UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE GRADUAÇÃO EM BIOMEDICINA

JULIANA NICHTERWITZ SCHERER

MODIFICAÇÃO DOS VALORES DE BDNF E TBARS EM USUÁRIOS DE CRACK INTERNADOS EM UM PROGRAMA ESPECIALIZADO

Porto Alegre
Novembro/2014

JULIANA NICHTERWITZ SCHERER

MODIFICAÇÃO DOS VALORES DE BDNF E TBARS EM USUÁRIOS DE CRACK INTERNADOS EM UM PROGRAMA ESPECIALIZADO

Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Bacharel(a) em Biomedicina.

Orientador: Prof. Dr. Flavio Pechansky
Co-orientadora: Dra. Lisia von Diemen

Porto Alegre

Novembro/2014

AGRADECIMENTOS

Aos meus pais, minhas fontes de amor e apoio incondicional. Aos meus irmãos, que inspiram meus melhores sorrisos. Aos tios, dindos, avós e primos, meus bens mais preciosos. Aos meus amigos, que colorem a minha vida nos mais belos momentos.

À equipe do Centro de Pesquisa em Álcool e Drogas, que me acolheu de braços abertos e me fez membro de sua família. Em especial aos colegas Cleide, Silvia S., Vanessa, Felipe, Mayra, Daiane, Graciela, Tanara, Silvia H, Anne e Félix, por todo o suporte, positividade e momentos de alegria em meio a uma rotina atarefada.

À pesquisadora Bianca Pfaffenseller e à amiga Giovanna Bristot, pelo auxílio e pelos ensinamentos dados durante a análise laboratorial dos biomarcadores.

Meus agradecimentos especiais ao Prof. Dr. Flavio Pechansky, a quem eu possuo profunda admiração, e cujas orientações foram fundamentais para o meu desenvolvimento profissional e pessoal.

À Dra Lisia von Diemen, minha mentora e co-orientadora, por estar sempre me guiando e por dividir comigo toda a sua sabedoria.

E, por fim, mas não menos importante, às minhas colegas de jornada e irmãs de coração Laís, Vanessa, Thayssa, Nathália e Laura, minhas cúmplices durante este longo percurso de faculdade e que fizeram meus dias na Biomedicina muito mais divertidos.

SUMÁRIO

Abreviaturas e Siglas

ATV – Área Tegumentar Ventral
BDNF – do inglês *Brain Deriveded Neurotrophic Factor*
BE – Benzoilecgonina
CPF – Córtex Pré-Frontal
DST – Doença Sexualmente Transmissível
MDA – Malodialdeído
NAc – *Nucleo Accumbens*
NTs – Neurotrofinas
SNC – Sistema Nervoso Central
SPAs – Substâncias Psicoativas
TUSP – Transtorno de Uso de Substâncias

1 RESUMO

O consumo de crack tem sido considerado como um problema de saúde pública tanto no contexto nacional quanto internacional. Mesmo com o aumento do uso dessa droga no Brasil nos últimos 20 anos, ainda existem poucas evidências sobre a toxicologia sistêmica induzida pelo crack e os mecanismos neurobiológicos envolvidos na dependência, abstinência e recaída. Devido à complexidade do tratamento da dependência de crack, a busca de marcadores biológicos que auxiliem na determinação do impacto e recuperação da dependência química tem se intensificado nos últimos anos. Portanto, o nosso principal objetivo foi avaliar a associação entre o BDNF e níveis de TBARS em usuários de crack durante o tratamento de internação, antes e depois da desintoxicação, e a associação desses biomarcadores com a evolução clínica durante o tratamento. Para isso, 66 usuários de crack do sexo masculino foram recrutados em uma unidade de tratamento, e duas amostras de sangue foram coletadas (uma na admissão e outra na alta), a fim de medir os níveis séricos de BDNF e TBARS. Para comparar os níveis de biomarcadores com a evolução do tratamento, os indivíduos foram divididos em dois grupos: não concluiram o tratamento (n=34) e tratamento completo (n=32). O padrão de uso de drogas e os dados socidemograficos foram avaliados através do instumento ASI-6. Como resultados, observamos que, no momento da alta, o grupo que concluiu o tratamento em regime de internamento teve níveis mais elevados de BDNF do que o grupo que não concluiu. Também foi observada uma correlação negativa entre os níveis de BDNF e TBARS no momento da internação. Além disso, vimos uma correlação entre os níveis de BDNF no momento da internação e anos de uso de crack. Estes resultados sugerem a associação de níveis mais elevados de BDNF e melhores resultados clínicos em usuários de crack após a desintoxicação, e

indicam que tempo de uso de crack pode estar envolvido com a intensidade dos danos em relação à neuroplasticidade. A correlação entre TBARS e BDNF em usuários de crack corroboram com a idéia de que o estresse oxidativo e a neuroplasticidade estão envolvidos com transtorno da dependência de crack. Portanto, esses resultados são importantes porque sugeram que estes biomarcadores podem futuramente ser utilizados para entender a fisiopatologia da dependência ao crack e ajudar a desenvolver tratamentos e acompanhamentos específicos para esse grupo de usuários.

2 INTRODUÇÃO

2.1 Cocaína e Crack

A cocaína é uma droga psicoestimulante com elevado potencial de abuso e que afeta diretamente o cérebro. Apesar de ter sido considerada a droga dos anos 80 e 90 em função do aumento de seu consumo nesse período, cabe dizer que não se trata de uma droga nova. Existem diversas evidências de que as folhas de coca vem sendo utilizadas por diversas civilizações e que, para os povos antigos, estas possuíam importância medicinal e espiritual. Há vestígios de que os povos andinos mascavam folhas de cocaína há cerca de 3000 a.C., tendo sido identificados seus vestígios através da pesquisa da substância em múmias dessa região (1). Ainda, no início de 1900, a cocaína purificada foi o principal ingrediente ativo na maioria dos tônicos e medicamentos que foram desenvolvidos para tratar uma ampla variedade de doenças – do *Vin Mariani* à Coca-cola (2).

A cocaína pura é originalmente extraída da folha do arbusto de *Erythroxylon coca*, uma planta naturalmente encontrada na vegetação do Peru e da Bolívia. Após a década de 1990, entretanto, a Colômbia se tornou o país com o maior cultivo de coca cultivada (3). Hoje, a cocaína é uma droga de Classe II, o que significa que tem alto potencial para abuso, mas pode ser administrada para usos médicos legítimos, como a anestesia local para algumas cirurgias dos olhos, ouvidos e garganta (4, 5).

Há duas formas químicas principais de cocaína que são comumente abusadas: a forma solúvel em água, o sal de cloridrato, e a forma insolúvel, conhecida como base de cocaína (ou base livre). De acordo com a **Figura 1**, observa-se que entre a folha de cocaína e a pedra de crack há uma série de etapas no processamento da droga. Nas fases iniciais do processamento, as folhas de cocaína são maceradas

em álcool junto com querosene ou gasolina e é adicionado ácido sulfúrico, formando a solução de cocaína. A partir da adição de cal e amoníaco e da filtração da solução de cocaína é formada a pasta de cocaína, a qual dará origem à pasta base através de novos aditivos e filtração. Da pasta base podem ser formados a merla, o cloridrato de cocaína (em forma de pó – usado na forma inalada ou injetável) ou a pedra de crack. A base livre é geralmente produzida a partir do cloridrato de cocaína, tratada com uma base líquida para remover o ácido hidroclorídrico, e então dissolvida em um solvente (em geral éter), gerando uma forma de cocaína cristalizada, após aquecimento elevado. Já o crack pode ser produzido a partir do cloridrato de cocaína ou da pasta base ao adicionar bicarbonato de sódio, amônia, água e um aquecimento leve. Uma das principais diferenças entre a base livre e o crack é que a primeira é convertida à forma de base após a remoção dos adulterantes, o que não acontece com o crack (6)

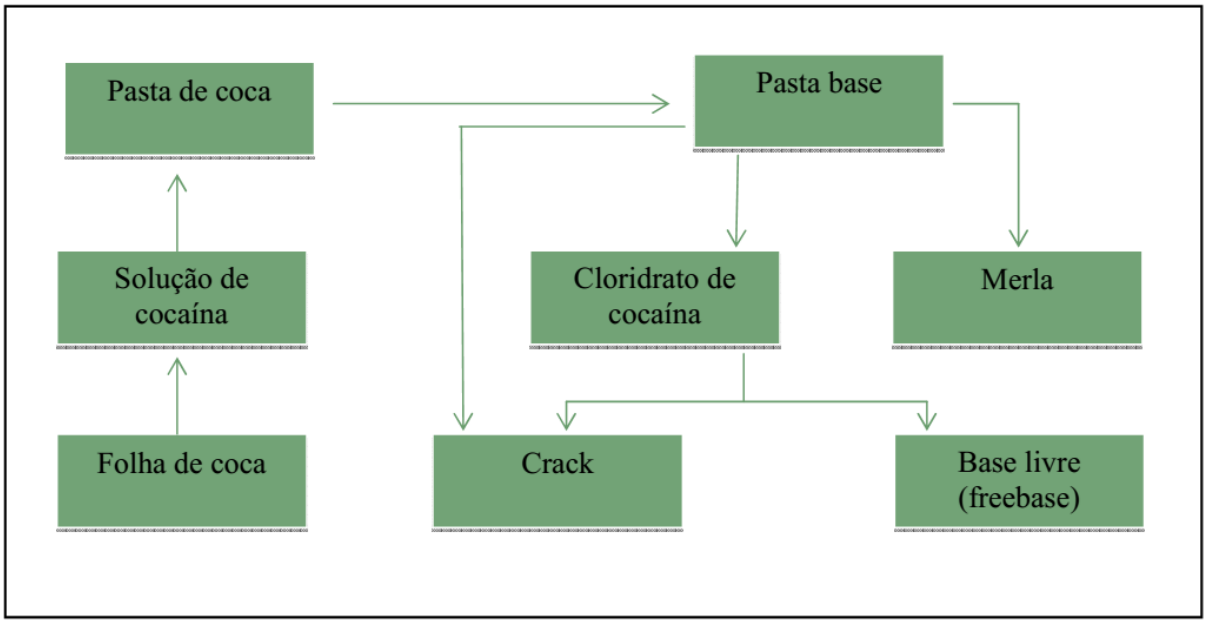

Figura 1. Etapas envolvidas na produção do crack (adaptado de von Diemen et al, 2013 (7)).

Outra diferença fundamental entre as apresentações de cocaína são suas formas de administração, o que implica diretamente nas características farmacocinéticas de cada droga. A cocaína pode ser administrada através de várias rotas: insuflação (cheirada), injeção intravenosa, inalação, ingestão ou aplicação tópica. A meia-vida da cocaína é de aproximadamente 0,7-1,5 horas e a maior parte da dose administrada é eliminada dentro de algumas horas (8).

Tanto a cocaína quanto o seu metabólito principal, a benzoilecgonina (BE), podem ser detectados na urina, sangue, saliva, cabelo, líquido amniótico e mecônio (9). O BE pode ser detectado na urina até 3 dias após o último consumo da droga através da técnica de imunoensaio de enzima multiplicada, ou por GC/MS, e até 7 dias por radioimunoensaio (10). Em usuários com uso crônico diário pesado, o metabólito pode ser detectado em até 10 dias após o último consumo (11).

A biodisponibilidade[1], portanto, também vai variar de acordo com a forma de administração da droga (tabela 1). O uso da cocaína na forma fumada difere das outras vias de uso principalmente pelo seu rápido início de ação (3 a 5 segundos), pouco tempo de efeito (5 a 15 minutos) e alta concentração plasmática (300 a 800 ng/mL). Como se observa na **Tabela 1**, o início da ação do crack e a duração do efeito são menores do que na via injetável e o pico plasmático é comparável, podendo ser maior (12). Dessa forma, o uso de crack se caracteriza por um efeito intenso, de curta duração, seguido por intensa fissura e desejo por nova dose. Quanto mais rápido, intenso e efêmero o efeito de uma substância, maior a chance de a droga ser consumida de novo. Portanto, a cocaína quando fumada tem um potencial de abuso e dependência maior do que as outras vias de uso (13).

---

[1]Biodisponibilidade: definida como a quantidade e velocidade na qual o princípio ativo da droga é absorvido, tornando-se disponível para a sua atuação no sítio de ação alvo.

Rota	Começo	Pico	Duração
Tópica	Dentro de 5 minutos	-	-
Intranasal	Dentro de 5 minutos	15-20 minutos	60-90 minutos
Intravenoso	10-60 segundos	3-5 minutos	20-60 minutos
Inalação	3-5 segundos	1-3 minutos	5-15 minutos
*Estes valores representam uso terapêutico			

Tabela 1. Início da ação, pico da ação e duração dos efeitos pelo uso de cocaína de acordo com a via de administração (retirado de Leikin, 2008 (14)).

2.2 Epidemiologia

O consumo de drogas no Brasil e no mundo, com óbvias especificidades regionais e locais, deve ser encarado não apenas como uma questão de saúde pública, mas também como algo que perpassa outros setores para além da saúde, como a segurança pública e a assistência e o desenvolvimento social de uma maneira mais ampla (15).

De acordo com o último Relatório Mundial sobre Drogas realizado pela *United Nations Office On Drugs And Crime* (UNDOC), o uso mundial da cocaína se estabilizou desde 2012, com 14-21 milhões de usuários tendo utilizado a droga pelo menos uma vez no último ano. Entretanto, quando analisadas regiões específicas, viu-se que na América do Norte ouve um aumento da prevalência do uso da droga entre 2011 e 2012, enquanto que na Europa Ocidental e Central houve uma redução do consumo (16).

Recentemente foi divulgado o relatório final da Pesquisa Nacional Sobre o Uso de Crack (15), que trouxe evidências sobre a prevalência de uso e sobre o perfil

sócio-demográfico dos usuários de crack no Brasil. Os principais achados sócio-demográficos podem ser visualizados na **Tabela 2** a seguir.

O estudo revelou que 366.598 brasileiros (aproximadamente 0,81% da população) faz uso regular de crack e/ou similares, como a merla e o oxi. Por mais que esses números representem pequena parte da população brasileira, a atenção à esses usuários é extremamente importante dados os inúmeros danos causados pelo seu consumo em uma grande variedade de esferas (sociais, psicológicas, biológicas). Assim, mesmo sendo observada uma baixa prevalência de consumo considerando a população absoluta, diversos autores já relatam o consumo de crack como um grande problema de saúde pública não apenas no Brasil (17, 18), mas no mundo (19-21).

Variável	**Média nas capitais**	**Média nas não capitais**	**Média Geral no país**
Idade (EP)	30,78 (0,45)	29,22 (0,35)	30,28 (0,33)
Sexo			
Homens (IC)*	-	-	78,68% (75,73 - 81,35)
Raça			
Caucasianos	22,27%	17,84%	20,85%
Não caucasianos	77,73%	82,16%	79,15%
Situação conjugal			
Solteiros (IC)*	-	-	60,64% (57,83 – 63,39)
Escolaridade			
Ensino Médio (IC)	-	-	16,45% (14,26 – 19,00)
Ensino Superior (IC)	-	-	2,35% (1,62 – 3,39)
Moradia			
Em situação de rua (IC)**	47,28% (42,85 – 51,76)	20% (15,19 – 29,64)	40% (34,18 – 44,14)

*Não se observou diferença significativa entre as proporções quando comparados os grupos de municípios. ** Houve diferença entre as proporções dos grupos de municípios, com p<0,05.

Tabela 2. Perfil dos usuário de Crack no Brasil (adaptado de Bastos & Bertoni, 2014 (15)).

Em geral, a pesquisa revelou que no Brasil os usuários de crack são majoritariamente adultos jovens – com idade média de 30,28 anos e predominantemente do sexo masculino (78,68%). Quanto à raça, foi observado que cerca de 20% dos usuários de crack e/ou similares no Brasil são de cor branca. Na população geral, segundo o Censo 2010 (IBGE), os “não-brancos” correspondiam a aproximadamente 52% da população brasileira, o que, de acordo com Bastos & Bertoni, sublinha a sobrerrepresentação de pretos e pardos (utilizando as categorias do IBGE) em contextos de vulnerabilidade social, como observado nas cenas de crack (15). Quanto à situação conjugal, a maioria dos usuários de crack e/ou similares do Brasil declarou ser solteira (60,64%). No que diz respeito à educação e moradia, foi observado que poucos usuários completarem o Ensino Médio (16%) e que uma expressiva quantidade de usuários se encontra em situação de rua (40%).

Outro achado importante desse estudo foi que os usuários de crack e/ou similares no Brasil são, basicamente, poliusuários - ou seja, o crack/similar é uma das drogas de um amplo “portfólio” de substâncias psicoativas que eles consomem, o que já havia sendo descrito anteriormente na literatura (22, 23). Logo, nota-se uma alta sobreposição do uso de crack e similares (pasta base, merla e oxi) com o consumo de drogas ilícitas, sendo o álcool e o tabaco as mais frequentemente consumidas (24, 25).

Acredita-se que, no Brasil, a dependência de crack seja atualmente a causa mais prevalente de internação por uso de cocaína. Em um estudo transversal realizado com 440 pacientes de seis hospitais psiquiátricos da Grande São Paulo, entre 1997-1998, 70% dos pacientes internados por problemas com cocaína eram usuários de crack (26). Além disso, os usuários de crack parecem buscar tratamento mais cedo do que os usuários de cocaína por via inalatória (27).

De modo geral, os usuários de crack em comparação com os usuários de cocaína inalada têm um número maior dos sintomas e maior gravidade de problemas em áreas diferentes da vida (28). Dados de um estudo multicêntrico realizado pelo nosso grupo demonstrou que aproximadamente 40% dos casos de pacientes internados e em ambulatórios especializados referiam que o crack era um dos seus principais problemas. Além disso, esses pacientes costumavam ter prejuízos graves nas áreas educacional/profissional, familiar, legal e psiquiátrica (29). Esses resultados, somados ao recente aumento da prevalência de uso de crack no país, destacam a gravidade dos sintomas e problemas relacionados com esta droga, e sugerem que as abordagens e planos de tratamento devem ser amplos e flexíveis (28).

2.3 Toxicidade

A toxicidade induzida pela cocaína vem sendo estudada e descrita por diversos autores e sendo alvo de estudos de revisão (9). Por mais que essa droga apresente toxicidade sistêmica, os principais estudos focam nos prejuízos cardíacos e neurológicos e, portanto, serão mais aprofundados a seguir. Na **Figura 2** são apresentados de forma resumida os principais fatores associados à fisiopatologia do consumo de cocaína.

O uso da cocaína leva a uma profunda toxicidade no SNC e no sistema cardiovascular. A cocaína bloqueia a recaptação de catecolaminas (dopamina, noradrenalina) e de serotonina (30). O aumento da atividade serotenérgica pode resultar em convulsões e pode estar envolvido na dependência e nos efeitos de recompensa da cocaína (30-33). Entretanto, é o excesso de atividade causado pela dopamina que se acredita ser a causa da maioria dos sintomas do SNC, tanto no

que se refere ao *craving*[2] quanto no que diz respeito aos efeitos tóxicos. Sintomas do SNC incluem euforia, aumento da autoconfiança e do estado de alerta em doses mais baixas, e agressividade, desorientação e alucinações em doses mais elevadas.

O uso repetitivo de cocaína resulta na depleção dos estoques de dopamina. Isto pode resultar tanto em um desejo intenso para a cocaína quanto no que é referido como uma síndrome de "*wash-out*" (30). Pacientes que experenciam "*wash-out*" apresentam síndrome de letargia e anedonia e têm dificuldade para a realização de movimentos musculares (34). A cocaína também afeta a regulação térmica central no hipotálamo e pode causar hipertermia (31). Acredita-se também que os efeitos sobre os aminoácidos/sistema glutamatérgico excitatório e sobre os receptores muscarínicos e sigma também contribuam para a toxicidade do SNC (30-32).

A inibição da recaptação de aminas biogênicas pela cocaína resulta em um poderoso efeito simpaticomimético. Como uma droga bloqueadora do canal de sódio, a cocaína é classificada como um agente antidisritimico do tipo I (30). Por apresentar cinética "*on-off*" lenta no canal de sódio, arritmias ventriculares e o alargamento do complexo QRS ou o prolongamento de QT/QTc podem ocorrer (31, 35). A cocaína também é conhecida por causar vasoconstrição, que pode resultar em hipertensão, acidente vascular cerebral (AVC), isquemia cardíaca, e enfartes de órgãos e de tecido. Entre os vários mecanismos e mediadores responsáveis pela vasoconstrição induzida por cocaína, podemos citar o aumento da norepinefrina neuronal, um efeito direto da benzoilecgonina sobre os vasos sanguíneos (possivelmente mediado por cálcio), o aumento dos níveis de endotelina 1 (um

[2] *Craving*: denominação pelo forte desejo pela droga, muitas vezes incontrolável.

potente vasoconstritor), e diminuição da produção de óxido nítrico (vasodilatador) (36).

Apesar da alta toxicidade relatada para a cocaína, pouca atenção tem sido dada a alterações centrais e periféricas associadas ao uso específico de crack. As drogas de abuso têm sido relacionadas com o aumento da carga alostática (37) através da exposição crônica ao stress e, a fim de compreender a toxicidade sistêmica causada pelo uso do crack, parâmetros de estresse oxidativo e de citocinas inflamatórias, entre outros, precisam ser investigados.

Um estudo recente do nosso grupo encontrou um aumento significativo nos níveis de BDNF, IL-β, TNF-α e IL-10 em pacientes usuários de crack quando comparados a controles; entretanto, não foi encontrada diferença nos parâmetros de estresse oxidativo entre os grupos (38). Esses resultados sugerem uma ativação dos sistemas imune, inflamatório e de recompensa no grupo de usuários. Assim, torna-se evidente a necessidade de estudos que investiguem parâmentros de toxicidade que possam contribuir para a avaliação de gravidadede uso de crack e gravidade de dependência ou mesmo que possam predizer fatores prognósticos e de recaída.

Estilo de vida do consumo de drogas		
Dieta pobre	Abuso de droga intravenosa	Criminalidade
Intoxicação		
Morte acidental	Homicídio	Suicídio
Método de abuso		
Inalação Queimaduras térmicas/químicas Perda do septo nasal	*Intravenoso* Endocardite infecciosa, HIV, hepatite, overdose aguda	
Efeito famacológico das drogas		
Cardiovascular Aumento de pressão arterial e da frequencia cardíaca Isquemia/infarto do miocárdio Dor no peito Trombose coronária Arritmia Miocardite, ruptura aórtica	*Respiratório* Pneumotórax Pnemomediastinum Infiltrados alveolares/intersticiais Edema pulmonar Infarto pulmonar Hemorragia pulmonar	*Gastroinstestinal* Gastrite/colite isquêmica Ulceração Infarto Hepatotoxicade
Cerebrovascular Hemorragia intracerebral Infarto intracerebral Vasculite	*Neurológico* Hipertermia Convulsões	*Psicológico* Euforia Vício e afastamento Depressão + fadiga (longo prazo) Psicose, esquizofrenia
Renal Rabdomiólise/Mioglobinuria Falha renal aguda Infarto renal	*Maternal* Aborto Rompimento de placenta Prematuridade Prisão cardíaca perinatal	*Fetal* Morte intrauterina Microcefalia Deficit neurocomportamental

Figura 2. Resumo das fisiopatologias associadas ao uso de cocaina (adaptado de Mendelson et al, 2011 (5)).

2.4 Dependência e Fatores Associados ao Uso de Crack

A expressão clínica da farmacocinética e da farmacodinâmica do crack em relação às outras formas de cocaína se dá através de diferentes aspectos. Os sintomas de dependência e os problemas relacionados ao consumo da droga se desenvolvem mais frequentemente e mais rapidamente nos usuários de crack, a mortalidade é mais elevada por diferentes causas, e a adesão e o prognóstico no tratamento são piores em relação ao uso inalado (39-42) Esses desfechos são acompanhados por outras consequências do uso de crack, como aumento da criminalidade, violência, doenças sexualmente transmissíveis (DST), infecções em geral, doenças físicas e impacto social, entre outros (13, 43).

Em particular, as taxas de infecção pelo vírus da imunodeficiência humana (HIV) e pelo vírus da hepatite C (HCV) são maiores em usuários de crack, sugerindo outras vias de infecção que não a injetável (44, 45). Há evidências de que o uso de cocaína está associado com alterações imunológicas nos linfócitos, incluindo as células *natural killer*, células T helper (CD4) e células T citotóxicas (CD8) (46). Estudos indicam que a cocaína pode inibir as funções de neutrófilos e macrófagos, interferindo com a habilidade do organismo de se defender contra infecções, bem como suprimir a produção de citocinas, diminuindo uma importante resposta imune (47, 48). Considerando que o uso de crack é a forma mais intensa de uso de cocaína, é possível que esses efeitos sejam maiores ainda nessa via de uso da cocaína.

O aumento da exposição a riscos para DSTs entre usuários de crack é bem documentado (49, 50). Entretanto, os mecanismos envolvidos são complexos e multifatoriais. No efeito agudo da droga, ocorre um prejuízo no processo de tomada de decisão e aumento de impulsividade, levando a avaliação inadequada dos riscos

envolvidos em determinados comportamentos (51, 52). Os intensos sintomas de abstinência observados durante a dependência de crack podem levar ao envolvimento em determinados comportamentos para obtenção da droga, como trocas envolvendo sexo, drogas e dinheiro, principalmente em mulheres, e atividades criminosas, mais prevalentes entre homens (24, 43, 50, 53). O uso crônico está associado à diminuição do desempenho cognitivo e alterações em partes específicas do cérebro que prejudicam ainda mais a capacidade de tomar decisões que avaliem adequadamente os riscos envolvidos em um comportamento (54, 55). Além disso, transtornos de personalidade e comorbidades psiquiátricas são fatores contribuintes nesse processo (56). Todos esses aspectos parecem ter peculiaridades entre os gêneros, mas tais diferenças ainda são pouco estudadas (57).

Quanto à neurobiologia da dependência, sabe-se que seu substrato neurológico está localizado no sistema límbico (58). Dentro do sistema límbico está alojado o circuito biologicamente primitivo para os estados de unidade, como a fome, o sexo, e sede, bem como o humor e a memória. A cocaína induz um estímulo muito intenso do sistema límbico, que é interpretado como um grande evento no organismo. O sistema límbico é composto de várias estruturas localizadas centralmente, frouxamente organizadas, mas ricamente interligadas por vias neuronais que utilizam neurotransmissores como o ácido gama-aminobutírico (GABA), serotonina, noradrenalina, substância P e encefalinas nas sinapses.

Uma parte específica do sistema límbico, o *nucleus accumbens* (NAC), parece ser o sítio de ação mais importante da cocaína. Quando estimuladas por dopamina, as células do NAC produzem sentimentos de prazer e satisfação (61, 62). A função natural desta resposta é ajudar a manter-nos focados em atividades que promovam

os objetivos biológicos básicos de sobrevivência e reprodução. Assim, a resposta das células receptoras de dopamina nos faz sentir bem e traz a vontade de repetir a atividade e reviver o prazer. Ao provocar artificialmente um acúmulo de dopamina no NAC, como descrito acima, a cocaína gera poderosos sentimentos de prazer.

O sistema límbico também inclui importantes centros de memória, localizados no hipocampo e amígdala. Estes centros de memória são responsáveis pela fixação de memórias relacionadas a comportamentos prazeirosos através da liberação de dopamina. Quando alguém experimenta cocaína, exite uma grande liberação de dopamina nesses centros (63), e por isso essas regiões estão envolvidas no processo de memória e reforço relacionados à adição (64, 65). A partir desta premissa é possível compreender a sensação que se desencadeia quando um indivíduo, ao voltar a um lugar onde utilizou a droga - ou simplesmente vendo as imagens de parafernália relacionada com cocaína - desencadeia memórias emocionalmente carregadas e desejo de repetir a experiência.

A terceira região límbica - o córtex frontal, é onde o cérebro integra informação e pesa diferentes cursos de ação. Ele age como um freio sobre as outras regiões do sistema límbico, quando é preciso renunciar a um prazer, a fim de evitar as suas consequências negativas. Uma vez que alguém se torna dependente químico, no entanto, o córtex frontal torna-se prejudicado, e há uma maior probabilidade dos impulsos não serem freados (66, 67).

Assim como em outras doenças, a vulnerabilidade para desenvolver problemas com drogas também é influenciada por uma combinação entre fatores genéticos e ambientais (68). Esses fatores, em conjunto com os efeitos diretos induzidos pelas drogas, irão influenciar a progressão da experimentação para o uso regular, deste para o abuso e para dependência, bem como para risco de recaída após período de

abstinência (69). Já foi visto na literatura que existe um forte componente genético no desenvolvimento de transtornos por uso de substâncias (TUSP), estimado em 50% da vulnerabilidade (70). Na dependência de cocaína, as estimativas variam de 42 a 79% (71). Entretanto, em vez de uma relação direta entre gene-doença*, "o papel da genética reflete o impacto combinado de fatores que operam em muitos níveis fenomenológicos, e é mediado através da codificação de múltiplos processos fisiológicos, comportamentais e de desenvolvimento, bem como suas interações com fatores ambientais igualmente poderosos, incluindo exposição à droga" (72)*.

De acordo com o modelo proposto por Volkow e Muenke, desenvolvimento cerebral, personalidade, comorbidades, sensibilidade e resiliência ao estresse, limiar entre prazer e aversão, neuroplasticidade e farmacogenômica são os principais intermediários propostos entre o ambiente e a genética (68). Nesta mesma linha, o impacto de cada um desses fatores é diferente nos estágios que levam do uso à dependência. Por exemplo, o papel dos efeitos induzidos pela droga seria mais importante na dependência, enquanto que a personalidade influencia mais o início do consumo e a transição para o uso regular (69). Além disso, vulnerabilidades individuais podem determinar que as disfunções cerebrais predominantes sejam diferentes nos sujeitos expostos à droga. Por exemplo, em um indivíduo a saliência do incentivo para a droga pode ser o mais importante, enquanto para outro a hiper-reatividade do sistema de estresse pode ser o principal (73).

Dentro de todas essas influências neurobiológicas, genéticas e ambientais, uma das hipóteses atualmente mais aceitas para a dependência de cocaína é que a droga induza neuroadaptações nos processos de memória e aprendizado relacionados ao sistema de recompensa cerebral, especialmente no sistema dopaminérgico mesocórtico-límbico e no circuito glutamatérgico córtico-límbico (74).

Por exemplo, os psicoestimulantes aumentam a complexidade e a densidade das espinhas dendríticas nos neurônios do NAC, ATV e CPF (75). De acordo com essa hipótese, essas adaptações causam uma hipersensibilidade aos estímulos associados com a cocaína e tornam o processo de tomada de decisão mais impulsivo, além de fazer com que os comportamentos disfuncionais associados à cocaína se tornem insensíveis às consequências negativas (69, 76, 77). Nesse sentindo, evidências apontam para um papel importante das neurotrofinas cerebrais na neuroadaptação induzida pela cocaína, podendo estar associadas ao desenvolvimento da dependência, fissura, dano cognitivo e predisposição para recaída (74, 78).

Considerando todos esses fatores, o crack é uma substância ainda mais danosa do que a cocaína (43, 79), e seus efeitos no corpo humano ainda são pouco conhecidos, pois há poucos estudos com seres humanos a respeito dessa droga.

2.5 Abordagens Terapeuticas

Atualmente várias abordagens de tratamento para dependência de cocaína e crack no Brasil vêm sendo discutidas, porém existem muitas controvérsias sobre qual demonstra maior efetividade na literatura científica. Entretanto, há um consenso de que a dependência de crack exige um tratamento difícil e complexo, pois a gravidade e tendência a recaídas sugere acompanhamento por longo tempo, associado a diferentes modalidades de tratamento interligadas, voltadas para as diferentes fases de recuperação (80).

Em virtude da gênese multifatorial da dependência química, o usuário precisa ser atendido nas diversas áreas afetadas, tais como: social, familiar, física, mental, além de ter atendidas suas questões legais, intensificação e melhora de sua

qualidade de vida, e receber enfoque especial nas estratégias de prevenção de recaída, acompanhamento educacional e reinserção social, com ênfase no mercado de trabalho e na recuperação de sua fragilizada rede social e familiar.

A abordagem apropriada dessas questões é tão importante quanto as estratégias dirigidas ao consumo de drogas. Além disso, ao definir o modelo técnico de abordagem terapêutica, é essencial ter o cuidado que ela seja adequada para a idade, gênero, etnia e cultura do paciente, devendo estar estruturada de modo quepossa ser reformulada conforme as necessidades mutantes dos sujeitos (81). Recentemente, uma revisão de 37 estudos randomizados demonstrou que os resultados mais relevantes com dependentes de psicoestimulantes eram provenientes do uso de diferentes técnicas de intervenção comportamental. Os desfechos apontam maior diminuição de uso de drogas, através de exames de urina negativos, nos estudos que utilizaram a técnica de gerenciamento de contingência. Contudo, novamente, a constatação foi de que não existe uma única técnica que abarque completamente a demanda multidimensional relacionada com a dependência de cocaína e crack (80).

Como dito anteriormente, os fatores que favorecem a dependência de crack são diversos e envolvem aspectos biológicos, psicológicos e socioculturais (82). Mesmo diante do progressivo aumento de consumo de crack no Brasil e no mundo, ainda pouco se sabe a respeito de mecanismos neurobiológicos da dependência, abstinência, evolução clínica, fissura e recaída, bem como dos aspectos genéticos relacionados. Dessa forma, para que intervenções mais efetivas sejam desenvolvidas para o tratamento do crack, a melhor compreensão desses aspectos é fundamental, já que os tratamentos convencionais para as outras substâncias têm tido resultados muito ruins nessa população (80, 83, 84). Nesse sentido, o

tratamento do usuário de crack é bastante complexo e há muito poucos estudos investigando técnicas terapêuticas para estes usuários.

2.6 Neurotrofinas

As NTs são responsáveis pelo controle de diversos processos importantes no desenvolvimento do sistema nervoso, como proliferação, migração, diferenciação, sobrevivência, apoptose e plasticidade sináptica (85). As NTs são sintetizadas em suas formas precursoras, como pró-neurotrofinas e posteriormente clivadas na sua forma madura (neurotrofinas). A ação das NTs se dá através de sua interação com um ou mais receptores tirosina-kinases (Trk) e todas se ligam ao receptor de neurtrofina de baixa afinidade, o p75.

2.6.1 *Brain Derived Neurotrophic Factor* (BDNF)

O BDNF é um membro da família das neurotrofinas, que inclui o fator de crescimento do nervo, a neurotrofina-3 e a neurotrofina 4/5. Ele é considerado a neurotrofina mais amplamente expressa e a mais abundante no sistema nervoso central (86). Como outros neuropeptidos, o BDNF é sintetizado como um pró-peptido (32 kDa) que é processado proteoliticamente em uma molécula final de menor tamanho (13 kDa). Alguns autores relatam que esta clivagem ocorre após sua secreção pelo tecido plasminogênio ativado por plasmina localizado extracelularmente (87). Outros autores, entretanto, relataram que os neurônios do SNC armazenam e secretam a molécula madura, e não na forma pro-BDNF, em resposta a estímulos excitatórios (88).

Dentre as neurotrofinas, o BDNF é o que possui maior evidência quanto a sua influência na plasticidade sináptica. O BDNF possui implicações nas bases

fisiopatológicas, de prognóstico e de resposta a tratamento em diversas desordens psiquiátricas, incluindo as adições (89-91).

A este respeito, tem sido reportado que o BDNF media em longo prazo a adaptação neuronal em condições patológicas por controlar a expressão do receptor de dopamina, induzindo, assim, a sensibilização comportamental (92). Mudanças tanto no mRNA quanto na proteína foram analisadas em várias regiões do cérebro, tais como a área tegmental ventral e o NAC após a administração e/ou a retirada de diversas substâncias aditivas (93, 94). Um estudo recente descobriu que a administração aguda de cocaína induz um aumento transitório nos níveis da proteína BDNF e ativa a sinalização mediada por TrkB no NAC em roedores (95). Além disso, infusões de BDNF no NAC são relacionadas ao aumento da auto-administração de cocaína, bem como o aumento da procura pela droga em situações de abstinência (95). Estes investigadores sugerem que a liberação de BDNF durante o consumo de cocaína é importante para o desenvolvimento e persistência do comportamento aditivo. Em ratos, o aumento do BDNF na área tegmentar ventral induz uma transição para um estado motivacional dependente de opiáceos (96).

Alguns resultados mostram que as alterações nos níveis de BDNF após a administração de cocaína são persistentes, sugerindo que modificações epigenéticas podem estar relacionadas com modificações de expressão genética induzidas pela droga (97).

Estudos em animais também demonstraram que os níveis de BDNF tem papel importante na instalação da dependência à cocaína, no processo de busca da droga, na sensibilização e na recaída (95, 98). A infusão exógena de BDNF pode melhorar o comportamento de procura de cocaína quando este é injetado na área tegmental ventral (99) ou suprimi-lo, quando injetado no córtex pré-frontal (100).

Além disso, alguns estudos investigaram a associação entre o cérebro e os níveis periféricos de BDNF, e encontraram uma forte correlação entre as duas medidas (101-103).

Neste cenário, a teoria da carga alostática surge como uma ferramenta útil para ajudar a elucidar resultados divergentes entre os níveis de BDNF em usuários de crack, e deve, portanto, receber certa atenção. A teoria, proposta inicialmente por McEwen, tenta explicar as adaptações que ocorrem no corpo e no cérebro em resposta a estressores, funcionando como um mecanismo de proteção de curto prazo (alostase), mas causando alterações que podem levar a doença a longo prazo (carga alostática) (104). Os fundamentos da teoria da carga alostática foram transpostos para a psiquiatria e ajudaram a alcançar uma melhor compreensão das doenças psiquiátricas (105), incluindo a adição (106).

A cocaína produz uma indução generalizada, mas transitória, da expressão da proteína BDNF em muitas áreas do cérebro relacionadas à adição e a recompensa (95, 107). Esta resposta pode estar desregulada como um resultado da exposição crônica à cocaína, agravada por fatores tais como o uso compulsivo da droga, presença de comorbidades psiquiátricas, abuso de outras drogas, vulnerabilidade genética e outros.

2.7 Estresse Oxidativo

Existem diversos achados na literatura que evidenciam a implicação do estresse oxidativo na patogenia de diversas desordens psiquiátricas (108-111). O estresse oxidativo é definido como um distúrbio no equilíbrio entre a produção de espécies reativas de oxigênio (radicais livres) e as defesas antioxidantes, o que

pode levar à lesão tecidual (112). Os radicais livres formam-se em grandes quantidades como um subproduto inevitável de muitos processos bioquímicos e, em alguns casos, deliberadamente, como em neutrófilos ativados. Entretanto, se a defesa antioxidante estiver prejudicada ou se houver um aumento do estresse oxidativo, podem ocorrer prejuízos nos tecidos.

Estudos em modelos animais mostraram que a administração de cocaína aumenta a produção de espécies reativas de oxigênio em diferentes estruturas cerebrais dopaminérgicas, tais como o córtex frontal e o estriado (113).

Estudos clínicos têm sugerido que marcadores de estresse oxidativo estão aumentados em sujeitos abusadores de álcool, cocaína, metanfetamina, nicotina e opióides, e que isso pode contribuir para o prejuízo causado pelo uso crônico dessas substâncias, levando a danos e apoptose nas células cerebrais (109, 114, 115). Entretanto, em relação à cocaína e ao crack ainda faltam informações sobre o envolvimento do estresse oxidativo na etiopatogênese e no prognóstico do uso e da dependência.

Existem diferentes maneiras de estimar o estresse oxidativo, uma vez que muitos compostos fazem parte deste processo. Substâncias reativas ao ácido tiobarbitúrico (TBARS) são substâncias de baixo peso molecular, que consistem amplamente em malodialdeído (MDA) e que são formados a partir da decomposição dos produtos de peroxidação lipídica instáveis e reagem com o ácido tiobarbitúrico para formar adutos fluorescentes (116). A peroxidação lipídica é o primeiro evento citotóxico que ocorre nas células, e por isso pode ser utilizado como um indicador de estresss oxidativo celular.

Os níveis de TBARS são elevados no plasma, plaquetas, eritrócitos e leucócitos em doenças cerebrais graves, tais como esquizofrenia (117). A

intensidade do estresse oxidativo está relacionada com a fase de algumas doenças, especialmente as mais avançadas. Além disso, o desenvolvimento de um transtorno de dependência, que engloba o comportamento compulsivo de procura de droga, pode aumentar a gravidade do estresse oxidativo, que ocorre no cérebro devido ao consumo da substância (115). Dessa forma, a associação entre o estado oxidativo e o comportamento induzido por cocaína durante a abstinência e o curso da doença está começando a ser estudada.

Um estudo clínico recente realizado em Porto Alegre mostrou uma correlação direta entre os níveis de TBARS e a gravidadedo uso de crack, onde os usuários de crack mais graves apresentaram maiores níveis de TBARS no momento da alta hospitalar (118). Além disso, estudos pré-clínicos indicaram o uso de antioxidantes como uma terapia promissora em diversos transtornos de adição, em substâncias como o álcool (119), heroína (120), e cocaína (121). Estudos clínicos pilotos também estão sendo realizados, e, até agora sugerem que o tratamento da dependência em usuários de cocaína com N-acetilcisteína é eficaz quanto à diminuição de sintomas de abstinência e *craving* (122) e de fissura induzida por objetos relacionados ao consumo de crack (123).

3 JUSTIFICATIVA

Mesmo havendo pouco conhecimento sobre os mecanismos específicos da toxicidade sistêmica do crack, alguns autores afirmam que ela é uma droga extremamente danosa para o organismo humano. Além disso, essa droga possui elevado potencial para abuso, e a instalação da dependência envolve fatores complexos e multivariados, abrangendo aspectos biológicos, psicológicos e socioculturais. Entretanto, existe pouca evidência científica no que diz respeito aos mecanismos neurobiológicos da dependência, abstinência, evolução clínica, fissura e recaída, bem como dos aspectos genéticos relacionados.

Sabe-se que o tratamento da dependência de crack é bastante complexo e há muito poucos estudos investigando técnicas terapêuticas para estes usuários. Nesse sentido, a busca por marcadores biológicos que possam auxiliar a compreender o desenvolvimento do processo da dependência e a descoberta de abordagens terapêutícas mais eficazes para o tratamento desses usuários se faz necessária.

4 OBJETIVOS

4.1 Objetivo Principal

Avaliar a associação dos níveis de BDNF e de TBARS em usuários de crack durante o tratamento de internação, antes e após a desintoxicação, com variáveis clínicas.

4.2 Objetivos Secundários

- Avaliar a associação das alterações dos níveis de BDNF após desintoxicação com a gravidade do uso de crack;

- Investigar se a alteração dos níveis de TBARS durante a desintoxicação está correlacionada com a alteração dos níveis de BDNF;

- Avaliar se os níveis de BDNF e de TBARS no início do tratamento estão associados com a adesão ao programa de tratamento na internação.

5 ARTIGO

Target Journal: Neuroscience Letters (Impact factor 2.026)

Title: Changes in BDNF and TBARS levels in crack-cocaine inpatients and its association with treatment evolution.

Authors: Juliana N. Scherer[a], Silvia Schuch[a], Felipe Ornell[a], Anne A. O. Sordi[a], Giovanna Bristot[b], Bianca Pfaffenseller[b], Felix H. P. Kessler[a], Lisia von Diemen[a], Flavio Pechansky[a].

[a] Center for Drug and Alcohol Research, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Rua Prof. Alvaro Alvim, 400, Porto Alegre, Brazil .

[b] Bipolar Disorders Program & INCT Translational Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul. Rua Ramiro Barcelos, 2350, Porto Alegre, Brazil.

Corresponding author: Juliana N. Scherer

Adress: Center for Drug and Alcohol Research, Hospital de Clínicas de Porto Alegre, Alvaro Alvim Unit, Rua Prof. Alvaro Alvim, 400, CEP: 90420-020, Porto Alegre, Brazil.

Phone number: + 55 51 33596488

E-mail: juliananscherer@gmail.com

5.1 Abstract

Introduction - There is very little evidence showing the systemic toxic effects induced by crack-cocaine and the neurobiological mechanisms involved in addiction, withdraw and relapse of this specific drug. Due to the complexity of crack addiction treatment, the search for biological markers that could help determine the impact or outcome of drug use has intensified in recent years. **Objective -** Our main objective was to evaluate the association between BDNF and TBARS levels in crack users during inpatient treatment, before and after detoxification, and the association of these biomarkers with treatment evolution**. Method -** A total of 66 male inpatient crack users were recruited in a treatment unit, and blood samples were collected at admission and discharge in order to measure BDNF and TBARS serum levels. In order to compare levels of biomarkers with treatment evolution, subjects were split into 2 groups: treatment non-completers (n=34) and treatment completers (n=32). Drug use pattern and socidemographic data were assessed using the ASI-6. **Results -** The group who completed inpatient treatment had higher levels of BDNF than non-completers at discharge. A negative correlation between BDNF and TBARS levels at admission was observed. We also saw a correlation between BDNF levels at admission and years of crack use. **Discussion -** Our findings suggest the association between higher levels of BDNF and better clinical outcomes in crack users after detoxification, and show that years of crack use can be involved in neuroplasticity damage. The correlation between TBARS and BDNF in crack users corroborates the idea that oxidative stress and neuroplasticity are involved with crack dependence. These findings are important because they suggest these biomarkers could be used to better understand the physiopathology of crack addiction, and help develop tailored monitoring and treatments of this specific subset of drug users.

Keywords: Crack-cocaine, BDNF, TBARS, treatment, addiction, biomarkers

Highlights

- Role of biomarkers for oxidative stress and neuroplasticity in the pathophysiology of crack addiction.
- Association between biomarkers and treatment evolution.
- Correlation between levels of BDNF and TBARS in crack users.
- Higher levels of BDNF found in subjects with better treatment evolution.

Conflict of interest: The authors declare no conflicts of interest.

5.2 Introduction

The development of crack addiction is a complex process which involves multiple and diverse factors in the biological, psychological and sociocultural dimensions. Even with the progressive increase in crack use in Brazil and in some parts of the world, the specific neurobiological mechanisms of addiction, abstinence, genetic aspects, craving, relapse, and clinical outcome are unknown. Thus, in order to develop tailored interventions for treatment, a better understanding of these aspects is fundamental, since conventional treatments for other substances have had very poor results in this population [1].

Due to the difficulties of treatment, the search for biological markers that could help determine the impact or outcome of drug use has intensified in recent years [2]. The Brain-Derived Neurotrophic Factor (BDNF) is one of the most abundant neurotrophins in the brain, and is involved in neurogenesis, neuroplasticity and cognitive functions [3]. Furthermore, BDNF is an important biomarker in many psychiatric disorders, including substance dependence [4-7]. Animal studies have demonstrated that levels of BDNF play an important role in the onset of addiction to cocaine, drug-seeking behavior, sensitization and relapse [8, 9]. Clinical trials have also evaluated the role of BDNF in crack-cocaine users, and the main results show increased levels of this neurotrophin during early withdrawal [10, 11]. Moreover, higher levels of BDNF after withdrawal appear to be associated with craving and a shorter time to relapse [12], and BDNF levels appear to be inversely correlated with the severity of crack cocaine use [11, 13]. Likewise, there are several findings in the literature showing the implication of oxidative stress in the pathogenesis of psychiatric disorders [14-17]. Clinical studies have suggested that oxidative stress markers are increased in subjects who abuse psychoactive substances such as

alcohol, cocaine, methamphetamine, nicotine and opioids, and this may contribute to the impairment caused by their repetitive use, leading to brain cell damage and apoptosis [18-22]. The analysis of the levels of thiobarbituric acid (TBARS) has been used in clinical studies as biomarkers of oxidative stress. The association between TBARS levels and cocaine-induced behaviors during withdrawal, as well as the course and development of addiction have been a focus of recent research. Our group has recently showed a correlation between the severity of crack consumption and levels of TBARS, where the most severe crack users had higher levels of TBARS at the time of hospital discharge [23]. However, there is still lack of evidence regarding oxidative stress induced by crack use and the physiopathology of dependence and clinical outcomes.

The main aim of this study was to evaluate the association between BDNF and TBARS levels in crack users during inpatient treatment, before and after detoxification, and the association of these biomarkers with treatment evolution in an inpatient sample.

5.3 Method

5.3.1 Sample selection

A total of 66 consecutive crack cocaine users were recruited at the Addiction Psychiatry Unit of Hospital de Clínicas de Porto Alegre, a large teaching hospital affiliated with the Federal University of Rio Grande do Sul, providing free public services. Inclusion criteria were: being a male crack cocaine user with the last intake reported at least 10 days before admission; being 18 years old or older; and agreeing to provide two blood samples during inpatient treatment. Subjects were excluded if

they were considered clinically and intellectually unable to participate, based on clinical evaluation.

5.3.2 Procedures

Patients were invited to enter the study as soon as they had the necessary clinical and mental conditions to understand the study objectives. One blood sample was collected with subjects in fasting during the first 24 hours of hospitalization, and the second was collected 24 hours preceding hospital discharge, also with subjects in fasting. Interviews were conducted by previously trained undergraduate. The non-completer group stayed a mean of 20.6 (9.75) days in the treatment, while the completer group stayed 33.31 (7.31). Psychology students between the 5th and 7th day of detoxification to circumvent potential cognitive impairment on the first days of hospitalization.

5.3.3 Ethics

The study was approved by the Institutional Review Board and Ethics Committee of Hospital de Clínicas de Porto Alegre. All subjects included in the analysis provided written informed consent.

5.3.4 Instruments

Drug use pattern and sociodemographic data were assessed using the Addiction Severity Index - 6th Version (ASI-6), validated for Brazilian Portuguese [24]. Clinical outcome was assessed through standard medical records.

5.3.5 Blood collection and processing

Ten milliliters of blood were collected by venipuncture into an anticoagulant free vacuum tube for each subject included in the study. Immediately after collection,

blood samples were centrifuged at 4,000 rpm for 10 minutes and the serum was aliquoted, labeled and stored at -80 °C until assay testing.

5.3.6 BDNF measurement

BDNF serum levels were measured by sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Milipore, USA). Briefly, microtiter plates (96-well flat-bottom) were coated overnight at 4 °C with the samples diluted 1:75 in sample diluent and standard curve ranging from 7.8 to 500 pg/ml of BDNF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BDNF monoclonal antibody (diluted 1:1000 in sample diluent), which was incubated for 3h at room temperature. After washing, a second incubation with streptavidin- horseradish peroxidase conjugate solution (diluted 1:1000) for 1h at room temperature was carried out. After addition of substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density and BDNF concentration.

5.3.7 TBARS assay

The levels of lipid peroxidation were measured by the method of TBARS (Thiobarbituric Acid Reactive Substances) using the TBARS assay kit (Cayman Chemical Company, Ann Arbor), according to the manufacturer's instructions. In this method, the quantification of lipid peroxidation products is performed by serum formation of substances reacting to 2-thiobarbituric acid (TBA), which is the analysis of the final products of lipid peroxidation (lipid peroxides, malondialdehyde and other aldehydes of low molecular weight) that react with TBA to form Schiff bases. These complexes exhibit color and its concentration can be determined

spectrophotometrically at 535 nm. The results are expressed in µM of malondialdehyde (MDA).

5.3.8 Statistical analysis

Variables with normal and asymmetrical distribution were compared using Student's T test and Mann-Whitney's test, respectively. Variables with a normal distribution are presented by the mean and standard deviation. Variables with an asymmetric distribution are presented by their median and interquartile range. Correlations between two variables with normal and asymmetrical distribution were performed through the Pearson and Sperman correlation test, respectively. Crack rocks used in the last 30 days were estimated by the equation: [mean of rocks used per day x number of days using crack in the previous 30 days]. Severity of crack use was estimated using previous described methodology [23]. BDNF levels at discharge were controlled for age and difference of days between blood collections through logistic regression.

5.4 Results

5.4.1 Demographic characteristics and drug use pattern

The demographic data of this sample, as well as its drug use pattern are shown in Table 1. The mean age of the sample was 33± 8.65 years, and the majority of the subjects were Caucasians (59%). Only 24% of the sample was married and 65% had 8 years or less of schooling. The mean of first age of crack use was 22 (18-30) years, with subjects consuming crack for approximately 9.33 ± 5.14 years. The number of crack rocks used in the last 30 days was around 300 (160-600), and the severity of use has median of 15 (11-23).

Variable	Crack users (66) N (%)
Age[a]	33.89 (8.65)
Caucasians	39 (59.1)
Married	16 (24.2)
8 years or less of schooling	43 (65.2)
Age of first crack use[b]	22 (18-30)
Years of crack use [a]	9.33(5.14)
Crack rocks used – last 30 days[b]	300 (160-600)
Severity[b]	15 (11-23)

[a]Mean (Standard Derivation); [b]Median (Interquartile Range)

Table 1. Sociodemographic and crack use pattern among subjects

5.4.2 BDNF and TBARS levels according to treatment evolution

Crack users were split into two groups (non-completers – n=34 vs. completers – n=32), according to their evolution in inpatient treatment. Inpatient treatment days and difference of days in blood collection were different among the groups (p<0,001) (see Table 2).

The levels of BDNF at discharge in the completer group were higher (17.76 ± 5,03) than non-completers(14.78 ± 5.36) (p=0.025). Even when this variable was corrected by age and difference of days in blood collection, its difference remained significant [p=0.039, B=1,141 IC (1.006; 1.294)].

The average difference between BDNF levels at admission and discharge was -0.097 (IC: -2.17; 1.97, p=0.924) in the non-completers;, completers showed a mean of 1.672 (IC: -0.39; 3.73 p=0.108).

Although we did not find a significant difference in TBARS levels between the two groups, we could observe a higher decrease of TBARS levels during inpatient treatment in the group of subjects who completed treatment.

Variable	**Incompleted Program (34)** Mean (SD)	**Completed Program (32)** Mean (SD)	***p***
Inpatien days	20.6 (9.75)	33.31 (7.31)	<0.001
Difference in blood collection (days)	14.74 (6.14)	24.7 (7.92)	<0.001
BDNF at admission	14.61 (4.76)	16.23 (3.33)	0.123
BDNF at discharge	14.78 (5.36)	17.76 (5,03)	0.025
BDNF variation[a]	2.21 (-20.17 – 23.87)	2.13 (-5.77 – 37.95)	0.428
TBARS at admission[a]	28.99 (17.81 – 53.58)	44.7 (28.1 – 58.0)	0.098
TBARS at discharge[a]	27.45 (17.63 – 50.3)	31.26 (22.7 – 48.59)	0.462
TBARS variation[a]	5.50 (-40.57 – 40.26)	-15.15 (-45.69 – 50.63)	0.582

[a]Median (Interquartile Range)

Table 2. BDNF and TBARS measures among groups of evolution in inpatient treatment

5.4.3 Correlations between biomarkers and drug use patterns

At admission, there was a negative correlation between BDNF and TBARS levels (Fig.1). These variables were not associated at discharge (Fig. 2).

There was no association in this sample between severity score of crack use and BDNF or TBARS levels (data not shown). However, the number of years of use by itself was associated with BDNF serum levels at admission (Fig.3).

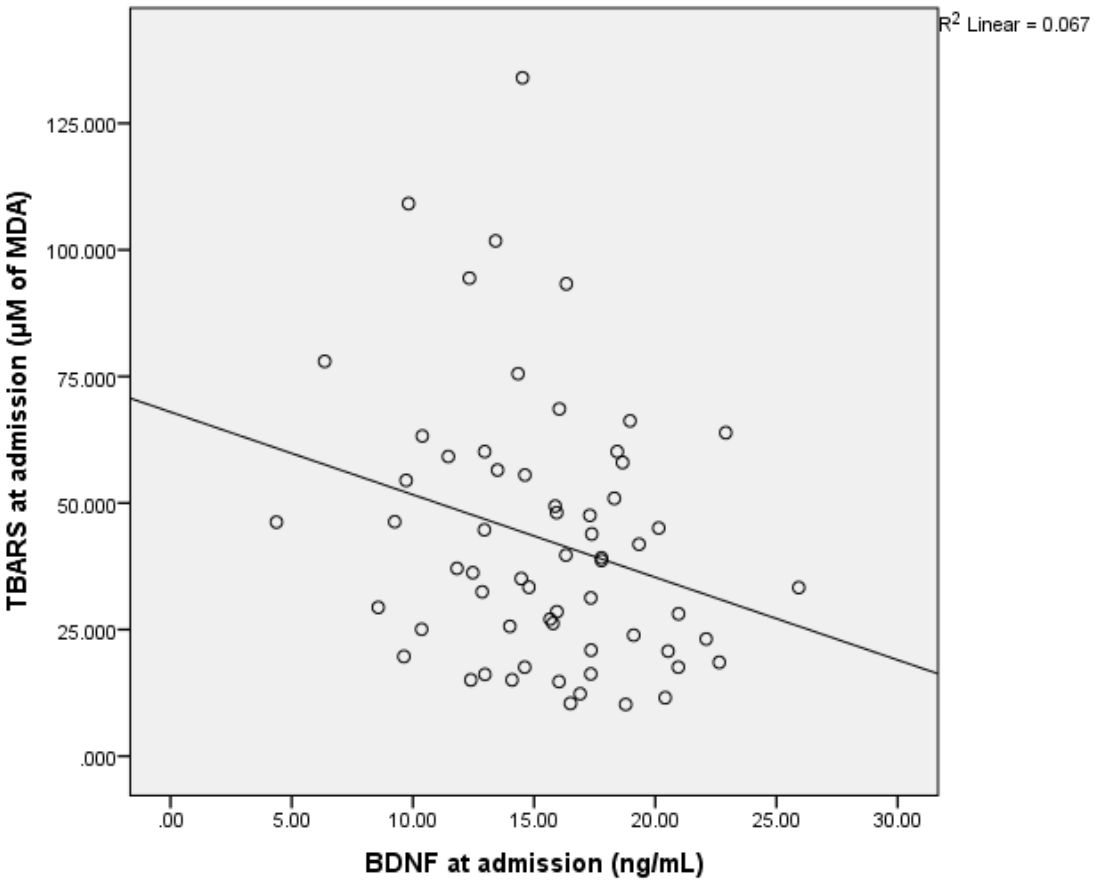

Figure 1. Correlation between BDNF levels at admission and TBARS levels at admission: partial correlation, -0.259; two-tailed significance, 0.042.

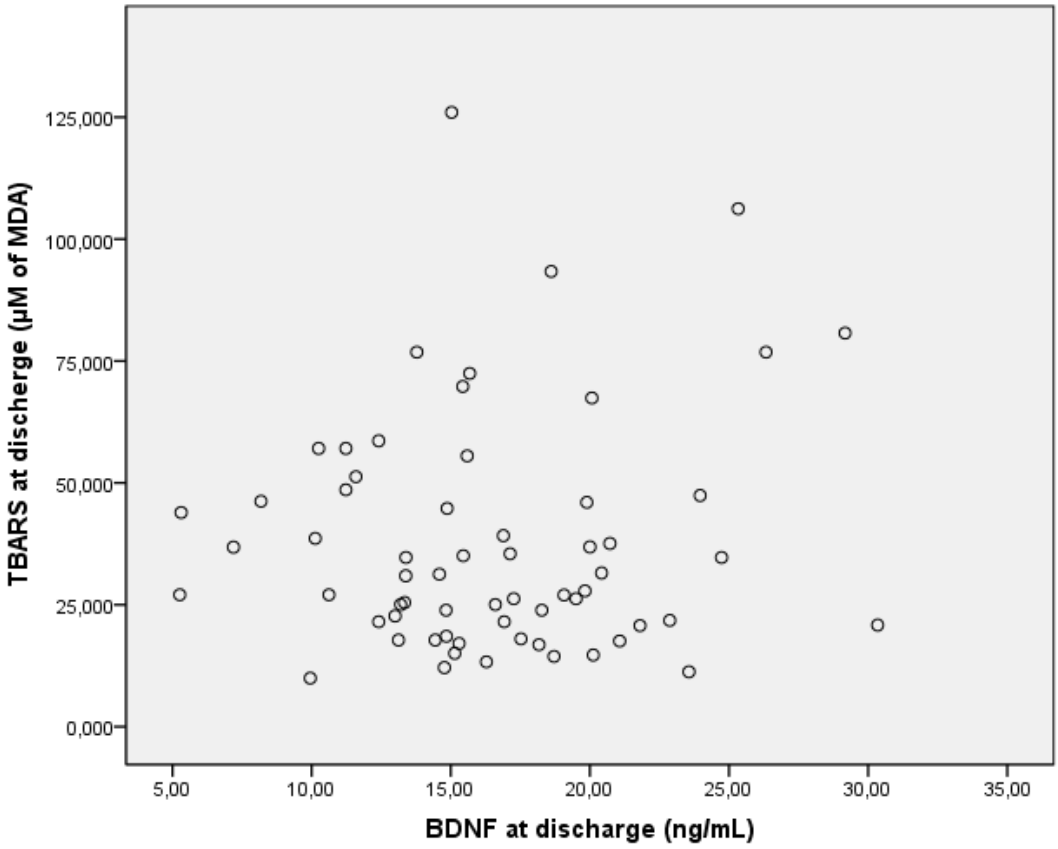

Figure 2. Distribution of BDNF and TBARS levels at discharge absence of correlation, -0.4, two-tailed significance, 0.756.

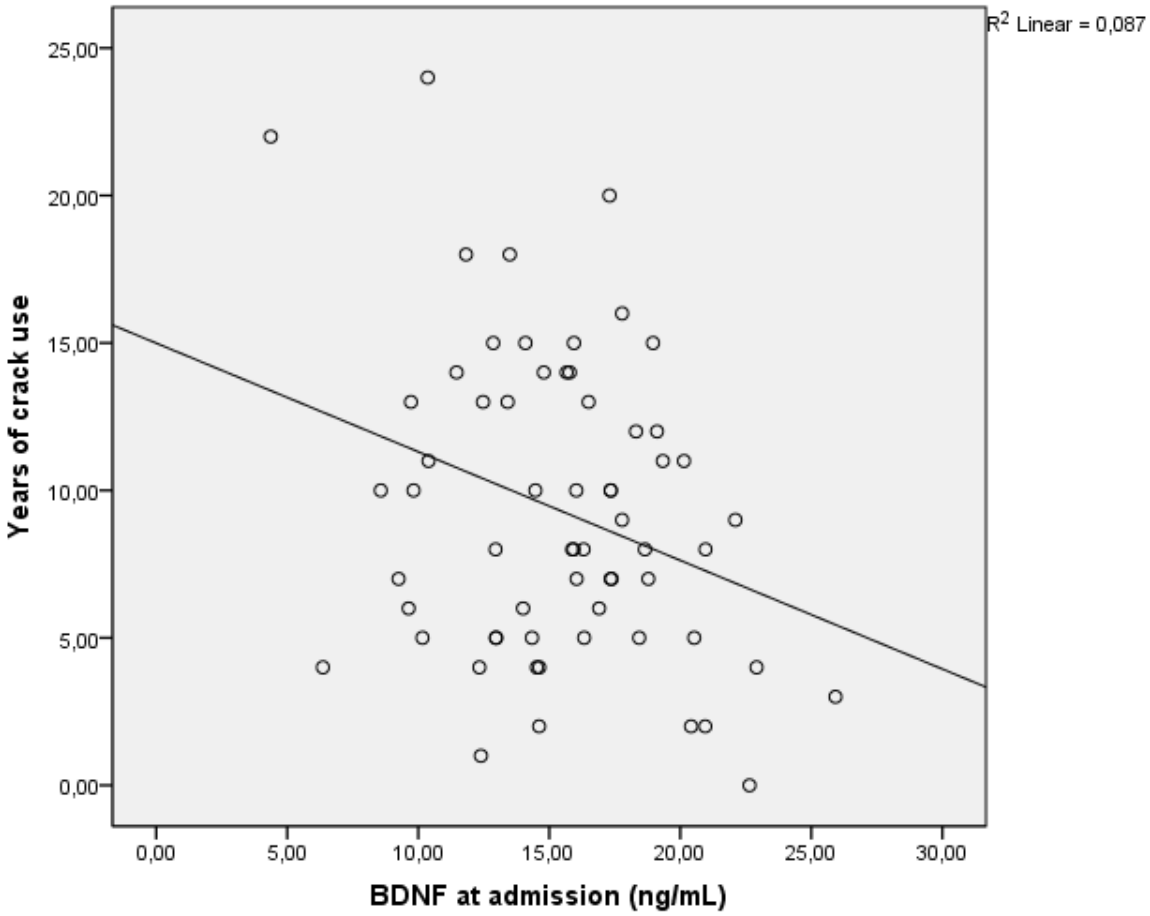

Figure 3. Correlations between BDNF levels in admission and years of crack use: partial correlation, -0.295; two-tailed significance, $p<0.05$.

5.5 Discussion

We observed a significant difference in the levels of BDNF at hospital discharge between the two groups of subjects with different evolution status in hospitalization program. We also evidenced a correlation between BDNF levels and TBARS on admission, and that the levels of BDNF at admission were associated with years of crack use.

Our results show that both BDNF and TBARS levels varied widely during the establishment of abstinence. Von Diemen *et al*. also found a large variation in the levels of BDNF in similar conditions to that of our sample, and suggested that the variation patterns were associated with the severity of crack use, where the number of crack rocks used in the past month and years of use was inversely correlated with the increase in BDNF during early withdrawal [11]. In our study we found that BDNF levels at admission were inversely associated with years of crack use only. It is known that chronic use of psychostimulants, including cocaine, increases the expression of dopamine in many brain structures and that continued administration may cause transient or permanent neuroadaptations in these areas [25, 26]. However, time of use and the amount of drug required to induce such changes is still unknown. These leads to another important point: our sample had more years of crack use than samples in previous studies - which have shown a significant increase of BDNF after initial withdrawal [11, 23]. Probably, due to the longer use of crack, our subjects may have greater neuroadaptive loss and thus lower capacity at varying levels of BDNF, even when the drug was removed.

In our sample, the levels of BDNF seem to be associated with the evolution of subjects in the stages of inpatient treatment, with those who completed the program having higher levels of BDNF at the beginning and significantly higher levels at the

end of treatment. Even when controlling for difference of days in blood collection and age, the significant difference in BDNF levels at discharge between groups was maintained. These results indicate that higher levels of BDNF could suggest greater neuroplasticity with possible association with prognosis. Studies with alcoholic subjects showed similar results, where BDNF increased after early withdrawal and the magnitude of increase was related to prognosis [27, 28].

Several studies described an increased in the levels of this neurotrophin during early withdrawal [10, 11]. When comparing BDNF levels before and after detoxification, we did not find a significant difference in the groups, but we could observe a tendency of higher increase on the subjects who completed the whole program. We believe that the small sample could have compromised the power of the study to appropriately identify a significant difference in this analysis.

In another recent study, our group found an inverse correlation between BDNF and TBARS levels at discharge [23]. These results revealed that while BDNF levels increase in the brain of crack cocaine patients, oxidative stress decreases, which could represent that those subjects with more preserved brain plasticity can overcome acute oxidative stress [23]. In this study we found a correlation between BDNF and TBARS levels upon admission, which corroborates the hypothesis that these markers are associated. However, we did not see a significant correlation between these markers in the initial abstinence.

Animal studies have evidenced that cocaine administration elevates the production of oxygen reactive specimens in different dopaminergic brain structures, such as frontal cortex and striatum [15] and increase lipid peroxidation [29]. In this study we could see that subjects who completed the program, and therefore stayed more days in abstinence, tend to decrease more TBARS levels at discharge

comparing to their admission. To the authors' knowledge, the lack of significance in these results is also due to the small sample size. The idea of time-dependent decrease in oxidative stress was observed by Huang *et al*. in an alcoholic sample, where they found that the level of MDA decreased gradually after one week of abstinence and appeared comparable to that of the control subjects after two weeks of detoxification [30]. Narvaez *et al*., when studying the peripheral toxicology in crack users, also found similar levels of TBARS when comparing at least one month abstinent users and controls [31].

One strength of this study is the fact the abstinence was induced in a controlled environment (inpatient unit) On the other hand, it is difficult to determine if alterations in BDNF and TBARS could be influenced by medications or inpatient activities (physical exercise, cue induced craving…) that the subjects are subjected during treatment. However, because the correlation we found between TBARS and BDNF was at the admission, and therefore before treatment initiation, these would not be confounding factors. This reinforces the hypothesis that BDNF could be a marker of brain plasticity and TBARS could be a marker of brain impairment caused by crack cocaine, and that these markers are associated in these users.

Other important aspect that may influence our results is that several studies indicate a relationship between BDNF and TBARS in the pathophysiology of psychiatric disorders [22, 32-36]. Von Diemen *at al.*, when studying alterations of BDNF levels in crack cocaine users during withdrawal, showed that comorbidities associated with drug use were not confounding factors [11]. However, regarding substance users, these kinds of comorbidities are only possible to be truly diagnosed whit users in abstinence for at least a month, which was not possible in the conditions of this study.

As for all psychiatric disorders, researches with biological markers that may help understand the establishment and treatment of substance dependence is still very incipient. Nevertheless, with respect to pre-clinical and clinical studies involving the use of crack, great advances are being made, and markers of neuronal plasticity, oxidative stress, inflammatory response - among others, are increasing and being associated with the physiopathology of addiction. In this study, we found important correlations between BDNF and TBARS, which corroborates the idea that oxidative stress and neuroplasticity are involved in crack addiction. We also saw that BDNF levels at discharge were different among groups of users with different clinical outcome, which could be a promising field of study for treatment management in the future. These findings are important because they can help in the development of tailored treatments according to the characteristics of each patient. Still, more studies are needed to strengthen these findings and to create concrete evidence that could help to improve and reinforce treatment and recovery of crack users.

5.6 References

[1] Lopez, A., et al. (2008) [What happens in a cocaine-dependent sample after 24 months of treatment?]. Adicciones 20, 347-355
[2] Sinha, R., et al. (2011) Translational and reverse translational research on the role of stress in drug craving and relapse. Psychopharmacology 218, 69-82
[3] de Lima, M.N., et al. (2011) Early life stress decreases hippocampal BDNF content and exacerbates recognition memory deficits induced by repeated D-amphetamine exposure. Behav Brain Res 224, 100-106
[4] Shim, S.H., et al. (2008) Increased levels of plasma brain-derived neurotrophic factor (BDNF) in children with attention deficit-hyperactivity disorder (ADHD). Progress in neuro-psychopharmacology & biological psychiatry 32, 1824-1828
[5] Mendelson, J., et al. (2011) Developing biomarkers for methamphetamine addiction. Current neuropharmacology 9, 100-103
[6] Pae, C.U., et al. (2012) Influence of BDNF variants on diagnosis and response to treatment in patients with major depression, bipolar disorder and schizophrenia. Neuropsychobiology 65, 1-11
[7] Cunha, A.B., et al. (2006) Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neuroscience letters 398, 215-219
[8] Graham, D.L., et al. (2007) Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. Nature neuroscience 10, 1029-1037
[9] Bahi, A., et al. (2008) Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. Psychopharmacology 199, 169-182
[10] Corominas-Roso, M., et al. (2013) Brain-derived neurotrophic factor serum levels in cocaine-dependent patients during early abstinence. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 23, 1078-1084
[11] von Diemen, L., et al. (2014) Increase in brain-derived neurotrophic factor expression in early crack cocaine withdrawal. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 17, 33-40
[12] D'Sa, C., et al. (2011) Increased serum brain-derived neurotrophic factor is predictive of cocaine relapse outcomes: a prospective study. Biological psychiatry 70, 706-711
[13] Sordi, A.O., et al. (2014) Oxidative stress and BDNF as possible markers for the severity of crack cocaine use in early withdrawal. Psychopharmacology 231, 4031-4039
[14] Kovacic, P. (2005) Unifying mechanism for addiction and toxicity of abused drugs with application to dopamine and glutamate mediators: electron transfer and reactive oxygen species. Medical hypotheses 65, 90-96

[15] Dietrich, J.B., et al. (2005) Acute or repeated cocaine administration generates reactive oxygen species and induces antioxidant enzyme activity in dopaminergic rat brain structures. Neuropharmacology 48, 965-974
[16] Valko, M., et al. (2007) Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology 39, 44-84
[17] Kovacic, P. (2005) Role of oxidative metabolites of cocaine in toxicity and addiction: oxidative stress and electron transfer. Medical hypotheses 64, 350-356
[18] Pomierny-Chamiolo, L., et al. (2013) Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine. Neurotoxicity research 23, 92-102
[19] Barr, J., et al. (2007) Nicotine induces oxidative stress and activates nuclear transcription factor kappa B in rat mesencephalic cells. Molecular and cellular biochemistry 297, 93-99
[20] Pan, J., et al. (2005) Oxidative stress in heroin administered mice and natural antioxidants protection. Life sciences 77, 183-193
[21] Yamamoto, B.K. and Zhu, W. (1998) The effects of methamphetamine on the production of free radicals and oxidative stress. The Journal of pharmacology and experimental therapeutics 287, 107-114
[22] Ng, F., et al. (2008) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 11, 851-876
[23] Sordi, A.O., et al. (2014) Oxidative stress and BDNF as possible markers for the severity of crack cocaine use in early withdrawal. Psychopharmacology
[24] Kessler, F., et al. (2012) Psychometric properties of the sixth version of the Addiction Severity Index (ASI-6) in Brazil. Revista brasileira de psiquiatria 34, 24-33
[25] Seger, D. (2010) Cocaine, metamfetamine, and MDMA abuse: the role and clinical importance of neuroadaptation. Clin Toxicol (Phila) 48, 695-708
[26] Jones, S.R., et al. (1995) Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. The Journal of pharmacology and experimental therapeutics 274, 396-403
[27] Huang, M.C., et al. (2008) Alterations of serum brain-derived neurotrophic factor levels in early alcohol withdrawal. Alcohol and alcoholism 43, 241-245
[28] Lee, B.C., et al. (2009) Relation between plasma brain-derived neurotrophic factor and nerve growth factor in the male patients with alcohol dependence. Alcohol 43, 265-269
[29] Kloss, M.W., et al. (1984) Biotransformation of norcocaine to norcocaine nitroxide by rat brain microsomes. Psychopharmacology 84, 221-224
[30] Huang, M.C., et al. (2009) Alterations in oxidative stress status during early alcohol withdrawal in alcoholic patients. Journal of the Formosan Medical Association = Taiwan yi zhi 108, 560-569

[31] Narvaez, J.C., et al. (2013) Peripheral toxicity in crack cocaine use disorders. Neuroscience letters 544, 80-84
[32] Hauck, S., et al. (2009) Serum levels of brain-derived neurotrophic factor in acute and posttraumatic stress disorder: a case report study. Revista brasileira de psiquiatria 31, 48-51
[33] Hauck, S., et al. (2010) Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Progress in neuro-psychopharmacology & biological psychiatry 34, 459-462
[34] Machado-Vieira, R., et al. (2007) Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. Biological psychiatry 61, 142-144
[35] Kauer-Sant'Anna, M., et al. (2007) Traumatic life events in bipolar disorder: impact on BDNF levels and psychopathology. Bipolar disorders 9 Suppl 1, 128-135
[36] Calabrese, V., et al. (2001) Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. Neurochemical research 26, 739-764

6 PERSPECTIVAS E CONSIDERAÇÕES FINAIS

Como perspectivas desse estudo, nosso grupo pretende continuar avaliando os fatores neurobiológicos e fisiológicos relacionados à dependência química, a fim de evidenciarmos e caracterizarmos mais precisamente a fisiopatologia da adição em usuários de substâncias psicoativas.

Nesse sentido, o estudo de biomarcadores como o BDNF e o TABRS faz parte de uma ampla linha de pesquisa desenvolvida pelo Centro de Pesquisa em Álcool e Drogas . Como demonstrado neste trabalho de conclusão de curso e em diversos outros estudos realizados pelo grupo, está cada vez mais evidente o envolvimento do estrese oxidativo e da neuroplasticidade no uso de drogas. Entretanto, a busca por biomarcadores que possam ajudar a compreender a evolução e instalação da dependência, assim como auxiliar no desenvolvimento de tratamentos mais eficientes e no acompanhamento desses usuários ainda é muito incipiente.

Para busca de resultados mais concretos nessa área, temos como perspectivas o aumento da nossa amostra, a busca por outros marcadores relacionados ao uso de substâncias, a análise de fatores que possam estar envolvidos com esses biomarcadores em usuários de crack - como estado nutricional e polimorfismos genéticos, e a utilização de outras técnicas, como por exemplo a neuroimagem, que possam ajudar a evidenciar achados confiáveis sobre estados de abstinência, predisposição à recaída, gravidade e vulnerabilidade. Além disso, a realização de um estudo de seguimento com usuários de crack seria ideal para avaliarmos todos esses fatores citados acima. Entretando, devido à dificuldade de seguimento dessa população, isto ainda não foi realizado e está como perspectiva futura.

7 REFERÊNCIAS

1. RIVERA M. A., AUFDERHEIDE A. C., CARTMELL L. W., TORRES C. M., LANGSJOEN O. Antiquity of coca-leaf chewing in the south central Andes: a 3,000 year archaeological record of coca-leaf chewing from northern Chile, Journal of psychoactive drugs 2005: 37: 455-458.
2. GAY G. R., INABA D. S., SHEPPARD C. W., NEWMEYER J. A. Cocaine: history, epidemiology, human pharmacology, and treatment. a perspective on a new debut for an old girl, Clinical toxicology 1975: 8: 149-178.
3. BOGHDADI M. S., HENNING R. J. Cocaine: pathophysiology and clinical toxicology, Heart & lung : the journal of critical care 1997: 26: 466-483; quiz 484-465.
4. VERLANDER J. M., JR., JOHNS M. E. The clinical use of cocaine, Otolaryngologic clinics of North America 1981: 14: 521-531.
5. WHITE S. M., LAMBE C. J. The pathophysiology of cocaine abuse, Journal of clinical forensic medicine 2003: 10: 27-39.
6. INCIARDI J. A. Crack-cocaine in Miami, NIDA research monograph 1991: 110: 263-274.
7. DIEMEN L. V. Avaliação de soroprevalência HCV/HIV em mulheres e de marcadores bioquímicos de toxicidade sistêmica em homens usuários de crack, Porto Alegre; 2013.
8. JEFFCOAT A. R., PEREZ-REYES M., HILL J. M., SADLER B. M., COOK C. E. Cocaine disposition in humans after intravenous injection, nasal insufflation (snorting), or smoking, Drug metabolism and disposition: the biological fate of chemicals 1989: 17: 153-159.
9. GOLDSTEIN R. A., DESLAURIERS C., BURDA A., JOHNSON-ARBOR K. Cocaine: history, social implications, and toxicity: a review, Seminars in diagnostic pathology 2009: 26: 10-17.
10. RK K. Cocaine Greenwood Village: Thomson Healthcare; 2008.
11. MAURER H. H., SAUER C., THEOBALD D. S. Toxicokinetics of drugs of abuse: current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine, heroin, morphine, and codeine, Therapeutic drug monitoring 2006: 28: 447-453.
12. ROMANO M R. M., MARQUES ACPR. Abuso e dependência de cocaína, São Paulo: Projetos Diretrizes: Associação Médica Brasileira eConselho Federal de Medicina; 2002.
13. DONATO EM R. E., RIBEIRO M, SILVA CJ. Farmacologia e neurobiologia do consumo de crack. O tratamento do usuário de crak, São Paulo: Casa Leitura Médica; 2010.
14. LEIKIN J P. F. Cocaine. Poisoning and Toxicology Handbook New York, NY: CRC Press; 2008.
15. FRANCISCO INÁCIO BASTOS N. B. Pesquisa Nacional sobre o uso de crack: quem são os usuários de crack e/ou similares do Braisl? quantos são nas capitais brasileiras?, Rio de Janeiro: ICICT/FIOCRUZ, 2014; 2014.
16. Drug World Report, Vienna: United Nations Publication; 2014.
17. BASTOS F. I., MENDES A., DUARTE PDO C., BERTONI N. Smoked crack cocaine in contemporary Brazil: the emergence and spread of 'oxi', Addiction 2011: 106: 1191-1192.

18. DIAS A. C., ARAUJO M. R., LARANJEIRA R. Evolution of drug use in a cohort of treated crack cocaine users, Revista de saude publica 2011: 45: 938-948.
19. HAASEN C., KRAUSZ M. Myths versus evidence with respect to cocaine and crack: learning from the US experience, European addiction research 2001: 7: 159-160.
20. WERB D., DEBECK K., KERR T., LI K., MONTANER J., WOOD E. Modelling crack cocaine use trends over 10 years in a Canadian setting, Drug and alcohol review 2010: 29: 271-277.
21. OLIVEIRA L. G., PONCE JDE C., NAPPO S. A. Crack cocaine use in Barcelona: a reason of worry, Substance use & misuse 2010: 45: 2291-2300.
22. SANCHEZ Z., NAPPO S. Sequência de drogas consumidas por usuários de crack e fatores interferentes, Rev Saúde Pública 2002: 36(4): 420-430.
23. GUINDALINI C., VALLADA H., BREEN B., LARANJEIRA R. Concurrent crack and powder cocaine users from São Paulo: do they represent a different group?, BMC Public Health 2006: 6:10.
24. NAPPO S. A., SANCHEZ Z., DE OLIVEIRA L. G. Crack, AIDS, and women in Sao Paulo, Brazil, Substance use & misuse 2011: 46: 476-485.
25. DUAILIBI L. B., RIBEIRO M., LARANJEIRA R. Profile of cocaine and crack users in Brazil, Cadernos de saude publica 2008: 24 Suppl 4: s545-557.
26. FERREIRA FILHO O., TURCH M., LARANJEIRA R., CASTELO A. Perfil sociodemográfico e de padrões de uso entre dependentes de cocaína hospitalizados, Rev Saúde Pública 2003: 37(6): 751-759.
27. DUNN J., LARANJEIRA R. Cocaine: profiles, drug histories, and patterns of use of patients from Brazil, Subst Use Misuse 1999: 34: 1527-1548.
28. KESSLER F., TERRA M., FALLER S., STOLF A., HOLMER B. P., PEUKER A. et al. Usuários de crack que procuram tratamento apresentam alta prevalência de personalidade antissocial e baixas taxas de uso de álcool: Centro de Pesquisa em Álcool e Drogas da UFRGS – CPAD
29. SORDI A. O., KREISCHE F., PECHANSKY F., KESSLER F. Qualidade de vida e problemas legais em Usuários de Crack e outras drogas que buscam tratamento em 4 Capitais Brasileiras: Centro de Pesquisa em Álcool e Drogas da UFRGS - CPAD; 2010.
30. SHANTI C. M., LUCAS C. E. Cocaine and the critical care challenge, Critical care medicine 2003: 31: 1851-1859.
31. KNUEPFER M. M. Cardiovascular disorders associated with cocaine use: myths and truths, Pharmacology & therapeutics 2003: 97: 181-222.
32. LASON W. Neurochemical and pharmacological aspects of cocaine-induced seizures, Polish journal of pharmacology 2001: 53: 57-60.
33. O'DELL L. E., GEORGE F. R., RITZ M. C. Antidepressant drugs appear to enhance cocaine-induced toxicity, Experimental and clinical psychopharmacology 2000: 8: 133-141.
34. SPORER K. A., LESSER S. H. Cocaine washed-out syndrome, Annals of emergency medicine 1992: 21: 112.
35. LIU D., HARIMAN R. J., BAUMAN J. L. Cocaine concentration-effect relationship in the presence and absence of lidocaine: evidence of competitive binding between cocaine and lidocaine, The Journal of pharmacology and experimental therapeutics 1996: 276: 568-577.
36. MCCORD J., JNEID H., HOLLANDER J. E., DE LEMOS J. A., CERCEK B., HSUE P. et al. Management of cocaine-associated chest pain and myocardial infarction: a

scientific statement from the American Heart Association Acute Cardiac Care Committee of the Council on Clinical Cardiology, Circulation 2008: 117: 1897-1907.

37. KOOB G. F., VOLKOW N. D. Neurocircuitry of addiction, Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 2010: 35: 217-238.
38. NARVAEZ J. C., MAGALHAES P. V., FRIES G. R., COLPO G. D., CZEPIELEWSKI L. S., VIANNA P. et al. Peripheral toxicity in crack cocaine use disorders, Neuroscience letters 2013: 544: 80-84.
39. DIAS A. C., ARAUJO M. R., DUNN J., SESSO R. C., DE CASTRO V., LARANJEIRA R. Mortality rate among crack/cocaine-dependent patients: a 12-year prospective cohort study conducted in Brazil, Journal of substance abuse treatment 2011: 41: 273-278.
40. RIBEIRO M., DUNN J., SESSO R., LIMA M. S., LARANJEIRA R. Crack cocaine: a five-year follow-up study of treated patients, European addiction research 2007: 13: 11-19.
41. SCHIFANO F., CORKERY J. Cocaine/crack cocaine consumption, treatment demand, seizures, related offences, prices, average purity levels and deaths in the UK (1990 - 2004), Journal of psychopharmacology 2008: 22: 71-79.
42. FALCK R. S., WANG J., CARLSON R. G. Among long-term crack smokers, who avoids and who succumbs to cocaine addiction?, Drug and alcohol dependence 2008: 98: 24-29.
43. PAIM KESSLER F. H., BARBOSA TERRA M., FALLER S., RAVY STOLF A., CAROLINA PEUKER A., BENZANO D. et al. Crack users show high rates of antisocial personality disorder, engagement in illegal activities and other psychosocial problems, The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions 2012: 21: 370-380.
44. STERN R. K., HAGAN H., LELUTIU-WEINBERGER C., DES JARLAIS D., SCHEINMANN R., STRAUSS S. et al. The HCV Synthesis Project: scope, methodology, and preliminary results, BMC medical research methodology 2008: 8: 62.
45. SCHEINMANN R., HAGAN H., LELUTIU-WEINBERGER C., STERN R., DES JARLAIS D. C., FLOM P. L. et al. Non-injection drug use and Hepatitis C Virus: a systematic review, Drug and alcohol dependence 2007: 89: 1-12.
46. BAUM M. K., RAFIE C., LAI S., SALES S., PAGE B., CAMPA A. Crack-cocaine use accelerates HIV disease progression in a cohort of HIV-positive drug users, Journal of acquired immune deficiency syndromes 2009: 50: 93-99.
47. BALDWIN G. C., ROTH M. D., TASHKIN D. P. Acute and chronic effects of cocaine on the immune system and the possible link to AIDS, Journal of neuroimmunology 1998: 83: 133-138.
48. IRWIN M. R., OLMOS L., WANG M., VALLADARES E. M., MOTIVALA S. J., FONG T. et al. Cocaine dependence and acute cocaine induce decreases of monocyte proinflammatory cytokine expression across the diurnal period: autonomic mechanisms, The Journal of pharmacology and experimental therapeutics 2007: 320: 507-515.
49. BREWER T. H., ZHAO W., METSCH L. R., COLTES A., ZENILMAN J. High-risk behaviors in women who use crack: knowledge of HIV serostatus and risk behavior, Annals of epidemiology 2007: 17: 533-539.
50. CARVALHO H. B., SEIBEL S. D. Crack cocaine use and its relationship with violence and HIV, Clinics 2009: 64: 857-866.

51. HOFFMAN J. A., KLEIN H., EBER M., CROSBY H. Frequency and intensity of crack use as predictors of women's involvement in HIV-related sexual risk behaviors, Drug and alcohol dependence 2000: 58: 227-236.
52. GARAVAN H., KAUFMAN J. N., HESTER R. Acute effects of cocaine on the neurobiology of cognitive control, Philosophical transactions of the Royal Society of London Series B, Biological sciences 2008: 363: 3267-3276.
53. KELLEY B. J., YEAGER K. R., PEPPER T. H., BEVERSDORF D. Q. Cognitive impairment in acute cocaine withdrawal, Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology 2005: 18: 108-112.
54. BOLLA K. I., ELDRETH D. A., LONDON E. D., KIEHL K. A., MOURATIDIS M., CONTOREGGI C. et al. Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task, NeuroImage 2003: 19: 1085-1094.
55. TUCKER K. A., POTENZA M. N., BEAUVAIS J. E., BROWNDYKE J. N., GOTTSCHALK P. C., KOSTEN T. R. Perfusion abnormalities and decision making in cocaine dependence, Biological psychiatry 2004: 56: 527-530.
56. KESSLER F., WOODY G., DE BONI R., VON DIEMEN L., BENZANO D., FALLER S. et al. Evaluation of psychiatric symptoms in cocaine users in the Brazilian public health system: need for data and structure, Public health 2008: 122: 1349-1355.
57. LEJUEZ C. W., BORNOVALOVA M. A., REYNOLDS E. K., DAUGHTERS S. B., CURTIN J. J. Risk factors in the relationship between gender and crack/cocaine, Experimental and clinical psychopharmacology 2007: 15: 165-175.
58. KOOB G. F., SANNA P. P., BLOOM F. E. Neuroscience of addiction, Neuron 1998: 21: 467-476.
59. KALIVAS P. W., MCFARLAND K. Brain circuitry and the reinstatement of cocaine-seeking behavior, Psychopharmacology 2003: 168: 44-56.
60. NESTLER E. J. Molecular basis of long-term plasticity underlying addiction, Nature reviews Neuroscience 2001: 2: 119-128.
61. CHANG J. Y., SAWYER S. F., LEE R. S., WOODWARD D. J. Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats, The Journal of neuroscience : the official journal of the Society for Neuroscience 1994: 14: 1224-1244.
62. CARELLI R. M., IJAMES S., KONSTANTOPOULOS J., DEADWYLER S. A. Examination of factors mediating the transition to behaviorally correlated nucleus accumbens cell firing during cocaine self-administration sessions in rats, Behav Brain Res 1999: 104: 127-139.
63. PONTIERI F. E., TANDA G., DI CHIARA G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens, Proceedings of the National Academy of Sciences of the United States of America 1995: 92: 12304-12308.
64. KOOB G. F. Drug addiction: the yin and yang of hedonic homeostasis, Neuron 1996: 16: 893-896.
65. KOOB G. F. Neural mechanisms of drug reinforcement, Annals of the New York Academy of Sciences 1992: 654: 171-191.
66. NESTLER E. J. Molecular mechanisms of drug addiction, Neuropharmacology 2004: 47 Suppl 1: 24-32.

67. VOLKOW N. D., FOWLER J. S., WANG G. J. Positron emission tomography and single-photon emission computed tomography in substance abuse research, Seminars in nuclear medicine 2003: 33: 114-128.
68. VOLKOW N. D., MUENKE M. The genetics of addiction, Human genetics 2012: 131: 773-777.
69. KREEK M. J., NIELSEN D. A., BUTELMAN E. R., LAFORGE K. S. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction, Nature neuroscience 2005: 8: 1450-1457.
70. MUSCHAMP J. W., NEMETH C. L., ROBISON A. J., NESTLER E. J., CARLEZON W. A., JR. DeltaFosB enhances the rewarding effects of cocaine while reducing the pro-depressive effects of the kappa-opioid receptor agonist U50488, Biological psychiatry 2012: 71: 44-50.
71. AGRAWAL A., VERWEIJ K. J., GILLESPIE N. A., HEATH A. C., LESSOV-SCHLAGGAR C. N., MARTIN N. G. et al. The genetics of addiction-a translational perspective, Translational psychiatry 2012: 2: e140.
72. VOLKOW N. D., WANG G. J., FOWLER J. S., TOMASI D. Addiction circuitry in the human brain, Annual review of pharmacology and toxicology 2012: 52: 321-336.
73. GEORGE O., KOOB G. F. Individual differences in prefrontal cortex function and the transition from drug use to drug dependence, Neuroscience and biobehavioral reviews 2010: 35: 232-247.
74. THOMAS M. J., KALIVAS P. W., SHAHAM Y. Neuroplasticity in the mesolimbic dopamine system and cocaine addiction, British journal of pharmacology 2008: 154: 327-342.
75. DIETZ D. M., DIETZ K. C., NESTLER E. J., RUSSO S. J. Molecular mechanisms of psychostimulant-induced structural plasticity, Pharmacopsychiatry 2009: 42 Suppl 1: S69-78.
76. VERDEJO-GARCIA A., BECHARA A. A somatic marker theory of addiction, Neuropharmacology 2009: 56 Suppl 1: 48-62.
77. KOOB G. F., AHMED S. H., BOUTREL B., CHEN S. A., KENNY P. J., MARKOU A. et al. Neurobiological mechanisms in the transition from drug use to drug dependence, Neuroscience and biobehavioral reviews 2004: 27: 739-749.
78. RUSSO S. J., MAZEI-ROBISON M. S., ABLES J. L., NESTLER E. J. Neurotrophic factors and structural plasticity in addiction, Neuropharmacology 2009: 56 Suppl 1: 73-82.
79. VON DIEMEN L., DE BONI R., KESSLER F., BENZANO D., PECHANSKY F. Risk behaviors for HCV- and HIV-seroprevalence among female crack users in Porto Alegre, Brazil, Archives of women's mental health 2010: 13: 185-191.
80. KNAPP W., SOARES B., FARREL M., LIMA M. Psychosocial interventions for cocaine and psychostimulant amphetamines related disorders., Cochrane Database Syst Rev 2007: CD003023.
81. PINHO P., OLIVEIRA M., ALMEIDA M. The psychosocial rehabilitation of individuals with alcohol and drug use disorders: a possible strategy?, Rev Psiquiatr Clín 2008: 35: 82-88.
82. LEITE M. E. A. Cocaína e Crack: dos fundamentos ao tratamento Porto Alegre: Artes Médicas; 1999.
83. LOPEZ A., BECOÑA E., VIEITEZ I., CANCELO J., SOBRADELO J., GARCIA J. et al. [What happens in a cocaine-dependent sample after 24 months of treatment?], Adicciones 2008: 20: 347-355.

84. KESSLER F., PECHANSKY F. A contemporary psychiatric view on the crack phenomenon, Rev Psiquiatr Rio Gd Sul 2008: 30: 1-3.
85. BARTKOWSKA K., TURLEJSKI K., DJAVADIAN R. L. Neurotrophins and their receptors in early development of the mammalian nervous system, Acta neurobiologiae experimentalis 2010: 70: 454-467.
86. THOENEN H. Neurotrophins and neuronal plasticity, Science 1995: 270: 593-598.
87. LU B. BDNF and activity-dependent synaptic modulation, Learning & memory 2003: 10: 86-98.
88. MATSUMOTO T., RAUSKOLB S., POLACK M., KLOSE J., KOLBECK R., KORTE M. et al. Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF, Nature neuroscience 2008: 11: 131-133.
89. CASTREN E. Neurotrophins as mediators of drug effects on mood, addiction, and neuroprotection, Molecular neurobiology 2004: 29: 289-302.
90. MCGINTY J. F., MENDELSON J. E. Is brain-derived neurotrophic factor a selective biomarker that predicts cocaine relapse outcomes?, Biological psychiatry 2011: 70: 700-701.
91. MENDELSON J., BAGGOTT M. J., FLOWER K., GALLOWAY G. Developing biomarkers for methamphetamine addiction, Current neuropharmacology 2011: 9: 100-103.
92. GUILLIN O., DIAZ J., CARROLL P., GRIFFON N., SCHWARTZ J. C., SOKOLOFF P. BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, Nature 2001: 411: 86-89.
93. NUMAN S., LANE-LADD S. B., ZHANG L., LUNDGREN K. H., RUSSELL D. S., SEROOGY K. B. et al. Differential regulation of neurotrophin and trk receptor mRNAs in catecholaminergic nuclei during chronic opiate treatment and withdrawal, The Journal of neuroscience : the official journal of the Society for Neuroscience 1998: 18: 10700-10708.
94. LU H., CHENG P. L., LIM B. K., KHOSHNEVISRAD N., POO M. M. Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition, Neuron 2010: 67: 821-833.
95. GRAHAM D. L., EDWARDS S., BACHTELL R. K., DILEONE R. J., RIOS M., SELF D. W. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse, Nature neuroscience 2007: 10: 1029-1037.
96. VARGAS-PEREZ H., TING A. K. R., WALTON C. H., HANSEN D. M., RAZAVI R., CLARKE L. et al. Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats, Science 2009: 324: 1732-1734.
97. GRIMM J. W., LU L., HAYASHI T., HOPE B. T., SU T. P., SHAHAM Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving, The Journal of neuroscience : the official journal of the Society for Neuroscience 2003: 23: 742-747.
98. BAHI A., BOYER F., CHANDRASEKAR V., DREYER J. L. Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats, Psychopharmacology 2008: 199: 169-182.
99. LU L., DEMPSEY J., LIU S. Y., BOSSERT J. M., SHAHAM Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal, The Journal of

neuroscience : the official journal of the Society for Neuroscience 2004: 24: 1604-1611.
100. WHITFIELD T. W., JR., SHI X., SUN W. L., MCGINTY J. F. The suppressive effect of an intra-prefrontal cortical infusion of BDNF on cocaine-seeking is Trk receptor and extracellular signal-regulated protein kinase mitogen-activated protein kinase dependent, The Journal of neuroscience : the official journal of the Society for Neuroscience 2011: 31: 834-842.
101. RASMUSSEN P., BRASSARD P., ADSER H., PEDERSEN M. V., LEICK L., HART E. et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise, Experimental physiology 2009: 94: 1062-1069.
102. SARTORIUS A., HELLWEG R., LITZKE J., VOGT M., DORMANN C., VOLLMAYR B. et al. Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats, Pharmacopsychiatry 2009: 42: 270-276.
103. KLEIN A. B., WILLIAMSON R., SANTINI M. A., CLEMMENSEN C., ETTRUP A., RIOS M. et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species, The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 2011: 14: 347-353.
104. MCEWEN B. S. Stress, adaptation, and disease. Allostasis and allostatic load, Annals of the New York Academy of Sciences 1998: 840: 33-44.
105. KAPCZINSKI F., VIETA E., ANDREAZZA A. C., FREY B. N., GOMES F. A., TRAMONTINA J. et al. Allostatic load in bipolar disorder: implications for pathophysiology and treatment, Neuroscience and biobehavioral reviews 2008: 32: 675-692.
106. GEORGE O., LE MOAL M., KOOB G. F. Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems, Physiology & behavior 2012: 106: 58-64.
107. FUMAGALLI F., DI PASQUALE L., CAFFINO L., RACAGNI G., RIVA M. A. Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex, The European journal of neuroscience 2007: 26: 2756-2763.
108. CALABRESE V., SCAPAGNINI G., GIUFFRIDA STELLA A. M., BATES T. E., CLARK J. B. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity, Neurochemical research 2001: 26: 739-764.
109. KOVACIC P. Role of oxidative metabolites of cocaine in toxicity and addiction: oxidative stress and electron transfer, Medical hypotheses 2005: 64: 350-356.
110. VALKO M., LEIBFRITZ D., MONCOL J., CRONIN M. T., MAZUR M., TELSER J. Free radicals and antioxidants in normal physiological functions and human disease, The international journal of biochemistry & cell biology 2007: 39: 44-84.
111. KOVACIC P. Unifying mechanism for addiction and toxicity of abused drugs with application to dopamine and glutamate mediators: electron transfer and reactive oxygen species, Medical hypotheses 2005: 65: 90-96.
112. BETTERIDGE D. J. What is oxidative stress?, Metabolism: clinical and experimental 2000: 49: 3-8.
113. DIETRICH J. B., MANGEOL A., REVEL M. O., BURGUN C., AUNIS D., ZWILLER J. Acute or repeated cocaine administration generates reactive oxygen species

and induces antioxidant enzyme activity in dopaminergic rat brain structures, Neuropharmacology 2005: 48: 965-974.
114. HUANG M. C., CHEN C. H., PENG F. C., TANG S. H., CHEN C. C. Alterations in oxidative stress status during early alcohol withdrawal in alcoholic patients, Journal of the Formosan Medical Association = Taiwan yi zhi 2009: 108: 560-569.
115. POMIERNY-CHAMIOLO L., MONICZEWSKI A., WYDRA K., SUDER A., FILIP M. Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine, Neurotoxicity research 2013: 23: 92-102.
116. FUKUNAGA K., YOSHIDA M., NAKAZONO N. A simple, rapid, highly sensitive and reproducible quantification method for plasma malondialdehyde by high-performance liquid chromatography, Biomedical chromatography : BMC 1998: 12: 300-303.
117. NG F., BERK M., DEAN O., BUSH A. I. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications, The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 2008: 11: 851-876.
118. SORDI A. O., PECHANSKY F., KESSLER F. H., KAPCZINSKI F., PFAFFENSELLER B., GUBERT C. et al. Oxidative stress and BDNF as possible markers for the severity of crack cocaine use in early withdrawal, Psychopharmacology 2014.
119. AMANVERMEZ R., AGARA E. Does ascorbate/L-Cys/L-Met mixture protect different parts of the rat brain against chronic alcohol toxicity?, Advances in therapy 2006: 23: 705-718.
120. ZHOU W., KALIVAS P. W. N-acetylcysteine reduces extinction responding and induces enduring reductions in cue- and heroin-induced drug-seeking, Biological psychiatry 2008: 63: 338-340.
121. BAKER D. A., MCFARLAND K., LAKE R. W., SHEN H., TODA S., KALIVAS P. W. N-acetyl cysteine-induced blockade of cocaine-induced reinstatement, Annals of the New York Academy of Sciences 2003: 1003: 349-351.
122. LAROWE S. D., MARDIKIAN P., MALCOLM R., MYRICK H., KALIVAS P., MCFARLAND K. et al. Safety and tolerability of N-acetylcysteine in cocaine-dependent individuals, The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions 2006: 15: 105-110.
123. LAROWE S. D., MYRICK H., HEDDEN S., MARDIKIAN P., SALADIN M., MCRAE A. et al. Is cocaine desire reduced by N-acetylcysteine?, The American journal of psychiatry 2007: 164: 1115-1117.
124. LOPEZ A., BECONA E., VIEITEZ I., CANCELO J., SOBRADELO J., GARCIA J. M. et al. [What happens in a cocaine-dependent sample after 24 months of treatment?], Adicciones 2008: 20: 347-355.
125. SINHA R., SHAHAM Y., HEILIG M. Translational and reverse translational research on the role of stress in drug craving and relapse, Psychopharmacology 2011: 218: 69-82.
126. DE LIMA M. N., PRESTI-TORRES J., VEDANA G., ALCALDE L. A., STERTZ L., FRIES G. R. et al. Early life stress decreases hippocampal BDNF content and exacerbates recognition memory deficits induced by repeated D-amphetamine exposure, Behav Brain Res 2011: 224: 100-106.
127. SHIM S. H., HWANGBO Y., KWON Y. J., JEONG H. Y., LEE B. H., LEE H. J. et al. Increased levels of plasma brain-derived neurotrophic factor (BDNF) in

children with attention deficit-hyperactivity disorder (ADHD), Progress in neuro-psychopharmacology & biological psychiatry 2008: 32: 1824-1828.

128. PAE C. U., CHIESA A., PORCELLI S., HAN C., PATKAR A. A., LEE S. J. et al. Influence of BDNF variants on diagnosis and response to treatment in patients with major depression, bipolar disorder and schizophrenia, Neuropsychobiology 2012: 65: 1-11.
129. CUNHA A. B., FREY B. N., ANDREAZZA A. C., GOI J. D., ROSA A. R., GONCALVES C. A. et al. Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes, Neuroscience letters 2006: 398: 215-219.
130. COROMINAS-ROSO M., RONCERO C., EIROA-OROSA F. J., GONZALVO B., GRAU-LOPEZ L., RIBASES M. et al. Brain-derived neurotrophic factor serum levels in cocaine-dependent patients during early abstinence, European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 2013: 23: 1078-1084.
131. VON DIEMEN L., KAPCZINSKI F., SORDI A. O., DE MAGALHAES NARVAEZ J. C., GUIMARAES L. S., KESSLER F. H. et al. Increase in brain-derived neurotrophic factor expression in early crack cocaine withdrawal, The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 2014: 17: 33-40.
132. D'SA C., FOX H. C., HONG A. K., DILEONE R. J., SINHA R. Increased serum brain-derived neurotrophic factor is predictive of cocaine relapse outcomes: a prospective study, Biological psychiatry 2011: 70: 706-711.
133. SORDI A. O., PECHANSKY F., KESSLER F. H., KAPCZINSKI F., PFAFFENSELLER B., GUBERT C. et al. Oxidative stress and BDNF as possible markers for the severity of crack cocaine use in early withdrawal, Psychopharmacology 2014: 231: 4031-4039.
135. KESSLER F., CACCIOLA J., ALTERMAN A., FALLER S., SOUZA-FORMIGONI M. L., CRUZ M. S. et al. Psychometric properties of the sixth version of the Addiction Severity Index (ASI-6) in Brazil, Revista brasileira de psiquiatria 2012: 34: 24-33.
136. KUMAR A., CHOI K. H., RENTHAL W., TSANKOVA N. M., THEOBALD D. E., TRUONG H. T. et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum, Neuron 2005: 48: 303-314.
137. SCHOENBAUM G., STALNAKER T. A., SHAHAM Y. A role for BDNF in cocaine reward and relapse, Nature neuroscience 2007: 10: 935-936.
138. SADRI-VAKILI G., KUMARESAN V., SCHMIDT H. D., FAMOUS K. R., CHAWLA P., VASSOLER F. M. et al. Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine, The Journal of neuroscience : the official journal of the Society for Neuroscience 2010: 30: 11735-11744.
139. KLOSS M. W., ROSEN G. M., RAUCKMAN E. J. Biotransformation of norcocaine to norcocaine nitroxide by rat brain microsomes, Psychopharmacology 1984: 84: 221-224.
140. YUKSEL N., UZBAY I. T., KARAKILIC H., AKI O. E., ETIK C., ERBAS D. Increased serum nitrite/nitrate (NOx) and malondialdehyde (MDA) levels during alcohol withdrawal in alcoholic patients, Pharmacopsychiatry 2005: 38: 95-96.

141. BLEICH S., SPILKER K., KURTH C., DEGNER D., QUINTELA-SCHNEIDER M., JAVAHERIPOUR K. et al. Oxidative stress and an altered methionine metabolism in alcoholism, Neuroscience letters 2000: 293: 171-174.
142. SOARDO G., DONNINI D., VARUTTI R., MORETTI M., MILOCCO C., BASAN L. et al. Alcohol-induced endothelial changes are associated with oxidative stress and are rapidly reversed after withdrawal, Alcoholism, clinical and experimental research 2005: 29: 1889-1898.

8 ANEXO

Guia da revista Neuroscience Letters para publicação de artigos

NEUROSCIENCE LETTERS

The rapid communication journal for the neurosciences.

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0304-3940

DESCRIPTION

Neuroscience Letters is devoted to the rapid publication of short, high-quality papers of interest to the broad community of neuroscientists. Only papers which will make a significant addition to the literature in the field will be published. Papers in all areas of **neuroscience** - **molecular**, **cellular**, **developmental**, **systems**, **behavioral** and **cognitive**, as well as **computational** - will be considered for publication. Submission of laboratory investigations that shed light on disease mechanisms is encouraged. Clinical studies will also be published if they provide new information about organization or actions of the nervous system, or provide new insights into the neurobiology of disease.

Papers that are primarily devoted to psychological or philosophical questions, that use unvalidated methodology, or that fall outside of the realm of neuroscience, will not be published.

Neuroscience Letters is published both on the web (in ScienceDirect - http://www.sciencedirect.com), and as a print journal. Publication in the web version of *Neuroscience Letters* will occur within five weeks of acceptance, and in the hard copy within seven weeks. Particularly important papers may be published more rapidly within a Plenary Article section of the journal.

Benefits to authors
We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our author services .

Please see our Guide for Authors for information on article submission. If you require any further information or help, please visit our support pages: http://support.elsevier.com

AUDIENCE

Neuroscientists, neurologists

IMPACT FACTOR

2013: 2.055 © Thomson Reuters Journal Citation Reports 2014

ABSTRACTING AND INDEXING

BIOSIS
Elsevier BIOBASE
Chemical Abstracts
Current Contents/Life Sciences
MEDLINE®
EMBASE
Pascal M
Reference Update
Science Citation Index
Scopus

EDITORIAL BOARD

GUIDE FOR AUTHORS

INTRODUCTION

Neuroscience Letters is devoted to the rapid publication of short, high-quality papers of interest to the broad community of neuroscientists. Only papers which will make a significant addition to the literature in the field will be published. Papers in all areas of neuroscience - molecular, cellular, developmental, systems, behavioral and cognitive, as well as computational - will be considered for publication. Submission of laboratory investigations that shed light on disease mechanisms is encouraged. Clinical studies will also be published if they provide new information about organization or actions of the nervous system, or provide new insights into the neurobiology of disease.

Papers that are primarily devoted to psychological or philosophical questions, that use unvalidated methodology, or that fall outside of the realm of neuroscience, will not be published.

The Neuroscience Peer Review Consortium

Neuroscience Letters is a member of the Neuroscience Peer Review Consortium (NPRC). The NPRC has been formed to reduce the time expended and, in particular, the duplication of effort by, and associated burden on reviewers involved in the peer review of original neuroscience research papers. It is an alliance of neuroscience journals that have agreed to accept manuscript reviews from other Consortium journals. By reducing the number of times that a manuscript is reviewed, the Consortium will reduce the load on reviewers and Editors, and speed the publication of research results.

If a manuscript has been rejected by another journal in the Consortium, authors can submit the manuscript to *Neuroscience Letters* and indicate that the referees' reports from the first journal will be made available to the Editors of Neuroscience Letters.

It is the authors' decision as to whether or not to indicate that a set of referee's reports should be forwarded from the first journal to *Neuroscience Letters*. If an author does not wish for this to happen, the manuscript can be submitted to *Neuroscience Letters* without reference to the previous submission. No information will be exchanged between journals except at the request of authors. However, if the original referees' reports suggested that the paper is of high quality, but not suitable for the first journal, then it will often be to an author's advantage to indicate that referees' reports should be made available.

Authors should revise the original submission in accordance with the first journal's set of referee reports, reformat the paper to *Neuroscience Letters*' specification and submit the paper to *Neuroscience Letters* with a covering letter describing the changes that have been made, and informing the Editors that the authors will ask for the referees' reports to be forwarded from the first Consortium journal. The authors then must contact the first journal, and ask that reviews be forwarded, indicating they have submitted to *Neuroscience Letters*, and providing the new manuscript ID number.

The Editors of *Neuroscience Letters* will use forwarded referees' reports at their discretion. The Editors may use the reports directly to make a decision, or they may request further reviews if they feel such are necessary
.

Visit http://nprc.incf.org for a list of Consortium journals, as well as further information on the scheme.

BEFORE YOU BEGIN

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

Policy and ethics

The work described in your article must have been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* http://www.wma.net/en/30publications/10policies/b3/index.html; *EC Directive 86/609/EEC*

for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; *Uniform Requirements for manuscripts submitted to Biomedical journals* http://www.icmje.org. This must be stated at an appropriate point in the article.

For other policy issues, authors are referred to the policy guidelines of the Society for Neuroscience (see their website http://www.jneurosci.org/misc/itoa.shtml).

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Contributors

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

Addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts

Before the accepted manuscript is published in an online issue

Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include:

The reason the name should be added or removed or the author names rearranged. Written confirmation (email, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that:

Journal Managers will inform the Journal Editors of any such requests. Publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who

must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.
After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

This journal offers authors a choice in publishing their research: Open access and Subscription.

For subscription articles
Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For open access articles
Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Retained author rights

As an author you (or your employer or institution) retain certain rights. For more information on author rights for:
Subscription articles please see http://www.elsevier.com/journal-authors/author-rights-and-responsibilities.
Open access articles please see http://www.elsevier.com/OAauthoragreement.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

Open access

This journal offers authors a choice in publishing their research:

Open access
• Articles are freely available to both subscribers and the wider public with permitted reuse
• An open access publication fee is payable by authors or their research funder
Subscription
• Articles are made available to subscribers as well as developing countries and patient groups through our access programs (http://www.elsevier.com/access)
• No open access publication fee

All articles published open access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:
Creative Commons Attribution (CC BY): lets others distribute and copy the article, to create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.
Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA): for non-commercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY-NC-SA).
Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND): for non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

To provide open access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published open access.
Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The open access publication fee in this journal is **$1800**, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://support.elsevier.com) for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Submit your article
Please submit your article via http://ees.elsevier.com/nsl/
Authors who are unable to provide an electronic version or have other circumstances that prevent online submission must contact the journal Editorial Office (NSL@elsevier.com; Tel +1-619-699-6583; Fax +1-619-699-6801) prior to submission to discuss alternate options. The Publisher and Editors regret that they are not able to consider submissions that do not follow these procedures.

Referees
Authors must send the names, addresses and email addresses for 8-10 potential referees that meet the following criteria: potential referees must be experts or active workers in the field, must not be current or prior mentors or collaborators, and must have institutional email addresses (e.g., xx.yy@zz.edu) and not generic email addresses (e.g., xx.yy@163.com or xx.yy@gmail.com). Although the journal does not guarantee these reviewers will be used, the Editors take these suggestions under consideration. These recommendations help the journal speed the editorial process.

Additional information
Length of manuscripts will in no case be more than 6 printed pages (5000 words) of the journal. As an approximate guide to authors for judging the length of their paper, the following estimation may be used: heading + abstract = 0.5-0.6 pages; 3 type-written (double-spaced) pages = 1 printed page; (when using a word-processor) 850 words or 5300 characters = 1 printed page; 3 single-column wide or 2 double-column wide figures plus legends = 1 printed page; 3 single-column wide or 2 double-column wide tables = 1 printed page; 17 references = 0.5 printed page.

PREPARATION

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision
Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply " the text" .

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is in very occasional cases appropriate, although in general, Results and Discussion should be presented as distinct sections of the manuscript. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**

• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract
A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract
Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images and in accordance with all technical requirements: Illustration Service.

Highlights
Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords
Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units
Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Database linking
Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Artwork
Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Provide captions to illustrations separately.
• Size the illustrations close to the desired dimensions of the printed version.
• Submit each illustration as a separate file.
A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
• Supply files that are too low in resolution;
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications that can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style
Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations: http://www.issn.org/services/online-services/access-to-the-ltwa/.

Video data
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Supplementary data
Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should

submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address
- Phone numbers

All necessary files have been uploaded, and contain:
- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations
- Manuscript has been 'spell-checked' and 'grammar-checked'
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):

http://dx.doi.org/10.1016/j.physletb.2010.09.059

When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor.It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author, at no cost, will be provided with a personalized link providing 50 days free access to the final published version of the article on ScienceDirect. This link can also be used for sharing via email and social networks. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's

WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/booklets).

AUTHOR INQUIRIES

You can track your submitted article at http://help.elsevier.com/app/answers/detail/a_id/89/p/8045/. You can track your accepted article at http://www.elsevier.com/trackarticle. You are also welcome to contact Customer Support via http://support.elsevier.com.