UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA
DEPARTAMENTO DE PSIQUIATRIA

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Estudo de associação entre disfunção neurocognitiva, estresse oxidativo e polimorfismos em pacientes jovens com Transtorno Bipolar tipo I

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Psiquiatria
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Versão Revisada

São Paulo
2013

Dados Internacionais de Catalogação na Publicação (CIP)

Preparada pela Biblioteca da

Faculdade de Medicina da Universidade de São Paulo

Soeiro-de-Souza, Márcio Gerhardt

Estudo de associação entre disfunção neurocognitiva, estresse oxidativo e polimorfismos em pacientes jovens com Transtorno Bipolar tipo I / Márcio Gerhardt Soeiro de Souza. -- São Paulo, 2013.

Tese(doutorado)--Faculdade de Medicina da Universidade de São Paulo. Programa de Psiquiatria.

Orientador: Ricardo Alberto Moreno

Coorientador: Rodrigo Machado Vieira.

Descritores: 1.Transtorno bipolar 2.Cognição 3.Fator neurotrófico derivado do encéfalo 4.Polimorfismo de nucleotídeo único 5.Estresse oxidativo 6.Dopamina 7.Canais de cálcio

USP/FM/DBD-041/13

AGRADECIMENTOS

Agradeço aos meus pais pelo carinho, estímulo, lições e especialmente pela educação de alta qualidade que me foi oferecida.

Agradeço aos Professores Dr. Ricardo A. Moreno e Dra. Doris H. Moreno por terem desde o início acreditado em minhas capacidades como pesquisador e pelo incentivo e investimento a mim dedicados.

Agradeço ao Professor Dr. Rodrigo Machado-Vieira pelo companheirismo, paciência e por ter acreditado na qualidade do meu trabalho.

Agradeço a equipe do Programa de Transtornos Afetivos (GRUDA) por ter fornecido o ambiente ideal para um pesquisador desenvolver todas suas habilidades.

Agradeço à Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) pelo investimento neste projeto científico – auxílio regular 2010/06230-0.

Se um homem tem um talento e não tem capacidade de usá-lo, ele fracassou. Se ele tem um talento e usa somente a metade deste, ele fracassou parcialmente. Se ele tem um talento e de certa forma aprende a usá-lo em sua totalidade, ele triunfou gloriosamente e obteve uma satisfação e um triunfo que poucos homens conhecerão.

Thomas Wolfe

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EQUIPE

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Prof. Dr. Ricardo Alberto Moreno: Orientador e responsável pela coordenação deste projeto.

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Psicólogo Domingos Fernandes: Psicólogo, executou as avaliações neuropsicológicas.

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Nayara Rodrigues: Secretária executiva do projeto.

Élida B. Ojopi: Colaborou na idealização do projeto.

Carolina Martins do Prado: Responsável técnica pelo procedimento de genotipagem.

Ana Andreazza: Responsável técnica pelo procedimento de mensuração do 8-OHdG; colaborou com a elaboração do projeto.

Prof. Dr. Robert Post: Colaborador científico. Contribuiu especificamente com a temática da criatividade e genética.

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O Transtorno Bipolar (TB) tipo I é uma doença caracterizada por episódios de mania e depressão recorrentes com importante prejuízo do funcionamento global e comprometimento das funções cognitivas. Além disso, sabe-se que o número de episódios de humor patológico ao longo da vida pode também influenciar o funcionamento cognitivo destes sujeitos. Neste cenário, ocorreu a necessidade de se investigar marcadores genéticos para disfunção cognitiva no TB com o objetivo de estudar este fenômeno. Dentre os potenciais genes responsáveis por influenciar a cognição destacam-se os polimorfismos funcionais do fator neurotrófico derivado do cérebro (*BDNF*), da catecol-O-metiltransferase (*COMT*), da apolipoproteína-E (A*P*OE) e do canal de cálcio de baixa voltagem subunidade α1-C (*CACNA1C*). Sabe-se, também, que no TB os marcadores de estresse oxidativo estão aumentados durante todas as fases da doença, entretanto, não é claro qual impacto destes na disfunção cognitiva de indivíduos com TB. O objetivo dessa tese foi avaliar o desempenho cognitivo de pacientes jovens com bipolaridade tipo I e sua associação com o genótipo de *BDNF, COMT, APOE e CACNA1C* e também com os níveis plasmáticos de oxidação da guanosina (8-OHdG) e citosina (5-Mec) durante os episódios de humor, eutimia e em controles. Para investigar essa associação foram incluídos 116 pacientes (79 em episódio de humor patológico e 37 eutímicos) com diagnóstico de TB tipo I (DSMIV-TR); 97 controles saudáveis foram submetidos à avaliação neuropsicológica e coleta de sangue para extração de DNA visando genotipagem para *BDNF* (rs6265), *COMT* (rs4680; rs165599), *APOE* (rs429358 e rs7412), *CACNA1C* (rs1006737), 8-OhdG e 5-Mec. A análise dos dados obtidos revelou que pacientes portadores do genótipo Met/Met rs4680/rs165599 do *COMT* apresentam comprometimento cognitivo mais grave (função executiva, fluência verbal, memória e inteligência) comparado ao genótipo Val/Met ou Val/Val durante episódios maníacos ou mistos. Na mesma direção destes resultados, verificou-se que pacientes portadores do alelo Met rs4680 do *COMT* apresentam comprometimento do reconhecimento de emoções faciais em episódios de mania e depressão. Nenhum efeito do *COMT* foi observado em controles. O alelo de risco Met do *CACNA1C* se associou a um pior comprometimento executivo independente dos sintomas maníacos ou depressivos no TB, porém nenhum efeito se observou nos controles. O alelo Met do *BDNF* rs6265 ou a presença do alelo ε4 da *APOE* não representa um fator que identifique um grupo com desempenho cognitivo diferenciado durante as fases do TB ou em controles. Sujeitos com TB apresentaram níveis mais elevados de 8-OHdG e tais níveis eram diretamente proporcionais ao número de episódios maníacos ao longo da vida, sugerindo um papel dos episódios hiperdopaminérgicos na oxidação das bases de DNA. Concluiu-se que a genotipagem para *COMT* e *CACNA1C* em pacientes com TB pode identificar um grupo de pacientes associados a pior disfunção cognitiva durante as fases maníacas e mistas do TB. Tal dado pode ser um indicador do envolvimento do sistema dopaminérgico e dos canais de cálcio de baixa voltagem na fisiopatologia da disfunção cognitiva no TB e deve ser explorado em outros estudos.

Descritores: 1.Transtorno bipolar 2.Cognição 3.Fator neurotrófico derivado do encéfalo 4.Polimorfismo de nucleotídeo único 5.Estresse oxidativo 6.Dopamina 7.Canais de cálcio

ABSTRACT

Soeiro-de-Souza, M. G. *Genetic association study among neurocognitive dysfunction, oxidative stress and polymorphisms in young patients with bipolar I disorder* [Thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2013.

Bipolar I disorder (BD) is a disease whose main features include severe mood swings that cause severe impairment in global functioning and cognitive domains. Moreover, the number of mood episodes throughout patients' life is also associated with deterioration in cognitive functions. In this context, it is important to study genetic markers for the cognitive dysfunction observed in BD to elucidate the physiopathology of this phenomenon. The main candidates for genetic modulation of cognition are the genes brain derived neurotrophic factor (*BDNF*), catechol-o-methyltransferase (*COMT*), apolipoprotein E (*APOE*) and α1-C subunit of the L-type voltage-gated calcium channel (*CACNA1C*). Furthermore, elevated levels of oxidative stress have been reported in BD for all types of mood episodes but no data is available on their impact on cognitive functioning of BD patients. The aim of this thesis was to investigate whether cognitive functioning of BD patients is influenced by *BDNF, COMT, APOE, CACNA1C* genotypes or by levels of oxidative damage to the DNA base guanosine (8-OHdG) and cytosine (5-Mec). One hundred sixteen patients (79 during mood episode and 37 euthymic) with BD type I (mania, depression or euthymia) and 97 healthy controls were submitted to neuropsychological evaluation and blood collection for DNA analysis. All subjects were genotyped for *BDNF* (rs6265), *COMT* (rs4680; rs165599), *APOE* (rs429358 and rs7412), *CACNA1C* (rs1006737), DNA levels of 8-OHdG and 5-Mec were also measured. Our results revealed that BD subjects that carried the rs4680/rs165599 Met/Met genotype had more severe cognitive dysfunction (executive function, verbal fluency, memory and intelligence) than carriers of other genotypes during manic or mixed episodes. Moreover, patients carrying the *COMT* rs4680 Met allele had worse performance on facial emotion recognition tests during manic and depressive episodes. BD carriers of the Met allele of *CACNA1C* had more severe executive dysfunction than non-carriers, regardless of manic or depressive symptoms. No effect of *CACNA1C* or *COMT* genotypes was observed in controls. The genotypes of *BDNF* or *APOE* were not associated with cognitive dysfunction in BD patients or controls. The BD group exhibited higher levels of 8-OHdG than the control group and these levels were influenced by the lifetime number of manic episodes, suggesting that hyperdopaminergic episodes may influence the oxidation of DNA bases. In summary, the genotype of *COMT* and *CACNA1C* may represent a useful tool for identifying BD subjects at risk of developing more severe cognitive dysfunction in all mood states of the disease. This evidence associating dopamine catabolism and calcium channels to degree of cognitive dysfunction in BD should be further explored by future research

Descriptors: Bipolar disorder, Cognition, Brain derived neurotrophic factor, single nucleotide polymorphism, oxidative stress, dopamine, calcium channels.

1. INTRODUÇÃO

O transtorno bipolar (TB) é uma doença crônica, recorrente, frequente, com elevada morbidade e mortalidade (Kupfer et al., 2002; Kupfer, 2005), e na sua forma clássica (TB tipo I) afeta igualmente homens e mulheres (Goodwin & Jamison, 2007). Atualmente existem fortes evidências que pacientes com TB apresentam *déficits* cognitivos durante os episódios de mania ou depressão e mesmo quando em eutimia (Martinez-Aran et al., 2004; Martinez-Aran et al., 2008). Tal *déficit* cognitivo pode ter uma grande variação com relação à gravidade dentre os pacientes durante as fases de humor e mesmo após anos de evolução da doença (Lala & Sajatovic, 2012). Os principais *déficits* cognitivos observados em pacientes bipolares eutímicos afetam a memória verbal (Clark et al., 2002; Martinez-Aran et al., 2004; Ferrier & Thompson, 2002), função executiva (Ferrier & Thompson, 2002; Martinez-Aran et al., 2008) e atenção (Clark et al., 2002; S. K. Liu et al., 2002; McGrath et al., 1997). Estudos mostram que os *déficits* específicos apresentados por esses pacientes podem ser compartilhados em menor intensidade por familiares de primeiro grau não afetados pelo TB (Kieseppä et al., 2005; Kéri et al., 2001). Estudos recentes relatam que alguns fatores clínicos podem influenciar negativamente o funcionamento cognitivo de pacientes com TB - com um impacto negativo no desempenho de tarefas que envolvam memória, atenção ou abstração (McKay et al, 1995; Zubieta et al, 2001; Martinez-Aran et al, 2004a,b). Tais fatores são: o número de episódios de humor (especialmente manias), número de hospitalizações, a ocorrência de sintomas psicóticos ao longo da doença e a cronicidade da mesma, definida pela duração em anos.

Dessa forma a disfunção cognitiva no TB é uma das principais candidatas à classificação de endofenótipo, pois está presente em todas as fases da doença e em familiares de primeiro grau não afetados (Glahn et al., 2010; Bora et al., 2009; Gottesman & Gould, 2003). Apesar disso, não se sabe exatamente a etiologia da disfunção cognitiva dos pacientes com TB, mas recentemente alguns genes foram associados à modulação de domínios cognitivos, especialmente em pessoas saudáveis e em portadores de esquizofrenia (Savitz et al., 2006).

1.1. Cognição e Genética

A cognição humana, sendo uma função complexa, é provavelmente influenciada por uma série de genes, cada qual com um pequeno papel no desempenho final. O desempenho em cada domínio cognitivo pode ser associado à atividade de uma determinada proteína e por extensão a variações genéticas responsáveis por essas diferenças funcionais. Tais estudos são denominados estudos genéticos de associação.

Nos estudos mais recentes que buscam a relação entre o fenótipo cognitivo e os polimorfismos de nucleotídeo único (SNP) destacam-se os genes ligados a neurotrofinas (*BDNF*), catecol-O-metilfransferase (*COMT*), apolipoproteína E (*APOE*), canais de cálcio (*CACNA1C*) e a via do estresse oxidativo (Savitz et al., 2006; Wilson, Schneider, et al., 2002b; Thimm et al., 2011).

1.2. Neurotrofinas e Cognição

Para estudar o impacto das neurotrofinas na disfunção cognitiva no TB, escolheu-se o BDNF. O fator neurotrófico derivado do cérebro (BDNF) é um polipeptídio codificado por um gene no cromossomo 11, com importante papel na sobrevivência, diferenciação e crescimento de neurônios periféricos e centrais tanto na infância quanto na maturidade de seres humanos. O BDNF participa de mecanismos de plasticidade neuronal dependente do uso, tal como potencialização de longo prazo, aprendizado e memória (Malcangio & Lessmann, 2003). Este e outros estudos indicam que o BDNF é importante para o processo de aprendizado e melhora da função cognitiva, além de proteger contra os processos que potencialmente degradam os circuitos neuronais e de neuroinflamação (Berk et al., 2011; Berk, 2009).

Dentre as neurotrofinas, o BDNF parece implicar fortemente na fisiopatologia do TB (Post, 2007). Algumas evidências sugerem que uma variação em um polimorfismo de nucleotídeo único (SNP) funcional na posição 66 na sequência promotora do alelo do proBDNF ($Val^{66}Met$) poderia estar associada a uma menor secreção ativa de BNDF

(Egan et al., 2003). Dessa forma, o alelo Met tem sido considerado como o alelo de risco do *BDNF* por secretar ativamente menos BDNF em modelos animais. Estudos de associação genética na população saudável reportam que portadores do alelo Met do *BDNF* apresentam pior funcionamento de memória (Egan et al., 2003; Hariri et al., 2003), funcionamento executivo (Rybakowski et al., 2003) e inteligência (Tsai et al., 2004). Estudos de associação entre o SNP funcional do *BDNF* e a performance cognitiva de pacientes com TB são escassos e reportam resultados controversos. Dois estudos, de um mesmo investigador, reportam melhor funcionamento executivo em indivíduos Val/Val comparados com os demais genótipos (Rybakowski et al., 2003; Rybakowski et al., 2006), entretanto outro estudo não replicou esse achado (Tramontina et al., 2009).

1.3. Dopamina e Cognição

Para se estudar o papel da dopamina na disfunção cognitiva dos bipolares, escolheu-se a COMT. Ela é uma enzima codificada por um gene no cromossomo 22, com papel importante no metabolismo dos neurotransmissores dopamina e noradrenalina no córtex pré-frontal, onde a proteína de membrana transportadora da dopamina (DAT) é menos ativa. A COMT catalisa na presença de Mg^{2+} a transferência de um grupo metil da S-adenosilmetionina (SAM) para um grupo hidroxil de um substrato catecol, levando a conversão da dopamina em 3-metoxitiramina (Napolitano et al., 1995).

O polimorfismo $Val^{158}Met$ (rs4680) resulta da substituição do aminoácido valina por metionina no códon 158, resultando em uma catabolização de dopamina no córtex pré-frontal até quatro vezes menor nos portadores do alelo Met (Egan et al., 2001; Gogos et al., 1998) devido a uma maior instabilidade térmica. Em uma revisão recente, setenta e seis por cento dos trabalhos que avaliaram a relação do polimorfismo $Val^{158}Met$ com a função cognitiva encontraram associação positiva entre desempenho nos testes e presença do alelo Met (Savitz et al., 2006). A grande maioria de tais trabalhos foi feita em indivíduos saudáveis e reporta que o alelo de risco Met estaria associado a um melhor desempenho em tarefas cognitivas que avaliam a memória

operacional, provavelmente pelo incremento nos níveis de dopamina no córtex pré-frontal (Savitz et al., 2006).

Apesar dessas evidências, apenas um estudo avaliou a influência do genótipo do SNP funcional da *COMT* no desempenho cognitivo de pacientes com TB. Burdick et al (2007) reportaram em sua análise de 52 bipolares tipo l eutímicos medicados e de 102 controles que o alelo Val do polimorfismo nucleotídeo único rs165599 esteve associado, em teste de memória verbal, a um pior desempenho, o que estaria de acordo com os resultados de estudos com indivíduos sadios (Burdick et al., 2007).

1.4. Apolipoproteínas e Cognição

Para estudar o efeito das apolipoproteínas na cognição do TB, foi escolhido o gene da apolipoproteína E (APOE). A APOE é uma proteína codificada por um gene no cromossomo 19, que se combina no sangue para formar moléculas chamadas lipoproteínas, as quais são responsáveis por armazenar colesterol e outras gorduras e por transportá-las na corrente sanguínea até sua metabolização (Rocchi et al., 2003). Além disso, estudos *in vitro* sugerem que a APOE pode influenciar a formação sináptica (Mauch et al., 2001).

Existem três alelos que codificam essa proteína: epsilon ε2, ε3, ε4; O ε3 é o mais comum, sendo encontrado em até 75% da população (Lahiri et al., 2004). O alelo ε4 está associado a um maior risco e declínio cognitivo em doença de Alzheimer (DA) tardia e precoce (Hirono et al., 2003; Marra et al., 2004), além de ser capaz de influenciar a cognição de indivíduos saudáveis (Kamboh & DeKosky, 1995). Indivíduos normais portadores de ε4 apresentam dificuldade em tarefas cognitivas da área de memória episódica e funcionamento executivo, além de terem um declínio cognitivo mais rápido ao longo do tempo (Wilson, Schneider, et al., 2002b; Wilson, Bennett, et al., 2002a).

A relevância do genótipo da *APOE* para o TB é incerta devido a dados controversos relacionados à prevalência alélica e associações (Kessing & Jørgensen, 1999). O alelo mais estudado em transtornos de humor é o ε4 e até a presente revisão não existiam dados a respeito dos alelos ε2 e ε3. Não existe uma associação de maior

frequência do ε4 nos transtornos afetivos, porém alguns grupos particularmente de maior prevalência desse alelo já foram identificados (Kessing & Jørgensen, 1999). Nesse sentido, Bellivier et al. (1997) reportaram uma associação entre o alelo ε4 e TB de início precoce com sintomas psicóticos (Bellivier et al., 1997). Uma maior prevalência do alelo ε4 em transtorno depressivo unipolar de início tardio (Rigaud et al., 2001; Krishnan et al., 1996) também já foi reportada, mas nunca replicada. Recentemente, este grupo de pesquisa, em um estudo piloto, relatou um possível efeito cognitivo protetor do ε3 (melhor funcionamento executivo) em pacientes com TB (Soeira-de-Souza et al., 2010), o que motivou a inclusão deste SNP neste estudo. Ademais, têm sido reportados menores níveis de APOE em pacientes com esquizofrenia e TB (Dean et al., 2008).

1.5. Canais de Cálcio e Cognição

O influxo de cálcio através dos canais de baixa voltagem (tipo L) consiste em um dos mecanismos de sinalização transmembrana mais frequentes. Dessa forma, variações na atividade dos canais de cálcio podem afetar a transdução do sinal e a circuitaria cerebral, o que poderia influenciar no funcionamento cognitivo. Alterações na regulação da sinalização de cálcio e maiores níveis de cálcio intracelular são um dos achados mais replicados no TB (Machado-Vieira et al., 2011; Akimoto et al., 2007; Kato, 2008; Sourial-Bassillious et al., 2009).

Recentemente o alelo Met do SNP rs1006737 do gene que codifica os canais de cálcio voltagem dependente tipo 1.2 (*CACNA1C)* tem sido associado a um maior risco de TB (Sklar et al., 2008; Ferreira et al., 2008; Y. Liu et al., 2011; Psychiatric GWAS Consortium Bipolar Disorder Working Group et al., 2011). Bigos et al. (2010) relataram que tal alelo de risco estaria associado a um aumento da expressão de RNA do gene do *CACNA1C* no córtex pré-frontal dorso-lateral de controles saudáveis (Bigos et al., 2010). Alguns estudos reportam que o alelo Met estaria associado a um maior volume de substância cinzenta (Kempton et al., 2009; Wang et al., 2011), da amígdala direita ou do hipotálamo (Perrier et al., 2011). A maior parte dos estudos que investigou a relação entre esse SNP e o funcionamento cognitivo ocorreu em indivíduos normais e os

resultados são controversos. Dois estudos reportaram maior ativação da região frontal enquanto apenas um desses demonstrou um efeito negativo do alelo Met em testes de memória (Bigos et al., 2010; Krug et al., 2010). Outro estudo identificou uma associação entre pior nível de atenção e orientação em portadores do alelo Met (Thimm et al., 2011), enquanto outros dois não identificaram qualquer associação entre o alelo de risco e a performance cognitiva em indivíduos normais (Roussos et al., 2011; Hori et al., 2012). Até o momento apenas dois estudos avaliaram o impacto desse SNP do canal de cálcio-L em TB. Zhang et al (2011) reportaram um melhor funcionamento executivo durante episódio maníaco em portadores de Met *versus* Val/Val (Zhang et al., 2012). Arts et al. (2012) avaliaram 51 pacientes bipolares durante um período de 2 anos e relataram um efeito negativo do alelo Met em uma composição de medidas cognitivas que avaliavam memória, atenção e funcionamento executivo (Arts et al., 2012).

1.6. Estresse Oxidativo no TB

Recentemente o estresse oxidativo tem sido implicado nesta fisiopatologia do TB e um crescente número de evidências se acumulam nessa direção (Andreazza et al., 2008; Machado-Vieira et al., 2007; Maes et al., 2011; Ozcan et al., 2004; Kapczinski et al., 2008). Vários estudos relatam que pacientes com TB apresentam alterações significantes nas enzimas antioxidantes e também nas substâncias oxidantes (Berk, 2009; Andreazza et al., 2008).

Um maior estresse oxidativo neuronal causa efeitos deletérios na transdução de sinais, plasticidade estrutural e na resiliência celular, principalmente por levar à oxidação nas membranas das proteínas e nos genes (Mahadik et al., 2001). O estresse oxidativo pode levar a múltiplas formas de dano ao DNA, incluindo modificações de bases, deleções, rearranjos cromossômicos e até rupturas de filamentos de DNA (Valko et al., 2004; Valko et al., 2006). A produção de substâncias reativas ao oxigênio (ROS) está associada a um maior dano ao DNA e aos cromossomos com alterações tanto para hiper quanto para hipometilação do DNA (Berk, 2009; Lim et al., 2008; Swann et al., 2000; Robinson & Ferrier, 2006; Kauer-Sant'Anna et al., 2009).

As ROS reagem com a base guanosina formando 8-Hidroxi-2'-deoxiguanosina (8-OHdG) (Berk, 2009; Coryell et al., 2001; Swann et al., 2000; Guo et al., 2011; Robinson & Ferrier, 2006; Kauer-Sant'Anna et al., 2009). Dessa forma, a guanosina é a base mais propensa a sofrer dano oxidativo (Clark et al., 2002; Altieri et al., 2008; Gutteridge & Halliwell, 2000; Kryston et al., 2011; Cavanagh et al., 2002; Radak & Boldogh, 2010; Ferrier & Thompson, 2002; Spassky & Angelov, 1997). Mais recentemente os estudos têm focado na oxidação da base citosina [5-metilcitosina (5-Mec)], uma vez que tais bases são "ancoras" para o grupo metil no DNA - ilhas CpG - (Bora et al., 2010; Branco et al., 2012; Lenaz, 2001). A 5-Hidroxi-metilcitosina (5-HMec) é um indicador de oxidação da base citosina e tal oxidação pode prejudicar o processo epigenético de metilação, contudo, vale ressaltar, a influência dos ROS no 5-HMec é ainda desconhecida (Valko et al., 2004; Guo et al., 2011; Valko et al., 2006).

2. RACIONAL DO ESTUDO

Neste estudo, trabalhou-se com a hipótese de que existe um grupo, dentre os pacientes bipolares com características genéticas semelhantes, que sofre um maior prejuízo cognitivo durante as fases da doença, independente do tratamento. Dos genes mais associados à função neurocognitiva se destacam o *BDNF, COMT, APOE e o CACNA1C*. Pretendeu-se, aqui, avaliar o desempenho cognitivo de pacientes bipolares jovens durante as fases de humor e eutimia, bem como verificar a associação com a presença dos alelos que potencialmente modificam a cognição do *BDNF* (Met), *COMT* (Met), *APOE* (ε4) ou *CACNA1C* (Met). Além disso, considerando os consistentes dados acerca do estresse oxidativo no TB, optou-se por verificar, via marcadores de oxidação das bases de DNA: a sua modulação pelos alelos de risco cognitivo e a influência destes na cognição. Optou-se por avaliar pacientes durante os episódios de humor e sem o uso da medicação visando observar a influência dos sintomas depressivos e/ou maníacos na cognição sem a interferência medicamentosa.

3. JUSTIFICATIVA

Houve a necessidade de se investigar marcadores neurobiológicos para o TB visando não somente o diagnóstico, mas também o tratamento personalizado e um melhor conhecimento da fisiopatologia da doença. A existência de um grupo de pacientes com características fenotípicas, genéticas e com evidência de maior dano ao DNA forneceu ao estudo informações úteis para entender a fisiopatologia do *déficit* cognitivo observado no TB. Como a idade de início precoce, presença de sintomas psicóticos, qualidade da resposta ao lítio, ciclotimia e transtornos de pânico comórbido no TB constituem características de grupos de pacientes geneticamente similares (Goodwin & Jamison, 2007), é possível que aqueles com disfunção cognitiva diferenciada também o sejam. A identificação de tal grupo pode ser o primeiro passo para planejar o treinamento cognitivo direcionado e preventivo visando evitar o agravamento progressivo da perda cognitiva.

4. HIPÓTESES

4.1. Hipótese 0

Os pacientes bipolares tipo I não apresentam uma associação entre desempenho cognitivo e a presença dos alelos que potencialmente modificam a cognição do *BDNF* (Met), *COMT* (Met), *APOE* (ε4) ou *CACNA1C* (Met).

4.2. Hipótese 1

Os pacientes bipolares tipo I apresentam uma associação entre prejuízo no desempenho cognitivo e a presença alelos que potencialmente modificam a cognição do *BDNF* (Met), *COMT* (Met), *APOE* (ε4) ou *CACNA1C* (Met).

5. OBJETIVOS

5.1. Objetivo Primário

O objetivo foi investigar se a presença dos alelos do *BDNF* (Met), *COMT* (Met), *APOE* (ε4) ou *CACNA1C* (Met) em pacientes bipolares jovens durante episódios de humor permite identificar um grupo de pacientes com disfunção cognitiva mais intensa.

5.2. Objetivo Secundário

O presente estudo teve como objetivo secundário verificar se o nível de 8-OHdG ou 5-Mec periférico é semelhante entre TB e controles e ainda se tais níveis associam-se a algum grau de disfunção cognitiva ou a características clínicas da doença.

6. MATERIAL E MÉTODO

6.1. Instrumentos e Procedimentos

6.1.1. Avaliação Clínica

a) Entrevista Clínica: Entrevista padronizada, utilizada pela Associação Brasileira de TB (ABTB), realizada por psiquiatra e empregada para coleta de informações como identificação do indivíduo, dados demográficos, história médica geral, hábitos de vida, história pessoal de tratamento psiquiátrico prévio e história familiar de transtorno psiquiátrico;

b) Entrevista Clínica Estruturada para transtornos do eixo I do DSM IV (SCID-I/P) (First et al., 1996);

c) Escala de Hamilton para Avaliação de Depressão -HDRS (Hamilton, 1960): é a mais usada no mundo e considerada padrão ouro para validação de outras escalas. Inicialmente composta de 17 itens, foi reformulada e passou a conter 21 itens, na tentativa de discriminar o subtipo de depressão. Deve ser aplicada por um clínico treinado, apesar de entrevistas semiestruturadas estarem disponíveis para utilização, auxiliando o trabalho do clínico. Avalia os sintomas depressivos ocorridos na última semana e pretende mensurar a gravidade desses sintomas.

d) Escala de Young para Avaliação da Mania – YMRS (Young et al., 1978): é composta de 11 itens e aplicada por um clínico para avaliar a presença de sintomas maníacos, por meio de informações fornecidas pelo paciente, presentes nas últimas 48 horas, assim como aqueles observados pelo clínico no momento da entrevista. É apropriada para

averiguar tanto a gravidade dos sintomas como a sua modificação ao longo do tempo, porém não avalia a presença de sintomas depressivos, sendo necessária a utilização de outra escala para esse fim. Uma pontuação menor ou igual a 12, na escala de Young, indica remissão dos sintomas.

e) Escala de Montgomery para avaliar depressão - MADRS (Montgomery & Asberg 1979): aplicada por um clínico treinado, é composta por 10 itens, sendo 9 baseados no relato do paciente e 1 na aparência observada pelo examinador. Seus principais objetivos são acessar a mudança na sintomatologia que se segue ao tratamento antidepressivo e dar maior ênfase aos sintomas psicológicos que os físicos da depressão, comparando com a HDRS, por exemplo. Tem sido amplamente utilizada nos ensaios clínicos que avaliam a eficácia de antidepressivos e identificam o ponto de corte que define a remissão , correspondente a um valor menor ou igual a 10, apesar de outros valores terem sido considerados em determinados estudos.

6.1.2. Provas Neuropsicológicas

- Dígitos - WASI-DS - (Wechsler, 1999): este subteste da Escala de Inteligência é utilizado para avaliar a capacidade de amplitude atencional e a memória de trabalho. A amplitude atencional corresponde a uma medida de quanto os estímulos auditivos podem ser apreendidos pelo indivíduo em um dado momento. A memória de trabalho refere-se à manipulação de dados auditivos na memória imediata. É uma prova composta por uma folha de respostas na qual estão impressas duas tarefas. A primeira chamada de “dígitos diretos - FW” é composta por oito sequências de dígitos em grau crescente de dificuldade, sendo que a série inicial tem dois dígitos e a última apresenta nove. A segunda parte chamada de “dígitos inversos - BK” é composta por sete sequências de dígitos, com crescente grau de dificuldade, iniciando por dois dígitos e

terminando com oito. Cada uma destas sequências, em ambas as provas, contém duas tentativas diferentes. Nas duas provas, o examinador lê em voz alta a sequência de números para o indivíduo. Para cada item da "ordem direta" o indivíduo deverá repetir a sequência numérica na mesma ordem apresentada, nas duas tentativas de cada série. Para cada item da ordem inversa, o indivíduo deverá repetir a sequência numérica na ordem inversa à apresentada, nas duas tentativas da série. Para cada sequência poderá ser atribuída uma pontuação que varia entre 2, 1 e 0. Caso o indivíduo acerte as duas tentativas, 2 pontos serão atribuídos à sequência; caso acerte uma tentativa, 1 ponto será atribuído; e caso erre ambas as tentativas, será atribuído 0. A aplicação do teste é interrompida quando o sujeito obtém 0 nas duas sequências da mesma série.

- Número/Letra – WAIS-LNS - (Wechsler, 1999): avalia a atenção dividida e memória de trabalho, ou seja, requer que o sujeito responda a duas tarefas simultaneamente ou a múltiplos elementos de uma esfera mental complexa. Para tanto, é necessário que ele mantenha na memória os elementos que compõem a atividade em questão. A prova consiste na apresentação verbal de sequências compostas por números e letras misturados. As sequências aumentam progressivamente a quantidade de estímulos, sendo que a primeira possui dois estímulos e a última apresenta oito, além disso, há três tentativas diferentes para cada série de sequências. Os acertos são pontuados e a prova é interrompida quando ocorre fracasso na reprodução verbal das três tentativas de uma determinada sequência. O máximo de pontos obtidos é 21.

- WCST - 64 cartões - (Strauss, 2006): avalia a flexibilidade mental. O material deste teste consiste em 64 cartões e 4 cartões-modelo, contendo desenhos de determinadas formas, cores e quantidades. Os cartões-modelo são colocados à frente do sujeito e os restantes em uma pilha que é dada ao sujeito. Solicita-se que o indivíduo pegue os cartões da pilha, um de cada vez, seguindo a sequência, e combine com os modelos, utilizando o critério que julgar correto, sendo que ele não sabe, *a priori*, quais são as possíveis combinações. Cada categoria é formada por dez cartas, sendo que não poderá haver erros durante a formação da categoria para que seja considerada completa. A cada cartão colocado, o examinador diz se a combinação está certa ou errada. O sujeito deve compreender que existem alguns critérios possíveis para se combinar os cartões (cor, forma e quantidade), e que estes são mudados algumas vezes no decorrer do teste (a cada dez combinações corretas efetuadas pelo sujeito). Avalia a habilidade

para formar conceitos abstratos em situações que recrutam mudança rápida de opinião e flexibilidade mental para adaptar-se a *feedbacks* positivos ou negativos. Os escores são computados indicando o número de cada uma das medidas abaixo:

- Total de acertos (WCST-CONC): número total de vezes em que o indivíduo combinou corretamente os cartões de acordo com o critério de combinação requerido;
- Erros (WCST-E): número total de vezes em que o indivíduo não conseguiu combinar os cartões de acordo com o critério de combinação requerido;
- Respostas perseverativas (WCST-PR): número total de respostas em que o indivíduo persistiu na combinação das cartas de acordo com o critério anterior e não com o requerido no momento;
- Erros perseverativos (WCST-P): número total de tentativas consideradas incorretas, nas quais o sujeito persistiu na combinação das cartas de acordo com o critério anterior e não com o requerido no momento;
- Erros não perseverativos (WCSTE-NP): número de vezes em que o indivíduo combinou os cartões incorretamente, não seguindo o critério anterior e nem o requerido no momento;
- Categorias completadas (WCST-CC): número total de sequências de dez respostas corretas consecutivas. Cada uma destas sequências completa uma categoria;
- Perda de *set* (WCST-FMS): número total de vezes em que o sujeito não conseguiu manter as sequências de respostas após cinco combinações corretas.

• Stroop Color – Word Test (SCWT) - (Strauss, 2006): analisa a atenção e a manutenção do controle inibitório, a partir da supressão de resposta usual em favor de uma resposta não usual. É composto de três cartões: no primeiro cartão (SCWT-I) há a distribuição de quatro cores (verde, rosa, azul e marrom) pintadas em quadrados e distribuídas em seis séries de forma randômica. É solicitado ao sujeito que nomeie as cores o mais rápido possível. São anotados o tempo e o número de erros, sendo que estes últimos não são computados quando corrigidos pelo sujeito. No segundo cartão (SCWT-II) há a distribuição de quatro palavras curtas (cada, hoje, nunca, e todo) escritas em quatro cores diferentes (verde, rosa, azul, marrom) e distribuídas em seis séries de forma randômica. É solicitado ao sujeito que nomeie as cores das tintas com as quais as palavras são escritas, o mais rápido possível. São anotados o tempo e o número

de erros, sendo que estes últimos não são computados quando corrigidos pelo sujeito. No terceiro cartão (SCWT-III) há distribuição de nomes de cores (verde, rosa, azul, e marrom) escritos em outras cores (exemplo: verde escrito com rosa) e alocados em seis séries de forma randômica. É solicitado ao sujeito que nomeie as cores das tintas com as quais as palavras são escritas, o mais rápido possível. São anotados o tempo e o número de erros, sendo que estes últimos não são computados quando corrigidos pelo sujeito. Os três cartões são pontuados separadamente e o tempo de cada um registrado em segundos. As performances do sujeito são classificadas de acordo com o tempo de execução da tarefa e o número de erros da mesma.

- WASI - *Wechsler Abbreviated Scale of Intelligence* - (Wechsler, 1999): o QI foi obtido a partir da soma dos resultados brutos dos subtestes - Vocabulário (VOC), Cubos (BD), Semelhanças (S) e Raciocínio Matricial (MR) - convertidos para resultados ponderados de acordo com a idade do indivíduo, sendo que esses são finalmente convertidos em índice de quociente intelectual estimado para idade. O subteste vocabulário é composto de 42 palavras, ordenadas segundo o grau de dificuldade e impressas em uma folha de respostas. O sujeito é instruído a dizer o significado da palavra lida pelo examinador ou o seu sinônimo. Após cinco erros consecutivos o teste é interrompido. Todos os significados conhecidos em dicionários são aceitáveis e são pontuados de acordo com a qualidade da definição (2, 1 ou 0). Avalia a capacidade para definir palavras, o que requer o conhecimento do significado da palavra ouvida (memória semântica), como também abstração verbal. O subteste Raciocínio Matricial avalia o raciocínio abstrato e a capacidade de retenção e evocação imediata de elementos na esfera visuoespacial (memória de trabalho) a partir de 35 figuras abstratas incompletas ordenadas segundo o grau de dificuldade e impressas em um caderno de questões, de tal forma que o sujeito precisa descobrir qual é a relação envolvida em um determinado grupo de figuras geométricas para completá-las com a alternativa correta. Após quatro erros consecutivos ou quatro acertos em cinco tentativas o teste é interrompido. A pontuação oferecida é 1 ou 0.

- Teste de Fluência Verbal - F.A.S. - (Strauss, 2006): a proposta deste teste é avaliar a capacidade de produção espontânea de palavras sob restrição semântica e o controle mental. O teste é composto por uma folha de papel e caneta, os quais serão utilizados pelo examinador para anotar as respostas do indivíduo. O sujeito é instruído a

dizer o maior número de palavras que ele conseguir se lembrar com as letras F, depois com a letra A e depois com a letra S, desde que não sejam nomes próprios e palavras semelhantes ditas de modos diferentes. Para a produção de cada uma das listas é permitido o tempo de sessenta segundos. O número total de palavras produzidas nas três listas é somado e registrado.

- Figura Complexa de Rey (RCFT) (Rey, 1999): esta prova avalia a habilidade visuoconstrutiva, a capacidade de planejamento e de desenvolvimento de estratégias para solução de problemas. Nesta prova, o sujeito copia uma figura gráfica bidimensional complexa. A pontuação é feita a partir da exatidão da reprodução das partes que compõem a figura. e o escore máximo é de 36 pontos.

- *"Trail Making Test"* (TMT) (Strauss, 2006): o objetivo desta prova é avaliar a atenção alternada, ou seja, a capacidade do sujeito de alternar o foco atencional entre dois estímulos concorrentes. A prova é composta de duas partes, sendo que ambas possuem um treino anterior à execução.

- Memória Lógica (WMS-LM) - (subteste da escala Weschler Memory Scale-III 1997): composto por memória lógica I e II, o teste avalia a memória declarativa episódica, mais especificadamente a recuperação tardia (Memória Lógica II), e a capacidade de evocação. Além de avaliar memória imediata e tardia, fornece dados sobre o reconhecimento de estímulos (memória de fixação).

- Aprendizagem Verbal (WMS-LM) - (subteste da escala Weschler Memory Scale-III 1997): esse subteste avalia as capacidades de aprendizagem de novas palavras por repetição sistemática, através de uma lista de palavras. Esse subteste avalia também a recuperação tardia das informações verbais e a capacidade de estocagem e reconhecimento desses estímulos.

- Avaliação da Cognição Social - (*Facial Expressions of Emotion: Stimuli and Tests* – FEEST) (Young et al., 2002): é um teste computadorizado no qual aparecem estímulos visuais dos seis principais tipos básicos de emoções da série de Ekman e Friesen (1976): alegria, surpresa, medo, tristeza, nojo, e raiva, assim como outras faces neutras. As faces são apresentadas uma de cada vez, por 5 segundos cada uma, seguidas por uma tela em branco. O participante é solicitado a decidir qual das emoções nomeia

melhor ou mais bem descreve a expressão facial mostrada (felicidade, tristeza, surpresa, aversão, raiva, e medo). Os nomes destas seis emoções são visíveis na tela do computador durante todo o teste, a ordem em que os nomes das emoções são mostrados na tela é distribuída aleatoriamente a cada vez que o teste é aplicado. Antes de começar o teste, o aplicador deve certificar-se que seu participante compreendeu os significados das palavras que nomeiam as emoções com suficiente exatidão para que os resultados sejam significativos (por exemplo, pedindo exemplos das circunstâncias em que as pessoas experimentariam o medo, se irritariam, se repugnariam etc). O teste não é cronometrado – os participantes podem utilizar o tempo que desejarem após os 5 segundos em que a face aparece para decidir a emoção. O teste envolve um bloco de prática de 30 experimentações, seguido por 5 blocos de teste de 30 experimentações cada um. Em cada bloco das experimentações as 30 imagens são apresentadas uma vez cada uma, em ordem aleatória. Os dados do bloco de prática das experimentações não são analisados. Os dados dos 5 blocos de 30 experimentações são analisados pelo programa e podem ser representados na forma de gráfico ou numericamente.

- Avaliação da Criatividade - Barron-Welsh Art Scale (Welsh, 1949; Barron and Welsh, 1952) e Revised Art Scale (RAS): consiste em um teste autoaplicável no qual o sujeito avaliado é orientado a observar 86 figuras abstratas e classificá-las como algo do seu gosto ou não. O teste tem a capacidade de quantificar e agrupar os indivíduos em níveis de criatividade (Barron, 1963).

6.1.3. Extração de DNA

Cerca de 7-10 mL de sangue periférico coletados via punção venosa do antebraço foram destinados à extração do DNA genômico, obtido a partir dos leucócitos, e utilizando-se protocolo baseado em *salting-out* (Laitinen et al., 1994).

6.1.4. Análise dos Polimorfismos

Os pacientes foram genotipados para os polimorfismos de nucleotídeo único do *BDNF* (Val^{66}Met), *COMT* (rs4680; rs165599), *APOE* (rs429358 e rs7412) e *CACNA1C* (rs1006737). Os alelos foram identificados por discriminação alélica no aparelho de PCR em tempo real 7500 R*eal-Time* PCR *System* (A*pplied Biosystems,* Foster City, CA, USA) com o sistema TaqMan®.

6.1.5. Medida da Oxidação do DNA

A oxidação do DNA foi avaliada com base na medida da oxidação da base guanosina via níveis de 8-OHdG e da base citosina via 5-HMec. Os dois biomarcadores foram medidos com o teste de ELISA da *Stress Marq Biosciences Inc*. (Victoria, BC, Canada) e *Epigentek Group Inc*. (Farmingdale, NY, USA), respectivamente (Shen et al., 2007).

7. SUJEITOS

7.1. TB Tipo I Sintomáticos

Oitenta e cinco pacientes com TB foram entrevistados; deste total 1 retirou o Termo de Consentimento Livre e Esclarecido (TCLE), pois a família não concordou com sua participação no estudo e 5 pacientes estavam com sintomas maníacos muito intensos ao ponto de não terem condições de responderem toda a bateria de testes neuropsicológicos. Setenta e nove pacientes com diagnóstico de TB tipo I de acordo com o DSM-IV-TR, ambulatoriais ou internados, que preencheram os critérios que serão apresentados a seguir, foram incluídos. Todos os pacientes sintomáticos estavam sem uso de medicações no momento da avaliação (abrindo exceção para o uso de lorazepam para insônia na dose máxima de 1mg/dia), devido à retirada gradual total da medicação pré-inclusão no ensaio clínico "Licaval" (Campos et al., 2010). O ensaio clínico duplo cego randomizado "Licaval" visou estudar a eficácia da associação de lítio mais valproato *versus* lítio mais carbamazepina em pacientes com TB tipo I em mania, depressão ou estado misto (DSM-IV).

7.1.1. Critérios de Inclusão

- Diagnóstico de TB I segundo o DSM-IV-TR, apresentando-se em fase de humor baseado na avaliação clínica e confirmado pela entrevista clínica estruturada SCID-P (First et al.; 1996);
- Idade entre 18 e 35 anos;
- Alfabetizados e com capacidade de compreensão das tarefas solicitadas;
- Os pacientes e/ou representantes legais devem compreender a natureza do estudo e assinar o TCLE.

7.1.2. Critérios de Exclusão

- Foram excluídos pacientes com Esquizofrenia ou Transtorno Esquizoafetivo, diagnosticados por meio de entrevista clínica estruturada SCID-P (First et al.; 1996);
- Também foram excluídos pacientes portadores de doenças graves, instáveis, incluindo doença renal, gastroenterológica, respiratória, cardiovascular, endocrinológica, neurológica, imunológica ou hematológica.

7.2. TB Tipo I Eutímicos

Trinta e sete pacientes com TB tipo I, eutímicos e medicados, com idade entre 18-35 anos, foram incluídos.

7.2.1. Critérios de Inclusão

- Diagnóstico de TB I segundo o DSM-IV-TR, apresentando-se fora de fase de humor e sem mudança de humor há pelo menos 1 mês, baseado na avaliação clínica e confirmado pela entrevista clínica estruturada SCID-P (First et al., 1996);
- YMRS < 7 e HDRS < 8;
- Idade entre 18 e 35 anos;
- Alfabetizados e com capacidade de compreensão das tarefas solicitadas;
- Os pacientes e/ou representantes legais precisavam compreender a natureza do estudo e assinar o TCLE.

7.2.2. Critérios de Exclusão

- Foram excluídos pacientes com Esquizofrenia ou Transtorno Esquizoafetivo, com base na entrevista SCID-P (First et al.; 1996);
- Pacientes portadores de doenças graves, instáveis, incluindo doença renal, gastroenterológica, respiratória, cardiovascular, endocrinológica, neurológica, imunológica ou hematológica (avaliados através de história clínica e exames laboratoriais na consulta de triagem) também foram excluídos.

7.3. Controles sem Patologia Psiquiátrica

Foram incluídos 79 controles normais, os quais foram convidados através de mídia digital, desde que preenchessem os seguintes critérios:

- Idade entre 18-35 anos;
- Ausência de histórico de doenças psiquiátricas de transtorno de humor ou psicótico atual, ou ao longo da vida [*The Mini International Neuropsychiatric Interview* (MINI)] (Sheehan et al., 1998);
- Ausência de história psiquiátrica de transtorno de humor ou psicose ao longo da vida, ou atual, em familiares de primeiro grau, via dado obtido verbalmente do voluntário;
- Ausência do uso, nos últimos três meses, de medicação psiquiátrica ou abuso de drogas ilícitas ou de álcool nas ultimas 72 horas, via dado obtido verbalmente do voluntário;

8. ANÁLISE ESTATÍSTICA

O equilíbrio de Hardy–Weinberg foi calculado antes das análises e a partir disso verificou-se que a frequência tanto alélica quanto genotípica eram constantes. Os sujeitos foram divididos em grupos de acordo com a presença ou ausência dos alelos que potencialmente modificam a cognição do *BDNF* (Met), *COMT* (Met), *APOE* (ε4) ou *CACNA1C* (Met). A análise da covariância multivariada (MANCOVA) foi empregada usando o seguinte modelo: testes neuropsicológicos foram categorizados como variáveis dependentes, e idade, gênero, educação, episódio de humor patológico patológico, alelo de risco, interação do alelo de risco com episódio de humor foram categorizados como cofatores. As correções foram feitas por meio do modelo de correção por múltiplas comparações de Bonferroni pós-teste. Utilizou-se, sempre, um nível de significância de 5%. As análises foram conduzidas utilizando-se o programa SPSS 20.0 para Mac.

9. RESULTADOS

9.1. Produção Científica Publicada

1: **Soeiro-de-Souza MG;** Machado-Vieira R; Soares Bio D; Do Prado CM; Moreno RA. "*COMT* polymorphisms as predictors of cognitive dysfunction during manic and mixed episodes in bipolar I disorder". Bipolar Disord. 2012 Aug; 14(5):554-64. doi: 10.1111/j.1399-5618.2012.01030.x.

FATOR DE IMPACTO (2012) 5.28

2: **Soeiro-de-Souza MG**, Post RM, de Sousa ML, Missio G, do Prado CM, Gattaz WF, Moreno RA, Machado-Vieira R. "Does *BDNF* genotype influence creative output in bipolar I manic patients?" J Affect Disord. 2012 Jul; 139(2):181-6. doi: 10.1016/j.jad.2012.01.036.

FATOR DE IMPACTO (2012) 3.51

3: **Soeiro-de-Souza MG**, Otaduy MC, Dias CZ, Bio DS, Machado-Vieira R, Moreno RA. "The impact of the *CACNA1C* risk allele on limbic structures and facial emotions recognition in bipolar disorder subjects and healthy controls". J Affect Disord. 2012 Dec 1; 141(1):94-101. doi: 10.1016/j.jad.2012.03.014.

FATOR DE IMPACTO (2012) 3.51

4: **Soeiro-de-Souza MG**, Bio DS, David DP, Rodrigues dos Santos D Jr, Kerr DS, Gattaz WF, Machado-Vieira R, Moreno RA. "*COMT* Met (158) modulates facial emotion recognition in bipolar I disorder mood episodes". J Affect Disord. 2012 Feb; 136(3):370-6. doi: 10.1016/j.jad.2011.11.021.

FATOR DE IMPACTO (2012) 3.51

5: **Soeiro-de-Souza MG**, Bio DS, Dias VV, Vieta E, Machado-Vieira R, Moreno RA. "The *Cacna1c* Risk Allele Selectively Impacts on Executive Function in Bipolar Type I Disorder" (*in press* Acta Psychiatrica Scandinavica - ACP-2012-3313)
FATOR DE IMPACTO (2012) 4.22

6: **Soeiro-de-Souza MG**, Andreazza AC, Carvalho AF, Machado-Vieira R, Young LT, Moreno RA. "Number of manic episodes is associated with elevated dna oxidation in bipolar I disorder" (*in press* International Journal of Neuropsychopharmacology - IntJNP-12-0199)
FATOR DE IMPACTO (2012) 4.57

7: **Soeiro-de-Souza MG;**, Dias VV, Figueira ML, Forlenza OV, Gattaz WF, Zarate CA Jr, Machado-Vieira R. "Translating neurotrophic and cellular plasticity: from pathophysiology to improved therapeutics for bipolar disorder". Acta Psychiatr Scand. 2012 Nov; 126(5):332-41. doi: 10.1111/j.1600-0447.2012.01889.x.
FATOR DE IMPACTO (2012) 4.22

8: **Soeiro-de-Souza MG**, Dias VV, Bio DS, Post RM, Moreno RA. "Creativity and executive function across manic, mixed and depressive episodes in bipolar I disorder". J Affect Disord. 2011 Dec; 135(1-3):292-7. doi:10.1016/j.jad.2011.06.024.
FATOR DE IMPACTO (2012) 3.51

9.2. Dados Gerais não Publicados: Características Clínicas e Comparação entre os Grupos nos Testes Empregados

No grupo de pacientes com TB, 45 indivíduos foram avaliados durante um episódio de mania (YMRS média 18.1), 34 foram avaliados durante um episódio depressivo (HDRS média 21) e 37 foram avaliados fora de episódio de humor patológico (YMRS média 2.4 e HDRS média 4.1). Deste grupo 32% eram homens e 68% eram mulheres. A idade média foi de 28.5 anos, o tempo médio de anos de estudo foi de 12.3, o QI médio

era de 96.05. O tempo médio de duração da doença era de 5.9 anos (±5), o número médio de episódios maníacos ao longo da vida foi de 4.2 (±2.1) enquanto que o número de episódios depressivos ao longo da vida foi de 3.5 (±1.5). Ressalta-se que, apesar do fato de que está equipe tenha estudado uma amostra de sujeitos jovens com TB, a duração média da doença era de 6 anos com uma média de episódios maníacos superior a de episódios depressivos. Outra característica dessa amostragem foi que os sujeitos avaliados durante o episódio de mania apresentavam pontuações moderadas na YMRS, provavelmente pelo desenho do estudo que exigia o uso apenas do lorazepam pelo menos nas duas semanas anteriores a inclusão. A baixa média na YMRS ocorreu pois os pacientes com quadros maniformes mais intensos não conseguiam finalizar a bateria neuropsicológica e foram excluídos do estudo. Por outro lado, os sujeitos em episódio depressivo não enfrentaram dificuldade para responder a bateria neuropsicológica, mesmo quando a intensidade dos sintomas era maior.

O grupo controle compreendia 97 indivíduos, sendo 49% do sexo masculino e 51% do sexo feminino. A idade média foi de 24.4, a média de anos estudados foi de 13.8 e o QI médio era de 110.4.

A performance nos testes neuropsicológicos, como esperado, diferiu bastante entre os grupos de sujeitos estudados, principalmente no funcionamento executivo. No SCWT os pacientes em mania apresentaram os piores resultados (mais tempo) quando comparados os quadros de depressão, eutimia e controles. O mesmo foi observado no WCST-PR. No WCST-CONC os controles apresentaram desempenho semelhante ao grupo depressão e eutimia, e o grupo com pior desempenho foi aquele com os indivíduos com quadro de mania. No TMT os eutímicos tiveram desempenho semelhante ao grupo depressão e mania, mas todos foram piores que o grupo controle. No WAIS-DS os controles apresentaram desempenho semelhante aos deprimidos, mas superior ao grupo eutímico e mania. Nos testes de memória (RCFT, WAIS-LNS, WMS-LM) os controles foram mais uma vez superiores aos demais grupos, sendo que entre os bipolares não se observou diferença de performance entre os grupos mania, eutimia e depressão. Na fluência verbal o único grupo que diferiu em performance dos demais foi o grupo mania com pior desempenho. E, para finalizar, os controles apresentaram maior QI médio que os demais grupos, porém não houve diferença de QI entre os grupos de TB.

Em relação aos testes de reconhecimento de emoções faciais, foi observado um escore médio menor em todos os grupos bipolares em comparação aos controles. O

mesmo se observou no reconhecimento de faces de medo. Dentre as seis emoções faciais estudadas, observou-se uma diferença maior entre os grupos apenas no reconhecimento das faces de surpresa. Neste tipo de face os controles não diferiram dos eutímicos, porém reconheceram mais faces de surpresa do que pacientes em depressão ou em mania. Não houve diferença de reconhecimento de emoções faciais entre os grupos mania e depressão.

Na testagem da criatividade feita pela escala de BWAS verificou-se que todas as fases de humor do TB apresentaram maior pontuação do que os controles. Pode-se notar que os escores de criatividade não diferiram entre mania e eutimia e em ambos os grupos o escore foi superior ao apresentado pelo grupo depressão.

9.3. Dados Específicos não Publicados

9.3.1. BDNF

O alelo Met do *BDNF* demonstrou influenciar negativamente a cognição do grupo controle em testes de funcionamento executivo e inteligência (WASI-DS p=0.03; WASI-VOC p=0.01 e QI p=0.03). O alelo Met do *BDNF* não foi associado a nenhum tipo de desempenho específico nos testes de reconhecimento de emoções faciais ou criatividade.

Nos grupos de sujeitos com TB, a presença do alelo Met não influenciou o desempenho cognitivo na mania ou eutimia, porém no grupo depressão sua presença se associou a um pior desempenho no QI (p=0.03) e na velocidade psicomotora medida pelo TMT-A (p=0.04).

9.3.2. APOE

A presença do alelo de risco da *APOE* ε4 não se associou ao desempenho

cognitivo, reconhecimento de emoções faciais ou criatividade em nenhum dos grupos estudados.

9.3.3. COMT

A presença do alelo Met do *COMT* se associou a um maior escore na escala de criatividade no grupo controle. Nenhum efeito do alelo de risco Met do *COMT* foi observado no grupo com TB.

9.3.4. CACNA1C

A presença do alelo de risco Met do *CACNA1C* não influenciou os escores de criatividade em nenhum dos grupos estudados.

9.3.5. 8-OHdG e 5-HMec

Os níveis de 8OHdG ou de 5-HMec não demonstraram influenciar qualquer teste da bateria neuropsicológica, criatividade ou reconhecimento de emoções faciais em pacientes com TB. Em controles, os níveis de 8OHdG se associaram a um maior tempo no TMT-A ($p=0.02$), mas não influenciaram escores de reconhecimento de emoções faciais ou criatividade. Observou-se uma modulação dos níveis de 8-OHdG em indivíduos com TB na presença do alelo Met do *CACNA1C* ($p=0.01$).

10. DISCUSSÃO E CONCLUSÕES

10.1. BDNF

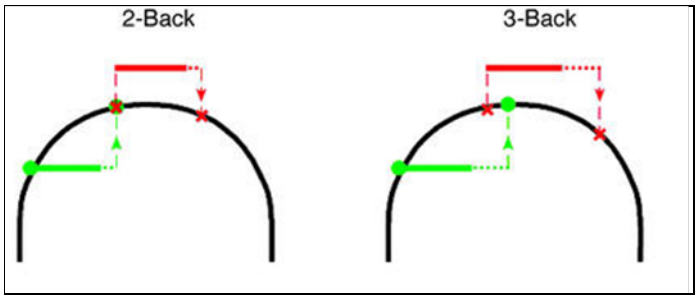
O *BDNF*, através da análise de seu polimorfismo funcional rs6265, não demonstrou ter um efeito consistente na modulação da cognição nas fases de humor do TB ou eutimia. Por outro lado, descreveu-se, pela primeira vez na literatura, um efeito do alelo de risco do *BDNF* na criatividade durante episódio de mania. Os dados do *BDNF* rs6265 observados na cognição de controles (influência negativa) estão de acordo com a literatura vigente que já demonstrou o efeito negativo do alelo Met na cognição de pessoas saudáveis. Assim sendo, pode-se considerar que esta neurotrofina não participa diretamente da modulação da disfunção cognitiva decorrente do TB, porém foi replicado o dado de que o alelo Met influencia o funcionamento cognitivo de pessoas normais.

10.2. COMT

A análise do polimorfismo funcional da *COMT* rs4680 forneceu a maior parte dos dados positivos dessa tese. Funcionamento executivo, memória, inteligência e reconhecimento de emoções faciais foram de alguma forma associados a desempenhos específicos pela presença ou ausência do alelo Met. Os dados obtidos com essa pesquisa revelaram que o alelo Met, associado, até então, na literatura a melhora do desempenho em algumas provas de funcionamento executivo, durante as fases do TB, apresenta um fenótipo distinto.

Principalmente durante a mania (e quadros mistos) observou-se que portadores desse alelo apresentavam pior desempenho em provas de inteligência, funcionamento executivo e memória. A explicação para compreender tal dado originou-se do modelo farmacológico do “U” invertido que descreve a ação das anfetaminas na cognição

humana (Mattay et al., 2003) (Figura 1).



FONTE: Adaptado de Mattay et al. 2003 (direitos autorais Academia Nacional de Ciências dos Estados Unidos).
Figura 1 - Modelo teórico do "U-invertido" descrevendo o desempenho no teste dos dígitos inversos (2-Back e 3-Back) antes e depois da administração de anfetamina em portadores do genótipo COMT rs4680 Val/Val (em verde) comparados a portadores do genótipo Met/Met (em vermelho).

Segundo tal modelo, essa droga dopaminérgica em pequenas doses pode incrementar o funcionamento cognitivo, entretanto, após ultrapassado um limiar de dose, ela passa a prejudicar a cognição. Partindo do princípio da existência de indivíduos com funcionamentos distintos do catabolismo da dopamina no córtex pré-frontal a partir da descrição do polimorfismo funcional da *COMT*, desenvolveu-se o seguinte modelo: Indivíduos portadores desse alelo, quando em vigência de um episódio potencialmente hiperdopaminérgico como a mania ou um quadro misto, ultrapassam o limiar de funcionamento cognitivo, no qual se observa algum benefício desse maior nível teórico de dopamina pré-frontal, e apresentam desempenho inferior quando comparados aos não portadores de Met (Soeiro de Souza, Machado-Vieira, et al., 2012b). Obviamente que para completar este modelo é necessário retestar os mesmos indivíduos fora de fase e em outras fases, além de aumentar consideravelmente a amostra. O tamanho pequeno da amostra usada neste estudo pode ser um dos motivos para não ter se observado nem em controles ou bipolares eutímicos a já descrita melhor performance dos indivíduos Met em alguns testes (Savitz et al., 2006; Burdick et al., 2007). Vale mencionar que os resultados de reconhecimento de emoções faciais diferem um pouco dos resultados de funcionamento cognitivo, até porque este estudo não considera que tal habilidade seja um domínio cognitivo. No reconhecimento de emoções faciais o prejuízo observado nos portadores do alelo Met não é específico para os episódios hiperdopaminérgicos (Soeiro de Souza, Bio, et al., 2012a). Isso sugere que o mínimo incremento de dopamina no córtex pré-frontal, por meio de uma menor catabolização descrita nos portadores do alelo Met, já seria suficiente para prejudicar tal

habilidade. Assim sendo, pode-se afirmar que provavelmente o reconhecimento de emoções faciais apresenta um limiar para melhora do funcionamento inferior aquele limiar referente aos domínios cognitivos ou talvez nem se beneficie da presença do estímulo dopaminérgico (Soeiro de Souza, Bio, et al., 2012a). Com base nos dados obtidos com essa pesquisa, é possível considerar o alelo Met do *COMT* como um alelo de "risco cognitivo". O reconhecimento de emoções faciais faz parte do conceito mais amplo de cognição social e teoria da mente, desta maneira, o fato de um indivíduo não ser capaz de reconhecer emoções básicas na face alheia acarreta um prejuízo social importante, potencialmente causador de desentendimentos. Dessa forma, seria possível classificar o alelo Met do *COMT* rs4680 também como um alelo de "risco social".

Os dados aqui obtidos referentes ao *COMT* fornecem a base para uma ampla pesquisa sobre o papel da dopamina na cognição e no reconhecimento de emoções faciais no TB. Obviamente, após a replicação destes dados em amostras maiores, deverá se investigar se tais indivíduos com o alelo de "risco cognitivo e social" poderiam se beneficiar do uso de medicações que possam aumentar a atividade do COMT. Tal intervenção poderia teoricamente atenuar o prejuízo cognitivo destes pacientes, especialmente dos homozigotos para Met, durante os episódios de humor ou talvez até mesmo melhorar o funcionamento social dos mesmos em todas as fases, por meio de um melhor reconhecimento de emoções faciais observado em indivíduos sem o alelo Met (Val/Val). Apesar destas hipóteses, reconhece-se que tanto a cognição como a habilidade de reconhecer as emoções faciais apresentam um funcionamento bem mais complexo e envolvem muitos outros neurotransmissores além da dopamina. De qualquer forma, acredita-se que pelo menos parte da complexidade que envolve o funcionamento destas habilidades parece ser influenciada pela dopamina e isso deveria ser mais explorado visando uma melhor compreensão do *déficit* nestas habilidades observado no TB. Por fim, há necessidade de estudos prospectivos com indivíduos homozigotos para o alelo de risco, a fim de verificar se existe associação entre a disfunção pontual destas habilidades e a perda cognitiva decorrente da idade e da evolução da doença.

10.3. APOE

O alelo de risco cognitivo ε4 não demonstrou influência na cognição, reconhecimento de emoções faciais ou criatividade de sujeitos com TB ou de controles. Considerando que o ε4 é de baixa prevalência na população, estudos com maiores amostras devem continuar investigando se tal alelo de risco influencia de alguma forma a cognição de pacientes com TB, como já foi descrito em controles e DA.

10.4. CACNA1C

O alelo Met do SNP rs1006737 da *CACNA1C*, conforme anteriormente dito, tem sido associado a um maior risco de TB (Sklar et al., 2008; Ferreira et al., 2008; Y. Liu et al., 2011; Psychiatric GWAS Consortium Bipolar Disorder Working Group et al., 2011), e a amostra utilizada neste estudo revelou dados significativos com relação ao COMT no que se refere a cognição e ao reconhecimento de emoções faciais.

Observou-se que o grau de disfunção no reconhecimento de emoções faciais em bipolares eutímicos é altamente relacionado ao número de alelos Met do *CACNA1C*, ao passo que nos controles nada foi observado (Soeiro de Souza, Otaduy, et al., 2012c). Os indivíduos homozigotos apresentaram os resultados mais inferiores, seguidos dos heterozigotos com performance intermediária e por fim os não portadores de Met (Val/Val), os quais apresentaram melhor desempenho. Tal associação foi tão ampla que acometeu 4 das 6 emoções básicas testadas. Isso foi descrito pela primeira vez na literatura, considerando que artigos relacionados a *CACNA1C* e emoções faciais se referiam apenas à ativação de estruturas cerebrais frente a um determinado estímulo facial (Jogia et al., 2011).

A análise do efeito do alelo de risco do *CACNA1C* na cognição foi mais ampla e incluiu pacientes em todas as fases da doença além da eutimia e revelou uma disfunção executiva importante (WAIS-LNS, WAIS-DS, TMT e WCST) entre os homozigotos para o alelo de risco e os homozigotos Val (Soeiro-de-Souza, Bio, et al., 2013b). Tais

dados revelam a influência dos canais de cálcio na modulação da cognição e do reconhecimento de emoções faciais no TB. Por outro lado, a falta de uma descrição clara da funcionalidade do SNP rs1006737 deixa em aberto via qual mecanismo específico ocorre tal efeito. O que se sabe é que o alelo de risco se associa a uma maior expressão do RNA-mensageiro no cérebro humano (Bigos et al., 2010), entretanto não se sabe se essa maior expressão resulta em uma alteração na função do canal de cálcio. Teoricamente se essa maior expressão levasse a uma maior atividade do canal, provocando um maior influxo celular de Ca^{2+}, haveria uma maior ativação da CREB e, por consequência, uma maior transcrição de uma série de genes (Wu et al., 2001; Soeiro-de-Souza et al., 2012). Os estudos anteriores relataram a ocorrência, em indivíduos saudáveis, de efeito deletério do alelo Met principalmente na atenção e orientação (Roussos et al., 2011; Thimm et al., 2011).

Observou-se também uma diferença entre o efeito do genótipo do *CACNA1C* na cognição e aquele observado no reconhecimento de emoções faciais, mais uma vez fortalecendo a hipótese de que são habilidades bem distintas. No reconhecimento de emoções observou-se uma graduação do efeito do *CACNA1C* proporcional ao número de cópias do alelo de risco, demonstrando uma maior sensibilidade a esse sistema (Soeiro de Souza, Otaduy, et al., 2012c). No funcionamento cognitivo a diferença foi observada apenas entre os homozigotos para Met e os homozigotos Val (Soeiro-de-Souza, Bio, et al., 2013b).

Da mesma forma já relatada no COMT, há a necessidade de estudos prospectivos com indivíduos homozigotos para o alelo de risco visando estudar se existe associação entre a disfunção pontual destas habilidades e a perda cognitiva decorrente da idade e da evolução da doença. Os dados, obtidos com este estudo, a respeito do papel dos canais de cálcio na cognição e reconhecimento de emoções faciais, levantaram a questão da possibilidade de uma intervenção farmacológica atenuar tais *déficits*. Por exemplo, sugiro que seria oportuno investigar a ação dos antagonistas dos canais de cálcio tipo L (verapamil, diltiazem e nifedipina) na cognição de indivíduos com TB homozigotos para Met, apesar da ação de tais medicações ser mais estudada quando se trata do sistema cardiovascular.

10.5. 8-OHdG e 5-HMec

O estudo do estresse oxidativo via 8-OHdG e 5-HMec nesta tese revelou dados importantes a respeito de um potencial dano progressivo decorrente da evolução do TB (Soeiro-de-Souza, Andreazza, et al., 2013a). Foram verificados níveis elevados de oxidação da guanosina em TB quando comparados com controles, entretanto não se observou diferença nos níveis de oxidação da citosina entre os grupos. Além disso, constatou-se que o nível de oxidação das bases de DNA guanosina era influenciado positivamente pelo número de episódios maníacos ao longo da vida. Isso revelou uma especificidade do dano oxidativo das bases de guanosina no TB e forneceu um dado importante acerca da consequência danosa dos episódios maníacos para o DNA.

Estudos sugerem que a guanosina parece ser a base com maior propensão a sofrer dano oxidativo (Clark et al., 2002; Altieri et al., 2008; Gutteridge & Halliwell, 2000; Kryston et al., 2011; Cavanagh et al., 2002; Radak & Boldogh, 2010; Ferrier & Thompson, 2002; Spassky & Angelov, 1997), uma vez que as ROS reagem com a base guanosina formando 8-OHdG (Berk, 2009; Coryell et al., 2001; Swann et al., 2000; Guo et al., 2011; Robinson & Ferrier, 2006; e Kauer-Sant'Anna et al., 2009). A consequência disso é a possibilidade de múltiplas formas de danos ao DNA, incluindo modificações de bases, deleções, rearranjos cromossômicos e até rupturas de filamentos de DNA (Valko et al., 2004; Valko et al., 2006). Isso pode ser uma das explicações para o fato de alguns pacientes com TB apresentarem um grande número de comorbidades clínicas (Kupfer, 2005). Da mesma forma, tais dados reforçam a hipótese de que o TB é uma doença que possui um dano progressivo ao organismo, por se tratar de um mal crônico e principalmente devido ao número de episódios maníacos ao longo da vida.

Além disso, verificou-se uma modulação positiva dos níveis de 8-OHdG na presença do alelo Met *CACNA1C*, que poderia ser uma primeira ponte entre um alelo de risco cognitivo e um maior dano oxidativo ao DNA. Tal dado será mais estudado em futuros trabalhos desta equipe.

Em suma, esta tese propôs que o catabolismo da dopamina no córtex pré-frontal e alterações nos canais de cálcio de baixa voltagem (tipo L) fazem parte da fisiopatologia do *déficit* cognitivo e de reconhecimento de emoções faciais observado no TB. Sugere-se, portanto, que indivíduos portadores destes alelos “de risco cognitivo”, especialmente

homozigotos, sejam alvos de futuros estudos visando esclarecer se tal grupo pode ser considerado de risco para disfunção cognitiva e de reconhecimento de emoções faciais. Dessa forma, seria possível traçar estratégias personalizadas de tratamento e prevenção baseadas no perfil genético de pacientes com TB.

10.6. Modelo Proposto

Ao se integrarem os resultados acerca da influência negativa do alelo Met do *COMT* e do alelo Met do *CACNA1C* na cognição e no reconhecimento de emoções faciais, propõe-se o seguinte modelo: na presença do alelo Met do *COMT* rs4680 ocorreria uma menor catabolização de DA no córtex pré-frontal, o que pode hipoteticamente levar a uma maior quantidade de DA na fenda sináptica (Egan et al. 2001), tendo como consequência um maior estímulo dos receptores D1 (Rankin et al. 2010). Além disso, sabe-se através de modelos animais que um maior agonismo com os receptores D1 pode levar a um aumento do cálcio intracelular por liberação de depósitos de inositol 1, 4, 5- trifosfato (Lezcano & Bergson 2002) e maior atividade dos canais de cálcio voltagem dependentes tipo 1.2 (Liu et al. 1992; Sumeier et al. 1995). Por fim, sabe-se que o estímulo dos canais de cálcio voltagem dependentes tipo 1.2 leva a um maior influxo de cálcio para dentro das células e isso pode acarretar uma maior liberação de DA (Okita et al. 2000). Dessa forma, indivíduos homozigotos para o alelo Met do rs4680 e do rs1006737 podem ser considerados como portadores, em sua base genética, de um duplo estímulo para maior quantidade de DA na fenda sináptica. Assim sendo, supõe-se que tais pessoas, quando em estado de humor maníaco, apresentariam-se com pelo menos 3 formas patofisiológicas que poderiam contribuir para um aumento de DA na fenda sináptica. Tal modelo permite propor que indivíduos homozigotos para

ambos alelos de risco do *CACNA1C* e do *COMT* sejam considerados como pertencentes a um grupo de risco cognitivo agudo durante as fases hiperdopaminérgicas do TB.

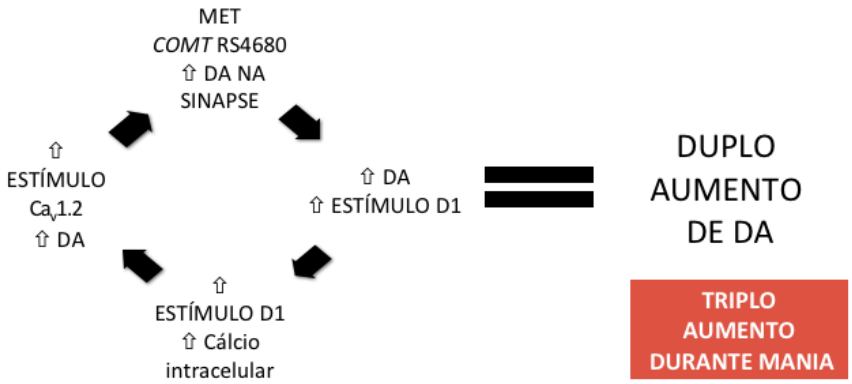


Figura 2- Proposta: Modelo de duplo estímulo para aumento de DA na fenda sináptica em indivíduos homozigotos para *COMT* rs4680 e *CACNA1C* Met.

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12. ANEXOS: ARTIGOS PUBLICADOS

12.1. COMT polymorphisms as predictors of cognitive dysfunction during maniac and mixed episodes in bipolar I disorder

BIPOLAR DISORDERS
AN INTERNATIONAL JOURNAL OF PSYCHIATRY AND NEUROSCIENCES

Bipolar Disorders 2012: 14: 554–564

BIPOLAR DISORDERS

Original Article

COMT polymorphisms as predictors of cognitive dysfunction during manic and mixed episodes in bipolar I disorder

Soeiro-de-Souza MG, Machado-Vieira R, Soares Bio D, Do Prado CM, Moreno RA. COMT polymorphisms as predictors of cognitive dysfunction during manic and mixed episodes in bipolar I disorder. Bipolar Disord 2012: 14: 554–564. © 2012 The Authors. Journal compilation © 2012 John Wiley & Sons A/S.

Objective: The dopaminergic system plays an important role in the prefrontal cortex (PFC) and is believed to mediate cognitive dysfunction (CD) in bipolar disorder (BD). The enzyme catechol-*O*-methyltransferase (COMT) is involved in the catabolism of dopamine in the PFC, and an association between COMT single nucleotide polymorphisms (SNPs) and BD has been reported. COMT SNPs have also been associated with executive and working memory performance in healthy subjects, patients with schizophrenia, and euthymic BD patients. The objective of this study was to investigate the association between COMT SNPs and acute CD during BD mood episodes.

Methods: Seventy-two symptomatic, medication-free subjects with bipolar I disorder (BD-I) and 76 healthy controls were evaluated using neuropsychological tests, and genotyped for COMT SNPs rs4680 and rs165599.

Results: Patients undergoing mania and mixed episodes carrying the COMT allele G had better performance on executive function, memory, verbal fluency, and intelligence tests. Moreover, an interaction was detected between the COMT allele G and the Young Mania Rating Scale in BD CD.

Conclusions: Allele G from COMT SNPs rs4680 and rs165599 may represent reliable state-dependent predictors of global CD during manic and mixed episodes in BD. Further studies in larger samples are necessary to confirm these findings.

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doi: 10.1111/j.1399-5618.2012.01030.x

Key words: bipolar disorder – catechol-*O*-methyltransferase – cognition – depression – dopamine – mania

Received 16 May 2011, revised and accepted for publication 23 March 2012

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Cognitive dysfunction (CD) is a common feature of bipolar disorder (BD) (1–4). The CD in subjects with BD is known to be influenced by the type of mood episode, severity of symptoms (5, 6), history of psychotic symptoms (7–11), number of previous manic episodes (12, 13), and use of medications (14, 15). CD aggregates in familial BD, suggesting an endophenotype that underlies genetic predisposition to both CD and BD (16), although little is known about the specific mechanisms involved. Recently, a single nucleotide polymorphism (SNP) of catechol-*O*-methyltransferase (COMT), an important regulator of prefrontal cortex (PFC) dopamine (DA), norepinephrine, and epinephrine levels (17), was shown to regulate cognitive function (18, 20), but its association with CD during mood episodes in BD has yet to be investigated.

Proposed CD mechanisms in BD involve DA metabolism in the PFC (21, 22). Dopaminergic pathways project into numerous brain areas

implicated in the pathophysiology of BD (23). Historically, dopaminergic models of BD have been dichotomous and support both DA excess in mania and deficiency in depression (22). However, most of these models were conceptualized, based on indirect evidence from pharmacological and animal studies (23). Studies investigating the cognitive effects of DA in the PFC have tended to focus on DA receptor 1 (D1), the predominant receptor type in the PFC. Insufficient (hypodopaminergic) or excessive (hyperdopaminergic) D1 receptor stimulation has been reported to impair PFC function (24–26), leading to CD. Therefore, PFC modulatory effects on cognition are believed to depend on an optimal level of DA to achieve normal function (27, 28). In pharmacological studies, these kinetics have been described by an inverted-U response function model (18, 27, 29). Under this model, the effects of amphetamine and other dopaminergic drugs on cognition are described as an inverted 'U'-shaped model, in which the peak is associated with better cognitive performance, with subsequent decline thereafter. This optimal threshold varies across cognitive domains and is modulated by baseline dopaminergic levels (18). BD is the only naturalistic disease model supporting the theory of both hyper- and hypodopaminergic states, in which cognitive disparities among patients in mania or depression are mediated by PFC-induced differences.

COMT is one of the major mammalian enzymes involved in the metabolic degradation of DA, norepinephrine, and epinephrine. It is an Mg^{2+}-dependent enzyme that catalyzes the transfer of methyl groups from S-adenosylmethionine (SAM) to a hydroxyl group of a catechol substrate. Based on this effect, DA is converted into 3-methoxytyramine (17, 30). Given the low levels of dopamine transporter (DAT) in the PFC, COMT plays an active role in the metabolism of DA (31, 32). Genetic studies have reported that COMT activity levels can vary considerably. COMT SNP rs4680 (also known as $Val^{158}Met$) has been reported to lead to a three- to fourfold reduction in COMT enzyme activity in individuals carrying allele A (Met) (30). COMT allele G carriers (Val) have higher enzyme activity, while heterozygous (Val/Met) carriers have an intermediate level of enzyme activity (30, 33, 34). Thus, COMT polymorphism rs4680 is responsible for genetically modulating dopaminergic levels in the PFC and for this reason is a likely candidate to play an important role in the neurobiology of CD observed during longitudinal changes in BD mood.

Given the well-established role of PFC DA in working memory and executive functions (27–29), it is unsurprising that COMT has been shown to regulate cognition (18, 20). Data suggest that the COMT rs4680 polymorphism exerts effects on cognition (35) in subjects with schizophrenia (SZ) and in healthy controls. In both SZ and healthy individuals (36–38), rs4680 allele G has been associated with worse performance in working memory, intelligence, and executive functions (39) compared to allele A. To date, only three studies have evaluated the effect of COMT rs4680 on BD cognition, all describing negative findings (36, 40, 41). Szöke et al. (40) administered attentional and executive functions tests [the Wisconsin Card Sorting Test (WCST) and the Trail Making Test (TMT)] in 94 medicated euthymic BD patients, genotyped for COMT rs4680, and found no association with performance in these tests. Wirgenes et al. (41) studied 87 euthymic BD patients genotyped for 21 COMT SNPs, including rs4680, and reported no differences in cognitive performance between genotypes. Another COMT SNP has been reported to have an effect on cognition. The SNP rs165599 is located in the non-coding region of the COMT gene (3′ untranslated region), and its allele G has been associated with lower relative RNA expression of the COMT protein (42, 43). In contrast to rs4680, the potential influence of COMT SNP rs165599 on enzyme activity remains unclear (44). Burdick et al. (36) evaluated 52 euthymic bipolar I disorder (BD-I) subjects and 102 healthy controls and reported no influence of rs4680 on cognition, but found that COMT rs165599 allele G carriers performed significantly worse than heterozygous and non-carriers on a test of verbal learning and memory (California Verbal Learning Test) compared to allele A in both groups.

The objective of this study was to investigate the influence of the COMT polymorphisms rs4680 and rs165599 on CD in BD-I patients experiencing a manic, mixed, or depressive episode. Given the inverted 'U-shaped' curve model of dopaminergic function and cognitive performance and the lack of studies evaluating the effects of COMT SNPs on cognition during mood episodes, we hypothesized that patients who were homozygous or heterozygous for allele G in a manic or mixed episode would have less CD than G non-carriers.

Material and methods

Subjects

The patient group comprised 72 individuals with BD-I (50 females, 22 males), with a mean age of 28.2 (± 5.4) years, mean schooling of 12.36

(± 3.11) years, and mean IQ of 95.8 (± 13.4). Twenty-two patients were undergoing a manic episode, 21 were in a mixed episode, and 29 were in a depressive episode. None of the patients had psychotic symptoms at the time of neuropsychological evaluation. These patients were participants in the LICAVAL (efficacy and tolerability of the combination of LIthium and CArbamazepine compared to lithium and VALproic acid in the treatment of young bipolar patients) clinical trial (45) and were evaluated immediately after the washout period (at least four weeks for antidepressants, mood stabilizers, or antipsychotic agents, or eight weeks for *depot* medications), prior to commencing use of medications. Diagnoses of BD mood episode were determined by trained psychiatrists based on the Structured Clinical Interview (SCID-I/P) (46) for DSM-IV TR (47). Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, currently abusing any substance, or submitted to electroconvulsive therapy in the preceding six months were excluded. The Young Mania Rating Scale (YMRS) (48), and the Montgomery–Åsberg Depression Rating Scale (MADRS) (49) were used to evaluate the severity of symptoms.

Control group

The control group included 76 individuals (37 females, 39 males), with a mean age of 23.4 (± 3.3) years, mean schooling of 14.1 (± 2.4) years, and a mean IQ of 115.1 (± 11.9) who were recruited by word of mouth at the University of São Paulo. All controls had no psychiatric diagnoses (present or past) according to the evaluation conducted by trained psychiatrists using The Mini International Neuropsychiatric Interview (M.I.N.I.) (50). All controls also had a negative family history of any mood or psychotic disorders (first-degree relatives) and had no recent use of psychotropic medicines or substance abuse over the previous three months.

Neurocognitive assessments

The neurocognitive battery was designed to assess the following domains: **Attention:** Wechsler Adult Intelligence Scale III (WAIS-III) subtest Digit Span [WAIS-DS forward (FW)], TMT – part A (TMT-A), Stroop Color–Word Test (SCWT); **Verbal memory:** Wechsler Memory Scale subtest – Logical Memory (WMS-LM) immediate (1) and delayed (2); **Visual memory:** Rey–Osterrieth Complex Figure Test (RCFT) delayed recall; **Visuospatial function:** Wechsler Abbreviated Scale of Intelligence (WASI) – Block Design (WASI-BD), RCFT-copy; **Language:** Controlled Oral Word Association Test (FAS), WASI–Vocabulary subtest (WASI-V); **Psychomotor speed:** TMT-A; **Executive function:** Letter–Number Sequence (WAIS-LNS), WAIS-DS backward (BK), SCWT, TMT-B, WASI Similarities (WASI-S), WASI Matrix Reasoning (WASI-MR), RCFT-copy, WCST – Conceptual level responses (WCST-CONC), WCST – Perseverative Responses (WCST-PR), WCST – Failure to Maintain Set (WCST-FMS), WCST – Corrected Categories (WCST-CC), WCST – Errors (WCST-E), WCST – Non-Perseverative Errors (WCST-NP), WCST – Perseverative Errors (WCST-P); **Intelligence:** WASI: Total Intelligence Quotient (IQ), Estimated IQ (EIQ), Execution IQ (EXIQ), Verbal IQ (VIQ). These are well-established and validated tests (51–54). Higher scores indicate better performance, with the exception of SCWT, TMT, WCST-PR, WCST-E, WCST-NP, and WCST-P.

Genotyping

Based on previous studies about COMT and cognition, we chose to genotype patients for rs4680 (the result of a guanine to adenine change at nucleotide location 472 in exon 4 in the coding region) and rs165599 (the result of a guanine to adenine change at location 1338 in the 3′ flanking region). DNA was extracted from peripheral blood according to the salting-out protocol (55) and then genotyped for COMT rs4680 and rs165599 using real-time polymerase chain reaction (PCR) allelic discrimination. PCR amplification for rs4680 and rs165599 was performed in 5 µl reactions with 5 ng of template DNA, 1X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA), 1X each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing at 60°C for 1 min. The allele-detection process and allelic discrimination was performed for 1 min at 60°C on a 7500 Real-Time System (Applied Biosystems). Quality control of real-time PCR results was done by direct sequencing on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Ethics

The research ethics board of *Hospital das Clínicas of the University of São Paulo* reviewed and approved the study. Informed consent was obtained from all subjects after a complete description of the study.

Statistical analyses

Subjects were classified into three groups according to type of mood episode (mania, mixed, depression), then into three groups according to COMT genotype (AA, AG, GG) or two groups according to the presence of COMT allele G [G+ (GG and AG) or G− (AA)]. We chose to perform the analysis by allele G because it has been reported to be a risk allele for schizophrenia (20, 56) and BD (36, 57) and also to influence cognitive function (20, 36, 38, 58). Descriptive analyses were carried out to evaluate distributions against the assumptions for each of the proposed analyses. The independent *t*-test and chi-square test were employed to evaluate differences between G+ and G−. A multivariate analysis of covariance (MANCOVA) design using Bonferroni post-hoc adjustments to protect against an inflated risk of type I errors was used to examine cognitive performance by genotype within each mood episode or control group. Finally, MANCOVA was used to evaluate the influence of the following variables on the cognitive performance of BD patients: gender, age, education, YMRS score, MADRS score, number of previous manic episodes, lifetime history of psychotic symptoms, and interaction between allele G*MADRS and allele G*YMRS. For all analyses, statistical significance was set at $p < 0.05$ (or Bonferroni adjusted for multiple comparisons) and partial eta-square effect sizes were calculated. The PASW statistics package, version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

In our sample, the allelic frequency of the rs4680 polymorphism was 48.8% (105) for allele A and 51.1% (110) for allele G. The allelic frequency of rs165599 was 53.5% (113) for allele A and 46.4% (98) for allele G. Both COMT SNPs were in Hardy–Weinberg equilibrium (HWE) (rs4680: $\chi^2 = 0.371$; rs165599: $\chi^2 = 0.143$).

No statistically significant differences ($p > 0.05$) in sociodemographic factor distribution were observed between allele G groups (rs4680 and rs165599) and in the control or BD group. In addition, no significant differences were detected between allele G groups in rs4680 and rs165599 for age, gender, years of schooling, or MADRS and YMRS scores in the BD group (Table 1).

Median scores on symptoms scales in each mood episode group were: mania YMRS 20.0 ± 8.3, MADRS 11.5 ± 7.0; mixed YMRS 15 ± 7, MADRS 22.0 ± 8.6; depression YMRS 7 ± 6, MADRS 24 ± 7.

The comparison between controls and BD patients in cognitive performance showed a global dysfunction in the BD sample in attention, executive function, intelligence, and on memory tests (as specified in the description of the neuropsychological battery).

Within the BD group, after performing tests comparing cognitive performance among mood symptom groups (manic, mixed, and depression group), significant differences in cognitive performance were revealed on WCST-PR [$F(2,67) = 3.62$, $p = 0.032$], WCST-E [$F(2,67) = 3.19$, $p = 0.047$), WCST-P [$F(2,67) = 4.50$, $p = 0.015$], and RCFT-recall [$F(2,67) = 3.69$, $p = 0.03$]. The manic group had the lowest mean scores, followed by the mixed mood group, while the depression group had the best performance among mood episodes across all reported tests. However, after Bonferroni post-hoc analyses, the only sustained differences were observed between the manic group and the depression group on WCST-PR ($p = 0.02$), WCST-P ($p = 0.01$), and RCFT-recall ($p = 0.03$).

In terms of genotype in the control group, no differences were observed in cognitive performance for genotype rs165599 and genotype rs4680. No significant differences emerged from any of the cognitive tests in AA, AG, and GG groups ($p > 0.05$).

Cognitive performance between COMT rs4680 genotypes in the manic group differed on WCST-PR [$F(2, 19) = 3.9$, $p = 0.03$], WCST-FMS [$F(19,2) = 4.23$, $p = 0.03$], WCST-P [$F(19,2) = 6.15$, $p = 0.009$], and FAS-F [$F(19,2) = 5.10$, $p = 0.01$]. In the mixed mood group, differences were observed between genotypes in EIQ [$F(17,2) = 5.96$, $p = 0.01$] and WMS-LM1 [$F(17,2) = 4.0$, $p = 0.03$].

Cognitive performance analysis for genotype rs165599 and rs4680 in the depressive mood group demonstrated no difference between scores in AA, AG, or GG groups ($p > 0.05$). Bonferroni's post-hoc test demonstrated sustained differences among the three genotypes only on WCST-P in the manic mood group, where scores in the AA group were higher than in the AG group, and GG group had the lowest cognitive performance (Fig. 1). In the mixed mood group, the EIQ test was the only measure that showed significant differences, where GG had the highest score, followed by AG and AA.

Significant differences in cognitive performance were also observed between COMT rs165599 genotype groups (AA, AG or GG) in the manic group on RCFT-recall [$F(19,2) = 7.51$, $p = 0.004$]. Differences were present in the mixed group in regard to EXIQ [$F(18,2) = 4.10$, $p = 0.03$] and

Table 1. Comparison of demographic and symptom scale score distribution in bipolar disorder subjects according to COMT allele G

	rs4680				rs165599			
	G+ (n = 54)	G− (n = 18)		Between-group differences	G+ (n = 47)	G− (n = 25)		Between-group differences
Variable	Median	Median	*t* (df)	Sig. (2-tailed)	Median	Median	*t* (df)	Significance (2-tailed)
Age (years)	28.3 (± 5)	27.8 (± 6.7)	0.27	0.78	27.8 (± 5.0)	29.25 (± 6.0)	−0.96	0.34
Gender (men/women)	19/35	3/15	$\chi^2 = 0.173$	0.17	14/33	17/8	$\chi^2 = 0.09$	0.76
Education	12.4 (± 3.3)	12.1 (± 2.3)	0.44	0.65	12.2 (± 3.1)	12.5 (± 3.1)	−0.36	0.71
MADRS	20.4 (± 8.3)	18.7 (± 9.4)	0.67	0.50	19.2 (±7.8)	21.4 (± 10)	−0.91	0.36
YMRS	14.5 (± 7.5)	13.0 (± 10.4)	0.58	0.58	13.6 (±7.9)	15.7 (± 8.5)	−1.04	0.30

COMT = catechol-*O*-methyltransferase; MADRS = Montgomery–Åsberg Depression Rating Scale; YMRS = Young Mania Rating Scale. Significance level: $p < 0.05$.

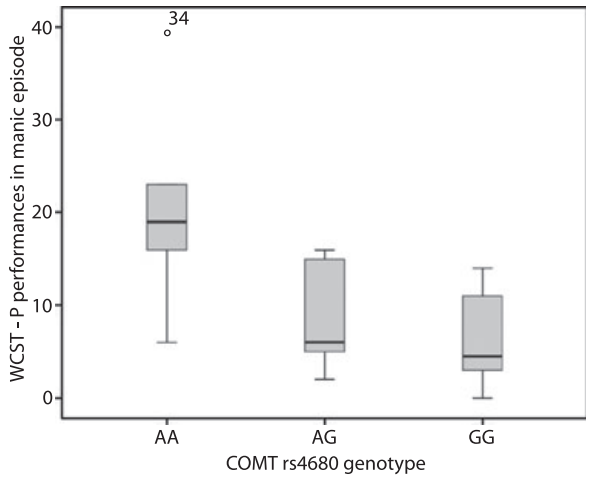

Fig. 1. WCST-P scores in manic patients with the COMT rs4680 genotype, demonstrating the highest perseverative error scores in the AA genotype group, intermediate scores in the AG group, and lowest scores in the GG group.

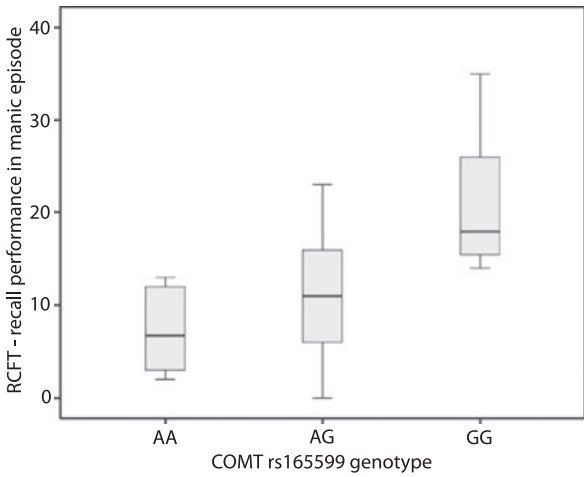

Fig. 2. RCFT-recall performance in manic episode patients with the COMT rs165599 genotype, demonstrating the highest scores in the GG group, intermediate scores in the AG group, and the lowest scores in the AA group.

TMT-B [$F(18,2) = 6.94$, $p = 0.006$]. No differences between genotypes were observed in the depression group ($p > 0.05$). The post-hoc test of RCFT in the manic group revealed that GG had the best performance, followed by AG and AA (Fig. 2), while in the mixed group the performance in TMT-B (sec) had the inverse pattern (AA > AG > GG).

Allele G rs4680 did not influence cognitive performance in healthy controls, while allele G rs165599 demonstrated a negative effect on TMT-B [$t(74) = -2.08$, $p = 0.04$]. Healthy G+ controls took longer than G− to complete TMT-B.

Patients in the manic group that were carriers of rs4680 allele G+ showed better performance than G− on WCST-P and FAS-F (Table 2). Cognitive performance differed in manic group patients for the presence of rs165599 allele G on SCWT-1, SCWT-2, and RCFT-recall. Similarly, G+ performed better on all tests (Table 3). In the mixed mood group, rs4680 G+ performed better than G− on WAISDS-BK, WCST-CONC, WCST-CC, WCST-E, EIQ, FAS-TOTAL, WMS-LM1, and RCFT-copy (Table 2). Analyses of the rs165599 G allele in the mixed group revealed higher performance by G+ on SCWT-2, SCWT-2 errors, WAIS-BD, WAIS-MR, EXIQ, and RCFT-copy (Table 3). The results of allele analysis of both SNPs for cognitive performance in the depressive mood group were not significant on any of the tests (all $p > 0.05$).

After controlling for the effect of covariates on the total BD sample, educational level was the sociodemographic variable that most influenced executive function WCST ($\eta^2 = 0.22$), WAIS-DS intelligence ($\eta^2 = 0.11$), and TMT verbal fluency

Table 2. Cognitive tests scores according to COMT rs4680 allele G presence in mania and mixed episodes

Episode	Neuropsychological test	rs4680 G allele presence	Mean	SD	*t*-test for equality of means		
					t	df	Significance (2-tailed)
Mania	WCST-P	G–	18.33	12.66	2.80	19.00	0.011
		G+	7.53	5.42			
	FAS-F	G–	8.33	3.50	–2.23	20.00	0.037
		G+	12.56	4.10			
Mixed	WAIS-DS-BK	G–	4.00	.00	–5.05	17.00	<0.001
		G+	6.56	2.15			
	WCST-CONC	G–	37.50	2.12	–3.21	6.84	0.015
		G+	45.67	8.71			
	WCST-CC	G–	1.50	.71	–2.16	17.00	0.045
		G+	3.53	1.28			
	WCST-E	G–	26.50	2.12	3.21	6.84	0.015
		G+	18.33	8.71			
	EIQ	G–	64.00	1.41	–3.55	18.00	0.002
		G+	96.61	12.66			
	FAS-TOTAL	G–	18.00	16.97	–2.32	18.00	0.033
		G+	35.28	9.44			
	WMS-LM1	G–	14.00	2.83	–2.23	18.00	0.039
		G+	24.72	6.60			
	RCFT-copy	G–	27.50	7.78	–2.25	17.00	0.038
		G+	33.82	3.36			

EIQ = Estimated IQ; FAS = Controlled Oral Word Association Test; RCFT = Rey–Osterrieth Complex Figure Test; WAIS-DS-BK = Wechsler Adult Intelligence Scale (WAIS-III) – Digit Span (Backward WAISDS-BK); WCST-CC = Wisconsin Card Sorting Test-Corrected Categories; WCST-CONC = Wisconsin Card Sorting Test – Conceptual level responses; WCST-E = Wisconsin Card Sorting Test-Errors; WCST-P = Wisconsin Card Sorting Test-Perseverative Errors; WMS-LM1 = Wechsler Memory Scale – Logical Memory immediate.

Table 3. Cognitive test scores according to COMT rs165599 allele G presence in mania and mixed episodes

Episode	Neuropsychological test	rs165599 allele G presence	Mean	SD	*t*-test for equality of means		
					t	df	(2-tailed)
Mania	SCWT-1	G–	19.57	4.04	2.17	20.00	0.042
		G+	15.80	3.69			
	SCWT-2	G–	30.86	12.94	2.39	20.00	0.027
		G+	19.93	8.40			
	RCFT-recall	G–	7.79	4.36	–2.19	20.00	0.041
		G+	15.93	9.30			
Mixed	SCWT-2	G–	30.00	11.94	2.59	18.00	0.019
		G+	19.93	5.69			
	SCWT-2 errors	G–	0.80	1.10	3.00	18.00	0.008
		G+	0.00	0.00			
	WASI-BD	G–	26.17	11.23	–2.63	19.00	0.016
		G+	40.73	11.54			
	WASI-MR	G–	20.67	6.09	–2.29	19.00	0.033
		G+	25.60	3.70			
	EXIQ	G–	84.67	13.13	–2.97	19.00	0.008
		G+	101.40	11.11			
	RCFT-copy	G–	29.60	6.66	–2.65	18.00	0.016
		G+	34.47	1.88			

EXIQ = Execution IQ; SCWT = Stroop Color–Word Test; RCFT = Rey–Osterrieth Complex Figure Test; SD = standard deviation; WASI-BD = Wechsler Adult Intelligence Scale – Block Design; WASI-MR = Wechsler Adult Intelligence Scale – Matrix Reasoning.

tests ($\eta^2 = 0.17$), in agreement with previous literature reports (Table 4).

The results also revealed an interaction between YMRS score and allele G rs4680 on SCWT-1 ($\eta^2 = 0.15$), SCWT-3-errors ($\eta^2 = 0.10$), FAS-TOTAL ($\eta^2 = 0.08$), FAS-A ($\eta^2 = 0.09$), WMS-LM1 ($\eta^2 = 0.09$), and TMT-B ($\eta^2 = 0.07$). For example, YMRS had a deleterious

Table 4. ANCOVA using cofactors gender, age, education, YMRS, MADRS, history of psychotic symptoms, number of previous manic episodes, allele G rs4680, and allele G rs165599

Source	Dependent variable	*B*	*F*	Significance	Partial eta-square (%)	Observed power (a) (%)
Gender						
	RCFT-copy	−2.401	5.551	0.022	9.02	63.89
	RCFT-recall	−4.553	5.334	0.025	8.70	62.16
Age						
	FAS-F	0.202	4.02	0.050	6.59	50.47
	RCFT-recall	−0.439	7.14	0.010	11.13	74.77
Education						
	WAIS-DS-FW	0.342	10.52	0.002	15.58	89.03
	WAIS-DS	0.475	6.88	0.011	10.78	73.22
	WCST-CONC	1.022	10.86	0.002	16.01	89.97
	WCST-CC	0.191	15.71	0.000	21.61	97.36
	WCST-E	−1.038	11.20	0.001	16.42	90.82
	WCST-NP	−0.606	5.73	0.020	9.14	65.31
	WASI-V	1.543	19.75	0.000	25.73	99.20
	WASI-S	0.833	11.75	0.001	17.09	92.07
	WASI-BD	1.219	6.86	0.011	10.74	73.06
	WASI-MR	0.471	4.86	0.032	7.85	58.19
	EIQ	1.813	12.45	0.001	17.93	93.43
	VIQ	1.994	9.07	0.004	13.72	84.14
	EXIQ	1.389	8.86	0.004	13.46	83.31
	FAS TOTAL	1.343	9.93	0.003	14.84	87.24
	FAS-F	0.415	6.51	0.013	10.25	70.82
	FAS-A	0.605	14.16	0.000	19.90	95.90
	FAS-S	0.376	6.01	0.017	9.54	67.37
	TMT-B	−4.936	11.63	0.001	16.94	91.81
YMRS						
	SCWT-1	0.169	1.760	0.190	3.05	25.64
	SCWT-3 errors	0.093	13.600	0.001	19.54	95.19
	FAS TOTAL	−0.381	1.180	0.282	2.06	18.74
	FAS-F	−0.123	0.909	0.344	1.60	15.51
	FAS-A	−0.152	1.259	0.267	2.20	19.68
	WMS-LM1	−0.113	0.348	0.558	0.62	8.93
	RCFT-copy	−0.434	17.566	0.000	23.88	98.45
	TMT-B	0.854	0.533	0.469	0.94	11.07
MADRS						
	SCWT-3 errors	0.046	4.376	0.041	7.25	53.82
	WCST-FMS	0.052	8.478	0.005	13.15	81.63
	FAS TOTAL	−0.069	0.051	0.822	0.09	5.57
	FAS-F	−0.081	0.527	0.471	0.93	11.00
	FAS-A	−0.063	0.293	0.590	0.52	8.30
	RCFT-copy	−0.202	5.050	0.029	8.27	59.82
Number of previous manic episodes						
	WCST-NP	−21.679	4.37	0.008	18.71	84.82
Lifetime psychotic symptoms						
	WCST-E	4.313	4.04	0.049	6.62	50.66
Allele G rs4680						
	SCWT-1	−7.026	−10.65	0.0019	15.75	89.41
Allele G rs4680* MADRS						
	FAS TOTAL	−0.975	5.251	0.026	8.57	61.49
	FAS-F	−0.362	5.339	0.025	8.70	62.21
	FAS-A	−0.381	5.310	0.025	8.66	61.97
Allele G rs4680* YMRS						
	SCWT-1	−0.411	−9.85	0.0027	14.74	86.99
	SCWT-3 errors	−0.66	−6.47	0.0137	10.19	70.54
	FAS TOTAL	0.731	−5.12	0.0275	8.24	60.45
	FAS-A	0.288	−5.42	0.0235	8.68	62.86
	WMS-LM1	0.453	−5.68	0.0205	9.07	64.94
	TMT-B	−2.215	−4.22	0.0446	6.89	52.35

Table 4. (Continued)

Source	Dependent variable	*B*	*F*	Significance	Partial eta-square (%)	Observed power (a) (%)
Allele G rs165599						
	FAS-F	−4.681	4.01	0.050	6.58	50.38
Allele G rs165599*MADRS						
	WCST-FMS	−0.058	5.696	0.020	9.23	65.01
Allele G rs165599*YMRS						
	FAS TOTAL	0.795	4.90	0.031	7.91	58.53
	FAS-F	0.326	5.64	0.021	9.01	64.62
	FAS-A	0.273	4.05	0.049	6.64	50.78
	RCFT-copy	0.250	4.02	0.050	6.58	50.40

Note that SCWT is the only test that is measured in sec. Higher scores indicate better performance, with the exception of SCWT and TMT (score measured in sec), WCST-PR, WCST-E, WCST-NP, and WCST-P.
Significance level: $p < 0.05$.
*Refers to the interaction between alleles.
ANCOVA = analysis of covariance; CC = corrected categories; CONC = conceptual level responses; E = errors; EIQ = estimated IQ; EXIQ = execution IQ; FAS = Controlled Oral Word Association Test; FMS = Failure to Maintain Set; IQ = Intelligence Quotient; NP = non-perseverative errors; P = perseverative errors; PR = perseverative responses; RCFT = Rey–Osterrieth Complex Figure Test; SCWT = Stroop Color–Word Test; TMT = Trail Making Test parts A & B; VIQ = Verbal IQ; WAIS, Wechsler Adult Intelligence Scale (III) – Digit Span (forward = WAISDS-FW; backward = WAISDS-BK); WAIS-LNS = Wechsler Adult Intelligence Scale-Letter-Number Sequence; WASI-BD = Wechsler Abbreviated Scale of Intelligence – Block Design; WASI-MR = Wechsler Abbreviated Scale of Intelligence – Matrix Reasoning; WASI-S = Wechsler Abbreviated Scale of Intelligence – Similarities; WASI-V = Wechsler Abbreviated Scale of Intelligence – Vocabulary; WCST = Wisconsin Card Sorting Test; WMS-LM1 = Wechsler Memory Scale – Logical Memory immediate; WMS-LM2 = Wechsler Memory Scale – Logical Memory delayed.

effect ($\eta^2 = 0.19$, $B = 0.093$) on SCWT-3 errors, but the interaction of COMT allele G rs4680 attenuated this dysfunction ($\eta^2 = 0.10$, $B = -0.6$) (Table 4).

In addition, an interaction between YMRS score and allele G rs165599 was observed on FAS-TOTAL ($\eta^2 = 0.08$), FAS-F ($\eta^2 = 0.09$), FAS-A ($\eta^2 = 0.07$), and RCFT-copy ($\eta^2 = 0.07$). In all of these interactions, COMT allele G attenuated the cognitive impairment of manic symptoms measured by the YMRS. For example, YMRS influenced RCFT-copy ($\eta^2 = 0.23$, $B = -0.43$) but the interaction between allele G rs165599 and YMRS reduced the deleterious effect of YMRS ($\eta^2 = 0.06$, $B = 0.25$) (Table 4).

The MANCOVA results also showed that MADRS had a negative effect on verbal fluency [FAS Total ($\eta^2 = 0.00009$, $B = -0.06$), FAS-F ($\eta^2 = 0.0009$, $B = -0.08$) and FAS-A ($\eta^2 = 0.0005$, $B = -0.06$)] that was intensified by interaction with allele G rs4680 [FAS Total ($\eta^2 = 0.08$, $B = -0.9$), FAS-A ($\eta^2 = 0.08$, $B = -0.38$)]. The opposite was observed with WCST-FMS, in which the interaction between COMT allele G rs165599 and MADRS tended to attenuate negative performance ($\eta^2 = 0.09$, $B = -0.005$) (Table 4).

Discussion

To the best of our knowledge, this is the first study reporting an association between COMT SNPs and CD in BD mood episodes. Our data showed that COMT G+ subjects (rs4680 and rs165599) had less CD than G− individuals during manic and mixed episodes. In our sample, G+ patients in a manic episode outperformed G− patients on executive function (SCWT, WCST) verbal fluency (FAS), and memory (RCFT-recall) tests, while G+ patients in a mixed episode performed better on executive function (WAIS-DS, SCWT, WCST), memory (WMS-LM1, RCFT-copy), verbal fluency (FAS), and intelligence tests (WASI). Furthermore, we report an interesting interaction between COMT allele G and YMRS scores on the tests of verbal fluency (FAS), memory (WMS-LM1, RCFT-copy), and executive function (SCWT), in which allele G attenuated the CD caused by manic symptomatology. Moreover, no influence of COMT SNPs on the cognitive function of patients undergoing a depressive episode or of controls was observed.

The cognitive performances of patients undergoing mania and mixed episodes were clearly modulated by allele G (rs4680/rs165599), a phenomenon that we attribute to the higher levels of DA in the PFC theoretically found in these mood states. Moreover, the lack of association between genotype or allele G (rs4680/rs165599) and cognitive performance in depressive subjects is very clear in our results, and is likely explained by no reports of DA alterations in depressive episodes. The impact of DA level alterations on cognition has been

extensively investigated. Studies have shown that low doses of D1 agonists improve working memory and attention regulation (25), while high levels of DA release impair PFC function (18). Early studies reported that at low psychostimulant doses, hyperkinetic children showed a significant improvement in short-term memory, whereas at higher doses a significant decline in performance was observed (59). Mattay et al. (18) reported similar findings in healthy volunteers using dextroamphetamine, a drug that potentiates dopaminergic activity. These authors observed that subjects carrying the COMT genotype AA (rs4680) had poorer working memory and executive function, while GG carriers improved performance after a single dose of dextroamphetamine. This phenomenon has also been described in pharmacological studies as an inverted-U response function (18, 27, 29). The inverted-U shape theory, whereby the effect of pharmacological agents on the PFC depends on the baseline level of PFC function, is presumed to reflect baseline dopaminergic levels (18). Hence, an optimal level of DA is required for good cognitive functioning, and this is coordinated by the PFC. Based on these findings, we suggest that in mania and mixed states, the inverted-U-shaped graph of cognitive performance would apply in the same way as in the pharmacological studies using amphetamine. We propose that the DA level in G− patients in mania or mixed episodes (hyperdopaminergic states) exceeds that required for the optimal level of PFC function, thus potentially impairing cognitive stability. On the other hand, G+ subjects receiving an extra DA load due to mania or mixed episode perform cognitively better than G− subjects because they have lower basal DA levels in the PFC. This model could also be verified based on the MANCOVA analysis, in which we observed an interaction between allele G and YMRS scores. Hence, allele G serves as a protective factor against the deleterious effects of mania and mixed episodes on cognition.

Evidence that DA levels vary among mood states is indirect but highly significant, since most of these models were conceptualized based on indirect evidence from pharmacological and animal studies (23). Also, pharmacological studies have shown that dopaminergic agonists trigger hypomanic/manic episodes, and that drugs that reduce dopaminergic throughput have anti-manic effects (60–62). Amphetamine, for instance, which promotes DA release and inhibits its uptake, can precipitate hypomania in BD patients and induce a hypomania-like state in normal individuals (63). Antipsychotic agents that block DA receptors are effective in the treatment of mania (61), while drugs that inhibit DA reuptake are effective for treating depression (62). Homovanillic acid (HVA) (the main metabolite of DA) showed a significant decrease in the cerebrospinal fluid (CSF) of depressive patients (64). By contrast, CSF HVA levels were increased in patients with mania compared to control and depressive patients (65).

Previously, there have been only three main studies about COMT and BD cognition. The first study about COMT and BD cognition evaluated attentional and executive functions in 94 medicated euthymic BD patients, genotyped for COMT rs4680, and found no association with performance (40). Burdick et al. (36) showed no association between rs4680 and cognition in 52 BD subjects, but found that rs165599 allele G homozygous and heterozygous carriers performed worse than non-carriers in a verbal learning and memory test (36). Recently, the effect of 21 COMT SNPs on working memory, executive function, and IQ were evaluated in a sample of 315 SZ, BD, and control individuals. The authors described no association between COMT and BD cognition (41). Importantly, in all three studies BD subjects were under pharmacological treatment and in euthymia. Interestingly, our findings are the opposite of those reported in euthymic BD subjects. We consider that this difference arises from the fact that our sample was medication free and experiencing a manic, mixed, or depressive episode, which characterizes a unique neurobiological setting for studying and analyzing the COMT genotype effect on cognition in the longitudinal change of mood presented in BD.

The main limitation of our study was the small sample size. The strengths of this study included the absence of medication interference on the results, the application of an extensive neuropsychological battery, and a uniform sample of young BD-I subjects. These results allow us to propose that COMT allele G+ can protect cognition from the deleterious effects of manic symptoms. Without a follow-up study, it is not possible to conclude that the cognition of a given patient works differentially across longitudinal changes in mood. Reaching this conclusion would require the evaluation of cognitive performance before, during, and after each kind of episode in the same individual. Nevertheless, we consider it plausible to hypothesize that when G− patients are in a manic episode, some cognitive abilities may improve.

Conclusions

The present study is the first to suggest a genetic explanation as to why CD varies among subjects

within the same BD episode. We propose that the COMT genotype might explain why some patients become more cognitively dysfunctional than others during mania and mixed episodes. The interaction between manic symptomatology and COMT rs165599 and rs4680 allele G during mood episodes is a dynamic example of how trait and state modulate BD cognition. Further studies in larger samples will be necessary to confirm these findings and analyze the long-term effects of repeated manic episodes on the cognition of G− subjects.

Acknowledgements

The São Paulo Research Foundation (Fundo de Apoio a Pesquisa do Estado de São Paulo: FAPESP 2010/06230-0 and 2010/12286-2) financed this research. We would like to thank the team from the Institute of Psychiatry at the University of São Paulo, especially the members of the Mood Disorders Unit (GRUDA) and Laboratory of Neuroscience (LIM27), for their dedication and hard work.

Disclosures

The authors of this paper do not have any commerical associations that might pose a conflict of interest in connection with this manuscript.

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12.2. Does BDNF genotype influence creative output in bipolar I manic patients?

Journal of Affective Disorders 139 (2012) 181–186

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Preliminary communication

Does BDNF genotype influence creative output in bipolar I manic patients?

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 2 January 2012
Accepted 30 January 2012
Available online 6 April 2012

Keywords:
Creativity
Brain-derived neurotrophic factor
Bipolar disorder
Cognition
Depression
Neuroprotection

ABSTRACT

Introduction: Creativity is a complex human ability influenced by affective and cognitive components but little is known about its underlying neurobiology. Bipolar Disorder (BD) is highly prevalent among creative individuals. Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophic factor, and has been implicated in the pathophysiology of BD. In contrast to the better functioning of the BDNF polymorphism (Val^{66}Met) Val *allele*, the Met *allele* decreases BDNF transport and has been associated with worsened performance on several cognitive domains in euthymic BD subjects and controls. We hypothesized that the Val *allele* is associated with increased creativity in bipolar disorder.
Materials and methods: Sixty-six subjects with BD (41 in manic and 25 in depressive episodes) and 78 healthy volunteers were genotyped for BDNF Val^{66}Met and tested for creativity using the Barrow Welsh Art Scale (BWAS) and neuropsychological tests.
Results: Manic patients with the Val *allele* (Met−) had higher BWAS scores than Met+ carriers. This relationship was not observed among patients in depressive episodes or among control subjects. BDNF Met *allele* status showed no association with cognitive function in any of the groups.
Conclusion: As postulated, these findings suggest that the better functioning *allele* of BDNF may selectively facilitate creative thinking in subjects with manic episodes, but not in controls or depressives. Further studies exploring the role of BDNF in the neurobiology of creativity in BD and in euthymic phases are warranted.

1. Introduction

Previous studies have suggested an increased prevalence of bipolar disorder (BD) among creative individuals (Akiskal et al., 2005; Andreasen and Glick, 1988; Jamison, 1994). In this sense, creativity has been studied as both a trait (Akiskal et al., 2005) and recently, as a state variable in BD (Soeiro de Souza et al., 2011). The neurobiological of creativity remains unknown. Furthermore subtle cognitive alterations associated with BD episodes can co-occur with alterations in creativity (Soeiro de Souza et al., 2011).

Neurotrophic factors mediate neuronal differentiation, proliferation, synaptogenesis, learning, memory and cell survival (Poo, 2001). Over the last decade, neurotrophins have been associated with cognitive function (Egan et al., 2003; Hariri et al., 2003; Woo and Lu, 2006) and potentially linked to the pathophysiology of depression and BD (Post, 2007). Brain-derived neurotrophic factor (BDNF) is one of the most abundant neurotrophic factors (Zigova et al., 1998) and expressed throughout the brain, particularly in the hippocampus and prefrontal cortex (PFC) (Pezawas et al., 2004), exerting long-term effects on neuronal survival, migration, and growth (Pang and Lu, 2004).

Importantly, a single nucleotide polymorphism (SNP) in the BDNF gene (rs6265) produces a Val to Met amino acid

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doi:10.1016/j.jad.2012.01.036

substitution at codon 66 (Val[66]Met) in the pro-BDNF sequence, affecting the activity-dependent secretion of BDNF (Egan et al., 2003). Regarding functional effects, depolarization-dependent secretion of BDNF is impaired by the presence of Met *allele* (Met+) (Egan et al., 2003). The Met *allele* is also associated with deficient intracellular transport of BDNF to dendrites and reduced magnitude of long term potentiation (LTP) (Kleim et al., 2006). This *allele* has also been associated with cognitive deficits in patients with BD (Rybakowski et al., 2003; Savitz et al., 2006) and schizophrenia (Rybakowski et al., 2006), as well as in healthy controls (Hariri et al., 2003; Pezawas et al., 2004).

Previous studies have shown that BDNF Met+ carriers have impaired performance in memory (Egan et al., 2003; Hariri et al., 2003), executive function (Rybakowski et al., 2003) and intelligence (Tsai et al., 2004). Few studies have evaluated the potential role of BDNF in regulating cognition in BD. Some studies have reported better executive function among BD euthymic medicated BDNF Met− patients (Rybakowski et al., 2003), which was subsequently not confirmed (Rybakowski et al., 2006; Tramontina et al., 2009). Serum BDNF levels are lower in both mania (Machado-Vieira et al., 2007) and depression (Cunha et al., 2006) and tend to be associated with episode severity. Recent meta-analyses support these observations and suggest that levels return to normal in euthymia, but not in all instances (Monteleone et al., 2008). Also, BDNF levels have been shown to increase after lithium treatment in mania (de Sousa et al., 2011). To the best of our knowledge there are no studies evaluating the effects of BDNF SNPs on cognitive function during mood episodes in BD patients or medication-free subjects.

High levels of creativity are observed in mania along with one of its cardinal features namely, increases in word production and associativity (loose associations) (Goodwin and Jamison, 2007). Based on the link of BD to increased creativity, we postulated that the better functioning Val *allele* of BDNF (Met−), and its related increases in the magnitude of LTP, is associated with greater in creativity in patients with BD compared to those with the less well functioning Met+ *allele*. In addition this study sought to further explore the previously reported associations of the Met+ *allele* to poorer cognitive functioning in a variety of medication-free patients and normal controls. This approach would allow the discrimination of the possible contributions of cognitive functioning to the putative relationship of BDNF with creativity.

2. Material and methods

The patients sample included sixty-six medication-free individuals with BD I (44 females), aged between 18 and 35 years old and currently in manic (n = 41) or depressive (n = 25) episodes according to DSM-IV TR criteria (DSM-IV, 2000). All patients were participants in the *LICAVAL* (*lithium plus carbamazepine versus lithium plus valproate*) clinical trial (Campos et al., 2010) and were evaluated immediately after a four week wash-out period for antidepressants, mood stabilizers or antipsychotics, and after eight weeks for *depot* medications. Diagnosis was determined by trained psychiatrists using the Structured Clinical Interview (SCID-I) (First et al., 1997) for DSM-IV TR (DSM-IV, 2000). The Young Mania Rating Scale (YMRS) (Young et al., 1978), and the Montgomery–Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) were used to evaluate the severity of symptoms. The cut-off point for mania was YMRS ≥ 12 and for depression was MARDS ≥ 15. Mean YMRS was 17.41 (±6.39) in mania, while MADRS mean score was 24.28 (±7.17) for depression. Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, current substance abuse, or that had undergone electroconvulsive therapy in the preceding six months, were excluded.

The control group comprised of healthy subjects (n = 78), age 18–35 years, recruited at the University of São Paulo (mostly medical students). Inclusion criteria for controls included absence of any psychiatric diagnosis (present or past) according to the evaluation by trained psychiatrists using The Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). Also, subjects with a positive family history of mood or psychotic disorders in first degree relatives, use of any psychopharmacological agent, and/or substance abuse over the last three months, were excluded.

Neurocognitive and creativity tests were carried out under standard conditions and scored by two trained neuropsychologists. Given the known high correlation between intelligence and creativity (Cropley and Field, 1969), it was necessary to exclude a potential association between creativity and SNPs that could merely reflect a relationship to intelligence. The neurocognitive battery was designed to assess the following domains: *Attention*: Wechsler Adult Intelligence Scale III (WAIS-III) subtest Digit Span (WAIS-DS), Trail Making Test-part A (TMT-A), Stroop Color-Word Test (SCWT); *Verbal Memory*: Wechsler Memory Scale subtest-Logical Memory (WMS-LM) immediate (1) and delayed (2); Visual Memory: Rey-Osterrieth Complex Figure Test (RCFT) delayed recall; *Visuospatial Function*: Wechsler Abbreviated Scale of Intelligence (WASI)-Block Design (WASI-BD), RCFTcopy; *Language*: Controlled Oral Word Association Test (FAS), WASI-Vocabulary subtest (WASI-V); *Psychomotor Speed*: Trail Making Test-part A (TMT-A); *Executive Function*: Letter-Number Sequence (WAIS-LNS), WAIS-DS, SCWT, TMT-B, WASI Similarities (WASI-S), WASI Matrix Reasoning (WASI-MR), RCFT copy, WCST (Wisconsin Card Sorting Test)-Conceptual level responses (WCST-CONC), Perseverative Responses (WCST-PR), Failure to Maintain Set (WCST-FMS), Corrected Categories (WCST-CC), Errors (WCST-E), Non-Perseverative Errors (WCST-NP), Perseverative Errors (WCST-P); *Intelligence*: WASI: Total Intelligence Quotient (IQ), Estimated IQ (EIQ), Execution IQ (EXIQ), Verbal IQ (VIQ).These are well-established and validated tests (Lezak, 2004; Strauss et al., 2006; Wechsler, 1981, 1999). Higher scores indicate better performance, with the exception of the SCWT, TMT, WCST-PR, WCST-E, WCST-NP and WCST-P.

Creativity was assessed using the Barrow Welsh Art Scale (BWAS). The BWAS (Barron, 1963) is an empirically-derived metric consisting of 86 black and white images that individuals rate as “like” or “dislike”, with higher scores reflecting preference for more asymmetrical and complex figures over more symmetrical and simple figures. Preference for more asymmetrical and complex figures is higher among artists than non-artists according to BWAS scores (Gough and Hall, 1996). The BWAS scale may also reflect cognitive/affective contributions to creativity, as it involves not only visual

processing, but also affective processing (like or dislike). Indeed, BWAS scores have been linked not only to creativity as measured by other means but also to emotionality (King and Curtis, 1991).

The research ethics board of the *Hospital das Clínicas of the University of São Paulo* approved this study. Written informed consent was obtained from all subjects.

2.1. Genotyping

DNA was extracted from peripheral blood according to the salting-out protocol (Laitinen et al., 1994) and then genotyped for BDNF rs6265 (Val^{66}Met) using real-time PCR allelic discrimination. PCR amplification for rs6265 was performed in 5 μl reactions with 5 ng of template DNA, 1× TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 1× each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. The *allele*-detection process and allelic discrimination were performed for 1 min at 60 °C on a 7500 Real-Time System (Applied Biosystems, Foster City, CA).

2.2. Statistical analyses

Subjects were grouped according to BDNF genotype into Met carriers (Met+) {Val^{66}Met and Met^{66}Met} and non-carriers (Met−) {Val^{66}Val} and compared using the Chi-square test for categorical data (*e.g.* gender), and Student's *t*-test for continuous data (*e.g.* BWAS, age, IQ). BWAS mean scores (total, like, dislike) were compared according to BDNF *allele* Met status using the *t*-test. Scores in mania, depression and control groups were compared for BDNF Met+ and Met−. The influence of IQ, age, education, gender, YMRS and MADRS on the results was assessed by backward regression analysis. All statistical analyses were carried out using version 18.0 of the PASW statistics software (SPSS Inc., Chicago, Illinois).

3. Results

BDNF rs6265 genotype distribution in the experimental samples of men and women were in accordance with the Hardy–Weinberg equilibrium ($p=0.65$) indicating that the samples were representative. Allelic frequency was 72.7% Met− and 27.3% Met+ in the patient groups *versus* 52.56% Met− and 47.44% Met+ in the control group. Thus, Met carrier prevalence was higher in the control group (n=37) compared to the BD group (n=18) ($p=0.01$).

No significant differences in sociodemographics were observed between genotypes or *allele* Met in terms of age, gender or years of schooling in the patient or control groups (Table 1).

In agreement with previous findings (Soeiro de Souza et al., 2011), subjects in mania had higher BWAS total scores than depressives (t=3.67 $p=0.001$). Manic patients had a mean BWAS total score of 27.02 (±11.38) while depressed patients' mean score was 16.76 (±10.97).

The BD group (mania + depression) subjects had worse cognitive performance than controls across all tests from the battery, with the exception of the WCST-FMS ($p=0.35$) in which no difference was observed. The manic patient group had lower scores on the WCST-CONC ($p=0.014$) and higher scores on the WCST-PR ($p=0.011$), WCST-ERRORS ($p=0.006$) and WCST-P ($p=0.007$) when compared to the depressed group, indicating worse executive function.

3.1. Manic (but not depressive or control) BDNF Met− carriers had higher BWAS scores than Met+ carriers

As hypothesized, BDNF Val^{66}Val (Met−) was associated with high creativity scores on the BWAS. However, this relationship was not observed among patients in the depressive phase or among controls.

Analysis of all subjects with BD (mania + depression) continued to show that BDNF Met− subjects (mean 25.14 ± 12.59) had higher BWAS total scores (t=2.45; $p=0.019$) compared to Met+ carriers (mean 17.88 ± 9.89) (Table 2). In controls, no difference in BWAS scores was found between Met+ (mean 27 ± 13.57) and Met− carriers (mean 24.88 ± 12.75) (t=0.69; $p=0.48$) (Table 2).

BDNF Met− carriers in manic episodes had higher BWAS total scores (30.03 ± 10.72), then Met+ carriers (18.81 ± 9.15), (t=3.31; $p=0.003$) (Fig. 1). Moreover, BWAS dislike scores were higher in BDNF Met− carriers (15.41 ± 9.89) in manic episode (t=2.55; $p=0.024$) compared to Met+ carriers (7.33 ± 5.95). No differences in BWAS scores (total, like, dislike) were observed between Met+ and Met− in patients with depressive episodes (Table 3). Backward linear

Table 1
Sociodemographic variables and symptoms scales in patients and controls according to BDNF rs6265 genotype.

	Bipolar disorder (N=66)				Between-groups differences[a]	Healthy controls (N=78)				Between-groups differences[a]
	Met+ (N=18)		Met− (N=48)			Met+ (N=37)		Met− (N=41)		
	Mean	SD	Mean	SD	*Sig. (2-tailed)*	Mean	SD	Mean	SD	*Sig. (2-tailed)*
Age (years)	29	5.70	27.60	4.90	0.36	23.81	3.93	23.34	3.20	0.56
Gender (men/women)[b]	8/10		14/34		0.25[b]	23/14		16/25		0.07[b]
Years of education	11.72	3.06	12.63	3.18	0.30	14.29	2.36	14.13	2.39	0.76
YMRS	12.72	7.04	13.64	6.82	0.63	–	–	–	–	–
MADRS	21.83	9.81	19.57	8.48	0.39	–	–	–	–	–

YMRS: Young Mania rating Scale; MADRS: Montgomery–Asberg Depression rating Scale.
[a] *t*-test, significance level $p<0.05$.
[b] Chi square test $p<0.05$.

Table 2
Comparison of intelligence and creativity according to BDNF rs6265 genotype in patients and controls.

	Bipolar disorder (N = 66)						Controls (N = 78)					
	Met+ (N = 18)		Met− (N = 48)		*t*-test		Met+ (N = 37)		Met− (N = 41)		*t*-test	
	Mean	SD	Mean	SD	t	*p*	Mean	SD	Mean	SD	t	*p*
IQ	94.44	11.50	96.27	14.17	0.53	0.59	115.81	14.20	113.73	11.16	−0.71	0.47
BWAS like	8.77	4.69	10.94	8.20	0.99	0.99	12.50	6.54	11.39	6.22	−0.74	0.45
BWAS dislike	8.55	7.40	14.11	9.26	1.89	0.07	14.50	9.69	13.56	9.49	−0.42	0.67
BWAS total	17.88	9.89	25.14	12.59	2.45	0.019	27	13.57	24.88	12.75	−0.69	0.48

BWAS: Barrow Welsh Art Scale; IQ: Intelligence Quotient (Wechsler Abbreviated Scale of Intelligence). Significance level $p<0.05$.

regression analysis using age, IQ, education, the YMRS, and MADRS as predictors revealed no influence on BWAS scores in manic Met− subjects.

No differences in BWAS scores (total, "like", "dislike") were observed between Met+ and Met− patients in depressive episode (Table 3).

3.2. Met allele did not influence cognitive function in manic, depressive or control subjects

The presence of the Met *allele* was found not to influence cognitive function (attention, verbal memory, visual memory, visuospatial function, language, psychomotor speed, executive functions and intelligence) in manic subjects or controls. In depressive episodes, only executive function (WCST-NP) showed better performance for Met+ ($t=-3.18$; $p=0.004$).

3.3. BWAS score was correlated with cognitive function in depression and control groups but not in mania group

In the manic group, creativity scores were not correlated with any of the cognitive tests. In depressives, BWAS total score was weakly correlated with performance on the SCWT-2 ($r=0.45$; $p=0.02$). In the control group, BWAS total score was correlated with performance on both the WAIS-DS ($r=0.23$; $p=0.05$), and WAIS-LNS ($r=0.28$; $p=0.014$).

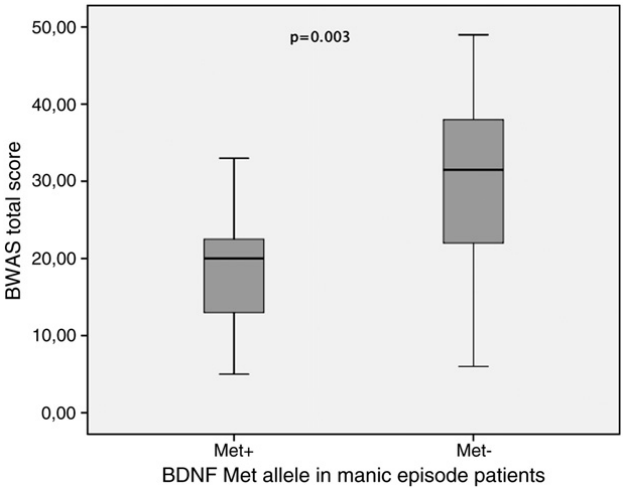

Fig. 1. Comparison of BWAS total score between manic patients with and without Met *allele*.

4. Discussion

To the best of our knowledge, this is the first study investigating the relationship of common BDNF *alleles* to creativity in BD. As hypothesized, the better functioning Val *allele* of proBDNF (Met−) was associated with greater creativity in BP patents. However, this association was selective for patients in manic phases whose BWAS scores were higher but was not seen among patients in the depressed phase or in normal controls.

Carriers of the Met *allele* Met (Met+) of the BDNF functional SNP rs6265 are known to have lower activity-dependent secretion of BDNF as well as a lesser magnitude of long-term potentiation (LTP) (Egan et al., 2003). In contrast, Met− subjects have higher BDNF levels and better executive function in euthymia (Rybakowski et al., 2003). Nonetheless, the Met *allele* has been associated with decreased cortex and hippocampal volume in bipolar patients (Chepenik et al., 2009; Matsuo et al., 2009) as well as normal controls (Hajek et al., 2011). BD Met+ patients exhibit poorer executive function in euthymia (Rybakowski et al., 2003). Similarly, Met+ normal controls and patients with schizophrenia had lower scores on measures of prefrontal cortical memory functioning (Dempster et al., 2005; Egan et al., 2003; Hariri et al., 2003). The lack of relationship of Met+ to cognition in manics and controls is puzzling, but supports the view that higher BWAS scores in mania are not simply secondary to cognitive functioning.

BD subjects with BDNF rs6265 Met− had higher creativity scores during manic episodes. This higher creativity score probably indicates that creativity has different underlying neurobiology when compared to cognition *per se*. This indicates that the potentially higher BDNF levels of Met− might be involved in the modulation of creative thinking. The explanation for the specificity in mania may involve higher dopamine (DA) levels postulated to occur in mania, a phenomenon previously associated with creativity (Burch et al., 2006; Folley and Park, 2005; Richards et al., 1988). Also, the ability to generate many different ideas about a topic in a short period of time (divergent thinking), a key aspect of creativity (Gundlach and Gesell, 1979), is influenced by dopaminergic function (Reuter et al., 2006). We speculate that higher DA function associated with the better functioning BDNF (Met−) *allele* in mania may lead to higher creativity scores. Given cognitive functioning was, if anything, lower in manic individuals than controls, coupled with the fact that creativity was not correlated with cognitive measures,

Table 3
Comparison intelligence and creativity in Bipolar Disorder manic and depressive episodes according to BDNF rs6265 genotype.

	Mania (N=41)						Depression (N=25)						Controls (N=78)					
	Met+ (N=11)		Met− (N=30)		*t*-test		Met+ (N=7)		Met− (N=18)		*t*-test		Met+ (N=37)		Met− (N=41)		*t*-test	
	Mean	SD	Mean	SD	t	*p*	Mean	SD	Mean	SD	t	*p*	Mean	SD	Mean	SD	t	*p*
IQ	94.09	12.46	94.87	14.82	0.16	0.86	95	10.73	98.61	13.10	0.70	0.49	115.81	14.20	113.73	11.16	−0.71	0.47
BWAS like	9.16	5.81	12.95	7.23	1.35	0.20	8	3.60	6.10	8.71	−0.55	0.59	12.50	6.54	11.39	6.22	−0.74	0.45
BWAS dislike	7.33	5.95	15.41	9.89	2.55	0.024	11	10.81	11	7.03	0	1	14.50	9.69	13.56	9.49	−0.42	0.67
BWAS total	18.81	9.15	30.03	10.72	3.31	0.003	16.42	11.55	17	11.39	0.76	0.91	27	13.57	24.88	12.75	−0.69	0.48

BWAS: Barrow Welsh Art Scale; IQ: Intelligence Quotient (Wechsler Abbreviated Scale of Intelligence). Significance level $P<0.05$.

the higher BWAS scores observed in BDNF (Met−) do not appear to stem from superior cognitive abilities.

Regarding healthy subjects, few studies have addressed the genetics of creativity. A recent study described an association between divergent thinking and DA receptor SNPs. Higher creativity scores were observed in the A1 *allele* of DA receptor D2 (rs1800497) (Reuter et al., 2006), which has a 30–40% reduction in DA-D2 receptor density (Ritchie and Noble, 2003). Similar findings were observed for the A *allele* carriers of the serotonin SNP TPH1 A779C (Reuter et al., 2006). In another study, Kéri (2009) found that the T *allele* in the promoter region of neuregulin 1 was associated with higher creativity scores in healthy volunteers (Kéri, 2009). Neuregulin 1 affects neuronal development, synaptic plasticity, glutamatergic neurotransmission, and glial functioning (Harrison and Law, 2006) in actions which have many parallels to BDNF.

Meta-analyses have shown lower peripheral BDNF levels in patients with depression and mania compared to controls (Lin, 2009). The mechanism of interaction of these alterations in brain or peripheral levels has yet t to be fully explored. One study reported that excellent lithium responders have higher BDNF levels as well as better working memory and attention (Rybakowski and Suwalska, 2010), while another reported negative results (Dias et al., 2009). However, the relationship to BDNF rs 6265 SNP was not studied.

While this is the first report describing a positive association between creativity and the better functioning *allele* of BDNF (Val), whether this association is also seen in euthymia (and thus, if it might help account for the general link between increased creativity and BD) remains a topic for future investigations. Clearly, further studies are required to assess the relationships of Val and BD vulnerability and creativity.

A limitation of this study is that the state-trait relationship of BDNF (Met−) cannot be dissected and larger samples including euthymic patients need to be studied. It would also be valuable to utilize other measures of creativity that do not depend on a measure of increased (loose) association which is so closely linked to manic symptomatology. On the other hand, the strength of this study was that it included unmedicated subjects, which allowed a clear observation of the phenotypic boundaries in mania and depression. Moreover, the inclusion of comprehensive measures of cognition allowed the inference that the observed relationships to creativity were not dependent on superior cognitive abilities.

5. Conclusion

Creativity scores were selectively influenced by the functional BDNFrs6265 SNP in manic BD (but not depressive or control) subjects. The effect of BDNF rs6265 Met− in improving creativity during mania may involve neuroprotective mechanisms. No evidence that this *allele* modulates cognitive function during mood episodes was found. The known alterations in the monoaminergic system associated with mood episodes in BD may also contribute to these effects on creativity. Future studies exploring the neurobiology of creativity in BD are warranted.

Role of funding source
Sao Paulo research foundation (Fapesp) is an independent public foundation with the mission to foster research and the scientific and technological development of the State of São Paulo.

Conflict of interest
The authors do not have any conflict of interest to report.

Acknowledgments
We would like to thank the Institute of Psychiatry at the University of Sao Paulo, especially the members of the Mood Disorders Unit (GRUDA) and Laboratory of Neuroscience (LIM27) for their dedication and hard work, and the volunteers for their collaboration in this study.

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12.3. The Impact of the CACNA1C risk allele on limbic structures and facial emotions recognition in bipolar disorder subjects and healthy controls

Journal of Affective Disorders 141 (2012) 94–101

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Preliminary communication

The impact of the CACNA1C risk allele on limbic structures and facial emotions recognition in bipolar disorder subjects and healthy controls

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ARTICLE INFO

Article history:
Received 13 February 2012
Accepted 6 March 2012
Available online 30 March 2012

Keywords:
Bipolar disorder
Calcium channel
CACNA1C
Social cognition
Facial emotions recognition

ABSTRACT

Introduction: Impairments in facial emotion recognition (FER) have been reported in bipolar disorder (BD) during all mood states. FER has been the focus of functional magnetic resonance imaging studies evaluating differential activation of limbic regions. Recently, the α1-C subunit of the L-type voltage-gated calcium channel (CACNA1C) gene has been described as a risk gene for BD and its Met allele found to increase CACNA1C mRNA expression. In healthy controls, the CACNA1C risk (Met) allele has been reported to increase limbic system activation during emotional stimuli and also to impact on cognitive function. The aim of this study was to investigate the impact of CACNA1C genotype on FER scores and limbic system morphology in subjects with BD and healthy controls.
Material and methods: Thirty-nine euthymic BD I subjects and 40 healthy controls were submitted to a FER recognition test battery and genotyped for CACNA1C. Subjects were also examined with a 3D 3-Tesla structural imaging protocol.
Results: The CACNA1C risk allele for BD was associated to FER impairment in BD, while in controls nothing was observed. The CACNA1C genotype did not impact on amygdala or hippocampus volume neither in BD nor controls.
Limitations: Sample size.
Conclusion: The present findings suggest that a polymorphism in calcium channels interferes FER phenotype exclusively in BD and doesn't interfere on limbic structures morphology.

1. Introduction

Impaired regulation of calcium signaling is considered the most reproducible cellular abnormality in bipolar disorder (BD) research (Akimoto et al., 2007; Kato, 2008; Machado-Vieira et al., 2011; Sourial-Bassillious, 2009). Recently, the α1-C subunit of the L-type voltage-gated calcium channel (CACNA1C) gene was strongly associated to BD risk in genome-wide association studies (Ferreira et al., 2008; Psychiatric GWAS Consortium Bipolar Disorder Working Group et al., 2011; Sklar et al., 2008). In healthy controls, the CACNA1C risk Met allele has been reported to modulate limbic activity during facial emotional stimuli (Bigos et al., 2010; Jogia et al., 2011; Wessa et al., 2010). There have also been several reports of altered limbic activation in BD subjects during facial emotional stimuli (Chen et al., 2006; Dickstein et al., 2007; Foland et al., 2008; Lelli-Chiesa et al., 2011; Malhi et al., 2007). Interestingly, despite this body of evidence linking CACNA1C to the limbic system and BD, no studies investigating the impact of this polymorphism on facial emotion recognition scores (FER) in BD have yet been conducted.

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doi:10.1016/j.jad.2012.03.014

Impairments in FER as part of social cognition have been reported in BD (Harmer et al., 2002; Lembke and Ketter, 2002; Summers et al., 2006; Venn et al., 2004) and have been the focus of fMRI studies disclosing differentiated activation of the limbic region (Chen et al., 2006; Dickstein et al., 2007; Foland et al., 2008; Lelli-Chiesa et al., 2011; Malhi et al., 2007). FER deficits in BD include enhanced recognition of faces expressing disgust (Harmer et al., 2002), impaired recognition of faces showing fear (Lembke and Ketter, 2002; Venn et al., 2004), as well as a selective effect of mood state (Rich et al., 2008; Rocca et al., 2009) on recognition of surprise. In a recent meta-analysis, Kohler et al. (2011) concluded that FER impairment in BD represents a moderate and stable deficit. Impairments in FER have been the focus of many functional magnetic imaging studies (fMRI) in BD, showing altered activation of the ventromedial prefrontal cortex (PFC), cingulate, hippocampus, amygdala and limbic region (Chen et al., 2006; Dickstein et al., 2007; Foland et al., 2008; Lelli-Chiesa et al., 2011; Malhi et al., 2007).

Social cognition refers to the mental operations underlying social interactions, which can be relatively independent from other aspects of cognition, and is not assessed by traditional neurocognitive tasks (Pinkham et al., 2003). One of the key aspects of social cognition is the ability to discriminate accurately between different facially expressed emotions. Recently, it was reported that limbic system volume correlates with social functioning in humans. Bickart et al. (2011) reported that a larger amygdala was associated with a more complex social network (Bickart et al., 2011). A larger amygdala improves identification and recognition of socioemotional cues in individuals of the same species (Whalen and Phelps, 2009), allowing humans to develop complex strategies to cooperate and compete (Silk, 2007).

Calcium channels are important to convert electrical activity into biochemical events. Calcium channel variations can affect signal transduction as well as brain circuitry and may result in cognitive changes. The α1-subunit of the L-type voltage-gated calcium channel has been reported to influence emotional behavior through enhanced neurotransmission via the lateral amygdala pathway, and its expression is reported to be elevated in fear-conditioned animals (Shinnick-Gallagher et al., 2003). The CACNA1C risk allele for BD consists of a single-nucleotide polymorphism (SNP) (rs1006737, Val➔Met) shown to increase CACNA1C mRNA levels in the human brain (Bigos et al., 2010). Recent fMRI studies have revealed that the Met allele influences brain morphology and modulates activation in the limbic region during emotion processing tasks. Most fMRI studies on CACNA1C have found higher activity in the amygdala and hippocampus of Met carriers during different tasks. Wessa et al. (2010) observed elevated left amygdala activity in healthy Met carriers during a reward task. Also, Bigos et al. (2010) found the Met allele to be associated with increased hippocampal activity on emotional processing tasks (Bigos et al., 2010). Jogia et al. (2011) reported less activation in the ventrolateral prefrontal cortex of Met carriers with BD (Jogia et al., 2011). Moreover, carriers of the Met allele were shown to have greater volumes of gray matter, (Kempton et al., 2009) (Wang et al., 2011), brainstem (Franke et al., 2010), right amygdala and right hypothalamus grey density (Perrier et al., 2011), compared to non-carriers. These findings support the effects of rs1006737 on frontotemporal neural function and morphology implicated in the pathophysiology of BD (Wang et al., 2011).

The objective of the present research was to investigate the impact of the CACNA1C risk allele on FER scores and limbic system volume (amygdala and hippocampus) in BD subjects and healthy controls.

2. Material and methods

2.1. Subjects

BD group: Thirty-nine euthymic subjects with bipolar I disorder were included. Diagnoses were determined by trained psychiatrists based on the Structured Clinical Interview (SCID-I/P) (First et al., 1996) for DSM-IV TR (DSM-IV, 2000). Criteria for inclusion were patients not currently in a mood state (DSM-IV, 2000) and stable for at least 2 months. Subjects presenting with neurological disorders, history of previous head trauma or any illness requiring medical intervention, currently abusing any substance, or submitted to electroconvulsive therapy in the preceding six months, were excluded. The Young Mania Rating Scale (YMRS) (Young et al., 1978), and the Hamilton Depression Rating Scale (HDRS-21) (Hamilton, 1960) were used to evaluate subsyndromal symptoms. In the BD group, 78.6% of subjects were in use of lithium, 52.4% anticonvulsants, 23.8% second-generation antipsychotics, 16.7 antidepressants and 4.8% benzodiazepines, at neuropsychological evaluation.

Control group: forty healthy volunteers aged between 18 and 35 years old were recruited from the University of São Paulo. All controls had no current or past history of psychiatric disorder according to the evaluation conducted by trained psychiatrists using The Mini International Neuropsychiatric Interview (MINI) (Sheehan, 1998). Similarly, all subjects had no family history (first-degree relatives) of mood or psychotic disorders and had not been in recent use of psychotropic medicine or had indulged in substance abuse over the past three months.

2.2. Procedures

Subjects completed image acquisition, FER tests and genotyping in the same day.

1) *Image acquisition*: magnetic resonance imaging (MRI) was carried out using an Intera Achieva 3.0-T system with an eight-channel head coil (Philips, Best, The Netherlands). Sagittal three-dimensional T1-weighted anatomical images covering the whole brain were obtained using a fast-field echo pulse sequence (TR = 7 ms; TE = 3.2 ms; TI = 900 ms; flip angle = 8º) with isotropic 1 mm^3 resolution. MR images were processed offline with the program "FreeSurfer" v.5.1.0 (http://surfer.nmr.mgh.harvard.edu/) to obtain (automatically and non-interactively) volumes for structures in both right and left hemispheres. Intracranial volume was also measured with the same software for normalization purposes.
2) *FER tests*: all subjects included in this study underwent FER tests. Facial emotion recognition was tested using

the Ekman 60 Faces Test (EK) employing a range of photographs from the Ekman and Friesen (1976) series of Pictures of Facial Affect, the most widely used and validated series of photographs in facial expression research. From this series, the faces of 10 actors (6 female, 4 male) were chosen, each displaying six basic emotions ("happiness", "sadness", "disgust", "fear", "surprise" and "anger"). The EK can be used to assess recognition of facial expressions conveying basic emotions. The maximum test score (indicating best performance) is 60 for all six emotions, with 10 points designated for each basic emotion. The computer software for the test was available on CD-ROM. Patients were allowed unlimited time to respond. Immediately prior to testing, it was verified that patients and healthy controls semantically understood the words anger, disgust, fear, happiness, sadness and surprise. Patients and healthy controls were asked to provide an example for each emotion by answering the questions: "Describe a situation when you feel happiness, fear, etc." Incorrect answers were grounds for subject exclusion from this study. However, correct answers were given by all participants. The Emotion Hexagon Test (HX) is a test of facial emotion recognition utilizing pictures of emotional faces derived from Ekman and Friesen's (1976) Pictures of Facial Affect. Ekman and Friesen's original pictures were modified using computer manipulation techniques to generate stimuli of varying levels of difficulty. Each emotional face was merged with a picture depicting another emotion with which it was most likely to be confounded. Three levels of intensity were created for each emotion: 90%, 70% and 50%. Each face was presented for 5 s, after which time, participants were asked to decide which of the six emotions (happiness, sadness, surprise, disgust, anger and fear) best described the face. Participants completed a practice block followed by 5 test blocks of 30 trials each. Faces were presented in random order. Data from the practice block, and stimuli at the 50% intensity level, were not included in the analysis.

3) *Genotyping*: DNA was extracted from peripheral blood according to the salting-out protocol (Laitinen et al., 1994) and then genotyped for CACNA1C rs1006737 using real-time PCR allelic discrimination. PCR amplification for rs1006737 was performed in 5 μl reactions with 5 ng of template DNA, 1× TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 1× each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. Fluorescence detection occurred in the annealing step. Amplification and allelic discrimination were performed on a 7500 Real-Time System (Applied Biosystems, Foster City, CA). Quality control of Real time PCR results was done by direct sequencing on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.3. Statistical analysis

Groups of subjects were classified into two groups (BD and control). The Chi-square test was used for comparison of categorical data (gender, genotype and allelic frequency) whereas the *t*-test was employed for continuous data (age, education, amygdala and hippocampus volume). A multivariate analysis of covariance (MANOVA) model, in which FER scores of EK and EX were entered as dependent variables, was used to investigate the impact of the CACNA1C genotype on FER scores, while age, gender, education, group, CACNA1C genotype and group*CACNA1C interaction were input as covariates. The impact of the CACNA1C genotype on limbic system morphology was then determined using a MANOVA model in which left/right hippocampus and amygdala volumes were entered as dependent variables, and age, gender, group, CACNA1C genotype and group*CACNA1C interaction were input as covariates.

2.4. Ethics

The research ethics board of the *Hospital das Clínicas of the University of São Paulo* approved the study. Written informed consent was obtained from all participants.

3. Results

The CACNA1C genotype distribution in the experiment was in accordance with the Hardy–Weinberg equilibrium ($\chi^2 = 0.09$ $p = 0.75$) indicating that the samples were representative. The prevalence of Met/Met was 8%, Val/Met 44% and Val/Val 48%. CACNA1C genotype frequency was similar in both groups. (Table 1) Sociodemographic data of both groups are given in Table 1. The BD group had a higher mean

Table 1
Comparison of sociodemographic characteristics and CACNA1C genotype prevalence in controls and bipolar disorder.

	Gender (female/male)	Age	Education	CACNA1C genotype	YMRS	HDRS	Amygdala volume	Hippocampus volume
Controls (N = 40)	20/20							
Mean		25.9	14.1	Met/Met = 3			0.0045	0.0071
Std. deviation		5.8	2.8	Val/Met = 15			0.001	0.001
				Val/Val = 22				
Bipolar (N = 39)	24/15							
Mean		32.9	12.6	Met/Met = 4	2.3	4.1	0.0047	0.0063
Std. deviation		10.9	3.1	Val/Met = 20	1.8	2.0	0.001	0.001
				Val/Val = 15				
t-test		0.01	0.1					
Chi-squared	0.21			0.38			0.64	0.08

Table 2
MANCOVA analysis of covariance, in which FER scores of EK and EX were entered as dependent variables and age, gender, education, group and CACNA1C genotype, were entered as covariates.

Dependent variable	Covariate	B	Std. error	t	Sig.	Partial eta squared	Observed power
Ex total score	Intercept	95.518	11.348	8.417	0	51.8%	100.0%
	Age	−0.386	0.174	−2.215	**0.03**	6.9%	58.8%
	Gender	3.312	2.967	1.116	0.268	1.9%	19.6%
	Education	1.387	0.52	2.664	**0.01**	9.7%	74.7%
	Group	−27.786	9.132	−3.043	**0.003**	12.3%	85.0%
	[CACNA1C = Val/Met]	2.639	7.492	0.352	0.726	0.2%	6.4%
	[CACNA1C = Val/Val]	0.304	7.222	0.042	0.967	0.0%	5.0%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	26.141	9.999	2.614	**0.011**	9.4%	73.1%
	[CACNA1C = Val/Val]*group	27.01	9.77	2.765	**0.007**	10.4%	77.8%
	[CACNA1C = Met/Met]*group	0[a]					
Ex anger	Intercept	13.22	5.084	2.601	0.011	9.3%	72.7%
	Age	−0.118	0.078	−1.51	0.136	3.3%	31.9%
	Gender	0.658	1.329	0.495	0.622	0.4%	7.8%
	Education	0.329	0.233	1.412	0.163	2.9%	28.5%
	Group	−2.428	4.091	−0.593	0.555	0.5%	9.0%
	[CACNA1C = Val/Met]	1.899	3.356	0.566	0.573	0.5%	8.6%
	[CACNA1C = Val/Val]	1.066	3.235	0.329	0.743	0.2%	6.2%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	2.868	4.479	0.64	0.524	0.6%	9.7%
	[CACNA1C = Val/Val]*group	3.83	4.376	0.875	0.385	1.1%	13.9%
	[CACNA1C = Met/Met]*group	0[a]					
Ex disgust	Intercept	19.58	3.271	5.986	0	35.2%	100.0%
	Age	0	0.05	0.006	0.995	0.0%	5.0%
	Gender	1.158	0.855	1.354	0.18	2.7%	26.6%
	Education	−0.023	0.15	−0.154	0.878	0.0%	5.3%
	Group	−7.218	2.632	−2.742	**0.008**	10.2%	77.1%
	[CACNA1C = Val/Met]	−1.189	2.16	−0.551	0.584	0.5%	8.4%
	[CACNA1C = Val/Val]	−3.165	2.082	−1.52	0.133	3.4%	32.2%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	6.743	2.882	2.34	**0.022**	7.7%	63.5%
	[CACNA1C = Val/Val]*group	8.404	2.816	2.984	**0.004**	11.9%	83.7%
	[CACNA1C = Met/Met]*group	0[a]					
Ex fear	Intercept	10.793	3.635	2.969	0.004	11.8%	83.3%
	Age	−0.115	0.056	−2.061	**0.043**	6.0%	52.9%
	Gender	−0.903	0.95	−0.95	0.346	1.3%	15.5%
	Education	0.344	0.167	2.064	**0.043**	6.1%	52.9%
	Group	−3.349	2.925	−1.145	0.256	1.9%	20.4%
	[CACNA1C = Val/Met]	4.333	2.4	1.806	0.076	4.7%	42.8%
	[CACNA1C = Val/Val]	4.487	2.313	1.94	0.057	5.4%	48.1%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	2.042	3.202	0.638	0.526	0.6%	9.6%
	[CACNA1C = Val/Val]*group	1.877	3.129	0.6	0.551	0.5%	9.1%
	[CACNA1C = Met/Met]*group	0[a]					
Ex happiness	Intercept	18.475	1.546	11.947	0	68.4%	100.0%
	Age	−0.05	0.024	−2.116	**0.038**	6.4%	55.0%
	Gender	0.955	0.404	2.363	0.021	7.8%	64.4%
	Education	0.202	0.071	2.852	**0.006**	11.0%	80.2%
	Group	−3.544	1.244	−2.848	**0.006**	10.9%	80.1%
	[CACNA1C = Val/Met]	−0.87	1.021	−0.853	0.397	1.1%	13.4%
	[CACNA1C = Val/Val]	−0.98	0.984	−0.995	0.323	1.5%	16.5%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	3.686	1.362	2.705	**0.009**	10.0%	76.0%
	[CACNA1C = Val/Val]*group	3.398	1.331	2.552	0.013	9.0%	71.1%
	[CACNA1C =						
Ex sadness	Intercept	19.171	2.255	8.503	0	52.3%	100.0%
	Age	−0.126	0.035	−3.634	**0.001**	16.7%	94.7%
	Gender	1.058	0.589	1.795	0.077	4.7%	42.4%
	Education	0.216	0.103	2.085	0.041	6.2%	53.8%
	Group	−5.166	1.814	−2.848	**0.006**	10.9%	80.1%
	[CACNA1C = Val/Met]	−0.601	1.488	−0.404	0.688	0.2%	6.8%
	[CACNA1C = Val/Val]	−0.136	1.435	−0.095	0.925	0.0%	5.1%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	5.64	1.986	2.839	**0.006**	10.9%	79.9%
	[CACNA1C = Val/Val]*group	4.957	1.941	2.554	**0.013**	9.0%	71.1%
	[CACNA1C = Met/Met]*group	0[a]					

(continued on next page)

Table 2 *(continued)*

Dependent variable	Covariate	B	Std. error	t	Sig.	Partial eta squared	Observed power
Ex surprise	Intercept	14.596	2.643	5.523	0	31.6%	100.0%
	Age	0.013	0.041	0.315	0.754	0.2%	6.1%
	Gender	0.349	0.691	0.505	0.615	0.4%	7.9%
	Education	0.318	0.121	2.625	**0.011**	9.5%	73.4%
	Group	−5.963	2.127	−2.804	**0.007**	10.6%	78.9%
	[CACNA1C = Val/Met]	−0.965	1.745	−0.553	0.582	0.5%	8.5%
	[CACNA1C = Val/Val]	−0.971	1.682	−0.577	0.566	0.5%	8.8%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	5.002	2.329	2.148	**0.035**	6.5%	56.2%
	[CACNA1C = Val/Val]*group	4.466	2.275	1.963	0.054	5.5%	49.0%
	[CACNA1C = Met/Met]*group	0[a]					
EK total score	Intercept	51.084	5.796	8.814	0	54.1%	100.0%
	Age	−0.178	0.089	−2	0.05	5.7%	50.4%
	Gender	0.582	1.515	0.384	0.702	0.2%	6.7%
	Education	0.4	0.266	1.505	0.137	3.3%	31.7%
	Group	−13.06	4.664	−2.8	**0.007**	10.6%	78.8%
	[CACNA1C = Val/Met]	−3.103	3.826	−0.811	0.42	1.0%	12.6%
	[CACNA1C = Val/Val]	−4.631	3.688	−1.256	0.214	2.3%	23.6%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	12.725	5.107	2.492	**0.015**	8.6%	69.0%
	[CACNA1C = Val/Val]*group	15.094	4.99	3.025	**0.004**	12.2%	84.6%
	[CACNA1C = Met/Met]*group	0[a]					
EK anger	Intercept	8.183	1.749	4.68	0	24.9%	99.6%
	Age	−0.013	0.027	−0.497	0.621	0.4%	7.8%
	Gender	0.589	0.457	1.288	0.202	2.5%	24.5%
	Education	0.126	0.08	1.574	0.12	3.6%	34.1%
	Group	−3.913	1.407	−2.781	**0.007**	10.5%	78.2%
	[CACNA1C = Val/Met]	−2.131	1.155	−1.846	0.069	4.9%	44.4%
	[CACNA1C = Val/Val]	−2.48	1.113	−2.229	**0.029**	7.0%	59.3%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	4.629	1.541	3.004	**0.004**	12.0%	84.1%
	[CACNA1C = Val/Val]*group	5.034	1.505	3.344	**0.001**	14.5%	90.9%
	[CACNA1C = Met/Met]*group	0[a]					
EK disgust	Intercept	11.21	1.915	5.854	0	34.2%	100.0%
	Age	−0.046	0.029	−1.571	0.121	3.6%	34.0%
	Gender	0.168	0.501	0.336	0.738	0.2%	6.3%
	Education	−0.017	0.088	−0.197	0.845	0.1%	5.4%
	Group	−2.297	1.541	−1.491	0.141	3.3%	31.2%
	[CACNA1C = Val/Met]	−2.478	1.264	−1.96	0.054	5.5%	48.9%
	[CACNA1C = Val/Val]	−2.488	1.219	−2.042	**0.045**	5.9%	52.1%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	3.153	1.687	1.869	**0.066**	5.0%	45.3%
	[CACNA1C = Val/Val]*group	2.52	1.649	1.529	0.131	3.4%	32.5%
	[CACNA1C = Met/Met]*group	0[a]					
EK fear	Intercept	9.531	2.337	4.079	0	20.1%	98.0%
	Age	−0.108	0.036	−3.008	**0.004**	12.1%	84.2%
	Gender	0.025	0.611	0.041	0.967	0.0%	5.0%
	Education	−0.05	0.107	−0.47	0.64	0.3%	7.5%
	Group	−2.678	1.88	−1.424	0.159	3.0%	28.9%
	[CACNA1C = Val/Met]	0.833	1.543	0.54	0.591	0.4%	8.3%
	[CACNA1C = Val/Val]	0.858	1.487	0.577	0.566	0.5%	8.8%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	2.063	2.059	1.002	0.32	1.5%	16.7%
	[CACNA1C = Val/Val]*group	2.462	2.012	1.224	0.225	2.2%	22.6%
	[CACNA1C = Met/Met]*group	0[a]					
EK happiness	Intercept	9.182	1.229	7.47	0	45.8%	100.0%
	Age	−0.006	0.019	−0.34	0.735	0.2%	6.3%
	Gender	−0.074	0.321	−0.23	0.819	0.1%	5.6%
	Education	0.052	0.056	0.931	0.355	1.3%	15.1%
	Group	0.081	0.989	0.082	0.935	0.0%	5.1%
	[CACNA1C = Val/Met]	0.252	0.812	0.311	0.757	0.1%	6.1%
	[CACNA1C = Val/Val]	−0.479	0.782	−0.612	0.542	0.6%	9.3%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	−0.27	1.083	−0.249	0.804	0.1%	5.7%
	[CACNA1C = Val/Val]*group	0.662	1.058	0.626	0.534	0.6%	9.5%
	[CACNA1C = Met/Met]*group	0[a]					
EK sadness	Intercept	6.602	1.976	3.341	0.001	14.5%	90.9%
	Age	−0.023	0.03	−0.763	0.448	0.9%	11.7%
	Gender	−0.271	0.517	−0.524	0.602	0.4%	8.1%
	Education	0.113	0.091	1.248	0.216	2.3%	23.4%
	Group	−3.405	1.59	−2.141	**0.036**	6.5%	56.0%

Table 2 *(continued)*

Dependent variable	Covariate	B	Std. error	t	Sig.	Partial eta squared	Observed power
	[CACNA1C = Val/Met]	0.57	1.305	0.437	0.664	0.3%	7.2%
	[CACNA1C = Val/Val]	0.431	1.258	0.343	0.733	0.2%	6.3%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	3.058	1.741	1.756	0.084	4.5%	40.9%
	[CACNA1C = Val/Val]*group	3.579	1.701	2.104	**0.039**	6.3%	54.5%
	[CACNA1C = Met/Met]*group	0[a]					
EK surprise	Intercept	18.563	5.372	3.455	0.001	15.3%	92.6%
	Age	−0.173	0.082	−2.094	**0.04**	6.2%	54.1%
	Gender	1.699	1.405	1.209	0.231	2.2%	22.2%
	Education	−0.351	0.246	−1.423	0.159	3.0%	28.9%
	Group	−0.957	4.323	−0.221	0.825	0.1%	5.5%
	[CACNA1C = Val/Met]	−1.525	3.547	−0.43	0.669	0.3%	7.1%
	[CACNA1C = Val/Val]	−1.579	3.419	−0.462	0.646	0.3%	7.4%
	[CACNA1C = Met/Met]	0[a]					
	[CACNA1C = Val/Met]*group	4.512	4.733	0.953	0.344	1.4%	15.6%
	[CACNA1C = Val/Val]*group	1.661	4.625	0.359	0.721	0.2%	6.4%
	[CACNA1C = Met/Met]*group	0[a]					

Text in bold indicates significance level $p<0.05$.

[a] This parameter is set to zero because it is redundant.

age than the control group. Mean YMRS and HDRS scores of euthymic BD patients were 2.3 (±1.8) and 4.1 (±2), respectively. No difference in amygdala or hippocampus volume was found between BD subjects and controls (Table 1).

3.1. BD subjects had a global deficit on FER scores compared to controls

FER scores were lower in the BD group than the control group on the following tests: EX total score (B = −27.7 t = −3.0 p = 0.003), EX "disgust" (B = −7.21 t = −2.7p = 0.008), EX "happiness" (B = −3.5 t = −2.8 p = 0.006), EX "sadness" (B = −5.16 t = −2.8 p = 0.006), EX "surprise" (B = −5.9 t = −2.8 p = 0.007), EK total score (B = −13.0 t = −2.8 p = 0.007) EK "anger" (B = −3.9 t = −2.7 p = 0.007) and EK "sadness" (B = −3.4 t = −2.1 p = 0.036) (Table 2).

3.2. The CACNA1C genotype influenced FER only in BD

An influence of the CACNA1C genotype on FER was observed only in the BD group. Carriers of the Met/Met genotype in this group had lower EX and EK total scores compared to carriers of the Val/Met or Val/Val genotypes (Table 2). This finding indicated a global dysfunction in FER among those subjects with BD homozygous for the CACNA1C risk allele (power > 70%). Moreover, Met/Met BD subjects were found to have poorer recognition of "disgust" faces (EX and EK disgust) compared to individuals with other genotypes. The same phenomenon was observed for recognition of "sadness" faces (EX and EK sadness). Furthermore, scores for EX "happiness", EX "surprise" and EK "anger" were also lower scores in Met/Met carriers (Table 2).

3.3. CACNA1C exerted no influence on amygdala or hippocampus volumes

The CACNA1C genotype had no influence on amygdala or hippocampus volumes in either of the groups. By contrast, an influence of gender and age on amygdala and hippocampus volumes was observed (Table 3).

4. Discussion

To the best of our knowledge, this is the first study to report an association of the CACNA1C risk allele for BD to global dysfunctions in FER among BD subjects. Moreover, the study results showed no impact of CACNA1C on amygdala or hippocampus volumes in BD or controls.

This data suggests that calcium channel dysfunction contributes in part to the genetic regulation of FER in BD, probably mediated by alterations in the functional activity of brain circuitries implicated in this condition. This report of a candidate risk allele for BD influencing FER is analogous to previous reports linking other genes associated with BD diagnosis to impaired FER performance. Recently, our group has demonstrated that catechol-O-methyltransferase (COMT), considered a susceptibility gene for BD (Shifman et al., 2004), also influences FER scores in BD (Soeiro de Souza et al., 2012). Thus, associating a BD risk gene to specific alterations in FER implies that the underlying neural system mechanisms involved in the neuropathology of BD are similar to the mechanisms controlling the FER phenotype. This conclusion is in agreement with the results of previous studies showing that FER deficits in BD are a characteristic of the disease observable in all mood states and even in first-degree relatives not affected by BD (Kohler et al., 2011; Samamé et al., 2012; Seidel et al., 2012).

Previous studies have not explored the impact of CACNA1C on FER scores in BD patients or controls. Some studies however, have associated the CACNA1C genotype with cognitive function in controls and schizophrenia (SZ) subjects but not in BD. The majority of studies evaluating the potential role of the CACNA1C risk allele in cognitive function have evaluated healthy volunteers. One study reported impaired performance in attention and orientation (Thimm et al., 2011) while another found impaired verbal fluency (Krug et al., 2010) in Met carriers. The largest study sample investigating the impact of CACNA1C on cognition (n = 700 healthy

Table 3
Multivariate analysis of covariance using hippocampus and amygdala volume as dependent variables and age, gender, group, and CACNA1C genotype as covariates.

Covariate	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared	Observed power
Age	Left hippocampus	8.64E−07	1	8.64E−07	4.68	0.03	6.3%	56.8%
	Left amygdala	1.14E−07	1	1.14E−07	1.88	0.17	2.7%	27.3%
	Right hippocampus	7.84E−07	1	7.84E−07	3.35	0.07	4.6%	43.8%
	Right amygdala	4.85E−08	1	4.85E−08	0.71	0.40	1.0%	13.2%
	Total amygdala	2.47E−09	1	2.47E−09	0.00	0.98	0.0%	5.0%
	Total hippocampus	5.87E−06	1	5.87E−06	1.88	0.18	2.6%	27.1%
Gender	Left hippocampus	4.22E−07	1	4.22E−07	2.29	0.14	3.2%	32.0%
	Left amygdala	3.49E−07	1	3.49E−07	5.79	0.02	7.7%	66.0%
	Right hippocampus	1.43E−06	1	1.43E−06	6.12	0.02	8.1%	68.4%
	Right amygdala	3.94E−07	1	3.94E−07	5.73	0.02	7.7%	65.6%
	Total amygdala	7.41E−08	1	7.41E−08	0.02	0.88	0.0%	5.2%
	Total hippocampus	7.80E−06	1	7.80E−06	2.49	0.12	3.5%	34.4%
Group	Left hippocampus	8.78E−09	1	8.78E−09	0.05	0.83	0.1%	5.5%
	Left amygdala	3.97E−10	1	3.97E−10	0.01	0.94	0.0%	5.1%
	Right hippocampus	5.53E−07	1	5.53E−07	2.36	0.13	3.3%	32.8%
	Right amygdala	6.84E−08	1	6.84E−08	1.00	0.32	1.4%	16.6%
	Total amygdala	1.32E−08	1	1.32E−08	0.00	0.95	0.0%	5.0%
	Total hippocampus	9.30E−07	1	9.30E−07	0.30	0.59	0.4%	8.4%
CACNA1C	Left hippocampus	9.62E−09	2	4.81E−09	0.03	0.97	0.1%	5.4%
	Left amygdala	4.35E−09	2	2.18E−09	0.04	0.97	0.1%	5.5%
	Right hippocampus	4.23E−07	2	2.11E−07	0.90	0.41	2.5%	20.0%
	Right amygdala	3.10E−08	2	1.55E−08	0.23	0.80	0.6%	8.4%
	Total amygdala	1.42E−05	2	7.12E−06	2.11	0.13	5.8%	42.0%
	Total hippocampus	1.30E−05	2	6.51E−06	2.08	0.13	5.7%	41.4%
CACNA1C*group	Left hippocampus	2.54E−07	2	1.27E−07	0.69	0.51	2.0%	16.1%
	Left amygdala	3.87E−08	2	1.94E−08	0.32	0.73	0.9%	9.9%
	Right hippocampus	1.21E−08	2	6.06E−09	0.03	0.97	0.1%	5.4%
	Right amygdala	1.20E−08	2	5.99E−09	0.09	0.92	0.3%	6.3%
	Total amygdala	7.78E−06	2	3.89E−06	1.16	0.32	3.2%	24.6%
	Total hippocampus	8.29E−06	2	4.15E−06	1.33	0.27	3.7%	27.7%

subjects) found no association of the CACNA1C Met allele with performance on memory, attention, or executive function tests (Roussos et al., 2011). Only one study has evaluated the impact of CACNA1C on cognition of BD (manic episode), and finding no association with working memory (Zhang et al., 2012).

Previous studies have reported that CACNA1C risk allele would influence brain morphology in controls. Met allele showed to increase gray matter volume, (Kempton et al., 2009) (Wang et al., 2011), brainstem volume (Franke et al., 2010), right amygdala and right hypothalamus grey density (BD and controls) (Perrier et al., 2011) compared to non-carriers. We did not confirm that CACNA1C risk allele influences amygdala or hippocampus volumes in controls or BD.

The explanation for our findings showing that a high-risk gene for BD is associated to worse FER in BD is highly plausible. Furthermore, knockout mice models have demonstrated that L-type calcium channels play a critical role on cognition (White et al., 2008). Calcium influx through L-type voltage-gated calcium channels activates calcium-dependent calmodulin kinase IV and Ras/mitogen activated kinase which, in turn, phosphorylate the transcription factor CREB at serine 133 (Wu et al., 2001). Once phosphorylated, CREB becomes part of an active transcriptional complex that binds to cAMP-response element DNA sequences to regulate transcription of a number of gene products. Importantly, calcium mediated activation of CREB has been implicated as a key subcellular signaling cascade in a wide range of behavioral processes including long-term memory consolidation (Deisseroth et al., 2003; West et al., 2002).

Limitations of this study include the relatively small sample size in both groups, and the fact that subjects were genotyped for only one CACNA1C polymorphism (the only functional one). The strengths of this study include that it is the first to report data about the impact of CACNA1C on FER and that it also explored CACNA1C impact on amygdala and hippocampus volumes. Moreover our BD sample compromised exclusively Bipolar type I disorder.

5. Conclusion

In summary, we reported evidence that a polymorphism in L-type calcium channels (CACNA1C rs1006737) correlates with FER scores but not with limbic system morphology. The risk Met allele had a negative impact on FER scores in BD subjects only. Further studies exploring how this CACNA1C risk allele for BD impacts on the neurobiological basis of the illness associated with behavior and cognition may provide further insights into the role of this calcium channel mutation in the biobehavioral model of BD.

Role of funding source
Sao Paulo research foundation (Fapesp) is an independent public foundation with the mission to foster research and the scientific and technological development of the State of São Paulo.

Conflict of interest
The authors do not have any conflict of interest to report.

Acknowledgments

We would like to thank the Institute of Psychiatry at the University of Sao Paulo, especially the members of the Mood Disorders Unit (GRUDA) and Laboratory of Neuroscience (LIM27) for their dedication and hard work, as well as all volunteers for their collaboration.

Financial disclosures: The São Paulo Research Foundation (Fundo de Apoio a Pesquisa do Estado de Sao Paulo—FAPESP) financed this research.

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12.4. COMT Met (158) modulates facial emotion recognition in bipolar I disorder mood episodes

Journal of Affective Disorders 136 (2012) 370–376

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

COMT Met (158) modulates facial emotion recognition in bipolar I disorder mood episodes

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ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form 25 October 2011
Accepted 14 November 2011
Available online 4 January 2012

Keywords:
Dopamine
Facial emotions
Catechol-O-methyltransferase
Bipolar disorder
Mania
Depression

ABSTRACT

Background: One of the many cognitive deficits reported in bipolar disorder (BD) patients is facial emotion recognition (FER), which has recently been associated with dopaminergic catabolism. Catechol-O-methyltransferase (COMT) is one of the main enzymes involved in the metabolic degradation of dopamine (DA) in the prefrontal cortex (PFC). The *COMT* gene polymorphism rs4680 (Val^{158}Met) Met *allele* is associated with decreased activity of this enzyme in healthy controls. The objective of this study was to evaluate the influence of Val^{158}Met on FER during manic and depressive episodes in BD patients and in healthy controls.
Materials and methods: 64 BD type I patients (39 in manic and 25 in depressive episodes) and 75 healthy controls were genotyped for *COMT* rs4680 and assessed for FER using the Ekman 60 Faces (EK60) and Emotion Hexagon (Hx) tests.
Results: Bipolar manic patients carrying the Met *allele* recognized fewer surprised faces, while depressed patients with the Met *allele* recognized fewer "angry" and "happy" faces. Healthy homozygous subjects with the Met *allele* had higher FER scores on the Hx total score, as well as on "disgust" and "angry" faces than other genotypes.
Conclusion: This is the first study suggesting that COMT rs4680 modulates FER differently during BD episodes and in healthy controls. This provides evidence that PFC DA is part of the neurobiological mechanisms of social cognition. Further studies on other COMT polymorphisms that include euthymic BD patients are warranted.
ClinicalTrials.gov Identifier: NCT00969.

1. Introduction

There is a growing body of evidence pointing to impaired facial emotion recognition (FER) in bipolar disorder (BD) during mood episodes and euthymia (Harmer et al., 2002; Lembke and Ketter, 2002; Summers et al., 2006; Venn et al., 2004). Recently, the dopaminergic system, through catechol-O-methyltransferase (COMT), has been implicated in the neurobiology of FER by genetic association studies with functional magnetic resonance imaging (fMRI) in healthy subjects and euthymic BD patients (Lelli-Chiesa et al., 2011; Mier et al., 2010). Most data from healthy subjects have provided system-level evidence supporting a behavioral dissociation by showing an effect of the COMT single nucleotide polymorphism (SNP) rs4680 (Val^{158}Met) on amygdala activation during tasks with emotional processing components. Despite the available data, it is unclear if or how the COMT functional polymorphism modulates FER capacity in healthy populations and BD patients during episodes.

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doi:10.1016/j.jad.2011.11.021

Social cognition refers to the mental operations underlying social interactions, which can be relatively independent from other aspects of cognition and is not assessed by traditional neurocognitive tasks (Pinkham et al., 2003). One of the key aspects of social cognition is the ability to discriminate accurately between different facially expressed emotions. The ability to process and identify facial emotions is an essential component of human communication and social interaction. Although social interaction may vary according to cultural norms and customs, cross-cultural studies have repeatedly provided evidence in favor of the universality of facial emotions. Six universal emotions have since been established, including happiness, sadness, anger, fear, disgust and surprise, each of which corresponds to a specific arrangement of facial muscles and has partially separable neurocircuitry processes (Ekman and Friesen, 1971; Gosselin and Kirouac, 1995). BD patients, even during remission, have psychosocial problems caused not only by residual symptoms, but also by cognitive deficits and difficulties in social cognition (Burdick et al., 2010; Jabben et al., 2010; Rocca et al., 2009; Solé et al., 2011).

Evidence for BD deficits in FER varies in the literature from reports of no alterations and improved recognition for disgust (Harmer et al., 2002), isolated fear recognition impairment (Lembke and Ketter, 2002; Venn et al., 2004), to a selective effect of mood state (Rich et al., 2008; Rocca et al., 2009) on surprise recognition. In a recent meta-analysis however, Kohler et al. (2011) concluded that FER impairment in BD represents a moderate and stable deficit that appears to be moderated by a limited number of demographic and clinical factors such as self-reported depression, age at time of testing and years of education. BD patient impairments in FER have been the focus of intense study with fMRI disclosing differentiated activation of ventromedial prefrontal cortex (PFC), cingulate, hippocampus, amygdala and limbic region (Chen et al., 2006; Dickstein et al., 2007; Foland et al., 2008; Lelli-Chiesa et al., 2011; Malhi et al., 2007). Chen et al. (2006) reported a significant increase in amygdala activity among BD patients versus control subjects emotion labeling tasks. In this regard, Foland et al. (2008) showed that compared to healthy subjects, manic patients had significantly reduced ventrolateral PFC regulation of amygdala response during the emotion labeling task.

COMT is an important regulator of PFC dopaminergic (DA) levels (Lachman et al., 1996). This role renders COMT one of the main enzymes involved in the metabolic degradation of extraneuronal DA in glial cells and postsynaptic neurons (Lachman et al., 1996). Genetic studies have shown that COMT activity levels can vary considerably. The rs4680 SNP in the *COMT* gene leads to a 3 to 4-fold reduction in COMT enzyme activity in A *allele* (Met) carriers (Lachman et al., 1996). As a result, they have high levels of PFC DA due to lower enzyme activity while heterozygous subjects have an intermediate level of enzyme activity (Lachman et al., 1996; Weinshilboum et al., 1999). Thus, the COMT polymorphism rs4680 is responsible for genetically modulating DA levels in PFC. Recently, genetic association fMRI studies confirmed that COMT SNP rs4680 influenced emotion stimulus processing, showing that the Val *allele* was associated with greater amygdala activation and that signal change was greater for the Met *allele* in the ventromedial PFC and ventrolateral PFC (Lelli-Chiesa et al., 2011). Furthermore, studies in healthy carriers of the Val *allele* reported impaired performance (Bosia et al., 2007; Egan et al., 2001; Joober et al., 2002) coupled with increased dorsal PFC activation during executive function tasks (Bertolino et al., 2006; Blasi et al., 2005; Mattay et al., 2003; Mier et al., 2010; Schott et al., 2006; Winterer et al., 2006). Nonetheless, healthy subjects with the Met *allele* are associated with greater reactivity to emotionally negative stimuli, as evidenced by increased activation in the ventral PFC and associated limbic regions (Drabant et al., 2006; Mier et al., 2010; Smolka et al., 2005). Despite all this information, it is unclear if/how the COMT polymorphism impacts FER in healthy controls or in BD patients during manic and depressive episodes.

Based on the potential association revealed by fMRI studies between COMT and FER, the objective of this research was to investigate how/if the lower activity of the Met *allele* of COMT influenced FER scores in BD patients (mania and depression states) and healthy controls.

2. Materials and methods

2.1. Sample

The patient sample comprised sixty-four medication-free individuals with BD I, aged between 18 and 40 years old (28.16 ± 5.24 years) and currently in manic or depressive episodes according to DSM-IV TR criteria (DSM-IV, 2000). All patients were participants in the *LICAVAL* clinical trial (Campos et al., 2010) and were evaluated immediately after a wash-out period of four weeks for antidepressants, mood stabilizers and antipsychotics, or eight weeks for *depot* medications. Diagnoses were determined by trained psychiatrists using the Structured Clinical Interview (SCID-I) (First et al., 1997) for DSM-IV TR (DSM-IV, 2000). The Young Mania Rating Scale (YMRS) (Young et al., 1978) and Hamilton Depression Rating Scale (HDRS-21) (Hamilton, 1960) were used to evaluate the intensity of symptoms. The cut-off point for mania was YMRS ≥ 12 and for depression was HDRS ≥ 15. The 39 manic patients had a mean YMRS of 15.67 (± 3.44), while the 25 depressive patients had a mean HDRS score of 21.70 (± 7.18). Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, current substance abuse, or that had undergone electroconvulsive therapy in the preceding six months, were excluded.

2.2. Control group

Seventy-five healthy volunteers (predominantly medical students) aged between 18 and 35 years old (23.54 ± 3.53) were recruited from the University of São Paulo. All control subjects had no current or past history of psychiatric disorder according to the evaluation conducted by trained psychiatrists using The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Similarly, all subjects had no family history (first degree relatives) of mood or psychotic disorders and had not been in recent use of psychotropic medicine or indulged in substance abuse over the last three months. Only women with a regular menstrual cycle were included. The YMRS (Young et al., 1978) and HDRS-21 (Hamilton, 1960) instruments were used to evaluate

subclinical symptoms in controls, yielding a mean YMRS score of 0.67 (±1.05) and mean HDRS score of 0.75 (±1.21).

2.3. FER tests

Facial emotion recognition was tested using the Ekman 60 Faces Test (EK60) employing a range of photographs from the Ekman and Friesen series of Pictures of Facial Affect (Ekman and Friesen, 1976), the most widely used and validated series of photographs in facial expression research. From this series, the faces of 10 actors (6 female, 4 male) were chosen, each displaying six basic emotions ("happiness", "sadness", "disgust", "fear", "surprise" and "anger"). The EK60 can be used to assess recognition of facial expression of basic emotions. The maximum test score (indicating best performance) is 60 for all six emotions, with 10 points designated for each basic emotion. The computer software for the test was available on CD-ROM. Patients were allowed unlimited time to respond. Immediately prior to testing, it was verified that patients and healthy controls semantically understood the words anger, disgust, fear, happiness, sadness and surprise. Patients and healthy controls were asked to provide an example for each emotion by answering the questions: "Describe a situation when you feel happiness, fear, etc." Any incorrect answer would have led to exclusion from this study but all participants gave correct answers.

The Emotion Hexagon Test (Hx) is a test of facial emotion recognition utilizing pictures of emotional faces derived from Ekman and Friesen's Pictures of Facial Affect (1976). Ekman and Friesen's original pictures were modified using computer manipulation techniques to generate stimuli of varying levels of difficulty. Each emotional face was merged with a picture depicting another emotion, which it was most likely to be confused with. Three levels of intensity were created for each emotion: 90%, 70% and 50%. Each face was presented for 5 s, after which time, participants were asked to decide which of the six emotions (happiness, sadness, surprise, disgust, anger and fear) best described the face. Participants completed a practice block followed by 5 test blocks of 30 trials each. Faces were presented in random order. Data from the practice block and stimuli at the 50% intensity level were not included in the analysis.

2.4. Ethics

The research ethics board of the *Hospital das Clínicas of the University of São Paulo* approved the study. Written informed consent was obtained from all study participants.

2.5. Genotyping

DNA was extracted from peripheral blood according to the salting-out protocol (Laitinen et al., 1994) and then genotyped for COMT rs4680 using real-time PCR allelic discrimination. PCR amplification for rs4680 was performed in 5 μl reactions with 5 ng of template DNA, 1× TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA), 1× each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. Fluorescence detection occurred in the annealing step. The amplification and allelic discrimination were performed in a 7500 Real-Time System (Applied Biosystems, Foster City, CA). Quality control of Real time PCR results was done by direct sequencing on a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.6. Statistical analyses

Subjects were classified into three groups (bipolar subjects in mania or depressive episodes and healthy controls) then stratified according to COMT genotype into Met allele presence [Met+ ($Met^{158}Val$, $Met^{158}Met$)] or absence [Met− ($Val^{158}Val$)]. Groups were not analyzed by genotype ($Met^{158}Met$, $Val^{158}Met$ $Val^{158}Val$) because after stratification by mood episode and genotype some groups contained less than 10 individuals, which could decrease our statistic power. Analysis of the three groups was done using the ANOVA test while the Chi-square test was used for categorical data, and the Student's t-test for continuous data. EK60 and Hx scores were compared within each group using Student's t test. Bonferroni's post hoc test was performed for correction when three or more groups were compared. Pearson's correlation test was used to assess the relationship between symptoms scales and FER tests in each group. Version 18.0 of the PASW statistics software (SPSS Inc., Chicago, Illinois) was used for all analyses.

3. Results

The COMT genotype distribution in the experimental samples of men and women was in accordance with the Hardy–Weinberg equilibrium ($\chi^2 = 0.79$) indicating that the samples were representative. Allelic frequency was 32.31% for Met− and 67.69% for Met+. No statistically significant differences in sociodemographics were observed between genotypes in terms of age, gender or years of schooling (Table 1). The COMT genotype did not influence YMRS or HDRS during manic or depressive episodes.

3.1. Bipolar subjects had lower scores on facial emotion recognition than controls

ANOVA analysis among the three groups of subjects (mania, depression and controls) for scores on the EK60 and Hx tests revealed significant differences in the following tests: EK60 total score ($F = 10.68$; $p < 0.001$), EK60 "anger" ($F = 2.98$; $p = 0{,}05$), EK60 "fear" ($F = 7.63$; $p = 0.001$), Hx total score ($F = 10.32$; $p < 0.001$), Hx "fear" ($F = 13.63$; $p = < 0.001$), Hx "happiness" ($F = 3.75$; $p = 0.026$), and Hx "surprise" ($F = 7.55$; $p = 0.001$) (Table 2). Bonferroni post hoc analysis for multiple variables confirmed the relationship: controls > mania = depression; in all FER tests with the exception of Hx "happiness" and EK60 "anger", in which controls = depression > mania (Table 2).

3.2. Symptoms severity correlated with facial emotion recognition in mania and depression

In manic subjects, YMRS score correlated positively with EK60 "fear" ($r = 0.47$; $p = 0.002$) and Hx "fear" ($r = 0.42$;

Table 1
Sociodemographic variables for patients in mania or depression and healthy controls, carrying the COMT Met+/− or Val+/− *alleles*.

	Mania			Depression			Controls		
	Met+ Mean (SD)	Met− Mean (SD)	p	Met+ Mean (SD)	Met− Mean (SD)	p	Met+ Mean (SD)	Met− Mean (SD)	p
YMRS	15.5 (7.2)	15.4 (4.3)	0.94	10.2 (6.1)	9.1 (6.9)	0.70	0.7 (1.1)	0.4 (0.7)	0.15
HDRS	18.2 (8.1)	19.1 (7.4)	0.72	22.3 (7.2)	22.7 (6.4)	0.91	0.8 (1.3)	0.5 (0.7)	0.24
Years of study	12.3 (3.3)	12.1 (4.1)	0.90	12.6 (2.8)	12.5 (1.3)	0.88	14.2 (2.2)	14.2 (2.6)	0.99
Age	29.3 (5.1)	27.8 (4.8)	0.40	25.9 (5.0)	28.2 (5.4)	0.33	23.3 (3.4)	23.5 (3.2)	0.86

YMRS = Young Mania Rating Scale; HDRS = Hamilton Depression Rating Scale.

$p = 0.006$) and negatively with EK60 "surprise" ($r = -0.43$; $p = 0.005$). Also, HDRS correlated negatively with both Hx "disgust" ($r = -0.35$; $p = 0.031$) and Hx "happiness" ($r = -0.39$; $p = 0.015$). In the depression group, YMRS correlated negatively with EK60 total score ($r = -0.41$; $p = 0.041$) while HDRS correlated negatively with both EK60 "happiness" ($r = -0.42$; $p = 0.035$) and Hx "happiness" ($r = -0.46$; $p = 0.02$).

3.3. In mania or depression Met + subjects recognized less facial emotions than Met −

Met+ bipolar subjects (mania + depression) irrespective of mood state recognized fewer "surprise" faces on the EK60 test than did Met− subjects. Met+ individuals in mania recognized fewer "surprise" faces ($t = -2.17$; $p = 0.037$) on the EK60 test than Met− subjects (Table 3). Met+ subjects in depression scored lower than Met− on EK60 "happiness" ($t = -2.32$; $p = 0.036$), Hx "anger" ($t = -2.25$; $p = 0.035$) and Hx "happiness" ($t = -2.35$; $p = 0.034$). Regarding controls, no difference in FER test scores was found between Met+ and Met− subjects, which may indicate selective effects of COMT rs4680 for BD FER (Table 3).

3.4. Scales of depressive and manic symptoms severity interacted with Met allele on FER results

Regression analysis detected interaction between Met allele and HDRS only on EK60 "happiness" ($B = -0.06$ $t = -2.2$ $p = 0.02$ Partial Eta Squared = 0.08). Allele Met and YMRS interaction was observed in EK60 "fear" ($B = 0.26$ $t = 2.11$ $p = 0.039$ Partial Eta Squared = 0.07) and EK60 "surprise" ($B = -0.17$ $t = -2.07$ $p = 0.043$ Partial Eta Squared = 0.07).

4. Discussion

To the best of our knowledge, this is the first research to report the association between the COMT Met *allele* and FER scores in bipolar I disorder mood episodes. During mood episodes, Met+ bipolar subjects had lower FER scores compared to Met−. Met+ subjects in manic episode recognized fewer surprise faces, while those in a depressive

Table 2
FER scores for bipolar patients in mania or depression and healthy controls.

	Mania Mean (SD)	Depression Mean (SD)	Controls Mean (SD)	F	p	Bonferroni Post hoc
Ek60 total	45.7 (5.7)	47.4 (6.7)	50.6 (5.1)	10.68	<0.001	**m = d < c**
ANGER	7.6 (1.8)	8.0 (1.7)	8.3 (1.3)	2.98	0.05	m < d = c
DISGUST	7.2 (2.0)	7.8 (1.9)	7.7 (1.9)	0.81	0.44	m = d = c
FEAR	5.4 (2.7)	5.5 (3.0)	7.2 (2.3)	7.63	0.001	**m = d < c**
HAPPINESS	9.5 (1.1)	9.6 (0.9)	9.8 (1.0)	1.43	0.24	m = d = c
SADNESS	7.3 (2.0)	7.7 (1.9)	8.2 (1.6)	2.90	0.05	m = d = c
SURPRISE	8.6 (1.8)	8.6 (2.1)	9.1 (1.4)	1.88	0.15	m = d = c
Hx total	99.0 (17.2)	102 (14.6)	110.2 (9.7)	10.32	<0.001	**m = d < c**
ANGER	15.6 (5.0)	16.0 (5.7)	17.6 (3.9)	2.97	0.05	m = d = c
DISGUST	17.1 (3.7)	17.1 (3.5)	17.8 (3.5)	0.68	0.50	m = d = c
FEAR	13.0 (4.6)	14.3 (5.3)	17.3 (3.6)	13.63	<0.001	**m = d < c**
HAPPINESS	18.4 (4.0)	19.4 (1.3)	19.6 (0.9)	3.75	0.02	**m < d = c**
SADNESS	22.5 (9.5)	18.3 (2.7)	18.93 (2.35)	0.82	0.44	m = d = c
SURPRISE	16.8 (3.79)	16.7 (3.9)	18.7 (1.62)	7.55	0.001	**m = d < c**

FER = Facial emotion recognition; EK60 = Ekman 60 Faces Test; Hx = Emotion Hexagon Test; m = mania; d = depression; c = healthy controls. Text in bold indicates the tests with significant differences according to Bonferroni post hoc test.

Table 3
FER scores for bipolar patients in mania or depression and healthy controls, carrying the COMT Met+ or Met− *alleles*.

	Mania			Depression			Controls		
	Met+ Mean (SD)	Met− Mean (SD)	p	Met+ Mean (SD)	Met− Mean (SD)	p	Met+ Mean (SD)	Met− Mean (SD)	p
EK60 total	45.4 (5.8)	46.5 (5.3)	0.58	46.2 (7.7)	48.2 (5.0)	0.46	50.7 (4.9)	50.8 (4.5)	0.91
Anger	7.6 (1.8)	7.3 (1.9)	0.69	7.8 (1.6)	7.8 (2.0)	0.95	8.4 (1.3)	8.1 (1.0)	0.48
Disgust	6.9 (2.1)	7.7 (1.7)	0.25	7.3 (2.2)	8.4 (0.9)	0.12	7.7 (1.9)	7.8 (1.9)	0.81
Fear	5.4 (2.6)	5.2 (3.0)	0.80	5.3 (3.1)	5.4 (3.5)	0.95	7.2 (2.4)	6.8 (2.0)	0.64
Happiness	9.6 (0.8)	9.7 (0.4)	0.54	9.3 (1.1)	10.0 (0.0)	0.03	9.8 (1.2)	10.0 (0)	0.23
Sadness	7.6 (2.2)	7.0 (1.7)	0.37	7.8 (2.1)	7.7 (1.7)	0.86	8.1 (1.7)	8.6 (1.3)	0.25
Surprise	8.3 (2.0)	9.3 (0.7)	0.03	8.2 (2.4)	8.8 (1.4)	0.49	9.3 (0.9)	9.3 (0.8)	0.91
Hx total	97.8 (19.3)	100.6 (14.5)	0.62	96.7 (15.4)	107.8 (10)	0.05	110.7 (9.5)	108.0 (10.5)	0.27
Anger	15.6 (5.1)	15.3 (5.1)	0.86	14.6 (6.4)	18.5 (2.9)	0.03	17.8 (4.1)	17.3 (3.4)	0.67
Disgust	16.7 (4.2)	17.5 (3.0)	0.54	16.2 (4.1)	18.0 (1.9)	0.17	18.0 (3.3)	16.9 (4.0)	0.21
Fear	12.5 (4.9)	14.1 (3.9)	0.27	13.8 (5.6)	14.8 (5.9)	0.71	17.4 (3.5)	16.3 (4.2)	0.25
Happiness	18.1 (4.8)	18.7 (2.8)	0.61	19.0 (1.6)	20.0 (0.0)	0.03	19.5 (1.0)	19.8 (0.4)	0.11
Sadness	25.4 (7.5)	17.7 (2.8)	0.33	17.7 (3.2)	19.0 (1.8)	0.26	19.0 (2.4)	19.0 (1.9)	0.94
Surprise	16.9 (4.4)	17.0 (2.6)	0.89	15.8 (4.4)	17.4 (2.9)	0.34	18.9 (1.3)	18.0 (2.0)	0.11

EK60 = Ekman 60 Faces Test; Hx = Emotion Hexagon Test.

state recognized fewer anger and happy faces compared to Met− subjects. Notably, the comparison between healthy Met+/Met− controls showed no differences in FER scores.

Previous studies on FER suggest that the COMT Val^{158}Met polymorphism has a pleiotropic effect within the neural networks subserving emotional processing (Lelli-Chiesa et al., 2011). Similarly, Mier et al. (2010), in a meta-analysis of all available neuroimaging studies on rs4680 investigating the evidence for a neural substrate of this behavioral pleiotropy, found strong and opposing effects for executive cognition paradigms (favoring Met *allele* carriers) and emotional paradigms (favoring Val), providing meta-analytical evidence of a neural substrate for the pleiotropic behavioral effects of COMT genetic variation.

The explanation for DA influence on FER might be linked to its influence on the amygdala, a key brain structure associated to FER (Adolphs and Tranel, 2003; Adolphs et al., 1994; Morris et al., 1996). A human fMRI study reported that dopaminergic drug therapy, such as levodopa or DA agonists, partially restored amygdala response to an emotional task in Parkinson's disease subjects who showed no significant amygdala response during drug-off states (Tessitore et al., 2002). In addition, another fMRI study of healthy volunteers has demonstrated that amphetamine potentiated the response of the amygdala during an emotional task (Hariri et al., 2002). Moreover, Kienast et al. (2008) reported that DA storage capacity in human amygdala, measured with 6-[18F] fluoro-LDOPA positron emission tomography (PET), was positively correlated with fMRI signal changes in the amygdala. A more recent study has reported that DA D1 binding in the amygdala was positively correlated with amygdala signal change in response to fearful faces and concluded that DA D1 receptors might play a major role in enhancing amygdala response when sensory inputs are affective (Takahashi et al., 2010). On the basis of these findings, Mier et al. (2010) proposed that the imaging signals obtained on fMRI studies of emotional processing tasks in their study relate to this decreased cortical efficiency as a neural correlate of behavioral inflexibility. Additionally, the physiology of DA modulation of cortical function has been elucidated, where an effect of COMT rs4680 on the interaction of DA synthesis with brain activity, indexed by blood flow, was directly shown by Meyer-Lindenberg et al. (2005). In BD, the COMT Val *allele* was recently associated with greater amygdala activation during sad facial affect (Lelli-Chiesa et al., 2011). In the same study, the Met *allele* was associated to greater activation in the ventrolateral PFC in family members with affective morbidity compared against relatives without psychiatric conditions and healthy controls.

The impact of DA level alterations on cognition has been extensively investigated. Studies have shown that low doses of D1 agonists improve working memory and attention regulation (Granon et al., 2000), while high levels of DA release impair PFC function (Mattay et al., 2003). In 1977, Sprague and Sleator (1977), reported that at low psychostimulant doses, hyperkinetic children showed significant improvement in short-term memory, whereas at higher doses a significant decline in performance was seen. Mattay et al. (2003) reported similar findings in healthy volunteers using dextroamphetamine, a drug that potentiates DA activity. These authors observed that subjects carrying the COMT Met/Met genotype (rs4680) had poorer working memory and executive function, while Val carriers showed improved performance after a single dose of dextroamphetamine. Healthy Met+ (rs4680) subjects have shown decreased phasic, and increased tonic, DA transmission subcortically, and increased DA concentrations cortically (Bilder et al., 2004). This increases the stability of networks mediating sustained working memory representations, but makes it more difficult to switch or update the currently active networks that represent sustained working memory representations or ongoing behavioral programs (Bilder et al., 2004). However, when the D1/D2 binding potentials are disturbed due to an increase in DA, as occurs during BD episodes (Gonul et al., 2009), cognitive performance is theoretically impaired by excessive D1 stimulation. However, although euthymic subjects with BD may show impairments in neurocognitive domains, there is a lack of information on social cognition skills in this population.

Strengths of the present study include being the first report of a positive association between FER and the COMT genotype in BD and the fact that the sample was comprised

homogeneously of young BD type I subjects with no current use of medication, allowing a clear observation of the phenotypic boundaries of mania and depression in terms of social cognition. In addition, a validated measure of FER was used to assess these bipolar subjects. Limitations of the present study include the group size, which should ideally have been larger in order to show significant differences more clearly, and also the absence of euthymic subjects.

5. Conclusion

This is the first study suggesting that COMT rs4680 modulates facial emotion recognition differently during episodes of BD among subjects, and in healthy controls. This provides evidence that PFC DA is part of the neurobiological mechanisms of social cognition. DA receptor stimulation alterations during BD mood episodes might explain the contrasting results seen in BD subjects compared to controls. Further studies on other COMT polymorphisms that include euthymic BD subjects are warranted.

Role of funding source

The Sao Paulo Research Foundation (FAPESP 2010/06230-0 2010/12286-2) financed this study. The Sao Paulo research foundation (FAPESP) is an independent public foundation with the mission to foster research and the scientific and technological development of the State of São Paulo.

Conflict of interest

The authors do not have any conflict of interest to report.

Acknowledgments

We would like to thank the Institute of Psychiatry at the University of Sao Paulo, especially the members of the Mood Disorders Unit (GRUDA) and Laboratory of Neuroscience (LIM27), for their dedication and hard work, as well as the volunteers for their collaboration in this study.

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12.5. The CACNA1C risk allele selectively impacts on executive function in bipolar type I disorder

Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2013: 1–8

DOI: 10.1111/acps.12073

ACTA PSYCHIATRICA SCANDINAVICA

The *CACNA1C* risk allele selectively impacts on executive function in bipolar type I disorder

Soeiro-de-Souza MG, Bio DS, Dias VV, Vieta E, Machado-Vieira R, Moreno RA. The *CACNA1C* risk allele selectively impacts on executive function in bipolar type I disorder.

Objective: Calcium channels are important for converting electrical activity into biochemical events. A single nucleotide polymorphism (SNP) (rs1006737) in the *CACNA1C* gene has been strongly associated with increased risk for Bipolar disorder (BD) in genome-wide association studies. Recently, this same SNP has been reported to influence executive function in schizophrenia and controls, but it remains unclear whether this SNP affects behaviour, especially cognition in subjects with BD.
Method: A total of 109 BD type I subjects and 96 controls were genotyped for *CACNA1C* rs1006737 and assessed with an executive function tests battery [Wechsler Adult Intelligence Scale III (WAIS-III) Letter-Number Sequence subtest (WAIS-LNS), digit span (WAISDS), trail making test (TMT), and WCST (Wisconsin Card Sorting Test)].
Results: In patients with BD, the *CACNA1C* genotype Met/Met was associated with worse performance on all four executive function tests compared to Val/Val. No influence of *CACNA1C* was observed in the cognitive performance of healthy controls.
Conclusion: Our data indicate for the first time that the *CACNA1C* risk allele is likely associated with executive dysfunction as a trait in BD, as this association was found regardless the presence of mood symptoms. Larger studies should evaluate the potential influence of *CACNA1C* on other cognitive domains in BD.

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Key words: calcium channel; executive function; *CACNA1C*; mania; depression; bipolar disorder; cognition

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Accepted for publication December 5, 2012

Significant outcomes

- Met/Met genotype compared to Val/Val genotype had poorer executive function in BD patients.
- Executive function did not differ between rs1006737 genotypes in controls.

Limitations

- Small sample size.
- There was an age difference between groups.
- Only executive function was accessed.

Introduction

Calcium influx through L-type voltage-gated calcium channels has evolved as one of the most widely used transmembrane signaling mechanisms. Variations in calcium channels activity can therefore affect signal transduction and brain circuitry, which may result in alterations of several cognitive domains. Impaired regulation of calcium (Ca^{2+}) signaling along with increased Ca^{2+} intracellular levels are considered the most reproducible cellular abnormality in Bipolar disorder (BD) research

(1–4). Recently, the Met al.lele of single nucleotide polymorphism (SNP), rs1006737, from the α1-C subunit of the L-type voltage-gated calcium channel (*CACNA1C*) gene has been reported to increase the risk for BD in genome-wide association studies (5–8). Although variations in calcium channels can affect signal transduction and brain circuitry, little is known about how the genetics of calcium channels influence the cognitive profile of BD in mood episodes and during euthymia.

The *CACNA1C* gene encodes to the α1-C subunit of the L-type voltage-gated calcium channel. The α1-C has been associated to enhanced neurotransmission via the lateral amygdala pathway, and its expression has been found to be elevated in fear-conditioned animals (9). Available biological information on this gene suggests a potential molecular mechanism involving ion channel dysfunction. Bigos and colleagues (2010) reported that the Met risk allele increased m-RNA expression of *CACNA1C* in postmortem dorsolateral prefrontal cortex of healthy controls (10). Kempton et al. (2009) demonstrated an association between *CACNA1C* SNP rs1006738 and higher gray matter volume in patients with BD, highlighting a promising connection between this calcium L-type mutation and brain metabolism (11).

Cognitive dysfunction (CD) is a common feature of BD (12–15) and aggregates in familial BD, suggesting an endophenotype that underlies genetic predisposition to both CD and BD (16–18). Although variations in calcium channels can affect signal transduction and brain circuits (10), little is known about how the genetics of calcium channels influence the cognitive profile of BD in mood episodes and in euthymia.

Some recent studies have indicated that the *CACNA1C* risk allele influences brain morphology and also modulates both brain function and cognitive performance. Most studies evaluating the effects of the Met allele on brain morphology have found an increase in gray matter volume (11, 19), brainstem volume (20), right amygdala or right hypothalamus volume (21) in its carriers. Functional magnetic resonance studies (fMRI) have also revealed that the *CACNA1C* risk allele influences brain function by increasing amygdala activity during reward tasks (22), elevating hippocampal activity during emotional processing tasks, as well as enhancing right amygdala activation during fear-face recognition (23). Furthermore, Erk and colleagues (2010) reported that healthy carriers of the *CACNA1C* risk variant exhibit a pronounced reduction in bilateral hippocampal activation during episodic memory recall and diminished functional coupling between left and right hippocampal regions (24). Thimm and colleagues (2011) noted that *CACNA1C* Met carriers had reduced right inferior parietal lobe activity during orientation tasks and in the medial frontal gyrus during executive control of attention (25). Wang and colleagues (2011) found that the Met carriers compared with the Val/Val, had significantly lower functional connectivity within a corticolimbic frontotemporal neural system (19).

The majority of studies investigating the effect of the *CACNA1C* allele on cognitive function have involved healthy volunteers and results are controversial. Two independent fMRI studies of healthy volunteers consistently found that subjects carrying the risk allele Met had increased prefrontal activity when performing working memory tasks whereas only one of the studies reported impaired performance (10, 26). However, these studies used two different tasks to evaluate working memory: a spatial N-back task and a verbal fluency task (10, 26). Another study in healthy volunteers identified impaired performance in attention and orientation among Met carriers (25), although two other studies found no association between the *CACNA1C* Met allele and cognitive performance in healthy controls (27, 28).

To date, only three studies have evaluated the impact of *CACNA1C* on cognition in BD. The study, by Zhang and colleagues (2011), evaluated patients with schizophrenia (SCZ), bipolar patients in manic episode and control subjects, and found the clinical risk Met allele to be associated with impaired working memory among SCZ patients and healthy controls, although in a sample of 74 bipolar mania subjects Met carriers performed fewer errors than Val homozygotes (29). Arts and colleagues (2012) evaluated 51 BD patients over a period of 2 years and reported a negative effect of the Met allele on a composite cognitive measure based on the means of five domains (verbal memory, sustained attention, selective attention, attentional span, and working memory) (30). Moreover, our group recently reported that the *CACNA1C* Met allele negatively influenced the recognition of facial emotions in BD patients, but not in healthy controls (31).

Aims of the study

The aim of this study was to ascertain whether or not the *CACNA1C* Met allele influences cognitive performance in mood episodes, euthymic BD, and healthy controls. The main study hypothesis was that the *CACNA1C* risk allele for BD impairs executive function in subjects with the disorder.

Material and methods

Subjects

A total of 109 subjects with bipolar I disorder were included. Diagnoses were determined by trained psychiatrists based on the Structured Clinical Interview (SCID-I/P) (32) for DSM-IV TR (33). Euthymic patients (n = 37) had to be stable and with no medication adjustment for at least 2 months prior evaluation. In the euthymic group, 78.6% were in use of lithium, 52.4% anticonvulsants, 23.8% second-generation antipsychotics, 16.7% antidepressants, and 4.8% were using benzodiazepines at neuropsychological evaluation. Manic (n = 39) and depressive (n = 33) subjects (33) were medication free at neuropsychological evaluation. The symptomatic patients were participants in the LICAVAL clinical trial (34) and were evaluated immediately after the washout period (at least 4 weeks for antidepressants, mood stabilizers or antipsychotics, or 8 weeks for *depot* medications), prior to commencing use of medications. LICAVAL study consisted in a double-blind clinical trial to compare the efficacy of the association of lithium plus valproate compared with lithium plus carbamazepine (34). Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, currently abusing any substance, or submitted to electroconvulsive therapy in the preceding 6 months were excluded. The Young Mania Rating Scale (YMRS) (35) and the Hamilton Depression Rating Scale (HDRS-21) (36) were used to evaluate symptoms.

Control group

A total of 96 healthy volunteers (18–40 years) were recruited from the University of São Paulo. All control subjects had no current or past history of psychiatric disorder according to the evaluation conducted by trained psychiatrists using The Mini International Neuropsychiatric Interview (MINI) (37). Similarly, all healthy subjects had no family history (first degree relatives) of mood or psychotic disorders and had not been in recent use of psychotropic medicine or indulged in substance abuse over the last 3 months.

Cognitive assessment

The cognitive test battery was applied by experienced neuropsychologists and comprised four executive function tests:

i) Wechsler Adult Intelligence Scale III (WAIS-III) subtest Digit Span [WAIS-DS forward (FW)], WAIS-DS backward (BK) – requires attention, concentration, mental control, and working memory;
ii) Wechsler Adult Intelligence Scale III (WAIS-III) subtesLetter-Number Sequence (WAIS-LNS) – requires attention, concentration, mental control and working memory;
iii) WCST (Wisconsin Card Sorting Test) – was developed to assess abstraction ability and the ability to shift cognitive strategies in response to changing environmental contingences and it is considered a measure of executive function in that it requires strategic planning, organized searching, the ability to use environmental feedback to shift cognitive set, goal-oriented behaviour, and ability to modulate impulsive responding. WCST is composed of seven items: Conceptual level responses (WCST-CONC), Perseverative Responses (WCST-PR), Failure to Maintain Set (WCST-FMS), Corrected Categories (WCST-CC), Errors (WCST-E), Non-Perseverative Errors (WCST-NP), Perseverative Errors (WCST-P);
iv) Wechsler Abbreviated Scale of Intelligence (WASI) – produces an estimate of general intellectual ability [Total Intelligence Quotient (IQ)] based on four subtests: Vocabulary (WASI-V); Similarities (WASI-S), Block Design (WASI-BD),Matrix Reasoning (WASI-MR),
v) Trail making test (TMT).

These are well established and validated tests. Higher scores indicate better performance, with exception for TMT, WCST-PR, WCST-E, WCST-NP, and WCST-P (38–41).

Genotyping

DNA was extracted from peripheral blood according to the salting-out protocol (42) and then genotyped for *CACNA1C* rs1006737 using real-time PCR allelic discrimination. PCR amplification for rs1006737 was performed in 5 μl reactions with 5 ng of template DNA, 1 × TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA), 1 × each primer and probe assay, and H_2O. Thermal cycling consisted of initial denaturation for10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60° C for 1 min. Fluorescence detection occurred in the annealing step. Amplification and allelic discrimination were performed in a 7500 Real-Time System (Applied Biosystems). Quality control of Real-time PCR results was done by direct sequencing on a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Statistical analysis

The chi-squared test was used for comparison of categorical data, and the ANOVA for continuous data. Tukey's test was used for multivariable bias correction. Subsequently, a multivariate analysis of covariance (MANOVA) model was applied: cognitive tests were entered as dependent variables, while using age, gender, education, HDRS-21, and YMRS as covariates and *CACNA1C* genotype as fixed factor. The PASW statistics version 19.0 software (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Ethics

The research ethics board of the *Hospital das Clínicas of the University of São Paulo* approved the study. Written informed consent was obtained from all study participants.

Results

The *CACNA1C* genotype distribution in the experiment was in accordance with the Hardy–Weinberg equilibrium ($\chi^2 = 0.09$ $P = 0.75$), indicating that the samples were representative. Genotype distribution in the sample was 11.4% for Met/Met, 39% for Val/Met and 49.6% for Val/Val. Socio-demographic and clinical data of both groups are given in Table 1.

Table 1. Comparison of socio-demographic and clinical characteristics of bipolar disorder (BD)and controls groups by CACNA1C genotype

CACNA1C	Group	*N*	Mean	SD
Met/Met				
HDRS	BD	15	15.64	11.96
YMRS	BD	15	9.18	8.59
Age	BD	15	31.91	10.01
	Controls	11	25.36	4.65
Education	BD	15	11.27	2.90
	Controls	11	13.82	1.33
IQ	BD	15	86.64	12.49
	Controls	11	112	14.18
Val/Met				
HDRS	BD	54	13.17	9.40
YMRS	BD	54	9.31	8.75
Age	BD	54	29.78	8.94
	Controls	31	24.29	4.01
Education	BD	54	13.02	3.24
	Controls	31	14.16	2.90
IQ	BD	54	98.07	11.89
	Controls	31	110.1	15.45
Val/Val				
HDRS	BD	49	15.41	9.01
YMRS	BD	49	11.96	8.73
Age	BD	50	29.9	6.42
	Controls	54	23.63	3.97
Education	BD	50	12.36	2.70
	Controls	54	13.67	2.08
IQ	BD	50	98.16	13.75
	Controls	54	110.37	16.95

A MANOVA model was tested in BD and controls separately. In the BD group database, results revealed that *CACNA1C* Met/Met genotype compared with Val/Val genotype had worse cognitive performance in: WAI-SDS-FW ($B = -1.9$ $P = 0.03$ Partial η^2 4.3% power 57.6%), WCST-CONC ($B = -7.9$ $P = 0.01$ Partial η^2 6.3% power 74.2%), WCST-PR ($B = 6.6$ $P = 0.01$ Partial η^2 5.8% power 70.8%), WCST-E ($B = 8.1$ $P = 0.008$ Partial η^2 6.6% power 76.9%), WCST-P ($B = 4.4$ $P = 0.02$ Partial η^2 5.1% power 65.1%), WAIS-BD ($B = -11.0$ $P = 0.007$ Partial η^2 6.8% power 77.7%), WAIS-MR ($B = -6.1$ $P = 0.02$ Partial η^2 4.6% power 59%), IQ ($B = -9.2$ $P = 0.02$ Partial η^2 5% power 64.3%), TMT-A ($B = 16.7$ $P = 0.004$ Partial η^2 7.8% power 83.4%) (Table 2). Val/Met genotype performed worse than Val/Val genotype only on TMT-A ($B = 8.3$ $P = 0.01$ Partial η^2 6.1% power 73.1%) and TMT-B ($B = 19.0$ $P = 0.008$ Partial η^2 6.7% power 77%). In the control group, the same MANOVA model revealed no influence of *CACNA1C* genotype on cognitive performance (supplemental to Table S1).

Discussion

To the best of our knowledge, this is the first investigation describing the influence of *CACNA1C* on executive performance in BD type I subjects. We observed a marked negative effect of the Met/Met on the executive function of BD subjects regardless of manic or depressive symptoms. No effect of this allele on executive function was observed in healthy controls.

Genetic variation in voltage-gated calcium channel genes has been associated with several other complex polygenic neuropsychiatric disorders, including autism (43), epilepsy and migraine (44), and schizophrenia (45). Most data linking *CACNA1C* to BD originate from genome-wide association studies, which indicated that calcium channels dysfunctions may be part of the underlying neural system mechanisms involved in the pathophysiology of BD. This report on a candidate risk allele for BD impacts on cognitive function is analogous to the behaviour of other genes associated with BD diagnosis and cognitive dysfunction, such as brain-derived neurotrophic factor (BDNF) (46) and Catechol-O-methyltransferase (COMT) (47, 48). Hence, as demonstrated here with *CACNA1C* by associating a BD risk gene to specific alterations in cognitive function, the underlying neural system mechanisms involved in the neuropathology of BD

Table 2. Multivariate analysis of covariance using cognitive tests as dependent variables, CACNA1C genotype as fixed factor and HAMD21, YMRS, gender, age and education as covariates in bipolar disorder subjects

Dependent variable	*B*	SE	*t*	Sig.	Partial η^2 (%)	Observed power (%)
WAIS-DS-FW						
Intercept	6.233	1.628	3.829	0.000	12.4	96.7
HAMD21	0.072	0.028	2.585	0.011	6.0	72.6
YMRS	−0.032	0.031	−1.043	0.299	1.0	17.8
Gender	−1.010	0.511	−1.976	0.051	3.6	49.9
Age	0.001	0.031	0.043	0.966	0.0	5.0
Education	0.273	0.080	3.419	0.001	10.1	92.3
[cacna1c genotype Met/Met]	−1.912	0.880	−2.171	0.032	4.3	57.6
[cacna1c genotype Val/Met]	−0.433	0.502	−0.863	0.390	0.7	13.7
[cacna1c genotype Val/Val]	0†					
WCST-CONC						
Intercept	42.356	5.599	7.565	0.000	35.5	100.0
HAMD21	0.083	0.096	0.862	0.391	0.7	13.7
YMRS	−0.313	0.105	−2.971	0.004	7.8	83.7
Gender	−1.493	1.758	−0.849	0.398	0.7	13.4
Age	−0.075	0.106	−0.704	0.483	0.5	10.7
Education	0.971	0.274	3.538	0.001	10.7	93.9
[cacna1c genotype Met/Met]	−7.978	3.029	−2.634	0.010	6.3	74.2
[cacna1c genotype Val/Met]	−2.030	1.727	−1.176	0.242	1.3	21.4
[cacna1c genotype Val/Val]	0†					
WCST-PR						
Intercept	12.364	4.503	2.746	0.007	6.8	77.6
HAMD21	−0.124	0.077	−1.600	0.113	2.4	35.4
YMRS	0.361	0.085	4.265	0.000	14.9	98.8
gender	−0.630	1.414	−0.446	0.657	0.2	7.3
Age	0.011	0.085	0.127	0.899	0.0	5.2
Education	−0.635	0.221	−2.880	0.005	7.4	81.4
[cacna1c genotype Met/Met]	6.165	2.436	2.531	0.013	5.8	70.8
[cacna1c genotype Val/Met]	2.577	1.389	1.855	0.066	3.2	45.2
[cacna1c genotype Val/Val]	0†					
WCST-E						
Intercept	20.656	5.549	3.723	0.000	11.8	95.8
HAMD21	−0.090	0.095	−0.938	0.350	0.8	15.3
YMRS	0.332	0.104	3.185	0.002	8.9	88.4
Gender	1.434	1.743	0.823	0.412	0.6	12.9
Age	0.107	0.105	1.017	0.312	1.0	17.2
Education	−0.993	0.272	−3.651	0.000	11.4	95.1
[cacna1c genotype Met/Met]	8.165	3.002	2.720	0.008	6.6	76.9
[cacna1c genotype Val/Met]	2.275	1.712	1.329	0.187	1.7	26.1
[cacna1c genotype Val/Val]	0†					
WCST-P						
Intercept	11.307	3.468	3.261	0.002	9.3	89.8
HAMD21	−0.060	0.060	−1.004	0.317	1.0	16.9
YMRS	0.263	0.065	4.038	0.000	13.6	97.9
Gender	−0.032	1.089	−0.029	0.977	0.0	5.0
Age	0.000	0.066	0.004	0.997	0.0	5.0
Education	−0.601	0.170	−3.538	0.001	10.7	93.9
[cacna1c genotype Met/Met]	4.443	1.876	2.369	0.020	5.1	65.1
[cacna1c genotype Val/Met]	1.704	1.070	1.593	0.114	2.4	35.2
[cacna1c genotype Val/Val]	0†					
WASI-BD						
Intercept	50.779	7.626	6.658	0.000	29.9	100.0
HAMD21	−0.124	0.131	−0.943	0.348	0.8	15.4
YMRS	−0.472	0.143	−3.289	0.001	9.4	90.3
Gender	−4.939	2.395	−2.062	0.042	3.9	53.3
Age	−0.530	0.144	−3.678	0.000	11.5	95.4
Education	1.201	0.374	3.214	0.002	9.0	89.0
[cacna1c genotype Met/Met]	−11.330	4.125	−2.746	0.007	6.8	77.7
[cacna1c genotype Val/Met]	−3.715	2.352	−1.579	0.117	2.3	34.7
[cacna1c genotype Val/Val]	0†					
WASI-MR						
Intercept	32.352	5.077	6.372	0.000	28.1	100.0
HAMD21	−0.025	0.087	−0.286	0.776	0.1	5.9
YMRS	−0.245	0.095	−2.569	0.012	6.0	72.1

Table 2. (Continued)

Dependent variable	*B*	SE	*t*	Sig.	Partial η^2 (%)	Observed power (%)
Gender	−3.186	1.594	−1.998	0.048	3.7	50.8
Age	−0.289	0.096	−3.010	0.003	8.0	84.7
Education	0.422	0.249	1.695	0.093	2.7	39.0
[cacna1c genotype Met/Met]	−6.132	2.746	−2.233	0.028	4.6	59.9
[cacna1c genotype Val/Met]	1.784	1.566	1.139	0.257	1.2	20.4
[cacna1c genotype Val/Val]	0†					
IQ						
Intercept	89.241	7.292	12.238	0.000	59.0	100.0
HAMD21	−0.061	0.125	−0.487	0.627	0.2	7.7
YMRS	−0.300	0.137	−2.185	0.031	4.4	58.1
Gender	−3.673	2.290	−1.604	0.112	2.4	35.6
Age	−0.192	0.138	−1.395	0.166	1.8	28.2
Education	1.710	0.357	4.784	0.000	18.0	99.7
[cacna1c genotype Met/Met]	−9.265	3.945	−2.349	0.021	5.0	64.3
[cacna1c genotype Val/Met]	−1.513	2.249	−0.673	0.503	0.4	10.2
[cacna1c genotype Val/Val]	0†					
TMT-A						
Intercept	37.248	10.446	3.566	0.001	10.9	94.2
HAMD21	0.029	0.180	0.160	0.873	0.0	5.3
YMRS	0.049	0.196	0.251	0.802	0.1	5.7
Gender	3.219	3.281	0.981	0.329	0.9	16.3
Age	−0.004	0.198	−0.018	0.986	0.0	5.0
Education	−0.826	0.512	−1.613	0.110	2.4	35.9
[cacna1c genotype Met/Met]	16.712	5.651	2.958	0.004	7.8	83.4
[cacna1c genotype Val/Met]	8.375	3.222	2.599	0.011	6.1	73.1
[cacna1c genotype Val/Val]	0†					
TMT-B						
Intercept	125.289	22.725	5.513	0.000	22.6	100.0
HAMD21	0.318	0.391	0.814	0.417	0.6	12.7
YMRS	−0.136	0.427	−0.319	0.751	0.1	6.1
Gender	5.098	7.137	0.714	0.477	0.5	10.9
Age	0.040	0.430	0.093	0.926	0.0	5.1
Education	−4.406	1.114	−3.957	0.000	13.1	97.5
[cacna1c genotype Met/Met]	15.707	12.293	1.278	0.204	1.5	24.4
[cacna1c genotype Val/Met]	19.087	7.010	2.723	0.008	6.7	77.0
[cacna1c genotype Val/Val]	0†					

Wechsler Adult Intelligence Scale III (WAIS-III) subtest Digit Span [WAIS-DS forward (FW)], WAIS-DS backward (BK); WCST (Wisconsin Card Sorting Test) - Conceptual level responses (WCST-CONC), Perseverative Responses (WCST-PR), Failure to Maintain Set (WCST-FMS), Corrected Categories (WCST-CC), Errors (WCST-E), Non-Perseverative Errors (WCST-NP), Perseverative Errors (WCST-P);Wechsler Abbreviated Scale of Intelligence (WASI) -WASI -Vocabulary subtest (WASI-V); WASI Similarities (WASI-S) Block Design (WASI-BD),WASI Matrix Reasoning (WASI-MR),Total Intelligence Quotient (IQ) Letter-Number Sequence (WAIS-LNS), Trail making test (TMT).These are well-established and validated tests. Higher scores indicate better performance, with exception for TMT, WCST-PR, WCST-E, WCST-NP and WCST-P. Significance level $P < 0.05$.

†This parameter is set to zero because it is redundant.

appear to closely resemble the mechanisms that control cognitive phenotype.

No effect of the *CACNA1C* risk allele on the cognition of healthy subjects was observed. Previous studies have shown controversial results. Our findings in controls are in agreement with the previous studies that found no association between the *CACNA1C* Met allele and cognitive performance (executive function, working memory, and attention) (27, 28). Moreover, our results are in agreement with Arts et al. (2012), who showed cognitive dysfunction in BD Met/Met genotype (49). By contrast, our results failed to confirm previous findings of working memory dysfunction in healthy volunteers associated with *CACNA1C* Met (26), with Met carriers group having a poorer performance in working memory, although opposite results were observed in BD patients (29). In this study, four tests were used to assess executive function, all of which revealed that Met/Met genotype had a poorer performance compared with Val/Val regardless of mood episode type.

Even though rs1006737 is located in an intronic region and it is still not clear whether this SNP or another variant in linkage disequilibrium is causally linked to the risk of BD, we suggest that our findings reflect calcium channels abnormalities during euthymia in BD. Considering that this is a genetic association study, the main limitation is the relatively small sample size. Another limitation is the fact that our control group was younger and had more years of study than the bipolar subjects, but among the bipolar individuals in different mood episodes there was no difference of age or educa-

tion. The fact that the *CACNA1C* risk allele influenced executive function in BD, but not in control individuals reflects the specificity of calcium-signaling alterations to the cognitive function of BD.

In conclusion, we have described the effect of one of the most strongly associated risk genes for BD on executive function in this disorder. Considering the specificity of cognitive dysfunctions associated to the *CACNA1C* Met allele for BD, we believe that alterations in the calcium-signaling pathway should be a key focus of future studies on cognitive degeneration in BD.

Acknowledgements

We thank the Institute of Psychiatry at the University of Sao Paulo, especially the members of the Mood Disorders Unit (GRUDA) and Laboratory of Neuroscience (LIM27) for their dedication and hard work, as well as the volunteers for their collaboration. We also thank the Associação Beneficente Alzira Denise Hertzog da Silva (ABADHS)

Financial disclosures

The Sao Paulo Research Foundation financed this study. The authors report no conflict of interest for this particular study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Multivariate analysis of covariance using cognitive tests as dependent variables whereas age, gender, and education as covariates and CACNA1C as fixed factor in 96 healthy controls.

12.6. Number of manic episodes is associated with elevated DNA oxidation in bipolar I disorder

International Journal of Neuropsychopharmacology, Page 1 of 8. © CINP 2013
doi:10.1017/S1461145713000047

ARTICLE

Number of manic episodes is associated with elevated DNA oxidation in bipolar I disorder

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Abstract

Bipolar disorder (BD) is a major public health problem characterized by progressive functional impairment. A number of clinical variables have been associated with progression of the disease, most notably number of affective episodes and presence of psychotic symptoms, both of which correlate with greater cognitive impairment, lower response rates for lithium, and possibly lower levels of neurotrophic factors. Oxidative damage to cytosine and guanosine (8-OHdG) has been described as a modulator of DNA methylation, but the extent of DNA oxidative damage involvement in BD remains unclear. The aim of this study was to evaluate the extent of DNA oxidative damage to 8-OHdG and 5-methylcytosine (5-HMec), as well as global methylation (5-Mec), in BD patients and healthy controls. Potential association with clinical variables was also investigated. DNA levels of 8-OHdG, 5-HMec and 5-Mec were measured in 50 BD type I patients and 50 healthy controls. DNA 8-OHdG levels were higher in BD patients compared to healthy controls and found to be positively influenced by number of previous manic episodes. BD subjects had lower levels of 5-HMec compared to controls, whereas this measure was not influenced by the clinical features of BD. Number of manic episodes was correlated with higher levels of 8-OHdG, but not of 5-Mec or 5-HMec. Lower demethylation activity (5-HMec) but no difference in global 5-Mec levels was observed in BD. This finding suggests that oxidative damage to 8-OHdG might be a potential marker of disease progression, although further prospective cross-sectional studies to confirm neuroprogression in BD are warranted.

Received 10 August 2012; Reviewed 11 October 2012; Revised 27 November 2012; Accepted 11 January 2013

Key words: Bipolar disorder, 8-hydroxy-2-deoxyguanosine, 5-hydroxymethylcystosine, 5-methylcytosine, oxidative stress.

Introduction

Bipolar disorder (BD) is a major public health problem characterized by significant functional impairment and clinical co-morbidities (Wachsman, 1997; Kupfer, 2005). Although there are currently scant prospective longitudinal studies (mostly short duration) in BD patients demonstrating progressive dysfunction during the disease course, a number of clinical variables have been associated with disease progression in some cross-sectional studies (Swann et al., 2000; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009). These variables include number of affective episodes and presence of psychotic symptoms, both of which have been correlated with greater cognitive impairment, elevated levels of inflammatory markers, lower neurotrophic factors, brain atrophy and poorer response to treatment (Swann et al., 2000; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009). Despite recent advances, the molecular mechanisms underlying hypothetical neuroprogression remain unclear. However, cumulative evidence has shown that increased oxidative stress damage to biomolecules may be central to the pathophysiology of BD (Andreazza et al., 2008; Berk, 2009). Therefore maintaining the chemical integrity of DNA during assault by oxidizing agents represents a constant challenge in BD patients. In addition, the impact of features of the disease on DNA oxidation, such as number of affective episodes or psychotic symptoms, remains unclear.

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and hydroxyl radicals are produced

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as by-products of mitochondrial phosphorylation (Gutteridge and Halliwell, 2000; Cavanagh et al., 2002; Clark et al., 2002; Ferrier and Thompson, 2002). When mitochondrial and cytoplasmic enzymatic and non-enzymatic antioxidant systems are overwhelmed by elevated ROS levels, oxidative damage to DNA, lipids and proteins can ensue (Lenaz, 2001; Bora et al., 2010). Oxidative stress leads to multiple forms of DNA damage including base modifications, deletions, strand breakage and chromosomal rearrangements (Valko et al., 2004, 2006). Such damage to DNA has been shown to affect its ability to function as a substrate for DNA methyl transferases, resulting in global hypomethylation (Wachsman, 1997; Kupfer, 2005). ROS production is associated with increased DNA damage and chromosomal degradation and also with alterations in both hypermethylation and hypomethylation of DNA (Swann et al., 2000; Robinson and Ferrier, 2006; Lim et al., 2008; Berk, 2009; Kauer-Sant'Anna et al., 2009). More specifically, ROS react with guanosine DNA residues, leading to the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG; Swann et al., 2000; Coryell et al., 2001; Robinson and Ferrier, 2006; Berk, 2009; Kauer-Sant'Anna et al., 2009; Guo et al., 2011). 8-OHdG is a DNA adduct resulting from damage to DNA via ROS (Tohen et al., 1990; Kohen and Nyska, 2002; Klaunig and Kamendulis, 2004; Andreazza et al., 2008; Berk, 2009). Guanosine base is known to be the most susceptible to oxidative damage (Spassky and Angelov, 1997; Gutteridge and Halliwell, 2000; Cavanagh et al., 2002; Clark et al., 2002; Ferrier and Thompson, 2002; Altieri et al., 2008; Radak and Boldogh, 2010; Kryston et al., 2011). Recently, greater research attention has been dedicated to oxidation of cytosine residues in DNA, given that cystosines are the anchor for methyl groups in DNA (Lenaz, 2001; Bora et al., 2010; Branco et al., 2012). Determination of 5-methylcytosine (5-Mec) levels is a method for assessing global methylation; therefore hydroxylation of 5-Mec to 5-hydroxymethylcytosine (5-HMec) has become an important novel epigenetic marker (Valko et al., 2004, 2006; Guo et al., 2011). The role of ROS in the modulation of 5-HMec, however, remains unknown.

In the present study, we therefore investigated whether levels of DNA oxidative damage (8-OHdG), 5-Mec and 5-HMec, were altered in young BD type I subjects compared to healthy controls. The impact of number of affective episodes, disease duration and psychotic symptoms on DNA oxidation and methylation levels were also evaluated since these may reflect disease severity or progression.

Materials and method

A total of 50 symptomatic subjects (33 women) with BD type I (26 in mania and 24 in depressive episode), aged 18–40 yr, were included. Diagnoses were determined by psychiatrists using the Structured Clinical Interview (SCID-I; First et al., 1996) for DSM-IV-TR (DSM-IV, 2000). The patients included in this study were recruited from the 'LICAVAL clinical trial', designed to compare the efficacy of valproate + lithium *vs.* carbamazepine + lithium in treating BD type I (Campos et al., 2010). These patients were evaluated immediately after the wash-out period ($\geqslant$4 wk for antidepressants, mood stabilizers or antipsychotics, or $\geqslant$8 wk for depot medications) and prior to commencing use of medications. The Young Mania Rating Scale (Young et al., 1978) and Montgomery–Asberg Depression Rating Scale (Montgomery and Asberg, 1979) were used to evaluate severity of symptoms. Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, current substance abuse or that had undergone electroconvulsive therapy in the preceding 6 months, were excluded. As an indicator of illness severity (Berk et al., 2011), data on disease duration (age at first use of medication for mood symptoms), number of previous depressive and manic episodes and lifetime psychotic symptoms (hallucinations or delusions during mood episodes) were extracted from the SCID-I results. Subjects who proved unable to furnish sufficient information were excluded.

In the control group, 50 healthy volunteers (25 women) aged 18–40 yr were recruited at the University of São Paulo. All controls had no current or past history of psychiatric disorder according to the evaluation conducted by trained psychiatrists using the Mini International Neuropsychiatric Interview (Sheehan et al., 1998). Similarly, control subjects had no family history (first degree relatives) of mood or psychotic disorders and had no recent treatment or substance abuse over the previous 3 months.

The research ethics board of the Hospital das Clínicas of the University of São Paulo approved the study. Written informed consent was obtained from all study participants.

DNA oxidation and methylation

DNA blocks were collected using EDTA-coated tubes from whole blood according to the salting-out protocol (Laitinen et al., 1994). Oxidation to DNA was evaluated based on oxidation to guanosine, by assessing 8-OHdG levels, and to cytosine by measuring 5-HMec levels. The two markers were measured using competitive ELISA analysis kits from Stress Marq Biosciences Inc. (Canada) and Epigentek Group Inc. (USA), respectively (Shen et al., 2007). In order to evaluate whether oxidative stress through DNA oxidation induced lower levels of DNA methylation, global DNA methylation (5-Mec) was evaluated using an ELISA-based method (Tahiliani et al., 2009).

Statistical analyses

Subjects were classified into two groups (BD patients and healthy controls). The χ^2 test was used for categorical

Table 1. Sociodemographic and clinical characteristics of the sample

	Bipolar disorder ($n=50$)		Healthy controls ($n=50$)		
	Mean	S.D.	Mean	S.D.	p
Age	26.8	4.5	26.0	4.00	<0.32[a]
Gender (female/male)	33/17		25/25		0.08[b]
8-OHdG (pg/ml)	77.4	9.7	64.6	12.2	
5-HMec (ng/μl)	0.06	0.03	0.10	0.07	
5-Mec (ng/μl)	2.42	1.15	2.90	1.28	
Illness duration (yr)	5.0	3.7			
YMRS	14.06	8.3			
MADRS	20.08	9.04			
Number of manic episodes	4.04	2.12			
Number of depressive episodes	3.72	1.21			
Lifetime psychotic symptoms	50.00%				

8-OHdG, 8-Hydroxy-2′-deoxyguanosine; 5-Mec, 5-methylcytosine; 5-HMec, 5-hydroxymethylcytosine; YMRS, Young Mania Rating Scale; MADRS, Montgomery–Asberg Depression Rating Scale.
[a] t test.
[b] χ^2.
Significant level $p<0.05$.

Table 2. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), 5-methylcytosine (5-Mec) and 5-hydroxymethylcytosine (5-HMec) levels were entered as dependent variables in a multivariate analysis of covariance model using gender, age and group as covariates

Dependent variable	Parameter	B	d.f.	F	p	Partial η^2 (%)	Observed power (%)
8-OHdG	Group	13.36	1	35.3	<0.001	27.1	100.0
	Gender	−4.21	1	3.4	0.07	3.5	44.8
	Age	0.01	1	0	0.98	0.0	5.0
5-HMec	Group	−0.03	1	7.33	0.01	7.2	76.4
	Gender	−0.03	1	4.6	0.03	4.7	56.9
	Age	0	1	1.21	0.27	1.3	19.3
5-Mec	Group	−0.49	1	3.66	0.06	3.7	47.4
	Gender	0.05	1	0.04	0.84	0.0	5.5
	Age	0	1	0	0.95	0.0	5.0

Significant level $p<0.05$.

data and Student's t test for continuous data. First, concentrations of 8-OHdG, 5-Mec and 5-HMec were entered as dependent variables to a multivariate analysis of covariance (MANCOVA) model using age, gender and group as covariates. Subsequently, for the BD group only, 8-OHdG, 5-Mec and 5-HMec were input as dependent variables to a MANCOVA model using age, disease duration, number of manic episodes, number of depressive episodes as covariates, and current mood state, gender and lifetime psychotic symptoms as fixed factors. Finally, Pearson's correlation test was employed to investigate the influence of clinical variables on biochemical measures and to determine correlation among measures. Version 19.0 of the PASW statistics software package (SPSS Inc., USA) was used for all analyses.

Results

Sociodemographics and clinical characteristics of the sample are shown in Table 1. Groups did not differ according to gender or age characteristics.

The BD group presented higher 8-OHdG concentrations ($F=35.3$, d.f. $=1$, $B=13.3$, $p<0.001$) and lower 5-HMec concentrations ($F=7.3$, d.f. $=1$, $B=-0.03$, $p=0.008$) compared to the control group (Table 2; Fig. 1).

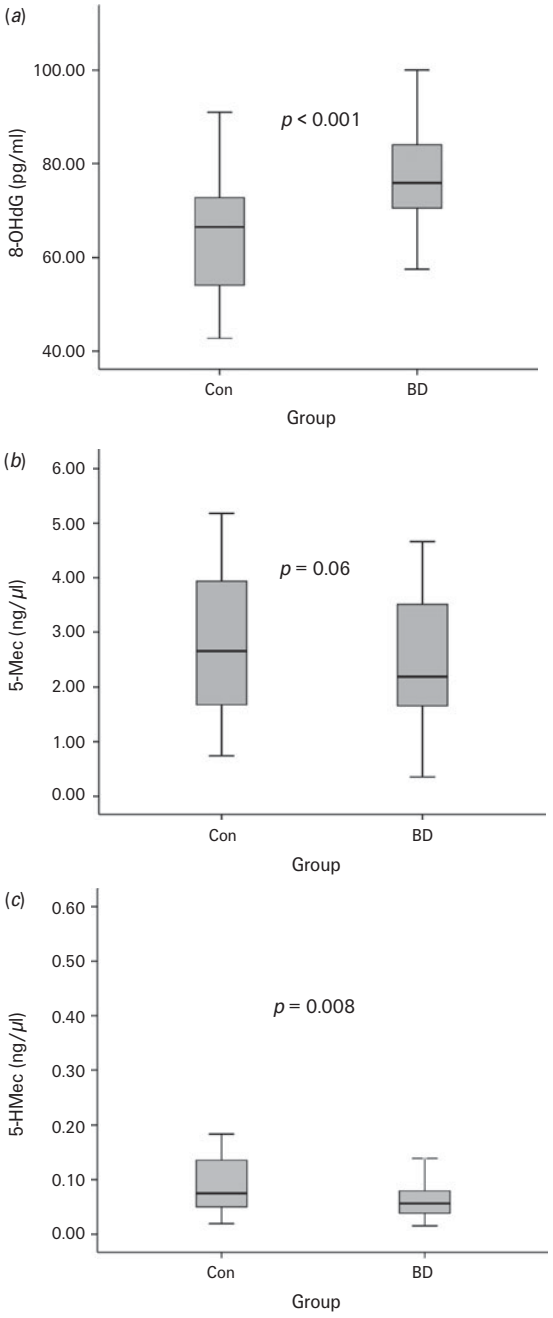

Fig. 1. Box plot graphic demonstrating simple comparison of mean levels and S.D. of (*a*) 8-hydroxy-2′-deoxyguanosine (8-OHdG), (*b*) 5-methylcytosine (5-Mec) and (*c*) 5-hydroxymethylcytosine (5-HMec) levels between healthy controls (Con) and bipolar disorder (BD) group.

Levels of 8-OHdG, 5-Mec and 5-HMec were not influenced by mood episode (manic or depressive; Table 3). In BD subjects, 8-OHdG levels were associated with higher number of past manic episodes ($F=14.2$, d.f. $=1$, $B=2.2$, $p<0.001$; Table 3). Disease duration showed a tendency to influence 8-OHdG concentrations ($F=4.06$, d.f. $=1$, $B=0.66$, $p=0.05$). Levels of 5-Mec and 5-HMec were not

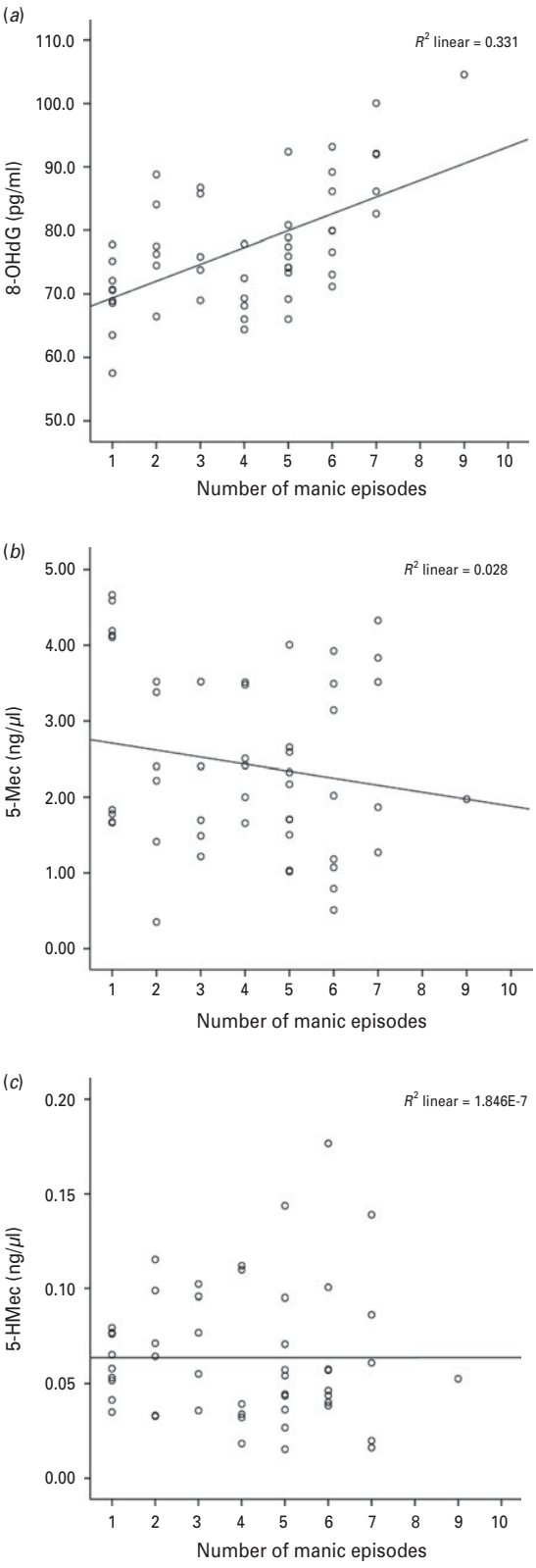

Fig. 2. Graphic demonstrating the correlation between number of manic episodes and (*a*) 8-hydroxy-2′-deoxyguanosine (8-OHdG; $p<0.001$), (*b*) 5-methylcytosine (5-Mec; $p=0.2$) and (*c*) 5-hydroxymethylcytosine (5-HMec; $p=0.9$) levels.

Table 3. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), 5-methylcytosine (5-Mec) and 5-hydroxymethylcytosine (5-HMec) levels were entered as dependent variables in a multivariate analysis of covariance model using gender, age, mood state, illness duration, number of manic episodes, number of depressive episodes and lifetime psychotic symptoms as covariates in the bipolar disorder group

Dependent variable	Covariate	*F*	d.f.	*t*	*p*	Partial η^2 (%)	Observed power (%)
8-OHdG	Gender	0.33	1	0.58	0.56	0.8	8.8
	Age	0	1	−0.01	0.99	0.0	5.0
	Mood episode	0.01	1	−0.12	0.91	0.0	5.2
	Illness duration	4.06	1	2.02	0.05	8.8	50.4
	Number of manic episodes	14.2	1	3.78	<0.001	25.4	95.8
	Number of depressive episodes	0.02	1	0.16	0.88	0.1	5.3
	Lifetime psychotic symptoms	4.08	1	−2.02	0.05	8.9	50.6
5-Mec	Gender	3.9	1	−1.98	0.06	8.5	48.9
	Age	0.3	1	0.55	0.59	0.7	8.3
	Mood episode	0.26	1	0.52	0.61	0.6	8.0
	Illness duration	0.08	1	0.29	0.78	0.2	5.9
	Number of manic episodes	2.5	1	−1.59	0.12	5.6	34.1
	Number of depressive episodes	0.2	1	0.45	0.66	0.5	7.2
	Lifetime psychotic symptoms	0.45	1	0.21	0.83	0.1	5.5
5-HMec	Gender	0.43	1	0.66	0.51	1.0	9.9
	Age	0	1	0.08	0.94	0.0	5.1
	Mood episode	0.35	1	0.6	0.56	0.8	9.0
	Illness duration	0.04	1	−0.2	0.84	0.1	5.4
	Number of manic episodes	0.04	1	0.22	0.83	0.1	5.5
	Number of depressive episodes	0.15	1	−0.39	0.7	0.4	6.7
	Lifetime psychotic symptoms	0.59	1	0.77	0.44	1.4	11.8

Significant level $p < 0.05$.

influenced by any of the clinical variables (Table 3). A positive correlation between number of manic episodes and 8-OHdG levels was also identified ($R^2 = 0.57$, $p < 0.001$) which remained significant even after controlling for disease duration (Fig. 2). No correlation was observed for other biochemical measures and lifetime number of depressive symptoms or disease duration.

Discussion

To the best of our knowledge, this is the first study to show increased levels of 8-OHdG in patients with BD compared to healthy controls. Our results indicated that levels of 8-OHdG, but not 5-HMec, were positively related with number of manic episodes. However, no influence of current mood state on 8-OHdG concentration was found in unmedicated subjects with BD. These results suggest that oxidative damage to guanosine, but not cytosine, might be associated with disease severity or represent a measure reflecting cumulative mood episodes over the course of the disease, rather than a state-dependent marker.

Previous studies have reported high levels of 8-OHdG in depressive disorder (Forlenza and Miller, 2006; Kupper et al., 2009; Maes et al., 2009; Wei et al., 2009) and to date, this is the first study reporting the same finding in BD. Our results suggest that 8-OHdG levels are positively related with the number of manic episodes in patients with BD (Fig. 2). In support of this theory, other studies have reported that greater progressive deterioration in treatment response, cognitive function and brain morphology are associated with a higher number of mood episodes (Swann et al., 1997, 2000; El-Badri et al., 2001; Strakowski et al., 2002; Lyoo et al., 2006; Robinson and Ferrier, 2006). The potential association between lifetime number of mood episodes and DNA damage was investigated since mood episodes were previously shown to be associated with reduced response to lithium in BD (Swann et al., 1999, 2000). Cognitive deficits (verbal memory and executive dysfunctions) have also been shown to be associated with number of manic and depressive episodes in patients with BD (El-Badri et al., 2001; Robinson and Ferrier, 2006). In the same context, larger ventricular volume and lower cortical thickness have been described in BD subjects with longer illness duration and multiple episodes (Strakowski et al., 2002; Lyoo et al., 2006). However, more prospective studies with larger sample sizes are needed to fully appreciate the progressive nature of this devastating illness.

An emerging body of data also points to accelerated ageing as a consequence of a high number of mood episodes or long duration of illness (McEwen, 2003;

Kapczinski et al., 2008; Juster et al., 2010). Additionally, depressive symptoms have been associated with elevated rates of ageing-related diseases such as cardiovascular disease and possibly cancer, even after adjusting for differences in health-related behaviours such as smoking and exercise (Penninx et al., 1998; Everson-Rose et al., 2004; Evans et al., 2005; Gump et al., 2005). Thus, our results showing correlation between DNA oxidation and number of manic episodes may further corroborate the associations observed between ageing and progression of BD in earlier studies. Moreover, it has been suggested that manic episodes contribute to persistent oxidative stress.

Accumulation of oxidative damage is thought to lead to neuronal cell death by apoptosis or as a consequence of aggregation of oxidized proteins, which may result in impairment of mood stabilizing mechanisms (Berk et al., 2011). This modification has been shown to be both cytotoxic and mutagenic (Wood et al., 1990; Chen et al., 1995; Choi et al., 1999; Klungland et al., 1999) and is followed by induction of the stress response as well as antioxidant and DNA repair enzymes (Lenaz, 2001; Bora et al., 2010). Recent studies have shown evidence of DNA damage in BD, findings corroborated by our results (Andreazza et al., 2007; Buttner et al., 2007; Mustak et al., 2010). Buttner et al. (2007) reported higher DNA fragmentation in post-mortem anterior cingulate cortex of BD patients (Buttner et al., 2007). Mustak et al. (2010) indicated that both single and double strand breakages were high in post-mortem brain tissues of BD patients who died from suicide compared with controls (Mustak et al., 2010). Andreazza et al. (2007) reported that higher DNA damage (comet assay) appeared to be associated with severity of manic or depressive episodes, suggesting that the high oxidative stress found in BD may be responsible for elevated DNA damage (Andreazza et al., 2007).

To date, only one published study has addressed peripheral global methylation levels in BD, reporting that leucocyte global DNA methylation did not differ between BD and controls (Bromberg et al., 2009). Other authors have studied methylation levels in specific promoter regions with mixed results (Abdolmaleky et al., 2006; Kuratomi et al., 2008; Rosa et al., 2008; Dempster et al., 2011; Nohesara et al., 2011). In the present study, no difference in 5-Mec was found between patient and control groups, but lower levels of 5-HMec were observed in BD, perhaps indicating lower active DNA demethylation activity. Conversely, as shown in Fig. 1, there is a total overlap between BD and controls for distribution of 5-Mec and 5-HMec, suggesting a lack of sensitivity or specificity for BD.

Limitations of this study include the fact that the analysis of DNA oxidation and methylation was based on peripheral blood. To date, there are no data associating the status of peripheral DNA with that of brain tissue DNA regarding DNA methylation or gene expression (Shen et al., 2001; Plume et al., 2012). Oxidative damage to DNA can be induced by several factors other than the pathophysiology of the illness. These factors were reviewed in the study by Gutteridge and Halliwell (2010) and include exposure to excessive UV radiation, pesticides and environmental toxins. It is important to note that control cases would be equally exposed to the same environmental factors, strengthening the case for attributing the results to the pathophysiology of the disorder. Another limitation is the study design (cross-sectional) which may allow bias in memory regarding the number of mood episodes reported. Moreover, the fact that we studied only young BD subjects who agreed to enrol on a clinical trial should be considered a recruitment bias.

In summary, we demonstrated that a higher number of manic episodes were associated with increased DNA oxidative damage to guanosine. The present findings highlight a significant role of DNA oxidation, predominantly involving guanosine, in severe mood disorders. Further studies supporting this model that involve other biological targets associated with progressive course in BD are warranted. Moreover, further studies to clarify the relationship between central and peripheral DNA methylation and oxidation are also needed.

Acknowledgements

We thank the Institute of Psychiatry at the University of Sao Paulo, particularly the members of the Mood Disorders Unit (GRUDA) for their dedication and hard work, and also the volunteers for their collaboration in this study. The São Paulo Research Foundation (Fundo de Apoio a Pesquisa do Estado de São Paulo – FAPESP 2010/06230-0) financed this study. L.T.Y. and A.C.A. were funded by the Canadian Institute for Health Research.

Statement of Interest

None.

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12.7. Translating neurotrophic and cellular plasticity: from pathophysiology to improved therapeutics for bipolar disorder

Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2012: 126: 332–341

DOI: 10.1111/j.1600-0447.2012.01889.x

ACTA PSYCHIATRICA SCANDINAVICA

Clinical overview

Translating neurotrophic and cellular plasticity: from pathophysiology to improved therapeutics for bipolar disorder

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Soeiro-de-Souza MG, Dias VV, Figueira ML, Forlenza OV, Gattaz WF, Zarate Jr CA, Machado-Vieira R. Translating neurotrophic and cellular plasticity: from pathophysiology to improved therapeutics for bipolar disorder.

Objective: Bipolar disorder (BD) likely involves, at a molecular and cellular level, dysfunctions of critical neurotrophic, cellular plasticity and resilience pathways and neuroprotective processes. Therapeutic properties of mood stabilizers are presumed to result from a restoration of the function of these altered pathways and processes through a wide range of biochemical and molecular effects. We aimed to review the altered pathways and processes implicated in BD, such as neurotrophic factors, mitogen-activated protein kinases, Bcl-2, phosphoinositol signaling, intracellular calcium and glycogen synthase kinase-3.
Methods: We undertook a literature search of recent relevant journal articles, book chapter and reviews on neurodegeneration and neuroprotection in BD. Search words entered were 'brain-derived neurotrophic factor,' 'Bcl-2,' 'mitogen-activated protein kinases,' 'neuroprotection,' 'calcium,' 'bipolar disorder,' 'mania,' and 'depression.'
Results: The most consistent and replicated findings in the pathophysiology of BD may be classified as follows: i) calcium dysregulation, ii) mitochondrial/endoplasmic reticulum dysfunction, iii) glial and neuronal death/atrophy and iv) loss of neurotrophic/plasticity effects in brain areas critically involved in mood regulation. In addition, the evidence supports that treatment with mood stabilizers; in particular, lithium restores these pathophysiological changes.
Conclusion: Bipolar disorder is associated with impairments in neurotrophic, cellular plasticity and resilience pathways as well as in neuroprotective processes. The evidence supports that treatment with mood stabilizers, in particular lithium, restores these pathophysiological changes. Studies that attempt to prevent (intervene before the onset of the molecular and cellular changes), treat (minimize severity of these deficits over time), and rectify (reverse molecular and cellular deficits) are promising therapeutic strategies for developing improved treatments for bipolar disorder.

Key words: brain-derived neurotrophic factor; Bcl-2; mitogen-activated protein kinases; neuroprotection; calcium; bipolar disorder; mania; depression

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Accepted for publication May 2, 2012

Clinical recommendations

- Neuroprotection and neurotrophic effects may represent a promising field for the development of improved treatments for bipolar disorder (BD).

- New therapeutic targets beyond monoamines are expected to involve intracellular signaling cascades involved in cell survival. Once the disrupted pathways are identified, therapeutic strategies would be aimed to prevent, treat, or rectify the identified altered cellular and molecular processes in BD
- Lithium represents an important tool to better understand the presumed targets involved in BD through its effects on neuroprotection, such as neurotrophic factors, mitogen-activated protein kinases, phosphoinositol signaling, intracellular calcium, energy metabolism (mitochondria and endoplasmic reticulum), and glycogen synthase kinase-3.

Additional comments

- The preclinical data showing neurotrophic and neuroprotective effects of mood stabilizers will need to be extended to the clinic to ascertain that they truly have a major role in the pathophysiology of BD.
- A major problem in neuropharmacological research is the difficulty in precisely ascribing therapeutic relevance to any observed biochemical finding, especially in the absence of suitable animal models (and unclear direct targets) for BD.

Introduction

Bipolar disorder (BD) likely arises from the complex interface among multiple susceptibility and protective genes and environmental factors. Its phenotype includes not only mood disturbances, but also a constellation of comorbidities, cognitive, motor, autonomic, neuroendocrine and neurovegetative alterations (1). This complex behavioural illness likely occurs through disturbances at multiple levels (systemic, cellular, molecular, and gene expression). On the basis of its polygenic origin, BD is characterized by the existence of diverse cellular and molecular targets associated not only with its pathophysiological basis, but also with its therapeutic profile. Most of these targets have been shown to critically regulate cellular plasticity and resilience (2). The objective of this study was to revise the evidences for the dysfunction of neurotrophic and cellular plasticity and resilience pathways in BD, with a particular focus on the disrupted process involved in neuroprotection.

Despite significant advances in the development of novel therapeutics during the last 20 years, the gold standard mood stabilizer lithium is still considered the most used and effective therapy for BD worldwide. Lithium exerts a wide range of effects at synapses and signal transduction pathways and has been used as an important tool in experimental paradigms of neuroprotection and neurotrophic effects; such paradigms are being used in the search of novel therapeutics for BD (1). It should be mentioned that most of these effects are associated with its chronic use at therapeutically relevant doses, which represents the key clinical paradigm when evaluating the predictive validity of lithium and other mood stabilizers.

Several direct targets of lithium have been identified and extensively studied, such as neurotrophic factors, mitogen-activated protein kinases, Bcl-2, phosphoinositol signaling, intracellular calcium, glutamate activity, and glycogen synthase kinase-3 (3–5). We proceed to review these targets of lithium in the following sections.

General concepts in neuroprotection and neuroplasticity: implications in BD

Neuroplasticity is characterized as the biological ability to induce and sustain important adaptative changes to internal and external stimuli in order to maintain the physiological functioning of the central nervous system (CNS). These changes aim to strengthen the synaptic signal and its efficacy through a direct regulation of neurotransmission (including receptor subunit phosphorylation and surface expression), intracellular signaling cascades in pre- and post-synaptic proteins as well as regulation of the expression of genes implicated in growth, survival, and synaptic transmission. These effects allow for a physiological remodeling of axonal and dendritic architecture. This remodeling is believed to be important to cellular resilience and at a clinical level, to mood stabilization.

Synaptic strength and cellular plasticity can be finely regulated over a short or even a long time scale by a combination of factors including previous activity of the network. Impairment of synaptic strength and cellular plasticity in mood disorders has been shown to involve changes in pathways regulating neurotrophic factors and neuroprotective proteins levels and expression. Neurotrophic factors, initially identified as

modulators of neuronal growth and differentiation, have been currently considered critical regulators of plasticity and cell resilience in adult neurons and glia (1, 6). Activation of neuroprotective and neurotrophic pathways has been also linked to the therapeutic effects of mood stabilizers. Even though lithium, valproate, and carbamazepine (CA) do not share similar chemical structure or all of the some biochemical targets, their neuroprotective effects have been shown to be the most replicated findings in both preclinical and clinical studies.

Aims of the study

This article will focus upon recent findings of cellular signaling abnormalities and impairments of cellular neurotrophic cascades that have been implicated in BD (Fig. 1).

Material and methods

We undertook a literature search of recent relevant journal articles, book chapter and reviews on this subject. Search words entered were 'brain-derived neurotrophic factor,' 'Bcl-2,' 'GSK3,' 'IP3,' 'mitogen-activated protein kinases,' 'neuroprotection,' 'neurotrophic,' 'calcium,' 'bipolar disorder,' 'mania,' and 'depression.'

Results

Neuroimaging and neuropathological human studies: evidence of cellular dysfunction in BD

Reductions in volume, density, number and/or size of neurons and glial cells have been described in the subgenual prefrontal cortex (PFC), orbital cortex, dorsal anterolateral PFC, amygdala, and basal

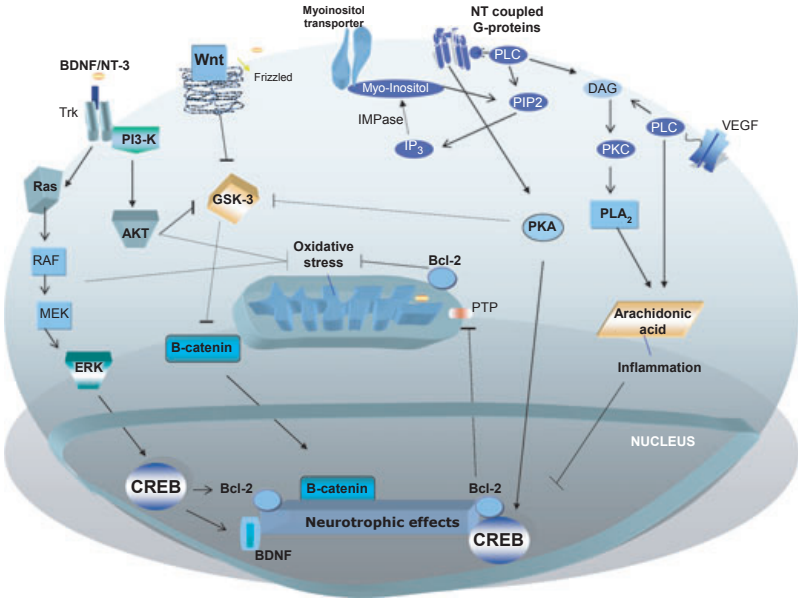

Fig. 1. Signaling abnormalities and impairment of neurotrophic cascades that underlie the neurobiological basis of bipolar disorder. Lithium increases their expression and/or levels, thus inducing neuroprotective and neurotrophic effects. Activation of brain neurotransmitter-coupled G-proteins induces PLC hydrolysis of PIP_2 to IP_3 and DAG (not shown), which activates PKC. IP_3 binds to the IP_3R, thus inducing the release of ER calcium stores. Elevated intracellular calcium levels have been described in bipolar disorder and may increase the risk of apoptosis. The neuroprotective protein Bcl-2 downregulates ER calcium release through an IP_3R-dependent mechanism. The same effect is induced by lithium treatment, which also increases Bcl-2 levels. IP_3 is recycled by IMPase, another of lithium's targets. Cellular signaling through Wnt glycoproteins and frizzled receptors result in GSK-3β inhibition, a critical cellular target and effector for diverse proteins. Inhibition of GSK-3β prevents β-catenin phosphorylation and stimulates its translocation to the nucleus, thus targeting transcription of specific genes activating neurotrophic effects and synaptogenesis. Activation of the BDNF receptor (Trk-B) activates the ERK/MAPK pathway, which inhibits GSK-3β and BAD. Activation of the extracellular signal-regulated kinase–mitogen-activated protein kinase pathway by BDNF increases the expression of nuclear CREB, which facilitates the expression of neurotrophic/neuroprotective proteins such as Bcl-2 and BDNF. BDNF also activates the PI3K pathway, which indirectly inhibits GSK-3β and BAD. Mitochondrial Bcl-2 and Bcl-xl also inhibit pro-apoptotic activation of BAD, as well as consequent mitochondrial increase in calcium influx and cytochrome C release. *Bcl-2 = B-cell lymphoma-2; BDNF = brain-derived neurotrophic factor; CREB = cAMP response element binding protein; DAG = diacylglycerol; ERK = extracellular regulated kinase; GSK = glycogen synthase kinase; IMPase = inositol monophosphatase; IP_3 = inositol 1,4,5-triphosphate 3; PI3K = phosphatidylinositol-3 kinase; PIP_2 = phosphoinositide 4,5-biphosphate; PKC = protein kinase C; PLC = phospholipase C; PTP = permeability membrane pore; TrkB = tyrosine receptor kinase B; RAS= RAt Sarcoma; AKT= B; PLA2 = Phospholipase A2; PLC= Phospholipase C; VEFG= Vascular endothelial growth factor; NT-3 = Neurotrophin-3.*

ganglia and dorsal raphe nuclei in BD (2, 7–9). Also, decreased glial cells density and increased nuclei size have been described in frontal cortical areas (1, 9–12). In parallel, reduced gray matter volumes in areas of the orbital and medial PFC, ventral striatum and hippocampus, and enlargement of third ventricles were observed in patients with BD. Significant reductions in the subgenual PFC glial number (41%) has also been reported in patients with a family history of BD (3–5, 10). A meta-analysis of imaging studies concluded that volumetric abnormalities of the subgenual PFC, striatum, hippocampus, and amygdala are present in first episode BD and children with BD (13). Other studies have found reductions in oligodendrocyte number and gene expression changes in the dorsolateral prefrontal cortex in individuals with BD (7–9, 14, 15). Interestingly, imaging data suggest that adolescents with BD who are taking mood stabilizers may be protected from the volume loss (16).

The presence of white matter abnormalities has been also described in imaging studies in BD. Although the cause of white matter hyperintensities in mood disorders is unknown, their presence particularly in the brains of young patients with BD suggests that importance in the pathophysiology of the illness (17, 18). In postmortem brain studies, reduced subcortical nuclei volumes in BD has been reported (19, 20). Neuronal density and size were found to be decreased in layers III, V, and VI in BD (8, 9, 11). Also, smaller pyramidal cell soma size was found in the hippocampal CA1 region (21).

Consistent with neurotrophic/neuroprotective properties of lithium and valproate, patients treated with chronic lithium or valproate exhibited subgenual PFC volumes that were significantly greater than non-treated patients, and not significantly different from controls (22). Also, in two elegant imaging studies, Moore et al. (23, 24) observed elevated N-acetyl-aspartate (NAA) (a mitochondrial marker of neuronal integrity) levels and gray matter volume in lithium-treated subjects. A subsequent MRI study by Sassi et al. (25) also showed an increased gray matter volume in lithium-treated BD subjects compared with untreated and healthy controls. Interestingly, the lithium-induced NAA increase showed a strong specificity to the gray matter. Other studies have noted similar effects with other mood stabilizers (16, 26).

Calcium dynamics, Bcl-2 regulation, and mitochondrial/endoplasmic reticulum (ER) activity

The calcium ion (Ca^{2+}) is a ubiquitous intracellular messenger controlling diverse critical biological functions in the human CNS. Calcium ions influence the synthesis and release of neurotransmitters and second-messenger cascades, thus affecting plasticity activation, intracellular signaling, energy metabolism, and synaptic consolidation (27–29). Mitochondria regulate different metabolic activities such as control of apoptosis, glutamate-mediated excitotoxic neuronal injury, and oxidative stress activity. Mitochondrial-encoded genes regulate synaptic activity related to long-lasting up-regulation of energy production (30, 31). Mitochondrial Ca^{2+} regulation also has a critical role in neuronal and glial activity, modulating both physiological and pathophysiological cellular responses (32). Decreased ATP and NAA levels in human studies have been associated with dysfunctions in mitochondrial metabolism. Inhibitors of the mitochondrial respiratory chain lead to a decrease in NAA levels, which is associated with deficits on ATP and oxygen availability (1, 33).

Impaired regulation of Ca^{2+} cascades is one of the most reproducible biological abnormalities described in BD research. It directly involves mitochondria and ER activity and has a critical role in neuroplasticity and cell survival. For instance, specific dysfunctions in store-operated calcium channel, ER function, and mitochondrial calcium uptake have been described in BD (2, 34). Elevated basal and agonist-stimulated intracellular Ca^{2+} levels in platelets and lymphocytes of bipolar I disorder (BD-I) have been widely described as well (1, 35–40). At therapeutically relevant doses, lithium attenuated agonist-stimulated intracellular Ca^{2+} responses (3–5, 41, 42). Furthermore, chronic lithium was shown to block an increase in calcium concentration, thus preventing oxidative stress and loss of mitochondrial membrane potential (6, 43, 44).

Mitochondrial dysfunction in BD was first proposed by Kato and Kato (45–47) and has been substantiated by others as well (see (9–12, 30). Konradi et al. (48) have also showed reduced expression of genes encoding mitochondrial proteins (including phosphorylation of mitochondrial inner membrane, subunits I and V). Decreased levels of NDUFV2 gene (a nuclear-encoded mitochondrial complex I subunit gene) was described in patients with BD (13, 49). Recently, Andreazza et al. reported a selective decrease in mitochondrial complex I subunit NDUFS7 activity in BD subjects (7–9, 14, 15, 50). Also, decreased pH and increased lactate, with altered oxidative phosphorylation (16, 51) were observed in the brains of BD subjects, giving further evidence for this model. Other related findings includes altered expression of antioxidant genes and its levels in patients with

BD (17, 18, 52, 53), as well as a significant increase in the number of proapoptotic genes in hippocampus from BD subjects (19, 20, 52). Interestingly, a downregulation in the proteins of the electron transfer chain in BD in glucose-deprived medium has also been described (8, 9, 11, 54).

Bcl-2 is a membrane-associated protein with both antiapoptotic and neuroprotective properties expressed preferentially in the limbic system (21, 55). Bcl-2 is mainly localized in the outer mitochondrial membrane and ER (22, 56–58) and activates neurotrophic pathways inducing neurite sprouting/outgrowth and axonal regeneration, thus preventing the deleterious effects of different insults (23, 24, 56, 57). Bcl-2 attenuates release of calcium from the ER and stabilizes mitochondrial membrane integrity thus limiting the release of cytochrome from mitochondria and activation of apoptotic pathways (25, 56, 59). Recently, the Bcl-2 rs956572 genotype AA was found to be associated with an abnormal Bcl-2 expression and to contribute to a dysfunctional Ca^{2+} homeostasis in BD, these effects were reversed by lithium (16, 26, 60). These effects seem to involve a direct regulation by inositol 1,4,5-triphosphate (IP_3) receptors (27–29, 60). Also, chronic lithium treatment increased Bcl-2 expression and protein levels in the frontal cortex, hippocampus and striatum (30, 31, 61, 62). Repeated electroconvulsive treatment (ECT) administration in primates increased precursor cell proliferation in the dentate gyrus (DG) because of increased expression of Bcl-2 (32, 63). Bcl-2 knockout mice were found to have increased anxiety-like behaviours (64), supporting the role of this gene in mediating emotional-like behaviours.

The ER has also been shown to be critically involved in the regulation of intracellular calcium levels. Ca^{2+} is released from the ER mostly through IP_3 receptors. XBP1, a pivotal gene in ER stress response, has been considered a genetic risk factor for BD and its variant XBP1C/G has been associated with higher stress responses in lymphoblastoid cells lines (65). So et al. (66) observed impaired ER stress responses in BD subjects. Moreover, Hayashi et al. (67) reported that the induction of the spliced form of XBP1 and total XBP1 was significantly attenuated in patients with BD. Interestingly, chronic valproate and lithium regulate expression of ER stress proteins (68, 69). More recently, it was suggested that XBP1 116C/G would predict response to treatment with valproate (70).

In addition, patients presenting Darier's disease, a disease that involves a mutation in the ER Ca^{2+} pump (SERCA) also have high rates of comorbid BD and/or the presence of manic-like symptoms.

Overall, changes in the expression of diverse mitochondrial/ER and plasticity genes regulated by calcium and Bcl-2 have been shown to significantly affect cellular viability and lithium seems to rescue cells from these deleterious effects.

Neurotrophic signaling cascades: BDNF and extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK/MAPK) pathway

Members of the neurotrophin family include nerve growth factor, *brain-derived neurotrophic factor* (BDNF), neurotrophins 3, 4, 5, and 6 and others. They bind to and activate a specific receptor tyrosine kinase (Trk) family. BDNF binds to the TrkB receptor with high affinity, thus activating diverse intracellular cascades involved in cellular survival and growth, such as the PI3K/Akt, MEK/ERK and phospholipase C (PLC)/PIP_2 signaling systems (71). BDNF exerts its biological effects through at least three key signaling pathways: phosphoinoside-3-kinase (PI3K)/Akt, PLC, or ERK–mitogen-activated kinase pathways. BDNF and the ERK pathways are considered key signaling cascades mediating neurotrophic actions and synaptic plasticity (7).

Altered BDNF levels and expression have been described in different animal models of depression and mania (72). Preclinical studies have also described that stress, which is involved in mood disorders, decreases the expression of BDNF (73). Interestingly, the interaction between BDNF and corticosteroids has been suggested to mediate the vulnerability to mood disorders (2). Chronic antidepressant treatment increases BDNF expression in rat prefrontal cortex and hippocampus (74).

Peripheral levels of BDNF showed a significant decrease during manic and depressive episodes in mood disorders, which in some cases were shown to be significantly associated with the severity of symptoms and therapeutic response to antidepressants (53, 75). Furthermore, we recently reported that lithium monotherapy increases BDNF in acutely manic patients (76). Based on these findings of altered BDNF levels during mood episodes in peripheral cells and plasma/serum, it has been proposed that this neurotrophin may be a useful surrogate outcome measure of clinical improvement in mood disorder patients undergoing treatment (72).

The ERK/MAPK pathway has also been implicated in mediating some behavioural and pathophysiological facets of BD, presumably by mediating long-term cell plasticity events (77). The ERK/MAPK pathway is a major intracellular signaling cascade mediating the biological effects of

neurotrophic factors. Activated ERK phosphorylates diverse proteins involved in cellular plasticity (78). The regulatory effects of mood stabilizers on cell survival and resilience have been shown to be mediated by activation of the MAPK cascade. One target of the ERK/MAPK cascade is ribosomal S6 kinase (RSK), which activates cAMP response element-binding (CREB). RSK phosphorylates CREB, thus increasing the expression of the neuroprotective proteins involved in BD pathophysiology (64, 79) (see Fig. 1). Recently, it was demonstrated that ERK1 KO mice exhibit a behavioural excitement profile in several models of depression and mania (78).

Regarding therapeutics, the potential involvement of ERK cascade in the effects of mood stabilizers has been shown. Chronic lithium and valproate activate a major neurotrophic signaling pathway—the ERK/MAPK cascade, and its downstream effectors RSK and CREB. Studies have shown that therapeutic dose of lithium and valproate upregulate the ERK/MAPK cascade in human neuroblastoma cells (77, 80). Also, chronic lithium and valproate at therapeutically relevant concentrations robustly increase the levels of activated ERK and RSK (measured by the phosphorylation of ERK and RSK) in the anterior cingulate cortex, hippocampus, rodent cerebellum, and cortex (61, 80–82). Taken together, these data support an integrated role for the BDNF and ERK pathways in the pathophysiology and therapeutic action of mood stabilizers.

Phosphoinositol signaling pathway (IP3/Inositol cascade)

The phosphoinositol signaling pathway (IP_3/Inositol cascade) has been linked to many cellular processes and pathological conditions (83). PLC produces diacylglycerol and IP_3, the latter of which binds IP3 receptors on the ER to generate calcium mobilization from internal stores (84, 85). Binding IP3 to its receptor induces release of ER calcium content. IP_3 binding sets forth downstream and is recycled back to PIP-2 by the enzymes IMPase and IPPase. It is important to mention that IMPA2 encoding IMPase is a candidate susceptibility gene for BD. One of the two human IMPase genes, *IMPA2* (86–89), was reported to be genetically associated with BD (87, 90). The 'inositol-depletion hypothesis' postulated that lithium, characterized as an uncompetitive inhibitor of inositol-1-phosphatase, generated its therapeutic effects by decreasing myoinositol levels. Lithium depletes IP_3 levels mediated by inhibiting of inositol monophosphatase and consequently decreases free inositol levels (87, 90).

Lithium has been shown to reduce inositol levels (1–4) by inhibiting IMPase *in vivo* (91), although the magnitude of inhibition is generally modest. However, it is not fully elucidated whether reduction in inositol is sufficient to limit PI or PIP2 synthesis. Williams et al. (2002), using a tissue-culture assay that measures sensory neuron growth-cone stability, have demonstrated that mood stabilizers (lithium, carbamazepine and valproate) share a similar mechanism of action. These findings reinforce the neural development hypothesis for lithium effects (92). Overall, the concept that lithium inhibits the dephosphorylation of IP_3 at therapeutic concentrations, depleting cells of free inositol and bringing about its therapeutic effects has been questioned, but still remains an important reference in this area.

Glycogen synthase kinase (GSK-3)

GSK-3 is a kinase that acts as an intermediary in numerous intracellular signaling pathways and is regulated by serotonin, dopamine, psychostimulants, and antidepressants (3). GSK-3 is a key regulator of apoptosis and cellular plasticity/resilience, and this role has been postulated to be a key molecular target for lithium and valproate effects (5, 93). Lithium is considered a direct inhibitor of GSK-3, via competition with magnesium for a binding site (94, 95). In mice, GSK-3 has also been shown to be inhibited by the structurally dissimilar valproate (96), and ECT, a non-pharmacologic therapy for mood disorders (97).

Animal behavioural data, from pharmacologic and genetic models, has shown that manipulation of GSK-3 produces both antidepressant and antimanic effects (3, 98). Similarly, lithium has been demonstrated to exert both such effects. Also, it was found that lithium-induced behaviours in wild-type mice are phenocopied by overexpression the GSK-3 target, β-catenin. GSK-3 inhibition results in a decrease in phosphorylation and degradation of its target β-catenin, and at therapeutically relevant concentrations, lithium increases β-catenin and Wnt-mediated gene expression in rodent brain. Notably, both lithium and β-catenin overexpression display mood stabilizing-like actions in prototypical animal models of mania (D-amphetamine hyperlocomotion) and depression (forced swim test) (99). It was therefore hypothesized that transgenic mice that overexpress a constitutively active form of β-catenin would phenocopy lithium's behavioural effects. Further studies have been carried out to identify the GSK-3 target most relevant to lithium's behavioural effects.

Interestingly, GSK-3 has been found to play a critical role in regulating circadian rhythm, in preclinical models (100, 101). Patients with BD often demonstrate circadian disturbances, and lithium has been shown to increase circadian period in humans and animals (92, 102, 103), consistent with a consistent decrease in GSK-3 activity. Studies showing that psychomimetic drugs (amphetamine, Lysergic acid diethylamide (LSD), and Phencyclidine (PCP)) increase GSK-3 phosphorylation in frontal cortex and striatum (104) provide circumstantial support for the possibility of an underlying GSK-3 abnormality in BD. However, direct evidence for the role of GSK-3 in the etiology of BD has not been reported yet, and genetic studies have not reproducibly found GSK-3 polymorphisms to be associated with the disease. Therefore, it remains to be determined whether the pathophysiology of BD involves dysfunction of GSK-3 itself, or of other signaling molecules regulated by GSK-3. Nevertheless, in view of the role of GSK-3 in neural plasticity, survival, and circadian rhythms, and its involvement in the action of mood stabilizers, development of new selective GSK-3 inhibitors is actively underway by numerous pharmaceutical companies.

Discussion

The search for strategies aiming to improve neuroprotection and neurotrophic effects may represent a promising field for the development of improved treatments for BD. Regional reductions in brain volume are most likely a consequence of impairments of structure plasticity and resilience. While there is preclinical data describing neurotrophic and neuroprotective alterations in BD and reversal of these deficits with mood stabilizers, data linking these alterations to patients is lacking. Overall, the most consistent and replicated findings in the pathophysiology of BD may be classified as follows: i) calcium dysregulation, ii) mitochondrial/endoplasmic reticulum dysfunction leading to, iii) glial and neuronal death/atrophy and iv) loss of neurotrophic/plasticity effects in brain areas critically involved in mood regulation. These changes may be interconnected and studies examining these potential associations are needed. Studies that attempt to prevent (intervene before the onset of the molecular and cellular changes), treat (minimize severity of these deficits over time), and rectify (reverse molecular and cellular deficits) are promising therapeutic avenues for developing improved treatments for BD. Investigations are ongoing in these areas and may provide further insights into the complex pathophysiological basis of this illness with the goal of improved therapeutics.

Acknowledgements

We thank Fapesp (2009/14891-9) and Associação Beneficente Alzira Denise Hertzog da Silva (ABADHS).

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12.8. Creativity and executive function across maniac, mixed and depressive episodes in bipolar I disorder

Journal of Affective Disorders 135 (2011) 292–297

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Preliminary communication

Creativity and executive function across manic, mixed and depressive episodes in bipolar I disorder☆

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ARTICLE INFO

Article history:
Received 21 June 2011
Accepted 23 June 2011
Available online 21 July 2011

Keywords:
Creativity
Bipolar
Executive function
Mania
Depression

ABSTRACT

Introduction: Creativity is a complex construct involving affective and cognitive components. Bipolar Disorder (BD) has been associated with creativity and is characterized by a wide range of affective and cognitive symptoms. Although studies of creativity in BD have tended to focus on creativity as a trait variable in medicated euthymic patients, it probably fluctuates during symptomatic states of BD. Since creativity is known to involve key affective and cognitive components, it is plausible to speculate that cognitive deficits and symptoms present in symptomatic BD could interfere with creativity.

Material and methods: Sixty-seven BD type I patients medication free, age 18–35 years and experiencing a maniac, mixed, or depressive episodes, were assessed for creativity, executive functioning, and intelligence.

Results: Manic and mixed state patients had higher creativity scores than depressive individuals. Creativity was influenced by executive function measures only in manic patients. Intelligence did not influence creativity for any of the mood episode types.

Conclusion: We propose that creativity in BD might be linked to the putative hyperdopaminergic state of mania and be dependent on intact executive function. Future studies should further explore the role of dopaminergic mechanisms in creativity in BD.

1. Introduction

Bipolar Disorder (BD) is a chronic, recurrent, affective disorder associated with manic and depressed states (Balanzá-Martínez et al., 2008). Despite the innumerous disadvantages of these mood swings, BD patients have been reported some advantages of the disease such as increased creativity (Jamison, 1996). To our knowledge, controlled studies on this theme to date have involved only medicated euthymic BD patients while no studies have investigated the differences in creativity across manic, depressive and mixed states. Even though creativity in BD has been studied largely in euthymic patients as a trait variable, creativity could also vary as a function of affective state. Since creativity is known to be a construct with affective (Jamison, 1996) and cognitive (Gundlach and Gesell, 1979) components it is plausible to speculate that cognitive deficits (CD) present in symptomatic BD could also interfere with the creative process.

The study of creativity in BD began with the description of increased rates of bipolarity in various groups of creative individuals (Akiskal et al., 2005; Andreasen and Glick, 1988; Jamison, 1989). Previous studies on creativity in BD have predominantly investigated the differences between euthymic BD patients and the general population. One of the major studies in this field examined creativity in a sample of bipolar

☆ ClinicalTrials.gov Identifier: NCT00969.

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doi:10.1016/j.jad.2011.06.024

and cyclothymic disorder patients (Richards et al., 1988). Using the Lifetime Creativity Scale, Richards et al. (1988) found greater overall creative achievement in a group of BD and cyclothymic patients, as well as in their healthy first-degree relatives, compared to healthy control subjects not at risk for affective disorders. More recent creativity studies in BD have focused on comparing creativity measures among medicated euthymic patients against those of controls, reporting higher creativity in BD (Santosa et al., 2007; Strong et al., 2007). Also some authors have demonstrated an important affective temperament/ personality component to creativity in BD (Srivastava et al., 2010b). Thus, creativity in BD has typically been studied as a trait variable. A few reports of higher creativity production in mania than in depression have emerged from biographical studies and empirical research (Jamison, 1996; Rothenberg, 2001). While little is known about the biological underpinnings of creativity, previous psychological, neuropsychological, and functional imaging studies suggest a potential role of the dopaminergic system (Burch et al., 2006; Folley and Park, 2005; Richards et al., 1988).

The objective of this research was to assess possible differences in creativity scores among manic, mixed, and depressive episodes of BD. Also, the influence of executive function on creativity scores in each type of episode was examined. To this end, a sample of young, medication-free bipolar I disorder patients during manic, depressive or mixed state, was recruited.

2. Materials and methods

The sample comprised individuals with BD I, aged between 18 and 35 years old. These patients were participants in the LICAVAL clinical trial (Campos et al., 2010) and were evaluated immediately after the wash out period prior to commencing use of medications. Diagnoses were determined by trained psychiatrists using the Structured Clinical Interview (SCID-I/P) (First et al., 1997) for DSM-IV TR (APA, 2000). The Young Mania Rating Scale (YMRS) (Young et al., 1978), and the Montgomery–Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) were used to evaluate the severity of symptoms. The Clinical Global Impression scale was used to measure illness severity (Guy, 1976). The cut-off for depression was 18 points on the MADRS while for mania was 12 points on the YMRS. Patients in use of any pharmacological treatment (at least four weeks for antidepressants, mood stabilizers or antipsychotics, or eight weeks for *depot* medications) were not included based on the assumption that these drugs could influence creativity scores. Subjects with neurological disorders, previous head trauma, any illness requiring medical intervention, currently abusing any substance, or undergoing electroconvulsive therapy in the preceding six months, were also excluded.

Neurocognitive and creativity tests were carried out under standard conditions and scored by two trained neuropsychologists. Executive function was assessed using the Wisconsin Card Sorting Test [(WCST)-Conceptual level responses (WCST-CONC), Perseverative Responses (WCST-PR), Failure to Maintain Set (WCST-FMS), Corrected Categories (WCST-CC), Errors (WCST-E), Non-Perseverative Errors (WCST-NP), and Perseverative Errors (WCST-P)] (Lezak et al., 2004). Intelligence Quotient (IQ) was assessed using Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999).

Creativity was assessed using the Barrow Welsh Art Scale (BWAS). The BWAS (Barron, 1963) is an empirically derived metric consisting of 86 black and white images that individuals rate as “like” or “dislike”, with higher scores reflecting preference for more asymmetrical and complex figures over more symmetrical and simple figures. Preference for more asymmetrical and complex figures is higher among artists than non-artists according to BWAS scores (Gough and Bradley, 1996). Creative individuals in disciplines other than the visual arts can also exhibit high BWAS scores (King et al., 1991). The BWAS scale could reflect cognitive/affective contributions to creativity, as it involves not only visual processing but also affective processing (like or dislike). Indeed, BWAS scores have been linked not only to creativity as measured by other means but also to emotionality (King et al., 1991).

The research ethics board of *Hospital das Clínicas of the University of São Paulo* approved the study. Written informed consent was obtained from all subjects.

3. Statistical analyses

Groups of subjects were compared using the Chi-square test for categorical data, and the ANOVA for continuous data. BWAS total sores were compared among mania, depression and mixed episodes using the ANOVA test and then by Tukey's multiple variables correction test. The influence of IQ, age, education, gender, age at diagnosis, as well as YMRS and MADRS scores, on the results from backward regression analysis was assessed. The PASW statistics version 18.0 software (SPSS Inc., Chicago, Illinois) was used for all analyses.

4. Results

4.1. Subjects

A total of 67 patients with bipolar I disorder (45 females) were included. Twenty patients were experiencing manic episodes; twenty-one mixed states and twenty-six depressive episodes. The mean age of the sample was 27.8 (±5.1) years old. Participants had 12.3 mean years (±3.1) of education and a mean IQ of 95.5 (±13.4). Regarding professional activity of individuals in the sample: 10 subjects were university students, 3 actors, 12 unemployed, 10 technicians, 10 health professionals and 22 subjects were working in business or legal professions. Subjects did not differ by episode type for age, sex, education, history of psychotic symptoms, number of previous manic episodes, clinical global impression score (CGI) or number of previous suicide attempts (Table 1).

4.2. Comparison of cognitive and creativity measures among groups

The ANOVA test showed that executive function scores, as rated by the WCST, differed between episodes (Table 2) on WCST PR, WCST Errors and WCST P. Post-Hoc analysis confirmed that the manic group had higher scores than the

Table 1
Sociodemographic and clinical variables.

	Bipolar disorder episodes						ANOVA[a]		Turkey post hoc test[a]
	Mania ($N=20$)		Mixed ($N=21$)		Depression ($N=26$)				
	Mean	SD	Mean	SD	Mean	SD	F	p	
Age (yrs)	28.90	5.13	28.67	5.00	26.46	5.17	1.64	0.201	
Gender (men/women)[b]	16/4		16/5		13/13		$p=0.06$		
Years of schooling	11.63	3.7	12.81	3.4	12.60	2.36	0.79	0.455	
MADRS	10.50	8.17	21.55	8.73	24.28	7.22	11.56	<**0.001**	Depre>Mania<Mixed
YMRS	18.05	6.73	13.86	5.79	9.60	6.33	7.95	**0.001**	Mania>Mixed
Age at diagnose	27.14	5.72	23.00	6.20	25.67	4.95	*2.47*	*0.094*	
Clinical Global Impression	3.67	0.88	4.05	1.22	3.25	0.85	*2.64*	*0.082*	
Psychotic symptoms[b]	60%		61.9%		34%		$p=0.08$		
>4 manic episodes[b]	45%		49%		53%		$p=0.34$		
Number of suicide attempts	1.64	3.15	1.73	1.77	2.24	3.05	0.249	0.780	

MADRS: Montgomery–Åsberg Depression Rating Scale; YMRS: Young Mania Rating Scale. The signs (>, <) indicate better or worse functioning and not actual scores on the tests. Bold indicates tests with statistic significance.
[a] Significance level $p<0.05$.
[b] Chi-square test, significance level $p<0.05$.

mixed group state across all neurocognitive tests (worse executive function). IQ scores did not differ among episode groups. ANOVA comparison among the three episode groups revealed that they differed in BWAS total score ($F=6.49$ $p=0.003$) (Fig. 1). Multiple comparisons analysis revealed that BWAS total score was higher in the mania group than in either the mixed ($p=0.006$) or depression ($p=0.014$) episode groups, but did not confirm the difference between mania and mixed groups. BWAS Like and Dislike scores showed no differences among episode types (Table 2).

4.3. Creativity measures and mood symptoms

Backward regression analysis revealed that YMRS, MADRS and CGI did not influence BWAS total scores in depressive ($F=1.06$ $p=0.40$) or mixed ($F=0.15$ $p=0.99$) episodes. In manic states however, the mood symptom scales influenced BWAS score ($R^2=0.76$, $F=8.67$, $p=0.007$). Separate analysis of these scales revealed that YMRS ($B=1.96$ $t=4.57$ $p=0.002$) and MADRS ($B=1.16$ $t=3.25$ $p=0.012$) were responsible for this interference. Both YMRS and MADRS in mania positively influenced BWAS total score.

4.4. Creativity measures and cognition

Executive function scores (WCST) did not influence BWAS total score in mixed ($F=0.92$ $p=0.51$) or depressive ($F=0.48$ $p=0.81$) episodes. However, in manic episodes, the subtests WCST CC ($B=12.96$ $t=2.3$ $p=0.036$ Partial Eta Squared 0.29) and WCST NP ($B=-2.34$ $t=-2.9$ $p=0.012$ Partial Eta Squared $=0.39$) influenced BWAS total score. The WCST CC positively influenced BWAS total score in mania while the WCST NP had a negative impact.

Table 2
Comparison of executive function, intelligence and creativity between episodes.

	Bipolar disorder episodes						ANOVA[a]		Turkey post hoc test[a]
	Mania ($N=20$)		Mixed ($N=21$)		Depression ($N=26$)				
	Mean	SD	Mean	SD	Mean	SD	F	p	
WCST CONC	42.74	11.54	45.33	8.69	49.27	6.57	3.08	0.053	
WCST PR	13.58	12.27	9.05	5.02	6.85	3.67	4.42	**0.016**	Mania<Depression
WCST FMS	0.37	0.59	0.10	0.30	0.58	0.98	2.44	0.095	
WCST CC	2.89	1.59	3.40	1.39	3.65	1.19	1.67	0.196	
WCST Errors	21.26	11.54	18.67	8.69	14.15	6.44	3.73	**0.029**	Mania<Depression
WCST P	11.32	9.45	8.10	3.76	5.92	3.39	4.57	**0.014**	Mania<Depression
WCST NP	9.95	8.59	10.20	6.99	8.15	5.74	0.58	0.559	
Intelligence Quotient	92.40	14.69	96.81	13.46	97.00	12.50	0.78	0.459	
BWAS Like	13.06	6.52	11.21	7.53	6.53	7.73	2.96	0.06	
BWAS Dislike	12.93	10.81	14.78	8.61	11.00	7.52	0.56	0.57	
BWAS total score	27.25	12.10	26.80	10.94	16.76	10.97	6.53	**0.003**	Mania>Mixed Mixed>Depress

WCST: Wisconsin Card Sorting Test – Conceptual level responses (CONC), Perseverative Responses (PR), Failure to Maintain Set (FMS), Corrected Categories (CC), Errors (E), Non-perseverative Errors (NP), Perseverative Errors (P); BWAS: Barrow Welsh Art Scale. The signs (>, <) indicate better or worse performance and not actual scores on the tests. Bold indicates tests with statistic significance.
[a] Significance level $p<0.05$.

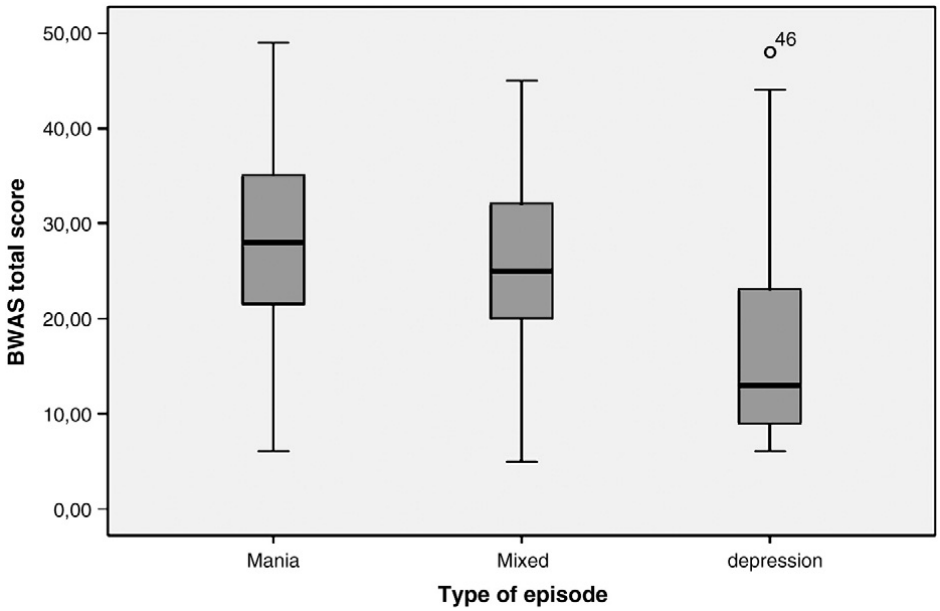

Fig. 1. Boxblot graphic comparing BWAS total scores in mania, mixed and depressive episodes (F = 6.49 $p = 0.003$).

IQ had no influence on BWAS scores for any type of episode (mania: F = 0.30 $p = 0.59$; mixed: F = 0.06 $p = 0.80$; depression: F = 0.24 $p = 0.62$).

4.5. Creativity measures and other variables

Regression analysis to evaluate the influence of gender, education, and age as cofactors, on BWAS total scores revealed no influence for any type of episode (mania F = 2.62 $p = 0.08$); (mixed F = 0.45 $p = 0.71$); (depression F = 1.26 $p = 0.31$).

5. Discussion

The present study reported that the validated measure of creativity (BWAS) differed among bipolar mood states and that executive function influenced creativity differently in each mood state. Manic patients were found to have higher creativity scores, in agreement with previous empirical observations (Jamison, 1996; Rothenberg, 2001), although this difference only reached statistical significant when compared with depressive episodes, but not with mixed episodes. Executive functioning was related to creativity scores only for mania, where WCST CC was positively, and WCST NP inversely, correlated to creativity score. Creativity scores in manic patients were shown to be positively influenced by executive function where the more creative the individual, the more categories on the WCST were completed, and the fewer non-perseverative errors made.

The manic stage of bipolar disorder shares the underlying common characteristic of an elevated mood, and is characterized by an increase in the quantity and speed of physical and mental activity. In our study, higher creativity scores were associated with better performance in executive function within the manic group, which is consistent with the concept that creativity requires the generation of multiple and appropriate stimulus (Lubart, 1994). In this sense, high creativity in mania is associated with better executive function. Nevertheless, compared to depression and mixed states, mania still had the worst executive function when analyzed individually.

To our knowledge, no previous studies have undertaken a combined analysis of creativity in each mood state of BD and analyzed how executive functions influence creativity during BD episodes. However, this topic has been studied in schizophrenic patients. In schizophrenics, performance on executive function tasks has been shown to play a mediating role in specific aspects of creative cognition, congruent with our results for mania. In fact, the study in schizophrenics reported that the performance of the schizophrenic group on measures of creativity elements of fluency were mediated by their performance on the executive control tasks (Abraham et al., 2007).

One possible explanation for the differences seen in creativity among episode types might involve dopamine (DA) variations. CD mechanisms in BD have been proposed to include deficits in DA in the prefrontal cortex (PFC) (Randrup and Braestrup, 1977; Williams and Goldman-Rakic, 1995). Historically, dopaminergic models of BD have been dichotomous and support dopamine (DA) excess in mania and deficiency in depression (Randrup and Braestrup, 1977). Insufficient (hypodopaminergic) and excessive (hyperdopaminergic) D1 receptor stimulation have been reported to impair PFC function (Arnsten and Li, 2005; Granon et al., 2000; Zahrt et al., 1997), leading to CD. For this reason it has been suggested that PFC cognition needs an optimal level of DA to achieve normal function (Goldman-Rakic et al., 2004; Mehta et al., 2000). DA has also been reported to influence mood and cognition (Cousins et al., 2009) while psychological, neuropsychological as well as functional imaging studies, indicate its potential role in the biology of creativity (Burch et al., 2006; Folley and Park, 2005; Richards et al., 1988). High DA has been reported to decrease inhibition of incoming stimuli from the surrounding

environment (latent inhibition) (Ellenbroek et al., 1996; Swerdlow et al., 2003), which is characteristic of creative individuals (Carson et al., 2003). Also, the ability to generate many different ideas about a topic in a short period of time (divergent thinking), a key aspect of creativity (Gundlach and Gesell, 1979), is influenced by the dopaminergic function (Reuter et al., 2006). Therefore, in a putative hyperdopaminergic state such as mania in which the optimal amount of DA for good executive functioning may be exceeded (Goldman-Rakic et al., 2004; Mehta et al., 2000), high creativity was observed in those with less compromised executive function. It is possible that to sustain the high creativity associated with high DA, a minimum of executive function is required, and if this optimal level is disrupted creativity then becomes impaired.

Previous controlled studies about creativity in BD have involved euthymic and medicated patients. Santosa et al. (2007) evaluated a mixture of euthymic medicated and unmediated BD I, II, and not otherwise specified and reported similar scores of creativity to creative controls and higher scores than non-creative controls. Data from this same cohort revealed that temperament and personality traits contributed to higher creativity in mood disorders (Srivastava et al., 2010b; Strong et al., 2007). Earlier studies have previously suggested a relationship between cyclothymia and creativity indicating the existence of an affective temperament/personality component to creativity (Akiskal and Akiskal, 1988; Akiskal et al., 2005). Furthermore some studies have indicated a cognitive temperament/personality component to creativity and suggest that intuitive cognitive processing may contribute to creativity by enhancing positive discrimination (Srivastava et al., 2010b). To our knowledge, there are no studies reporting correlations between affective and cognitive temperament/personality components and neuropsychological aspects in BD patients. Based on our results, creativity could be considered partially state dependent as opposed to solely a trait related to a BD diagnosis. We agree that probably the cognitive temperament/personality component to creativity may become indistinguishable in a sample that is medication free and full of symptomatology but this is undoubtedly the best way to evaluate magnificence of creativity in BD and its differences among episodes.

Among the limitations of the present study, the group sizes should ideally have been larger in order to demonstrate significant differences more clearly. Also, although the sample included many women, gender analysis by episode was not done due to the small sample size. The strengths of this study include its use of a validated measure of creativity in patients, without interference of medication, in a sample of young BD I patients in three different mood states. By contrast, most previous studies have involved euthymic and medicated patients (Santosa et al., 2007; Srivastava et al., 2010b; Strong et al., 2007). However, the general association of bipolar disorder to increased creativity suggests that some individuals may show altered creativity as a trait variable (Akiskal and Akiskal, 1988; Akiskal et al., 2005; Srivastava et al., 2010b; Srivastava and Ketter, 2010a), in addition to the differences among states seen in the present study. Tests on the same individual across different mood states, and in comparison to unipolar and nondepressed controls, may be indicated. Moreover, other measures of creativity and of creative accomplishments may yield different relationships to mood states.

6. Conclusion

This study is the first to report measures of creativity for three different types of mood episodes of BD, as well as their association with executive function. In agreement with clinical observations, mania was the mood state with the highest creativity score. Furthermore, high creativity in manic patients was shown to be associated with better executive function. We propose that creativity in BD episodes might be positively influenced by DA levels in the PFC, but may also be dependent on executive function. Future studies should attempt to replicate our findings and clarify the connection between DA, mania, and creativity in BD.

Role of funding source

Sao Paulo research foundation (Fapesp) is an independent public foundation with the mission to foster research and the scientific and technological development of the State of São Paulo.

Conflict of Interest

The authors do not have any conflict of interest to report.

Financial disclosures

The São Paulo Research Foundation (*Fundo de Apoio a Pesquisa do Estado de São Paulo* – FAPESP 2010/06230-0) financed this research.

Acknowledgments

We would like to thank the team of the Institute of Psychiatry at the University of São Paulo, especially the members of the Mood Disorders Unit (GRUDA) and São Paulo Research Foundation (FAPESP) for their dedication and hard work.

Team "Research on Research," Duke University Health System, for templates on writing introduction and discussion sections of the manuscript.

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