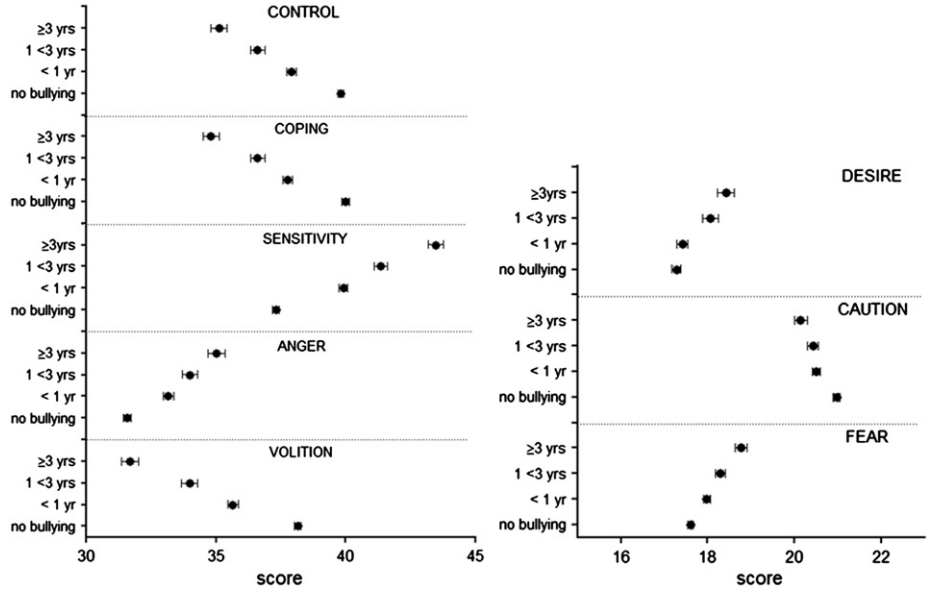

Fig. 1. Emotional traits according to bullying exposure. Data is shown as mean and 95% CI.

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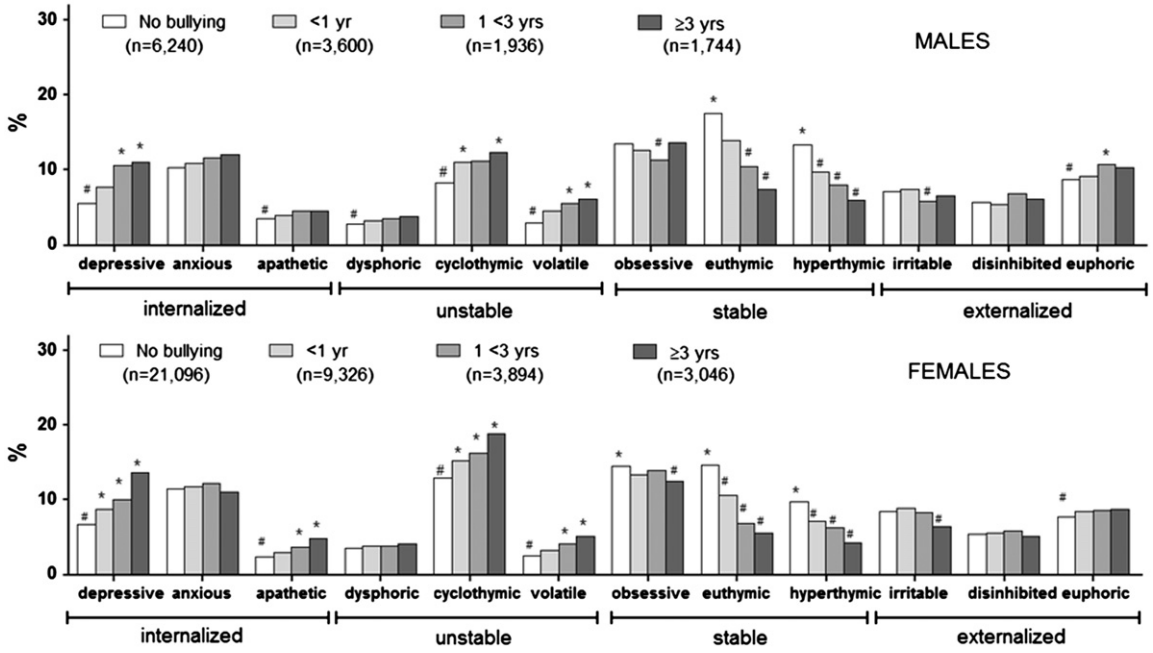

Fig. 2. Affective temperaments according to bullying exposure. Data is shown as a percentage of subjects in each category separated by gender. *=a higher proportion and #=a lower proportion, according to the chi-square test ($p < 0.05$).

victimization, suggesting a causal role of bullying. Another Person versus Environment interaction approach studied by Sugimura and Rudolph (2012) has recently shown that temperament predicts children's reaction to being victimized: development of overt aggression was associated with poor inhibitory control, and depression was linked to high and low negative emotionality in girls and boys, respectively. Our results suggest that the two major outcomes are the development/reinforcement of a depressive or an unstable (cyclothymic or volatile) temperament. Of note, our results show no major association between being bullied and externalized temperaments, which are less likely to play a submissive role.

In agreement with an increase in internalized and unstable traits, the development of a wide range of psychiatric disorders and symptoms has been associated with peer victimization. Several studies have reported an increase in internalizing symptoms, depression, anxiety, low self-esteem and suicidal ideation (Reijntjes et al., 2010; Gladstone et al., 2006; Salmon et al., 1998; Egan and Perry, 1998; Kaltiala-Heino et al., 1999; Kaltiala-Heino et al., 2000; Dake et al., 2003; Arseneault et al., 2010). However, bullying victimization has also been associated with binge eating (Striegel-Moore et al., 2002), paranoid symptoms and hallucinations (Richard et al., 2012), borderline personality disorders (Sansone et al., 2012; Wolke et al., 2010) and substance use (Carlyle and Steinman, 2007; Tharp-Taylor et al., 2009; Radliff et al., 2012). Such diversity of outcomes is possibly influenced by complex interactions between temperament and environment.

Face-to-face studies on sensitive issues use are prone to underreporting. However, data collected by computer can enhance the validity for sensitive, intimate, moral and personal issues when compared to anonymous pen and paper methods (Turner et al., 1998), face-to-face (Gosling et al., 2004), and telephone interviews (Cuijpers et al., 2008). Especially when assessing research websites from remote personal computers, respondents may feel more anonymous and private and less concerned about how they appear to others. Online data regarding other measures and topics is remarkably consistent with offline data (Buchanan and Smith, 1999; Hewson and Charlton, 2005) and Internet users are similar to nonusers when considering measures of adjustment, social interaction and personality traits (Gosling et al., 2004). Also, the Internet provides a means to enhance the motivation for participants (e.g., immediate personalized feedback) and to insert validity checks, which significantly increase the response rates preserving data quality (Edwards et al., 2009). Finally, almost all respondents prefer web-based versions to mailed questionnaires and telephone interviews, or they had no preference in such matters (Rankin et al., 2008; Touvier et al., 2010). Based upon this evidence, web-based questionnaires can even be considered the gold standard for issues prone to social desirability bias, especially in population studies.

The major limitations of this study were that the assessment of bullying, while practical and simple, was not undertaken with a standardized assessment tool, did not discriminate types of bullying and was retrospective. Also, only self-report instruments were used. The major strengths were the large sample size, the use of an internet system developed to optimize data validity and the fact that participants were not aware of the particular objective of this study. Since the AFECTS was the first instrument in the BRAINSTEP, their responses could not have been biased by emotional reactions produced by questions on bullying or other lifetime stressful events.

5. Conclusions

This study suggests clear time-dependent differences in temperament towards a dysfunctional profile in bully victims, helping to explain individual variation in children's reactions to peer victimization. Also, being bullied was associated with a broad and profound impact on emotional and cognitive domains in all dimensions of emotional traits, with implications for Person × Environment models of development. Furthermore, bullying was associated with internalized and unstable affective temperaments, which have been linked with several psychiatric disorders (Lara et al., 2006; Rihmer et al., 2010). Finally, this research highlights the need for targeted preventive and intervention programs for victimized youth.

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Conflict of interest

The authors declare no conflicts of interest.

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5 CONCLUSÃO

Esta tese sobre a avaliação das bases biológicas e sociais do temperamento foi baseada em três estudos. Referente às bases biológicas, o primeiro utilizou *microarrays* em regiões cerebrais de camundongos e o segundo avaliou o perfil de temperamento de pessoas com hiperuricemia. O terceiro estudo avaliou a relação entre *bullying* e suas implicações sobre o temperamento, referente às bases sociais. No estudo de *microarrays* com animais alto e baixo exploradores encontraram-se alguns genes diferencialmente expressos, tais como o receptor mGLU2, VGLUT2, CCK e do sistema de MCH em córtex e corpo estriado que podem servir de alvos para intervenção em novos estudos. Além disso, outras moléculas envolvidas em processos intracelulares e fenómenos epigenéticos (tradução e pós-tradução), tais como eIF2, H2B e CBX3, também apresentaram-se diferencialmente expressos e apontam a necessidade de maiores estudos a fim de avaliar sua implicação no temperamento e nos seus respectivos distúrbios.

Com relação ao estudo sobre a associação do ácido úrico e temperamento, pode-se concluir que os indivíduos hiperuricêmicos apresentam temperamentos mais externalizados e instáveis, além de sintomas de humor, tanto em homens quanto em mulheres. O papel do ácido úrico como causa/conseqüência nos traços emocionais e nos temperamentos afetivos, ainda não foi estabelecido, no entanto o efeito terapêutico do alopurinol na mania sugere pelo menos um papel importante das purinas em alguns pacientes. Dessa forma, deve-se investigar se a hiperuricemia em indivíduos com traços de externalização deriva da maior produção ou menor excreção de ácido úrico.

Na avaliação das bases sociais do temperamento nossos resultados sugerem que sofrer *bullying* está significativamente associado com diferenças claras e tempo-dependente de temperamento para um perfil disfuncional. Além disso, sofrer *bullying* está associado com um impacto significativo sobre os domínios cognitivos e emocionais em todas as dimensões de traços emocionais, e com implicações nos modelos pessoa x ambiente. Nossos resultados sugerem que *bullying* está associado com temperamentos afetivos internalizados e instáveis, que já foram associados com vários transtornos psiquiátricos (Lara et al., 2006; Rihmer et al., 2010). Sendo assim, nossos resultados destacam a necessidade de programas de prevenção e orientação a jovens que sofreram *bullying*.

Dessa forma, nossos resultados apontam para uma forte associação entre o ambiente social e o temperamento, assim como uma associação entre o temperamento e as variáveis biológicas. Os resultados de expressão gênica identificaram genes diferencialmente expressos mesmo em animais que não sofreram intervenções ambientais específicas, reforçando o papel de traços herdados. Além disso, a avaliação dos indivíduos hiperuricêmicos abre a possibilidade de se testar intervenções voltadas para o sistema purinérgico para a modulação de traços emocionais. Entretanto, de todos nossos resultados, o mais significativo foi a forte associação entre o ambiente social e o temperamento, observado no estudo das vítimas de *bullying.* Este é um resultado impactante e também preocupante, pois demonstra a magnitude e persistência dos danos ao temperamento observado nas vítimas de *bullying*. Destaca-se, aqui, a preocupação para a implantação de programas de prevenção ao *bullying* e de apoio às vítimas para prevenir a ocorrência de distúrbios de comportamento nas vítimas.

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