UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

TESE DE DOUTORADO

# EFEITOS DA N-ACETILCISTEÍNA EM MODELOS ANIMAIS DE ESQUIZOFRENIA

ANA PAULA HERRMANN

PORTO ALEGRE
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Tese apresentada ao Programa de Pós-graduação em Ciências Biológicas: Bioquímica, da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Doutora em Bioquímica.

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Co-orientadora: Prof. Dra. Clarissa Severino Gama

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*Nothing is real.*

Lennon/McCartney

*We do not have to visit a madhouse to find disordered minds; our planet is the mental institution of the universe.*

Johann Wolfgang von Goethe

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# SUMÁRIO

# APRESENTAÇÃO

Esta tese está organizada em três partes, conforme descrito abaixo:

**Parte I**: introdução e objetivos;

**Parte II**: resultados, apresentados em três capítulos no formato de artigos científicos;

**Parte III**: discussão, conclusão e referências bibliográficas citadas nas partes I e III.

Os trabalhos que compõem esta tese foram desenvolvidos entre 2011 e 2015 em três locais: no Laboratório de Etnofarmacologia do Departamento de Farmacologia da UFRGS, na Unidade de Experimentação Animal do Hospital de Clínicas de Porto Alegre, e no Laboratório de Fisiologia e Comportamento do *Swiss Federal Institute of Technology in Zurich* (ETH Zürich – doutorado-sanduíche sob co-orientação do Prof. Dr. Urs Meyer).

# PARTE I

*Onde a introdução é apresentada e os objetivos são delineados*

## RESUMO

A esquizofrenia é um transtorno mental crônico e incapacitante, que em geral inicia na adolescência ou início da vida adulta. O diagnóstico atual é baseado na presença de sintomas como delírios, alucinações, discurso e comportamento desorganizados, expressão emocional diminuída e declínio significativo no nível de funcionamento social, profissional ou acadêmico. Apesar da revolução no tratamento com o surgimento do primeiro antipsicótico no início da década de 1950, a esquizofrenia ainda é um dos transtornos mentais mais custosos em termos de sofrimento humano e encargos sociais. Os antipsicóticos disponíveis apresentam pouco ou nenhum efeito no controle dos sintomas negativos e, de modo geral, não há melhora das funções cognitivas. Sabe-se hoje que o primeiro episódio psicótico é precedido por uma fase prodrômica, e foram estabelecidos critérios para o reconhecimento precoce da doença, possibilitando a identificação de indivíduos em risco de converter para psicose. Assim, é necessário investigar a segurança e a eficácia de tratamentos precoces com potencial para adiar ou prevenir a transição para psicose. Na presente tese, investigamos nesse contexto o potencial da N-acetilcisteína (NAC), um precursor de cisteína com ação antioxidante e anti-inflamatória, e modulador da transmissão glutamatérgica. Utilizada como mucolítico e no manejo da intoxicação por paracetamol, estudos recentes levaram NAC a ser considerada uma promessa na psiquiatria. O objetivo dessa tese foi gerar dados relevantes ao uso clínico de NAC para intervenção terapêutica preventiva na esquizofrenia, analisando seus efeitos em modelos animais da doença. No modelo desenvolvimental de isolamento social pós-desmame, NAC preveniu o aumento da resposta locomotora a anfetamina. No modelo farmacológico de sensibilização a anfetamina, NAC atenuou a resposta locomotora a anfetamina mas não preveniu o déficit de inibição latente em animais sensibilizados; em animais controles, NAC induziu déficit de inibição latente. Em experimentos agudos, NAC não preveniu a hiperlocomoção induzida por anfetamina ou MK-801. Finalmente, testamos os efeitos de NAC em um modelo de “dois hits” que combina ativação imune pré-natal com estresse na puberdade. Nesse modelo, NAC preveniu o déficit de inibição por prepulso da resposta de sobressalto e o aumento da resposta locomotora a anfetamina. O mecanismo de ação de NAC nesses modelos ainda deve ser esclarecido, mas sabe-se que processos inflamatórios e estresse oxidativo estão implicados no aparecimento das alterações comportamentais em modelos animais e em humanos que convertem a esquizofrenia. A adolescência é uma fase crítica de vulnerabilidade, mas também representa uma janela de oportunidade para prevenção, e os dados apresentados nessa tese corroboram o potencial do uso de NAC como estratégia farmacológica com potencial para atenuar, adiar, ou mesmo prevenir o surgimento de alterações comportamentais características de transtornos psicóticos. Ensaios clínicos em indivíduos em risco de converter a psicose são necessários para avaliar a real eficácia e segurança desse fármaco.

## ABSTRACT

Schizophrenia is a chronic and disabling mental disorder that usually onsets in adolescence or early adulthood. The current diagnosis is based on the presence of symptoms such as delusions, hallucinations, disorganized speech and behavior, decreased emotional expression and significant decline in the level of social, professional or academic functioning. Despite the breakthrough in its treatment with the emergence of the first antipsychotic in the early 1950s, schizophrenia is still one of the most costly mental disorders in terms of human suffering and social costs. Currently available antipsychotics have little or no effect in controlling the negative symptoms and, in general, there is no improvement in cognitive function. It is now accepted that the first psychotic episode is preceded by a prodromal phase, and criteria were implemented for enabling the identification of individuals at risk of converting to psychosis. It is thus necessary to investigate the safety and efficacy of drug candidates with potential to delay or prevent the transition to psychosis. In this thesis, we investigated in this context the potential of N-acetylcysteine (NAC), a cysteine precursor with antioxidant and anti-inflammatory properties, and a modulator of glutamate transmission. Used as mucolytic and in the management of paracetamol intoxication, recent studies led NAC to be considered a promise in psychiatry. The aim of this thesis was to generate relevant data to the clinical use of NAC as a preventive therapeutic intervention in schizophrenia by investigating its effects in animal models of the disease. In the developmental model of post-weaning social isolation, NAC prevented the increase in locomotor response to amphetamine. In the pharmacological model of amphetamine sensitization, NAC attenuated locomotor response to amphetamine but did not prevent the latent inhibition deficit in sensitized animals; in control animals, NAC per se induced latent inhibition deficit. In acute experiments, NAC did not prevent amphetamine- or MK-801-induced hyperlocomotion. We also tested the effects of NAC in a "two-hit" model that combined prenatal immune activation with stress in puberty. In this model, NAC prevented the prepulse inhibition deficit and the increased locomotor response to amphetamine. The mechanism of action of NAC in these models remains to be clarified, but it is known that inflammation and oxidative stress are involved in the emergence of the behavioral abnormalities in animal models and in humans that convert to schizophrenia. Adolescence is a critical stage of vulnerability, but also represents a window of opportunity for prevention. The data presented in this thesis supports the potential use of NAC as a drug strategy with to attenuate, delay or even prevent the emergence of behavioral changes associated with psychotic disorders. Clinical trials in subjects at risk of converting to psychosis are required to assess the efficacy and safety of this drug.

## LISTA DE ABREVIATURAS

| | |
|---|---|
| **AMPH** | Anfetamina |
| **ANOVA** | Análise de variância |
| **CON** | Controle |
| **DOI** | 2,5-dimetoxi-4-iodoanfetamina |
| **i.p.** | Intraperitoneal |
| **LI** | Inibição latente |
| **mGluR** | Receptor metabotrópico de glutamato |
| **NAC** | N-acetilcisteína |
| **NMDA** | N-metil-D-aspartato |
| **NPE** | Não pré-exposto |
| **PE** | Pré-exposto |
| **p.o.** | Via oral |
| **POL** | Ácido poli-inosínico:policitidílico |
| **Poly(i:c)** | Ácido poli-inosínico:policitidílico |
| **PPI** | Inibição por prepulso da resposta de sobressalto |
| **S-** | Não estressado |
| **S+** | Estressado |
| **SAL** | Salina |
| **VEH** | Veículo |

# INTRODUÇÃO

O cineasta brasileiro Eduardo Coutinho morreu no dia 2 de fevereiro de 2014, aos 80 anos, minutos após ser golpeado com duas facadas desferidas pelo filho mais novo, Daniel Coutinho, 41 anos, diagnosticado posteriormente com esquizofrenia. A esposa de Eduardo e mãe de Daniel, Dora Coutinho, sobreviveu aos ferimentos infligidos pelo filho, que falhou ao tentar cometer suicídio depois dos ataques. Embora atos de violência por parte de pessoas acometidas por transtornos psicóticos sejam exceção – e não regra –, a triste tragédia familiar ilustra bem como a esquizofrenia pode impactar a vida do paciente e das pessoas que o cercam.

Os relatos sobre a história familiar e as circunstâncias que antecederam esse lamentável desfecho informam sobre diversos aspectos relevantes à biologia da esquizofrenia. Eduardo Coutinho tornou-se pai de Daniel aos 39 anos. Um grande número de estudos epidemiológicos indicam uma associação positiva entre idade paterna no momento da concepção e risco de esquizofrenia na prole (Brown et al., 2002; Dalman and Allebeck, 2002; Goriely et al., 2013; Kong et al., 2012; Lehrer et al., 2015; Malaspina et al., 2001; McGrath et al., 2014; Miller et al., 2011a, 2011b; Sipos et al., 2004; Zammit et al., 2003). Eduardo Coutinho foi um cineasta premiado, artista brilhante, considerado por muitos um gênio, criador de um estilo único de direção dos seus documentários, como o consagrado “Cabra Marcado para Morrer”. Diversos estudos mostram associação entre criatividade e transtornos mentais como a esquizofrenia, possivelmente mediada por fatores genéticos – criatividade e psicose compartilham, portanto, as mesma raízes genéticas (Andreasen, 2008; Jamison, 1989; Karksson, 1970; Kaufman and Paul, 2014; Kyaga et al., 2011; Ludwig, 1992, 1994; Post, 1994; Power et al., 2015). Dora Coutinho foi

diagnosticada com um quadro agudo e grave de toxoplasmose logo no início da gravidez de Daniel. Muitos estudos epidemiológicos associam infecção materna por vírus, bactérias e parasitas como o *Toxoplasma gondii*, principalmente nos primeiros trimestres de gestação, com risco aumentado de esquizofrenia na prole (Babulas et al., 2006; Brown and Derkits, 2010a; Brown et al., 2001, 2004, 2005, 2009; Buka et al., 2001; Mednick et al., 1988; Menninger KA, 1919; Mortensen et al., 2007; Sørensen et al., 2009; Suvisaari et al., 1999; Torrey and Peterson, 1973; Torrey et al., 1988, 2012). Apesar da gravidez de risco, Daniel foi uma criança relativamente normal, mas, a partir da adolescência, passou a viver cada vez mais isolado, e teve dificuldades para concluir a graduação em jornalismo. Isolamento social e déficits cognitivos são características centrais da esquizofrenia, e estudos mostram que, na maioria dos casos, esses sintomas precedem o primeiro surto psicótico (Cornblatt et al., 2012; Häfner et al., 1999; Haller et al., 2014; Keshavan et al., 2005, 2010; Tandon et al., 2008; Woodberry et al., 2010). Daniel também passou a fumar, beber e fazer uso de maconha e cocaína. São vários os estudos epidemiológicos que concluem que o consumo de maconha na adolescência aumenta o risco de desenvolver transtornos psicóticos (Andréasson et al., 1987; Arendt et al., 2005; Arseneault et al., 2002, 2004, 2004; D'Souza, 2007; Ferdinand et al., 2005; Fergusson et al., 2003; Henquet et al., 2004; Kuepper et al., 2011; Manrique-Garcia et al., 2012; Moore et al., 2007; Niemi-Pynttäri et al., 2013; van Os et al., 2002; Zammit et al., 2002). Aos 18 anos, numa série de fatalidades, Daniel perdeu três amigos em seis meses, o que limitou ainda mais sua vida social. Exposição a estresse psicológico é outro fator de risco associado a transtornos psicóticos, e eventos estressantes muitas vezes precipitam o início ou as recaídas da doença (Birley and Brown, 1970; Corcoran et al., 2003; Day et al., 1987; Morgan and Fisher,

2007; van Os et al., 2010; van Winkel et al., 2008). O risco constante de Daniel cometer suicídio instaurou um terror silencioso na casa da família. Estima-se que 50% das mortes em pessoas com esquizofrenia até os 39 anos de idade tem como causa o suicídio (Alaräisänen et al., 2009).

Assim como é o caso da maioria das pessoas com esquizofrenia, Daniel não trabalhava, e não teve condições de se manter nas poucas oportunidades de emprego que teve. Estimativas da Europa mostram que a taxa de emprego nesse grupo varia de 8 a 35% (Gaite et al., 2002; Marwaha et al., 2007).

Infelizmente, como tantos brasileiros, Daniel não foi diagnosticado nem recebeu qualquer tratamento antes do surto psicótico que culminou na morte de seu pai. Apesar dos dados epidemiológicos no Brasil serem escassos, estima-se que mais da metade dos 2 milhões de brasileiros com esquizofrenia não recebem tratamento – uma proporção alarmante (Ferri et al., 2004; Kohn et al., 2004; Leitão et al., 2006). Estudos também confirmam a noção de que os recursos alocados para a saúde no Brasil são insuficientes, e muito aquém do que é praticado em países desenvolvidos (em porcentagem do produto interno bruto), principalmente em relação à saúde mental (Leitão et al., 2006; World Health Organization, 2008, 2013).

Há muito que ser feito para reduzir o impacto social e econômico acarretado por transtornos psiquiátricos e melhorar a vida de pacientes e familiares. A presente tese visa a contribuir para modificar esse cenário, fornecendo evidências para responder à seguinte pergunta: poderia o tratamento precoce com N-acetilcisteína antes do primeiro surto psicótico evitar histórias como a de Daniel e de sua família no futuro?

## Esquizofrenia

A esquizofrenia é um transtorno mental crônico e incapacitante, que em geral inicia na adolescência ou início da vida adulta. O diagnóstico atual é baseado na presença de sintomas específicos, como delírios, alucinações, discurso e comportamento desorganizados, expressão emocional diminuída e declínio significativo no nível de funcionamento social, profissional ou acadêmico (American Psychiatric Association, 2014).

Os principais sintomas da esquizofrenia podem ser classificados em positivos, negativos e cognitivos. Os sintomas positivos incluem alucinações (visuais e auditivas), delírios (geralmente do tipo paranoide) e comportamento desorganizado. Os sintomas negativos referem-se a emoções embotadas ou impróprias à situação, timidez excessiva, isolamento social, anedonia, avolia, entre outros. A capacidade cognitiva das pessoas com esquizofrenia é severamente afetada, e são observados sintomas como rigidez de raciocínio e déficits de memória de trabalho, função executiva e linguagem. São os sintomas negativos e cognitivos que mais afetam a integração e o funcionamento social dos pacientes (Green, 1996; Häfner and an der Heiden, 1999; Perkins et al., 2005; Tandon et al., 2009).

Apesar da revolução no tratamento de pacientes com o surgimento do primeiro antipsicótico no início da década de 1950, a esquizofrenia ainda é um dos transtornos mentais mais custosos em termos de sofrimento humano e encargos sociais. O custo econômico decorrente da doença foi estimado em mais de US$ 60 bilhões nos Estados Unidos em 2002 (Wu et al., 2005), US$ 24 bilhões no Japão em 2008 (Sado et al., 2013), e mais de € 93 bilhões na Europa em 2010 (Gustavsson et al., 2011). Além disso, os antipsicóticos disponíveis atualmente apresentam pouco

ou nenhum efeito no controle dos sintomas negativos e, de modo geral, não há melhora das funções cognitivas (Keefe et al., 2007; Murphy et al., 2006). O tratamento crônico pode ainda induzir efeitos extrapiramidais, ganho de peso e alterações metabólicas, que eventualmente determinam a interrupção do tratamento (Ellenbroek, 2012; Gardner et al., 2005).

Além dos antipsicóticos (Leucht et al., 2009), a eficácia de outros medicamentos psiquiátricos é alvo de críticas e questionamentos. É o caso dos antidepressivos usados no tratamento da depressão maior (Kirsch et al., 2008), dos inibidores da acetilcolinesterase na Doença de Alzheimer (Kaduszkiewicz et al., 2005), e do lítio no controle do transtorno bipolar (Geddes et al., 2004; Moncrieff, 1995). Esse assunto foi pauta de diversas matérias veiculadas em jornais e revistas da mídia e motivou movimentos antipsiquiatria e livros sobre o tema (Breggin, 2009; Carlat, 2010; Kirsch, 2010; Whitaker, 2010). Entretanto, uma revisão de metanálises concluiu que a eficácia dos medicamentos psiquiátricos modernos é comparável à eficácia de medicamentos usados na medicina geral (Leucht et al., 2012). Apesar desse resultado, é consenso geral que ainda existe muito espaço para melhorias no tratamento de transtornos mentais, tanto em relação à eficácia quanto aos efeitos adversos dos medicamentos.

Além disso, observou-se nas últimas décadas uma redução drástica na morbidade e mortalidade associadas a câncer e doenças cardiovasculares, enquanto nenhum avanço prático foi obtido em relação à esquizofrenia e outros transtornos mentais (Insel, 2010; Jacka and Berk, 2014; McGorry, 2015). Para mudar esse cenário atual, é necessário “repensar a esquizofrenia” (Insel, 2010), pois é mais provável que os próximos grandes avanços serão alcançados por estratégias

de prevenção, diagnóstico e intervenção precoce do que pela descoberta de medicamentos dramaticamente mais eficazes.

*Prevenção e intervenção precoce*

Sabe-se hoje que o primeiro episódio psicótico é precedido por uma fase prodrômica, em que podem ser observados sintomas psicóticos atenuados, isolamento social, humor deprimido, ansiedade, irritabilidade e/ou déficits cognitivos (Klosterkötter et al., 2011; Yung and McGorry, 2007). Essas observações levaram ao desenvolvimento de programas de reconhecimento precoce da doença, possibilitando a identificação de indivíduos em risco de converter para psicose (Correll et al., 2010; de Koning et al., 2009; McGorry, 2011, 2015). São diversos os critérios e as denominações utilizadas para o estado prodrômico: '*ultra-high risk*' (UHR), '*at-risk mental state*' (ARMS), '*early initial prodromal state*' (EIPS) e '*clinical high risk*' (CHR), entre outros. Na última edição do Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-5), foi incluída uma nova categoria com critérios de diagnóstico para o que foi denominado de 'síndrome psicótica atenuada' (American Psychiatric Association, 2014). Essa mudança polêmica foi alvo de muita discussão e controvérsia, mas talvez possa contribuir de alguma forma para que possíveis estratégias de prevenção e intervenção precoce sejam estabelecidas. Dessa forma, seria possível de fato "repensar a esquizofrenia", como propôs Thomas Insel em seu artigo seminal publicado na revista Nature, em edição especial dedicada ao tema (Insel, 2010).

Além do reconhecimento dos sinais comportamentais da síndrome prodômica, um número crescente de estudos de imagem tem relatado alterações

estruturais progressivas no cérebro, como perda de matéria cinzenta, que surgem antes do primeiro episódio psicótico (Bois et al., 2015; Borgwardt et al., 2008; Fornito et al., 2008; Fusar-Poli et al., 2011; Job et al., 2005; Jung et al., 2011; Keshavan et al., 2007; Lawrie et al., 1999; Mechelli et al., 2011; Pantelis et al., 2005; Smieskova et al., 2010; Sun et al., 2009; Takahashi et al., 2009; Walter et al., 2012; Ziermans et al., 2012). Dessa forma, criou-se a expectativa de que seja possível prevenir o primeiro surto e o aparecimento da esquizofrenia em sua forma plena. Algumas estratégias potencialmente preventivas estão sendo avaliadas em sujeitos considerados em estado de risco. Entre as estratégias farmacológicas já propostas estão o tratamento precoce com antipsicóticos, antidepressivos, lítio e ácidos graxos ômega-3 (Amminger et al., 2010; Klosterkötter et al., 2011; McGorry et al., 2009). Entretanto, como os critérios de seleção ainda são baseados em sintomas comportamentais, que não são específicos da prodrome esquizofrênica, o número de casos falso-positivos é significativo: as taxas de conversão a psicose foram estimadas em 36% em três anos (Fusar-Poli et al., 2012). Portanto, o risco-benefício de usar tais medicamentos nessa população deve ser bem avaliado para evitar exposição a tratamentos desnecessários e potencialmente prejudiciais. Também é importante salientar que os resultados quanto à eficácia desse tipo de intervenção não são conclusivos (McGorry et al., 2008).

Achados recentes sugerem que processos inflamatórios e desregulação do estado redox são relevantes especialmente no período desenvolvimental que precede o primeiro surto psicótico (Cannon et al., 2015; Fournier et al., 2014; Khandaker et al., 2014; Pasternak et al., 2015; Perkins et al., 2015). Um estudo recente em particular sugere que a neuroinflamação pode estar envolvida na conversão de pessoas em estado de risco para psicose (Cannon et al., 2015). Os

dados mostraram que os níveis basais de marcadores pró-inflamatórios foram preditivos da taxa de perda de matéria cinzenta em indivíduos CHR que converteram. Os autores sugerem que a ativação da microglia é responsável pela perda tecidual, o que está de acordo com a teoria de que na esquizofrenia existe uma aceleração dos processos normais de *pruning* sináptico que se intensificam no final da adolescência/início da vida adulta – ver **Figura 1** (Faludi and Mirnics, 2011; Keshavan et al., 1994; Schafer et al., 2013). Entretanto, o papel ativo da microglia na eliminação de sinapses durante o desenvolvimento é alvo de controvérsias (Boksa, 2012). No entendimento do professor Hugh Perry não existem provas concretas, e a microglia é apenas "culpada por associação" (Perry and O'Connor, 2010). De qualquer forma, essas observações sugerem que intervenções precoces com medicamentos anti-inflamatórios e/ou antioxidantes são uma possibilidade promissora na prevenção dos processos patofisiológicos que antecedem a esquizofrenia.

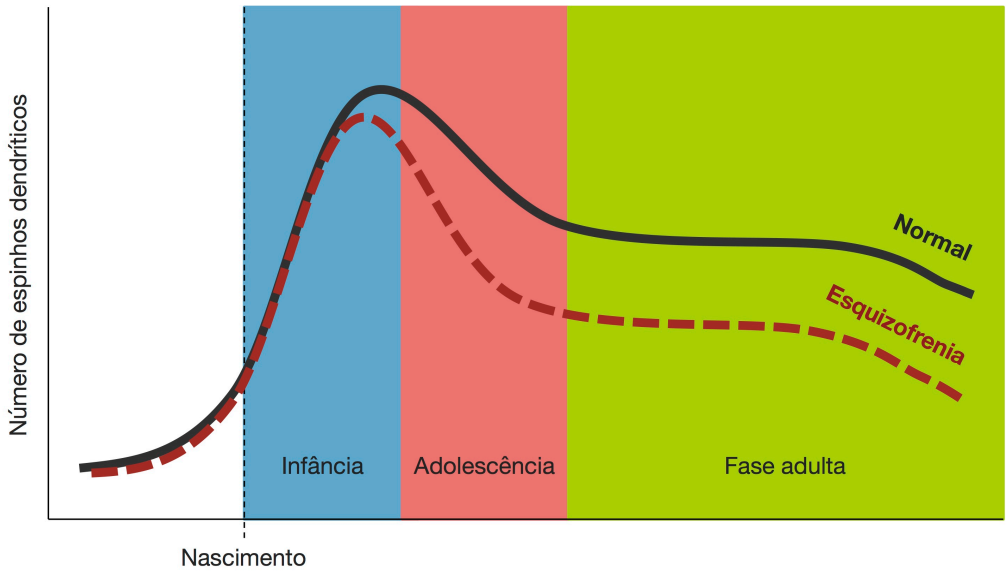


**Figura 1.** Alterações nos processos de maturação sináptica durante a transição da adolescência para a vida adulta precedem o primeiro episódio psicótico e o estabelecimento da esquizofrenia em sua forma plena. Acredita-se que processos inflamatórios, como ativação da microglia, e estresse oxidativo estejam implicados nas alterações estruturais observadas no cérebro de indivíduos considerados em estado de risco. Esse período de vulnerabilidade é também uma janela de oportunidade para possíveis intervenções preventivas. Adaptado de Penzes et al., 2011.

Infelizmente, estratégias de diagnóstico e intervenção precoce não são tarefa fácil. O Brasil já tem dificuldades para diagnosticar e tratar a maior parte dos casos de esquizofrenia (e outros transtornos mentais) mesmo após anos de manifestação de sintomas. Há muito que se avançar para chegar nos níveis desejados de assistência, mas exemplos podem ser observados na experiência de países como Austrália, Reino Unido e Canadá, pioneiros em programas de intervenção precoce (McGorry, 2015). Cabe dizer que investimentos em estratégias de prevenção e na melhoria dos serviços de atenção à saúde mental possibilitam a redução dos gastos do setor público com transtornos mentais a longo prazo.

Enquanto os critérios de diagnóstico e o entendimento do curso da doença avançam a cada dia, é necessário investigar a segurança e a eficácia de candidatos farmacológicos com potencial para adiar ou prevenir a transição para psicose. Modelos animais são ferramentas úteis para testar as possibilidades.

**Modelos animais de esquizofrenia**

Embora não seja possível recriar em animais uma condição exclusivamente humana como a esquizofrenia, modelos animais podem nos ajudar a entender a patofisiologia da doença e a investigar novos tratamentos e seus mecanismos (Powell, 2010; Powell and Geyer, 2007; Swerdlow et al., 1994). A seguir são descritos os três modelos animais que foram empregados na presente tese.

*Modelo de isolamento social pós-desmame*

Ratos e camundongos são os principais mamíferos utilizados como animal modelo na pesquisa científica. A estrutura social e hierárquica das colônias desempenha um papel importante no desenvolvimento dessas espécies (Hall, 1998; Powell, 2010). Privar ratos e camundongos de contato social após o desmame, que acontece em torno do dia pós-natal 21, altera o desenvolvimento do cérebro e o comportamento dos animais de forma comparável ao que se observa na esquizofrenia (Geyer et al., 1993; Powell and Geyer, 2002). A ausência precoce de estímulos sociais também modela o isolamento social que ocorre antes do primeiro surto psicótico e que é um fator de predição da conversão à psicose em indivíduos em risco (Cannon et al., 2008). Dessa forma, o isolamento social pós-desmame é considerado um modelo desenvolvimental de esquizofrenia, em que uma manipulação ambiental induz alterações neuroquímicas e comportamentais que se manifestam de forma plena apenas após a puberdade dos animais, replicando o curso da doença. Dependendo da duração e do período de isolamento, não é possível reverter os déficits comportamentais com a reintegração social (Hall, 1998; Pascual et al., 2006).

As alterações comportamentais observadas nesse modelo incluem déficits de processamento sensório-motor (inibição por prepulso da resposta de sobressalto), resposta aumentada à anfetamina, inflexibilidade cognitiva (déficit no aprendizado de reversão), déficits de memória (déficit no reconhecimento de objeto novo), entre outros; as alterações neuroquímicas e estruturais incluem hiperatividade dopaminérgica na via mesolímbica, redução do volume do córtex pré-frontal e perda de interneurônios gabaérgicos que expressam parvalbumina (revisado por: Fone and

Porkess, 2008; Jones et al., 2011; Powell, 2010; Powell and Geyer, 2007). Essas observações confirmam a validade desse modelo que mimetiza vários dos fenômenos comportamentais, neuroquímicos e neuropatológicos característicos da esquizofrenia.

*Modelo de sensibilização a anfetamina*

Em humanos, o caso mais bem documentado de sensibilização a psicoestimulantes refere-se à epidemia de abuso de metanfetamina no Japão após o final da Segunda Guerra Mundial. Os indivíduos que apresentaram um quadro de psicose induzida por abuso crônico de metanfetamina permaneceram vulneráveis aos efeitos psicotomiméticos da droga mesmo após anos de abstinência: quando expostos a baixas doses de metanfetamina ou eventos estressores, um episódio psicótico muito semelhante ao episódio original era desencadeado nesses indivíduos (Sato et al., 1983, 1992). Efeitos similares também foram observados nos Estados Unidos em usuários de cocaína (Satel and Edell, 1991).

Em animais, a sensibilização comportamental induzida pela administração repetida de psicoestimulantes é amplamente utilizada como um modelo de psicose (Jones et al., 2011; Robinson and Becker, 1986; Ujike, 2002), e considera-se que mimetiza a sensibilização endógena do sistema dopaminérgico observada em pacientes com esquizofrenia (Laruelle, 2000) e em indivíduos em risco de conversão a psicose (Howes et al., 2009, 2011). As principais alterações comportamentais observadas em modelos de sensibilização a anfetamina em roedores são resposta locomotora aumentada à anfetamina, déficits de processamento sensório-motor (inibição por prepulso da resposta de sobressalto), déficits de atenção seletiva

(inibição latente) e inflexibilidade cognitiva (aprendizado de reversão) (Jones et al., 2011; Peleg-Raibstein et al., 2008; Robinson and Becker, 1986). De forma geral, são observados déficits na execução de tarefas cognitivas dependentes do córtex pré-frontal, enquanto que a função hipocampal não é afetada (Featherstone et al., 2008; Russig et al., 2003). Como esse modelo induz basicamente alterações no sistema dopaminérgico, é mais responsivo a fármacos que afetam esse neurotransmissor, mas outras classes de fármacos, como por exemplo agonistas de receptores metabotrópicos de glutamato do tipo 2/3 ($mGluR_{2/3}$), também podem ter efeito na hiperlocomoção induzida por anfetamina (Kim and Vezina, 2002).

*Modelo de "dois-hits": ativação imune pré-natal + estresse na puberdade*

A observação epidemiológica de que infecção materna durante a gestação aumenta o risco de desenvolver esquizofrenia (Brown, 2012; Brown and Derkits, 2010b) levou ao desenvolvimento de diversos modelos animais em roedores baseados nessa possível etiologia (Meyer, 2014). Os primeiros estudos utilizaram vírus *Influenza*, que por razões práticas foi substituído por poly(i:c), um RNA dupla fita que mimetiza uma infecção viral, e por lipopolissacarídeo (LPS), uma endotoxina bacteriana (Kneeland and Fatemi, 2013; Meyer and Feldon, 2010; Meyer et al., 2005, 2009; Shi et al., 2003). Apesar de infecções no período pré-natal serem relativamente comuns, apenas uma fração dos indivíduos expostos desenvolve o transtorno (Mednick et al., 1988; Selten et al., 2010). Esse fato pode ser explicado pela hipótese de "dois-hits" da esquizofrenia, que postula que um primeiro *hit* pré-natal compromete processos de desenvolvimento cerebral, aumentando a vulnerabilidade a um segundo *hit* mais tardio (Bayer et al., 1999; Clarke et al., 2009;

Maynard et al., 2001). Dessa forma, apenas a combinação de dois insultos leva ao desenvolvimento da doença. Essa ideia foi testada diretamente em camundongos e resultou na publicação do artigo de Giovanoli e colaboradores (2013) na revista Science.

Nesse modelo, uma dose baixa de poly(i:c), administrada no 9º dia de gestação, combinada com estresse variado no período peripubertal (entre os dias pós-natal 30 e 40) resultou em alterações comportamentais e bioquímicas observadas apenas em animais adultos. Por outro lado, sinais transitórios de neuroinflamação, como ativação microglial e secreção de citocinas pró-inflamatórias, foram observados logo após o estresse, mas não em animais adultos (Giovanoli et al., 2013). O fato de que essas alterações neuroinflamatórias precedem os sintomas comportamentais reforça o potencial de intervenções preventivas e a validade do modelo para testar possíveis candidatos.

**N-acetilcisteína**

Nessa tese investigamos o potencial da N-acetilcisteína (NAC) como tratamento farmacológico para intervenção precoce em esquizofrenia. NAC é um precursor de cisteína com notada ação antioxidante, utilizada corriqueiramente na clínica como mucolítico e no manejo da intoxicação por paracetamol. Diversos estudos recentes levaram a NAC a ser considerada uma “promessa na psiquiatria” (Berk et al., 2013). A perspectiva do uso de NAC é também promissora pelo fato de já estar no mercado, possuir estudos toxicológicos (inclusive pós-comercialização, o que comprova a ampla margem de segurança) e farmacocinéticos e estar disponível em formas farmacêuticas de baixo custo, inclusive como medicamento genérico.

Algumas hipóteses têm sido levantadas para a ação central de NAC. Devido a sua ação antioxidante, NAC pode modular o sítio sensível ao estado redox do receptor NMDA, assim como quelar o íon $Zn^{2+}$ (Krezel et al., 2003). Como na esquizofrenia as alterações no estado redox existem antes mesmo do primeiro surto psicótico, o uso precoce de antioxidantes pode diminuir o dano oxidativo e melhorar o curso da doença (Mahadik et al., 2001). Além disso, NAC é capaz de modular o sistema glutamatérgico indiretamente: por ser precursor de cisteína, aumenta a liberação extra-sináptica de glutamato através do antiporter cistina/glutamato presente nos astrócitos (Baker et al., 2003a, 2008). Dessa forma, o glutamato liberado ativa receptores metrabotrópicos de glutamato (mGluRs) inibitórios, que por sua vez modulam a liberação de glutamato e outros neurotransmissores na fenda sináptica. Um estudo em fatias estriatais mostrou ainda que NAC pode modular a liberação de dopamina, facilitando em doses baixas e inibindo em concentrações milimolares (Gere-Pászti and Jakus, 2009). Em macacos, NAC preveniu a redução dos níveis do transportador de dopamina induzida pelos efeitos neurotóxicos da metanfetamina (Hashimoto et al., 2004). As propriedades anti-inflamatórias de NAC também são relevantes para o seu potencial terapêutico em transtornos psiquiátricos, sendo NAC capaz de reduzir os níveis de citocinas pró-inflamatórias e inibir processos de ativação microglial (Awad et al., 2011; Beloosesky et al., 2006, 2009, 2012; Chang et al., 2011; Haber et al., 2013; Karalija et al., 2012, 2014; Khan et al., 2004; Lanté et al., 2008; Tsai et al., 2009).

*N-acetilcisteína e esquizofrenia: estudos clínicos*

Os efeitos benéficos de NAC no tratamento de transtornos psiquiátricos já foram demonstrados para transtorno bipolar, depressão maior, abuso de substâncias e transtornos de ansiedade, entre outros (revisado por Deepmala et al., 2015). Os ensaios clínicos que analisaram os efeitos de NAC em pacientes com esquizofrenia estão listados na **tabela 1**. Um dos estudos (Berk et al., 2008) tem nível de evidência 1b, já que é um ensaio clínico randomizado controlado de alta qualidade, com número de participantes superior a 100.

*N-acetilcisteína e esquizofrenia: estudos pré-clínicos*

Diversos estudos pré-clínicos investigaram o potencial de NAC em modelos animais relevantes para depressão (Arent et al., 2012; Costa-Campos et al., 2013; Ferreira et al., 2008; Linck et al., 2012; Smaga et al., 2012) e abuso de substâncias (Achat-Mendes et al., 2007; Amen et al., 2011; Baker et al., 2003a, 2003b; Bauzo et al., 2012; Corbit et al., 2014; Frankowska et al., 2014; Kau et al., 2008; Madayag et al., 2007; Moussawi et al., 2009, 2011; Murray et al., 2012; Ramirez-Niño et al., 2013; Reichel et al., 2011; Schneider et al., 2015; Taracha et al., 2015; Weiland et al., 2015). Os estudos pré-clínicos que avaliaram os efeitos de NAC em parâmetros comportamentais relevantes à esquizofrenia estão listados na **tabela 2**.

**Tabela 1.** Estudos clínicos que avaliaram os efeitos de N-acetilcisteína em pacientes com esquizofrenia.

| Referências | Número de participantes | Desenho experimental | Tratamento | Resultados |
|---|---|---|---|---|
| Berk et al., 2008 | 140 | Duplo cego controlado por placebo, paralelo | 2 g/dia por 6 meses | Melhora nas escalas PANSS total, PANSS negativo, PANSS geral e CGI e diminuição de acatisia (um efeito extrapiramidal decorrente do uso de antipsicóticos) |
| Bulut et al., 2009 | 1 | Relato de caso | 0,6 g/dia por 2 meses | Melhora nas escalas PANSS e CGI |
| Farokhnia et al., 2013 | 42 | Duplo cego controlado por placebo, paralelo | 2 g/dia por 2 meses | Melhora nas escalas PANSS total e PANSS negativo |
| Lavoie et al., 2008 | 22 | Duplo cego controlado por placebo, cruzado | 2 g/dia por 2 meses | Melhora de *mismatch negativity* (MMN), uma medida de processamento sensorial auditivo |

CGI: *Clinical Global Impression*, PANSS: *Positive and Negative Syndrome Scale*

**Tabela 2.** Estudos pré-clínicos que avaliaram os efeitos de N-acetilcisteína em parâmetros comportamentais relevantes à esquizofrenia.

| Referências | Espécie | Modelo/Teste | Tratamento | Resultados |
|---|---|---|---|---|
| Baker et al., 2008 | Ratos Sprague-Dawley | Fenciclidina, agudo e sub-crônico | 90 mg/kg, i.p. | Prevenção dos déficits de WM e SI – dependente de $mGluR_{2/3}$ |
| Cabungcal et al., 2014 | Ratos Sprague-Dawley | Lesão hipocampal neonatal | 0,9 g/L, água de beber, PND5-50 e PND35-50 | Prevenção dos déficits de MMN e PPI |
| Chen et al., 2010 | Camundongos C57BL/6J | $mGluR_5$ KO | 50 e 100 mg/kg, i.p. | Redução do déficit de PPI – independente de $mGluR_{2/3}$ |
| Fukami et al., 2004 | Ratos Wistar | Metanfetamina, agudo e sensibilização | 30, 100 e 300 mg/kg, i.p. | Diminuição da hiperlocomoção e prevenção da estereotipia |
| Lee et al., 2014 | Camundongos NMRI | DOI (agonista 5-$HT_{2A}$) | 30 e 100 mg/kg, i.p. | Diminuição do número de espasmos da cabeça (*head twitch*) – dependente de $mGluR_{2/3}$ |
| Lutgen et al., 2013 | Ratos Sprague-Dawley | Fenciclidina | 100 µM, intra-PFC | Redução do déficit de PPI |
| Möller et al., 2013 | Ratos Sprague-Dawley | Isolamento social pós-desmame | 150 mg/kg, i.p., 14 dias | Redução dos déficits de SI, NOR e PPI |

5-$HT_{2A}$: receptor de serotonina do tipo 2A, DOI: 2,5-dimetoxi-4-iodoanfetamina, i.p.: intraperitoneal, KO: nocaute, mGluR: receptor metabotrópico de glutamato, MMN: *mismatch negativity*, NOR: reconhecimento de objeto novo, PFC: córtex pré-frontal, PND: dia pós-natal, PPI: inibição por prepulso da resposta de sobressalto, SI: interação social, WM: memória de trabalho,

# OBJETIVOS

O objetivo dessa tese foi gerar dados relevantes ao uso clínico da N-acetilcisteína na intervenção terapêutica preventiva da esquizofrenia, analisando seus efeitos em modelos animais relevantes à doença.

Os objetivos específicos foram:

i) investigar os efeitos da N-acetilcisteína no modelo desenvolvimental de isolamento social pós-desmame;

ii) investigar os efeitos da N-acetilcisteína no modelo farmacológico de sensibilização a anfetamina e na hiperlocomoção induzida por administração única de anfetamina ou MK-801;

iii) investigar os efeitos da N-acetilcisteína no modelo de “dois hits” que combina ativação imune pré-natal com estresse na puberdade.

# PARTE II

*Onde os resultados são apresentados na forma de artigos científicos*

# CAPÍTULO 1

*N-acetylcysteine Prevents Increased Amphetamine Sensitivity in Social Isolation-Reared Mice*

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# N-acetylcysteine prevents increased amphetamine sensitivity in social isolation-reared mice

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ABSTRACT

Treating individuals at risk to develop schizophrenia may be strategic to delay or prevent transition to psychosis. We verified the effects of N-acetylcysteine (NAC) in a neurodevelopmental model of schizophrenia. C57 mice were reared in isolation or social groups and treated with NAC from postnatal day 42–70; the locomotor response to amphetamine was assessed at postnatal day 81. NAC treatment in isolated mice prevented the hypersensitivity to amphetamine, suggesting neuroprotection relevant to striatal dopamine. Considering its safety and tolerability profile, complementary studies are warranted to further evaluate the usefulness of NAC to prevent conversion to schizophrenia in at-risk individuals.



## 1. Introduction

Schizophrenia is characterized by a chronic relapsing course and onset in late adolescence or early adulthood, and after sizable investments and decades of research is still one of the most debilitating and costly mental disorders (Tandon et al., 2010). The development of early detection strategies has recently raised interest in possible interventions prior to the onset of full-blown psychosis, in hope that it could yield substantial outcome improvements (McGorry et al., 2008; Tsuang et al., 2013). Emerging evidence indicates that psychological and pharmacological intervention strategies may be suitable to delay or prevent transition to psychosis (McGorry et al., 2009; Marshall and Rathbone, 2011; Stafford et al., 2013). However, the use of antipsychotic medication in prodromal stages is controversial due to uncertain risk-benefit ratio (Francey et al., 2010). The identification of safer and more effective candidates is thus warranted.

Clinical and pre-clinical data placed the antioxidant N-acetylcysteine (NAC) as a potentially useful drug for various psychiatric disorders, including schizophrenia (Dean et al., 2011; Berk et al., 2013). NAC is a precursor of cysteine that increases brain glutathione levels and modulates glutamate transmission through the astrocyte cystine-glutamate antiporter. NAC has also been suggested as a potential medication to counteract the pathological processes that precede full-blown schizophrenia (Asevedo et al., 2012), but studies addressing this possibility are lacking.

Rearing rodents in social isolation is an established neurodevelopmental model of schizophrenia, in which adult animals present a range of behavioral deficits relevant to various symptoms of the disease (Fone and Porkess, 2008; Pietropaolo et al., 2008; Jones et al., 2011; Niwa et al., 2011). The aim of this study was to verify whether NAC treatment in adolescent mice could prevent the consequences of post-weaning social isolation rearing later in adulthood. Specifically, we studied the increased sensitivity to amphetamine-induced hyperlocomotion, a behavioral correlate of the alterations in striatal dopamine release observed in schizophrenia.

## 2. Methods

### 2.1. Animals

C57BL/6 male mice were obtained from Universidade Federal de Pelotas and maintained under controlled environmental conditions (reversed 12-h light/dark cycle and constant temperature of 22 ± 1 °C) with free access to food and water. The study was approved by the University Ethics Committee (approval #23618), and followed institutional policies on experimental animal handling. All efforts were made to minimize the number of animals used and their suffering.

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### 2.2. Experimental design

Animals were weaned at postnatal day 21 and randomly assigned to isolation or social (3–5 animals per cage) rearing groups. Mice were treated (daily, i.p.) with saline (NaCl 0.9%) or NAC (60 or 120 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) during postnatal days 42 to 70. Locomotor activity testing was performed after a washout period, at postnatal day 81.

### 2.3. Locomotor sensitivity to amphetamine

The locomotor response to amphetamine challenge (D-amphetamine sulfate, Sigma-Aldrich, St. Louis, MO, USA) was assessed in open field arenas (40 × 40 × 40 cm). Animals were treated with saline and placed in the center of the arena; after 30 min they were briefly removed, treated with amphetamine (2.5 mg/kg, i.p.) and returned to the arena for another 60 min. Experiments were video recorded and analyzed with the ANY-Maze tracking software (Stoelting Co., Wood Dale, IL, USA).

### 2.4. Statistical analysis

Three-way ANOVA was used to identify the main effects of housing condition, treatment and bins, as well as their interactions. Fisher's least significant difference post hoc was used for comparisons. Significance was set at $p < 0.05$. SPSS 18.0 for Windows was used.

## 3. Results

Isolation-reared mice showed increased locomotor activity in response to amphetamine in comparison to social-reared controls. While NAC was devoid of effect in the social-reared group, it prevented, at both doses, the enhanced sensitivity to amphetamine in the isolated group (Fig. 1). Statistical support for these results was provided by three-way repeated measures ANOVA, which yielded a significant main effect of bins ($F_{11,440} = 59.4$, $p < 0.0001$) and a significant housing condition × treatment group × bin interaction ($F_{22,440} = 1.64$, $p < 0.05$). No significant differences in basal locomotor activity (the initial habituation period after saline administration) were observed between the groups.

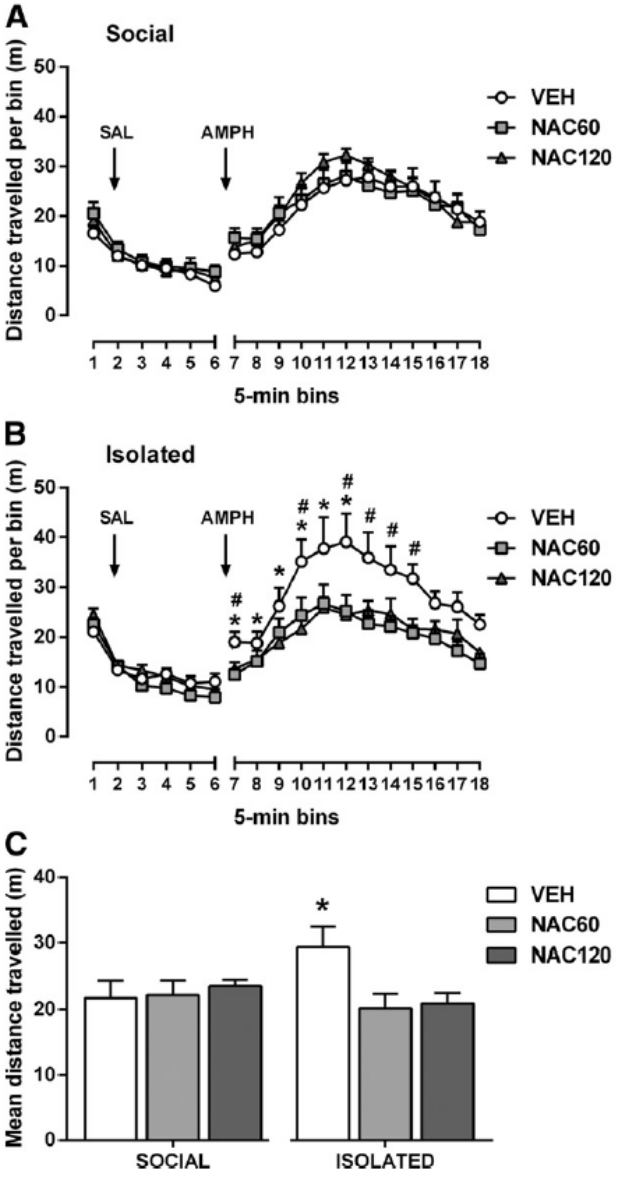


**Fig. 1.** Effects of N-acetylcysteine on sensitivity to amphetamine in social- and isolation-reared mice. Distance traveled per bin after saline (SAL) (bins 1–6) and amphetamine (AMPH) (bins 7–18) for (A) social and (B) isolated mice. (C) Mean distance traveled after AMPH. Data presented as mean + SEM. *$p < 0.05$ VEH-treated social compared to VEH-treated isolated mice; #$p < 0.05$ VEH-treated isolated compared to NAC-treated isolated mice, based on Fisher post hoc tests. n = 6 to 9.

## 4. Discussion

This study shows that treating adolescent mice with NAC prevents the hypersensitivity to amphetamine induced by social isolation rearing, supporting with experimental data the proposal that NAC can be useful for early intervention in individuals at risk to develop psychosis. The yet to be defined mechanisms underlying this preventive effect are likely to be multifaceted given that, adding to its antioxidant and glutamatergic properties, NAC also targets other relevant neurochemical pathways such as neurotrophic, apoptotic and inflammatory signaling (Oja et al., 2000; Dean et al., 2011; Palacio et al., 2011).

The psychoactive properties of NAC have been documented in animal models and clinical trials (Berk et al., 2008a, 2008b; Linck et al., 2012; Costa-Campos et al., 2013). Specifically regarding schizophrenia, clinical data suggest beneficial effects of NAC as adjunctive medication in regard to positive and negative symptoms, as well as adverse effects associated with antipsychotics (Berk et al., 2008a; Bulut et al., 2009; Farokhnia et al., 2013). Additionally, NAC was found to modulate neural synchronization (Carmeli et al., 2012), and to improve mismatch negativity in schizophrenia patients (Lavoie et al., 2008). NAC was also found to be effective in various rodent models relevant to psychosis (Chen et al., 2010; das Neves Duarte et al., 2012; Lutgen et al., 2013) and reported to reverse isolation rearing-induced behavioral and neurochemical changes in rats, including parameters of immune and mitochondrial function (Möller et al., 2013). However, these experimental designs preclude direct assumptions on the preventive effects of NAC. Though it has been suggested that NAC could be useful to prevent conversion to schizophrenia in at-risk individuals, to the best of our knowledge this is the first study supporting this suggestion.

Clinical studies are ultimately needed to investigate benefits to treat individuals in prodromal stages, but difficulties with prospective identification of the subjects and varying diagnostic criteria of the prodrome across research groups are major limitations (Addington et al., 2008). Moreover, considering the high rates of false-positive predictions, the identification of early intervention candidates devoid of serious adverse effects is crucial. Animal models are thus useful to explore potential medications capable of attenuating the progression to full-blown psychosis. NAC is an advantageous candidate since it is proved to be well tolerated and to present few side effects and contraindications (Whyte et al., 2007). Complementary data on the neuroprotective effects of NAC in the context of preventing psychosis are thus warranted to subsidize clinical trials.

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**Contributors**

Ana P. Herrmann designed the study, performed the experiments and the statistical analysis, and wrote the first draft of the manuscript. Radharani Benvenutti and Luísa K. Pilz participated in the experiments and revised the manuscript. Elaine Elisabetksy designed the study, analyzed the data and edited the manuscript. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

All the authors declare that there are no conflicts of interest to report.

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## References

Addington, J., Epstein, I., Reynolds, A., Furimsky, I., Rudy, L., Mancini, B., McMillan, S., Kirsopp, D., Zipursky, R.B., 2008. Early detection of psychosis: finding those at clinical high risk. Early Interv. Psychiatry 2, 147–153.

Asevedo, E., Cunha, G.R., Zugman, A., Mansur, R.B., Brietzke, E., 2012. N-acetylcysteine as a potentially useful medication to prevent conversion to schizophrenia in at-risk individuals. Rev. Neurosci. 23, 353–362.

Berk, M., Copolov, D., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I., Anderson-Hunt, M., Judd, F., Katz, F., Katz, P., Ording-Jespersen, S., Little, J., Conus, P., Cuenod, M., Do, K.Q., Bush, A.I., 2008a. N-acetyl cysteine as a glutathione precursor for schizophrenia—a double-blind, randomized, placebo-controlled trial. Biol. Psychiatry 64, 361–368.

Berk, M., Copolov, D.L., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I., Anderson-Hunt, M., Bush, A.I., 2008b. N-acetyl cysteine for depressive symptoms in bipolar disorder—a double-blind randomized placebo-controlled trial. Biol. Psychiatry 64, 468–475.

Berk, M., Malhi, G.S., Gray, L.J., Dean, O.M., 2013. The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol. Sci. 34, 167–177.

Bulut, M., Savas, H.A., Altindag, A., Virit, O., Dalkilic, A., 2009. Beneficial effects of N-acetylcysteine in treatment resistant schizophrenia. World J. Biol. Psychiatry 10, 626–628.

Carmeli, C., Knyazeva, M.G., Cuénod, M., Do, K.Q., 2012. Glutathione precursor N-acetylcysteine modulates EEG synchronization in schizophrenia patients: a double-blind, randomized, placebo-controlled trial. PLoS ONE 7, e29341.

Chen, H.-H., Stoker, A., Markou, A., 2010. The glutamatergic compounds sarcosine and N-acetylcysteine ameliorate prepulse inhibition deficits in metabotropic glutamate 5 receptor knockout mice. Psychopharmacology (Berl.) 209, 343–350.

Costa-Campos, L., Herrmann, A.P., Pilz, L.K., Michels, M., Noetzold, G., Elisabetsky, E., 2013. Interactive effects of N-acetylcysteine and antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 44, 125–130.

Das Neves Duarte, J.M., Kulak, A., Gholam-Razaee, M.M., Cuenod, M., Gruetter, R., Do, K.Q., 2012. N-acetylcysteine normalizes neurochemical changes in the glutathione-deficient schizophrenia mouse model during development. Biol. Psychiatry 71, 1006–1014.

Dean, O., Giorlando, F., Berk, M., 2011. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J. Psychiatry Neurosci. Jpn. 36, 78–86.

Farokhnia, M., Azarkolah, A., Adinehfar, F., Khodaie-Ardakani, M.-R., Hosseini, S.-M.-R., Yekehtaz, H., Tabrizi, M., Rezaei, F., Salehi, B., Sadeghi, S.-M.-H., Moghadam, M., Gharibi, F., Mirshafiee, O., Akhondzadeh, S., 2013. N-acetylcysteine as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia: a randomized, double-blind, placebo-controlled study. Clin. Neuropharmacol. 36, 185–192.

Fone, K.C.F., Porkess, M.V., 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci. Biobehav. Rev. 32, 1087–1102.

Francey, S.M., Nelson, B., Thompson, A., Parker, A.G., Kerr, M., Macneil, C., Fraser, R., Hughes, F., Crisp, K., Harrigan, S., Wood, S.J., Berk, M., McGorry, P.D., 2010. Who needs antipsychotic medication in the earliest stages of psychosis? A reconsideration of benefits, risks, neurobiology and ethics in the era of early intervention. Schizophr. Res. 119 (1–3), 1–10.

Jones, C., Watson, D., Fone, K., 2011. Animal models of schizophrenia. Br. J. Pharmacol. 164, 1162–1194.

Lavoie, S., Murray, M.M., Deppen, P., Knyazeva, M.G., Berk, M., Boulat, O., Bovet, P., Bush, A. I., Conus, P., Copolov, D., Fornari, E., Meuli, R., Solida, A., Vianin, P., Cuénod, M., Buclin, T., Do, K.Q., 2008. Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. Neuropsychopharmacology 33, 2187–2199.

Linck, V.M., Costa-Campos, L., Pilz, L.K., Garcia, C.R.L., Elisabetsky, E., 2012. AMPA glutamate receptors mediate the antidepressant-like effects of N-acetylcysteine in the mouse tail suspension test. Behav. Pharmacol. 23, 171–177.

Lutgen, V., Qualmann, K., Resch, J., Kong, L., Choi, S., Baker, D.A., 2013. Reduction in phencyclidine induced sensorimotor gating deficits in the rat following increased system $xc^-$ activity in the medial prefrontal cortex. Psychopharmacology (Berl.) 226, 531–540.

Marshall, M., Rathbone, J., 2011. Early intervention for psychosis. Cochrane Database Syst. Rev. 15 (6), (CD004718).

McGorry, P.D., Killackey, E., Yung, A., 2008. Early intervention in psychosis: concepts, evidence and future directions. World Psychiatry 7, 148–156.

McGorry, P.D., Nelson, B., Amminger, G.P., Bechdolf, A., Francey, S.M., Berger, G., Riecher-Rössler, A., Klosterkötter, J., Ruhrmann, S., Schultze-Lutter, F., Nordentoft, M., Hickie, I., McGuire, P., Berk, M., Chen, E.Y.H., Keshavan, M.S., Yung, A.R., 2009. Intervention in individuals at ultra-high risk for psychosis: a review and future directions. J. Clin. Psychiatry 70, 1206–1212.

Möller, M., Du Preez, J.L., Viljoen, F.P., Berk, M., Emsley, R., Harvey, B.H., 2013. Social isolation rearing induces mitochondrial, immunological, neurochemical and behavioural deficits in rats, and is reversed by clozapine or N-acetyl cysteine. Brain Behav. Immun. 30, 156–167.

Niwa, M., Matsumoto, Y., Mouri, A., Ozaki, N., Nabeshima, T., 2011. Vulnerability in early life to changes in the rearing environment plays a crucial role in the aetiopathology of psychiatric disorders. Int. J. Neuropsychopharmacol. 14, 459–477.

Oja, S.S., Janáky, R., Varga, V., Saransaari, P., 2000. Modulation of glutamate receptor functions by glutathione. Neurochem. Int. 37, 299–306.

Palacio, J.R., Markert, U.R., Martínez, P., 2011. Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. Inflamm. Res. 60, 695–704.

Pietropaolo, S., Singer, P., Feldon, J., Yee, B.K., 2008. The postweaning social isolation in C57BL/6 mice: preferential vulnerability in the male sex. Psychopharmacology (Berl.) 197, 613–628.

Stafford, M.R., Jackson, H., Mayo-Wilson, E., Morrison, A.P., Kendall, T., 2013. Early interventions to prevent psychosis: systematic review and meta-analysis. BMJ 346, f185.

Tandon, R., Nasrallah, H.A., Keshavan, M.S., 2010. Schizophrenia, "just the facts" 5. Treatment and prevention. Past, present, and future. Schizophr. Res. 122, 1–23.

Tsuang, M.T., Van Os, J., Tandon, R., Barch, D.M., Bustillo, J., Gaebel, W., Gur, R.E., Heckers, S., Malaspina, D., Owen, M.J., Schultz, S., Carpenter, W., 2013. Attenuated psychosis syndrome in DSM-5. Schizophr. Res. 150, 31–35.

Whyte, I.M., Francis, B., Dawson, A.H., 2007. Safety and efficacy of intravenous N-acetylcysteine for acetaminophen overdose: analysis of the hunter area toxicology service (HATS) database. Curr. Med. Res. Opin. 23, 2359–2368.

# CAPÍTULO 2

*Effects of N-acetylcysteine in Schizophrenia-like Behaviors Induced by a Progressive Regimen of Amphetamine Sensitization in Mice*

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# Effects of N-acetylcysteine in Schizophrenia-like Behaviors Induced by a Progressive Regimen of Amphetamine Sensitization in Mice

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**Running title:** Effects of N-acetylcysteine in Amphetamine Sensitization

**Key Words:** Schizophrenia; Early intervention; N-acetylcysteine; Amphetamine; Sensitization.

## ABSTRACT

A process of ‘endogenous sensitization’ is thought to mediate the hyperfunction of the mesolimbic dopamine system in patients with schizophrenia. This can be modeled in rodents by repeated exposure to psychostimulants such as amphetamine, which leads to a long-term enduring process where increased behavioral response to the drug is observed at a subsequent exposure. We investigated the effects of the antioxidant and glutamate modulator N-acetylcysteine (NAC) on amphetamine sensitization in an escalating dose protocol proposed to mimic the progression from the prodromal state to full-blown schizophrenia. Amphetamine was administered to C57BL/6 mice three times per week during 3 weeks, and the dose weekly increased from 1 to 3 mg/kg; NAC treatment (60 mg/kg, i.p.) started in the second week. Latent inhibition and locomotor response to amphetamine challenge were assessed after four weeks of washout. The progressive amphetamine regimen disrupted latent inhibition and induced locomotor sensitization to an amphetamine challenge. NAC disrupted latent inhibition in control animals, and failed to prevent the amphetamine-induced disruption. In sensitized animals, NAC attenuated the enhanced locomotor response to amphetamine. NAC was devoid of effects when given acutely before a single amphetamine or MK-801 challenge. This study supports the idea that NAC, an antioxidant, anti-inflammatory and glutamate modulator, may be useful in preventing the neurochemical changes that precede full-blown schizophrenia. The data may be particularly relevant for early intervention in subjects at risk to convert to psychosis.

## 1. Introduction

The long-standing dopamine hypothesis of schizophrenia postulates that mesolimbic dopamine hyperfunction underlies the psychotic symptoms of the disorder. A process of 'endogenous sensitization' is thought to be implicated in the development of such neurochemical abnormality (Laruelle, 2000; Lieberman et al., 1997). This idea is supported by evidence from neuroimaging studies showing enhanced striatal dopamine release in response to an acute amphetamine challenge in schizophrenia patients (Breier et al., 1997; Abi-Dargham et al., 1998; Laruelle et al., 1996, 1999). Moreover, repeated consumption of high doses of amphetamine or other stimulants often results in the progressive development of a psychotic state that closely resembles that of paranoid schizophrenia (Angrist and Gershon, 1970; Bell, 1973; Ellinwood et al., 1973; Snyder, 1973).

The heightened sensitivity to amphetamine in patients with schizophrenia can be recapitulated in rodents by repeatedly exposing them to psychostimulants. This leads to a long-term sensitization process where increased behavioral response to the drug is observed at a subsequent exposure. Sensitization is an enduring phenomenon, since sensitized animals display enhanced response to psychostimulants even months or years after drug withdrawal (Robinson and Becker, 1986). Amphetamine sensitization became a widely accepted animal model of schizophrenia because it induces behavioral and neurochemical abnormalities similar to those observed in the disorder (Peleg-Raibstein et al., 2008, 2009).

N-acetylcysteine (NAC) is a safe and well-tolerated drug, clinically used as mucolytic and as antidote against acetaminophen intoxication (Heard, 2008; Sadowska, 2012). NAC is a glutathione precursor that exerts antioxidant, anti-

inflammatory, anti-apoptotic and neurotrophic actions (Atkuri et al., 2007; Karalija et al., 2014; Palacio et al., 2011). It also modulates glutamate release through activation of the cystine-glutamate antiporter expressed by astrocytes in extra-synaptic sites (Baker et al., 2003, 2008). The effects of NAC as adjunctive treatment for neuropsychiatric disorders have been assessed in several clinical trials (reviewed by Deepmala et al., 2015), and the resulting data support the idea of NAC as a promising drug in psychiatry (Berk et al., 2013).

We previously showed that NAC prevents the increased sensitivity to amphetamine in social isolation-reared mice (Herrmann et al., 2014). Here we investigated the effects of NAC on amphetamine sensitization by using the escalating dose protocol proposed to mimic the progression from the prodromal state to full-blown schizophrenia (Tenn et al., 2005a). This amphetamine regimen induces behavioral abnormalities related to schizophrenia, including hyperlocomotion, latent inhibition disruption and prepulse inhibition deficits (Tenn et al., 2003, 2005a, 2005b). Additionally, we investigated the acute effects of NAC on hyperlocomotion induced by a single amphetamine or MK-801 challenge.

## 2. Methods

### *2.1. Animals*

C57BL/6 male mice (2-month old) were obtained from Universidade Federal de Pelotas and maintained under controlled environmental conditions (reversed 12-h light/dark cycle with lights on at 7:00 am and constant temperature of 22 ± 1 °C) with free access to food and water. All procedures were approved by the Ethics

Committee of Hospital de Clínicas de Porto Alegre (approval #13-0457), and are in agreement with the principles of laboratory animal care in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication, 2011). All efforts were made to minimize the number of animals used and their suffering.

*2.2. Drugs*

N-acetylcysteine (NAC), amphetamine and MK-801 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in saline (0.9% NaCl). Solutions were freshly prepared and injected intraperitoneally (i.p.) in a final volume of 5 ml/kg.

*2.3 Amphetamine sensitization protocol*

Animals were randomly assigned into 4 groups, as depicted in **Figure 1**. Injections were administered three times per week during 3 weeks. The doses of amphetamine were progressively increased from 1 to 3 mg/kg from the first to the third week. NAC (60 mg/kg) was given immediately before saline or amphetamine during the second and third weeks. Animals returned to their home cages immediately after the injections. Behavioral tests started after a 4-week washout from the last amphetamine administration.

*2.3.1. Latent inhibition*

Latent inhibition (LI) was assessed in a conditioned active avoidance procedure using a 2-way shuttle box (Insight Equipamentos Científicos, Ribeirão Preto, Brazil). In the first phase of the test, animals from each of the 4 treatment groups were allocated to a pre-exposure (PE) condition: conditioned stimulus (CS)-PE or non-pre-exposure (NPE). The PE subjects were placed in the apparatus and presented with 100 discrete exposures to a 5-s burst of white noise (85 $dB_A$) in a random interstimulus interval of 40 ± 15 s. The NPE subjects were placed in the apparatus for an equivalent period of time without any stimulus presentation. 24 h later (conditioning day), all subjects received a total of 100 avoidance trials presented with an intertrial interval of 40 ± 15 s; each trial began with the onset of the noise (CS). If the animal shuttled within 5 s of CS onset, the CS was terminated and the animal avoided the electric shock (unconditioned stimulus [US]) on that trial. Avoidance failure led to an electric foot shock (0.3 mA) presented in conjunction to the CS that could last for a maximum of 2 s but could be terminated by a shuttle response during this period (i.e., an escape response). Conditioned avoidance learning was indexed as the mean number of avoidance responses recorded on successive blocks of 10 trials.

*2.3.2. Locomotor activity*

Locomotor activity was assessed in an open field arena to verify the response after an amphetamine challenge (2 mg/kg, i.p.). The apparatus consisted of 4 identical square arenas (40×40×40 cm), located in a testing room under dim diffused lighting. A webcam connected to a computer was mounted directly above the 4 arenas, and the videos were analyzed with ANY-Maze tracking software (Stoelting

Co., Wood Dale, IL, USA). The animals were injected i.p. with vehicle solution and immediately placed in the apparatus to measure basal locomotor activity for 30 min; subsequently, they were treated with amphetamine and immediately returned to the same arena, where locomotor response was monitored for 90 min.

*2.4. Acute experiment*

To assess whether NAC presents acute antipsychotic-like effects, we tested the locomotor response after a challenge with amphetamine or MK-801, an antagonist of NMDA receptors. Naïve mice were treated (i.p.) with saline or NAC (60 mg/kg) and immediately returned to their home cages. One hour later subjects were placed individually in the open field arenas and baseline activity recorded for 30 min; they were then injected with saline, amphetamine (2.5 mg/kg) or MK-801 (0.3 mg/kg) and locomotor activity was recorded for 100 min. The time point for pretreatment with NAC was chosen based on Baker et al. (2008), who showed that NAC blocked the increase in extracelular glutamate when injected 100 min before phencyclidine.

*2.5. Statistical Analyses*

Data were analyzed using analysis of variance (ANOVA). Fisher's least significant difference (LSD) post-hoc tests were used whenever significant interactions were obtained by the initial ANOVAs. Statistical significance was set at $p<0.05$. Statistical analyses were performed using the statistical software StatView (version 5.0).

## 3. Results

### *3.1 N-acetylcysteine Impairs Latent Inhibition*

**Figure 2** shows the effects of amphetamine sensitization and NAC on latent inhibition. A latent inhibition (LI) effect was observed in control (SAL/SAL) animals (**Figure 2A**); consistent with previous studies, the sensitization protocol led to a disruption in latent inhibition (**Figure 2C**). Treatment with NAC not only failed to prevent the LI disruption induced by amphetamine sensitization (**Figure 2D**) but also abolished the LI effect in the saline-treated control animals (**Figure 2B**). The 2 × 10 (PE condition × 10-trial blocks) ANOVAs restricted to each of the 4 experimental groups revealed a significant main effect of PE condition only in the SAL/SAL group ($F_{1,11}$=13.9, p<0.01).

### *3.2 N-acetylcysteine Blunts Hyperlocomotion in Amphetamine-sensitized Animals*

**Figure 3** shows that NAC partially prevented the hyperlocomotion induced by the sensitization protocol (pretreatment × bins interaction: $F_{11,429}$=16.5, p<0.0001). The effects of NAC are independent of potential influences on basal locomotor activity given that no significant changes were observed in baseline activity.

### *3.3 N-acetylcysteine Does Not Present Acute Antipsychotic-Like Effects*

In a next series of investigations, we tested whether NAC could prevent the hyperlocomotion induced by an acute amphetamine (**Figure 4A**) or MK-801

challenge (**Figure 4B**). NAC did not affect locomotion per se nor influenced the response to amphetamine (pretreatment × treatment × bins interaction: $F_{13,208}$=0.7, p>0.05) or to MK-801 (pretreatment × treatment × bins interaction: $F_{13,208}$=0.6, p>0.05).

## 4. Discussion

Our study shows that mice treated with amphetamine in the progressive dose schedule became sensitized, as measured by the locomotor response to amphetamine challenge after four weeks of withdrawal. This sensitization reflects the development of long-term neuroadaptive changes, only partially prevented by NAC. NAC failed to prevent the amphetamine-induced latent inhibition disruption, and disrupted latent inhibition *per se* in control, non-sensitized animals. NAC acutely administered was devoid of effects against amphetamine- and MK-801-induced hyperlocomotion. Repeated amphetamine administration protocols are also used in the context of bipolar disorder and drug abuse. The experimental design used in our study adds graded dose progression to the repeated amphetamine administration used to model other mental disorders. Other than recapitulating the graded progression observed from prodrome to full-blown schizophrenia, this protocol has been shown to lead to changes that resemble several of the behavioral and neurochemical findings observed in patients with schizophrenia and at-risk subjects (Tenn et al., 2005). Further differentiating the repeated amphetamine protocol used in this study from the protocols used to model other mental disorders, this protocol induces long-lasting behavioral deficits relevant to schizophrenia (e.g., PPI and latent inhibition) even in the absence of an amphetamine challenge.

In the study by Valvassori et al. (2008), NAC (20 mg/kg three times per day) did not prevent the hyperactivity induced by one week of daily amphetamine injections in rats; however, sensitization was not evaluated since locomotion was assessed 2 hours after the last amphetamine administration. Fukami et al. (2004) showed that NAC (100 mg/kg) prevented behavioral sensitization to five consecutive daily injections of methamphetamine in rats; they also showed that NAC (100 mg/kg) attenuated the locomotor response to an acute methamphetamine challenge. In the present study NAC treatment was initiated after the first week of amphetamine, and it cannot be ruled out that more robust preventive effects would have been observed if treatment was initiated concurrent to the installment of the amphetamine protocol.

It is important to differentiate the two main paradigms of repeated amphetamine administration. In the paradigm used in this study, the phenomenon of behavioral sensitization is induced by repeated intermittent administration of relatively low doses amphetamine. In the neurotoxic paradigm, continuous delivery or multiple high dose injections maintain amphetamine levels in the brain constantly elevated for a few days, which damages dopamine terminals as characterized by decreased tyrosine hydroxylase and dopamine transporter activities in the striatum, dopamine depletion and increased levels of oxidative stress markers (Carvalho et al., 2012; Ellison and Eison, 1983; Ellison et al., 1978; Wagner et al., 1980; Yamamoto et al., 2010). Several studies reported the protective effects of NAC against the neurotoxic effects of psychostimulants, where its antioxidant properties are likely to play an important role (Achat-Mendes et al., 2007; Fukami et al., 2004; Hashimoto et al., 2004; Wan et al., 2006). The escalating and intermittent properties of the amphetamine regimen used in the present study protect against the neurotoxic

effects of subsequent amphetamine doses (Robinson and Becker, 1986; Robinson and Camp, 1987).

It is hypothesized that glutamate pathways are involved in the neurochemical mechanisms that underlie the sensitization phenomenon. Psychostimulants enhance glutamate release in the nucleus accumbens (Wolf et al., 2000; Xue et al., 1996), activating postsynaptic NMDA and AMPA receptors. Activation of NMDA receptors increases the intracellular calcium concentration, which is required for the induction of sensitization (Karler et al., 1991; Licata et al., 2000; Pierce et al., 1998). NAC increases extrasynaptic glutamate by activating the cystine-glutamate antiporter, resulting in stimulation of metabotropic glutamate receptors 2/3 ($mGluR_{2/3}$) with consequent decrease in synaptic glutamate release (Baker et al., 2003, 2008; Dean et al., 2011). This mechanism of action might account for the effects of NAC observed here against amphetamine sensitization.

Latent inhibition is a measure of selective associative learning in which repeated exposure to a non-reinforced stimulus retards subsequent conditioning with that stimulus. Latent inhibition has an established translational validity since it is disrupted in patients with schizophrenia (Lubow, 2005; Weiner, 2003; Weiner and Arad, 2009). While NAC attenuated the enhanced locomotor response to amphetamine in sensitized animals, it did not prevent the disruption in latent inhibition. As revealed by the use of different amphetamine schedules, the expression of behavioral sensitization is not required for disruption in latent inhibition, which suggests that deficits of information processing are mediated by different neuronal mechanisms than those underlying sensitization (Peleg-Raibstein et al., 2008). Divergent neurochemical basis for these two phenomena is congruent with

the differential effects of NAC on locomotor sensitization and latent inhibition. The effect of NAC disrupting latent inhibition *per se* remains to be elucidated.

NAC was devoid of effects when administered acutely before amphetamine and MK-801 challenges. This suggests that NAC is more effective in counteracting the long-term adaptations that follows repeated psychostimulant administration or developmental manipulations (Herrmann et al., 2014) than exerting acute antipsychotic-like actions in animal models mostly associated with positive symptoms. It is evident, however, that a range of doses and administration intervals have to be tested before a definite conclusion can be reached in this matter.

In conclusion, our study complements previous data advancing the idea that NAC may be useful in preventing the neurochemical changes that are thought to lead to full-blown schizophrenia. The results are especially relevant to at-risk subjects. Our study contributes to extend the increasing knowledge on the potential of NAC in the treatment of neuropsychiatric disorders and encourages clinical trials.

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## Contributors

Ana P. Herrmann designed the study, performed the experiments and the statistical analysis, and wrote the first draft of the manuscript. Roberta Andrejew and Radharani Benvenutti assisted in drug administration and behavioral testing. Clarissa Severino Gama revised the study design and the manuscript. Elaine Elisabetksy supervised the study design, data analysis and co-authored the manuscript. All authors contributed to and have approved the final manuscript.

## Conflict of interest

All the authors declare that there are no conflicts of interest to report.

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## References

Achat-Mendes, C., Anderson, K.L., and Itzhak, Y. (2007). Impairment in consolidation of learned place preference following dopaminergic neurotoxicity in mice is ameliorated by N-acetylcysteine but not D1 and D2 dopamine receptor agonists. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *32*, 531–541.

Angrist, B.M., and Gershon, S. (1970). The phenomenology of experimentally induced amphetamine psychosis--preliminary observations. Biol. Psychiatry *2*, 95–107.

Atkuri, K.R., Mantovani, J.J., Herzenberg, L.A., and Herzenberg, L.A. (2007). N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency. Curr. Opin. Pharmacol. *7*, 355–359.

Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Toda, S., and Kalivas, P.W. (2003a). N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. Ann. N. Y. Acad. Sci. *1003*, 349–351.

Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Tang, X.-C., Toda, S., and Kalivas, P.W. (2003b). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat. Neurosci. *6*, 743–749.

Baker, D.A., Madayag, A., Kristiansen, L.V., Meador-Woodruff, J.H., Haroutunian, V., and Raju, I. (2008). Contribution of cystine-glutamate antiporters to the psychotomimetic effects of phencyclidine. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *33*, 1760–1772.

Bell, D.S. (1973). The experimental reproduction of amphetamine psychosis. Arch. Gen. Psychiatry *29*, 35–40.

Berk, M., Malhi, G.S., Gray, L.J., and Dean, O.M. (2013). The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol. Sci. *34*, 167–177.

Breier, A., Su, T.-P., Saunders, R., Carson, R.E., Kolachana, B.S., de Bartolomeis, A., Weinberger, D.R., Weisenfeld, N., Malhotra, A.K., Eckelman, W.C., et al. (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography method. Proc. Natl. Acad. Sci. U. S. A. *94*, 2569–2574.

Carvalho, M., Carmo, H., Costa, V.M., Capela, J.P., Pontes, H., Remião, F., Carvalho, F., and Bastos, M. de L. (2012). Toxicity of amphetamines: an update. Arch. Toxicol. *86*, 1167–1231.

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R.M., Seibyl, J.P., Bowers, M., van Dyck, C.H., Charney, D.S., Innis, R.B., and Laruelle, M. (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am. J. Psychiatry *155*, 761–767.

Dean, O., Giorlando, F., and Berk, M. (2011). N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J. Psychiatry Neurosci. JPN *36*, 78–86.

Deepmala, null, Slattery, J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., and Frye, R. (2015). Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. Neurosci. Biobehav. Rev. *55*, 294–321.

Ellinwood, E.H., Sudilovsky, A., and Nelson, L.M. (1973). Evolving behavior in the clinical and experimental amphetamine (model) psychosis. Am. J. Psychiatry *130*, 1088–1093.

Ellison, G.D., and Eison, M.S. (1983). Continuous amphetamine intoxication: an animal model of the acute psychotic episode. Psychol. Med. *13*, 751–761.

Ellison, G., Eison, M.S., Huberman, H.S., and Daniel, F. (1978). Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. Science *201*, 276–278.

Fukami, G., Hashimoto, K., Koike, K., Okamura, N., Shimizu, E., and Iyo, M. (2004). Effect of antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. Brain Res. *1016*, 90–95.

Hashimoto, K., Tsukada, H., Nishiyama, S., Fukumoto, D., Kakiuchi, T., Shimizu, E., and Iyo, M. (2004). Effects of N-acetyl-L-cysteine on the reduction of brain dopamine transporters in monkey treated with methamphetamine. Ann. N. Y. Acad. Sci. *1025*, 231–235.

Heard, K.J. (2008). Acetylcysteine for Acetaminophen Poisoning. N. Engl. J. Med. *359*, 285–292.

Herrmann, A.P., Benvenutti, R., Pilz, L.K., and Elisabetsky, E. (2014). N-acetylcysteine prevents increased amphetamine sensitivity in social isolation-reared mice. Schizophr. Res. *155*, 109–111.

Karalija, A., Novikova, L.N., Kingham, P.J., Wiberg, M., and Novikov, L.N. (2014). The effects of N-acetyl-cysteine and acetyl-L-carnitine on neural survival, neuroinflammation and regeneration following spinal cord injury. Neuroscience *269*, 143–151.

Karler, R., Turkanis, S.A., Partlow, L.M., and Calder, L.D. (1991). Calcium channel blockers and behavioral sensitization. Life Sci. *49*, 165–170.

Laruelle, M. (2000). The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. Brain Res. Brain Res. Rev. *31*, 371–384.

Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., et al. (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc. Natl. Acad. Sci. U. S. A. *93*, 9235–9240.

Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L., and Innis, R. (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. Biol. Psychiatry *46*, 56–72.

Licata, S.C., Freeman, A.Y., Pierce-Bancroft, A.F., and Pierce, R.C. (2000). Repeated stimulation of L-type calcium channels in the rat ventral tegmental area mimics the initiation of behavioral sensitization to cocaine. Psychopharmacology (Berl.) *152*, 110–118.

Lieberman, J.A., Sheitman, B.B., and Kinon, B.J. (1997). Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *17*, 205–229.

Lubow, R.E. (2005). Construct Validity of the Animal Latent Inhibition Model of Selective Attention Deficits in Schizophrenia. Schizophr. Bull. *31*, 139–153.

Palacio, J.R., Markert, U.R., and Martínez, P. (2011). Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al *60*, 695–704.

Peleg-Raibstein, D., Knuesel, I., and Feldon, J. (2008). Amphetamine sensitization in rats as an animal model of schizophrenia. Behav. Brain Res. *191*, 190–201.

Peleg-Raibstein, D., Yee, B.K., Feldon, J., and Hauser, J. (2009). The amphetamine sensitization model of schizophrenia: relevance beyond psychotic symptoms? Psychopharmacology (Berl.) *206*, 603–621.

Pierce, R.C., Quick, E.A., Reeder, D.C., Morgan, Z.R., and Kalivas, P.W. (1998). Calcium-mediated second messengers modulate the expression of behavioral sensitization to cocaine. J. Pharmacol. Exp. Ther. *286*, 1171–1176.

Robinson, T.E., and Becker, J.B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. *396*, 157–198.

Robinson, T.E., and Camp, D.M. (1987). Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal locomotion. Pharmacol. Biochem. Behav. *26*, 821–827.

Sadowska, A.M. (2012). N-Acetylcysteine mucolysis in the management of chronic obstructive pulmonary disease. Ther. Adv. Respir. Dis. *6*, 127–135.

Snyder, S.H. (1973). Amphetamine psychosis: a “model” schizophrenia mediated by catecholamines. Am. J. Psychiatry *130*, 61–67.

Tenn, C.C., Fletcher, P.J., and Kapur, S. (2003). Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia. Schizophr. Res. *64*, 103–114.

Tenn, C.C., Fletcher, P.J., and Kapur, S. (2005a). A putative animal model of the “prodromal” state of schizophrenia. Biol. Psychiatry *57*, 586–593.

Tenn, C.C., Kapur, S., and Fletcher, P.J. (2005b). Sensitization to amphetamine, but not phencyclidine, disrupts prepulse inhibition and latent inhibition. Psychopharmacology (Berl.) *180*, 366–376.

Valvassori, S.S., Petronilho, F.C., Réus, G.Z., Steckert, A.V., Oliveira, V.B.M., Boeck, C.R., Kapczinski, F., Dal-Pizzol, F., and Quevedo, J. (2008). Effect of N-acetylcysteine and/or deferoxamine on oxidative stress and hyperactivity in an animal model of mania. Prog. Neuropsychopharmacol. Biol. Psychiatry *32*, 1064–1068.

Wagner, G.C., Ricaurte, G.A., Johanson, C.E., Schuster, C.R., and Seiden, L.S. (1980). Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. Neurology *30*, 547–550.

Wan, F.-J., Tung, C.-S., Shiah, I.-S., and Lin, H.-C. (2006). Effects of alpha-phenyl-N-tert-butyl nitrone and N-acetylcysteine on hydroxyl radical formation and dopamine

depletion in the rat striatum produced by d-amphetamine. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. *16*, 147–153.

Weiner, I. (2003). The “two-headed” latent inhibition model of schizophrenia: modeling positive and negative symptoms and their treatment. Psychopharmacology (Berl.) *169*, 257–297.

Weiner, I., and Arad, M. (2009). Using the pharmacology of latent inhibition to model domains of pathology in schizophrenia and their treatment. Behav. Brain Res. *204*, 369–386.

Wolf, M.E., Xue, C.J., Li, Y., and Wavak, D. (2000). Amphetamine increases glutamate efflux in the rat ventral tegmental area by a mechanism involving glutamate transporters and reactive oxygen species. J. Neurochem. *75*, 1634–1644.

Xue, C.J., Ng, J.P., Li, Y., and Wolf, M.E. (1996). Acute and repeated systemic amphetamine administration: effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. J. Neurochem. *67*, 352–363.

Yamamoto, B.K., Moszczynska, A., and Gudelsky, G.A. (2010). Amphetamine toxicities: classical and emerging mechanisms. Ann. N. Y. Acad. Sci. *1187*, 101–121.

## Figure Legends

**Figure 1.** Experimental design. Drugs were administered i.p. three times per week for three weeks. Amphetamine (AMPH) doses were weekly increased and ranged from 1 to 3 mg/kg. N-acetylcysteine (NAC, 60 mg/kg) started on the second week and was given immediately before saline or amphetamine. Animals were assigned to the following experimental groups: SAL/SAL, NAC/SAL, SAL/AMPH, NAC/AMPH. Animals returned to their home cages after the injections. Behavioral tests started after 4 weeks of withdrawal.

**Figure 2.** Effects of amphetamine sensitization and N-acetylcysteine (NAC) on latent inhibition (LI). LI was observed in the SAL/SAL group (**A**), and disrupted by amphetamine (**C**). NAC abolished the LI effect in control animals (**B**) and failed to prevent the LI disruption induced by amphetamine (**D**). **=$p<0.01$, reflecting the significant main effect of pre-exposure condition. *n*=7-9. Mean + SEM.

**Figure 3.** Effects of amphetamine sensitization and N-acetylcysteine (NAC) on locomotor activity. NAC partially prevented the increased locomotor response to amphetamine challenge in sensitized animals. *=$p<0.05$ compared to SAL/SAL; $^{\#}$=$p<0.05$ compared to NAC/AMPH. *n*=10-11. Mean + SEM.

**Figure 4.** Effects of N-acetylcysteine (NAC) on the locomotor response to acute amphetamine and MK-801 challenges. NAC did not influence the locomotor responses to amphetamine (**A**) or to MK-801 (**B**). *n*=6-8 per group. Mean + SEM.

**Figure 1**

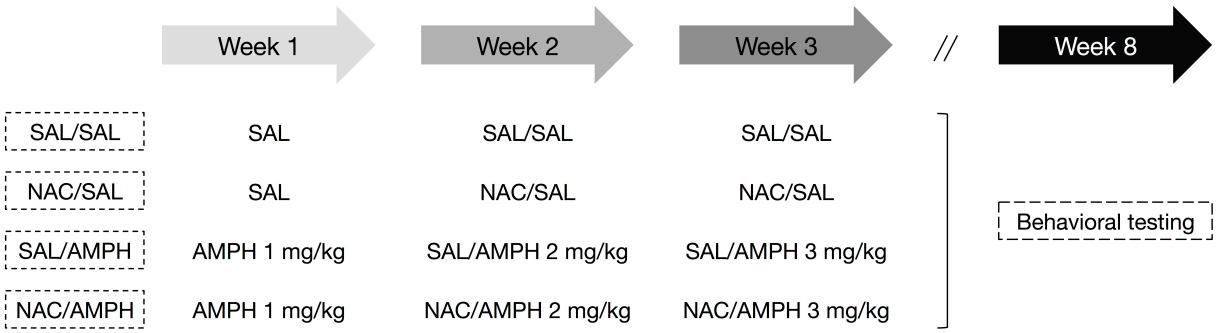



**Figure 2**

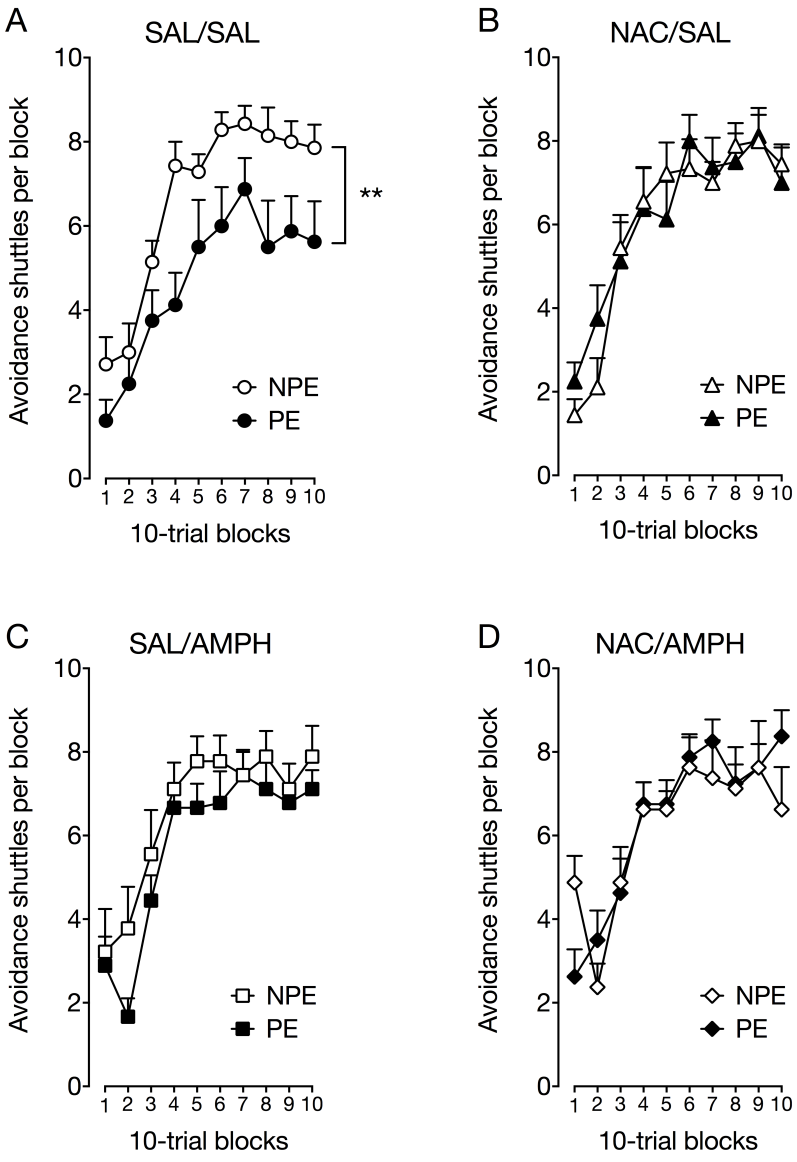
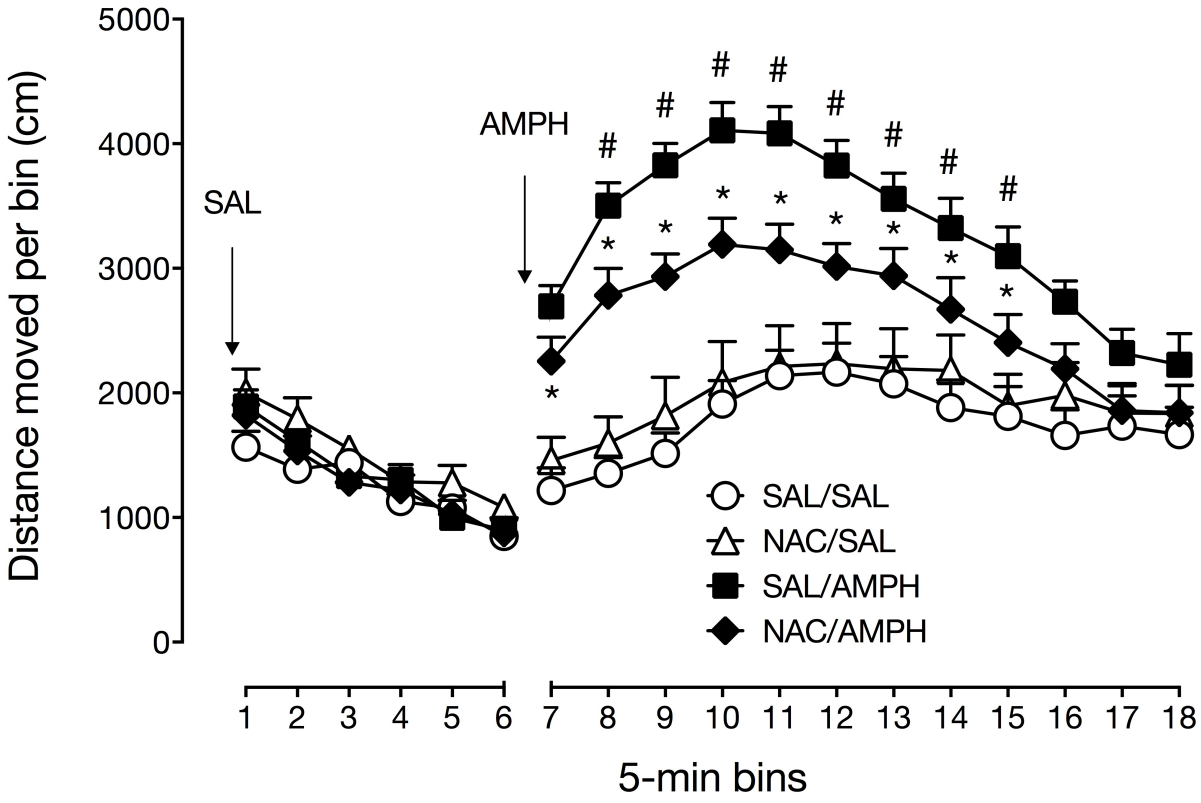


**Figure 3**


Distance moved per bin (cm)
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2000
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SAL
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SAL/AMPH
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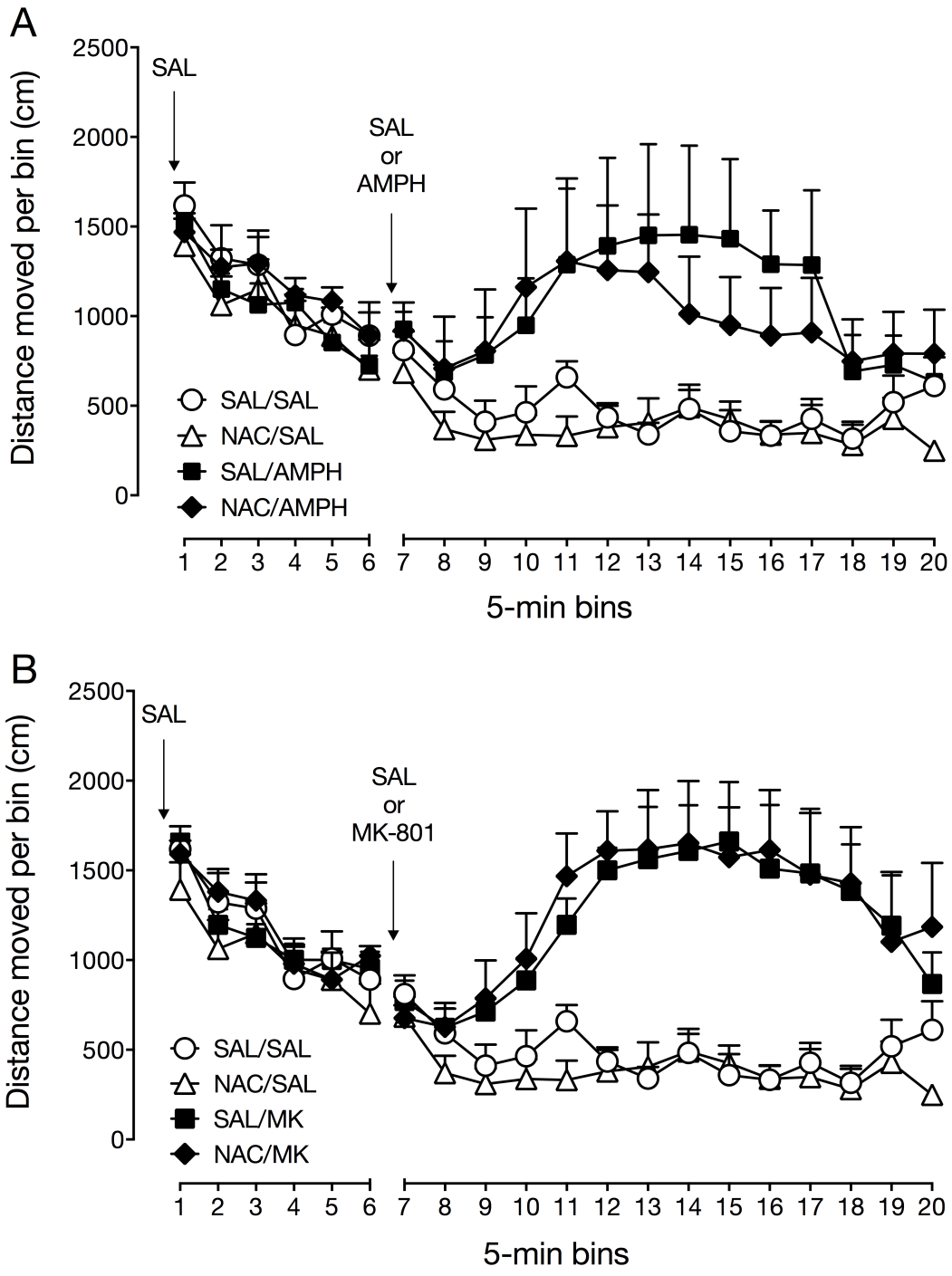
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
5-min bins

**Figure 4**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SAL/SAL
NAC/SAL
SAL/MK
NAC/MK
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
5-min bins

# CAPÍTULO 3

*N-acetylcysteine Prevents the Emergence of Schizophrenia Pathology in a Two-Hit Mice Model*

Ana Paula Herrmann, Sandra Giovanoli, Urs Meyer, Elaine Elisabetsky

Manuscrito em construção a ser submetido ao periódico *Biological Psychiatry*

# N-acetylcysteine Prevents the Emergence of Schizophrenia Pathology in a Two-Hit Mice Model

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**Running title:** Preventive Effects of N-acetylcysteine in a Schizophrenia Two-Hit Mice Model

**Key Words:** Schizophrenia; Prenatal infection; Peribubertal stress; Oxidative stress; Early intervention; N-acetylcysteine.

## ABSTRACT

**Background:** Therapeutic interventions during critical periods of vulnerability before the onset of evident symptoms have the potential to prevent conversion to psychosis in at-risk subjects. N-acetylcysteine (NAC), a glutathione precursor with antioxidant, anti-inflammatory and glutamate-modulating properties, is a promising drug in the treatment of various psychiatric disorders. Here we evaluated the effects of NAC in a developmental mouse model with translational validity in the context of schizophrenia.

**Methods:** We used an environmental two-hit model, in which prenatal maternal administration of the viral mimic poly(I:C) served as the first hit, and exposure to unpredictable stress during peripubertal maturation as the second. We investigated whether NAC administered through the drinking water during the stress exposure could prevent the subsequent emergence of behavioral abnormalities in adult male mice.

**Results:** The combination of prenatal immune activation and peripubertal stress induced significant deficits in prepulse inhibition and increased sensitivity to amphetamine in adult mice. NAC treatment during peribubertal stress exposure prevented the subsequent emergence of these behavioral alterations.

**Conclusions:** Our results replicate the previous report regarding the two-hit model and corroborate the relevance of combined prenatal immune activation and peripubertal stress in the emergence of behavioral changes relevant to schizophrenia in adult mice. Most importantly, the study shows the preventive effects of NAC against the pathological interactions between these two environmental factors associated with psychiatric disorders. This study has translational value in the context of early intervention in individuals at risk to develop schizophrenia.

## Introduction

Schizophrenia is arguably one of the worst ways biology can go awry. The incapacitating symptoms occur in a chronic relapsing course which, combined with onset often in late adolescence, leads to disability early in life and a substantial share of the global burden of disease (1). Unfortunately, the major advances accomplished in the last decades in understanding the neurobiology of schizophrenia have not yet translated into better treatment outcomes. “Rethinking schizophrenia” (2) is necessary to dramatically change the current scenario, and real progress will only be achieved with early diagnosis, early intervention and prevention – these strategies accounted for most of the reduction in mortality and morbidity seen in cancer and cardiovascular disease over recent years (3). Progress already started with the implementation of early intervention programs (4; 5), but investigating the safety and efficacy of pharmacological candidates is an important step to develop stage-specific treatments aiming to delay or prevent transition to psychosis.

It has long been recognized that schizophrenia is a neurodevelopmental disorder, in which disruptions in early life processes result in long-term abnormalities that only manifest many years later (6; 7). A prenatal event consistently linked to increased risk of schizophrenia is maternal infection, particularly during early pregnancy (8; 9). However, even though it has a relatively high frequency of occurrence, prenatal infection has a quite modest effect size (10; 11). It has thus been proposed that a prenatal first hit may disrupt brain development and increase the vulnerability to a second hit occurring later in life (12–14). According to this two-hit hypothesis, neither insult alone is sufficient to induce schizophrenia, but only their combination. We have recently tested this idea using a translational mouse model,

and showed that combined exposure to prenatal immune challenge and peripubertal stress act synergistically to induce pathological effects on adult behavior and neurochemistry (15). In this model we observed transient signs of neuroinflammation, manifested by microglia activation and hypersecretion of inflammatory cytokines in stress-sensitive brain areas. These neuroinflammatory abnormalities preceded the emergence of behavioral dysfunctions, reinforcing the potential for preventive interventions.

Oxidative stress is closely associated with inflammation, and a growing body of evidence implicates the cytotoxic effects of reactive oxygen species (ROS) in the maturational deficits observed in the brains of people with schizophrenia (16; 17). N-acetylcysteine (NAC) is a glutathione precursor that exhibits antioxidant and anti-inflammatory properties. It also modulates extra-synaptic glutamate transmission through the activation of the cystine-glutamate antiporter (18; 19). NAC is clinically used as a mucolytic and as an antidote for paracetamol intoxication, and, in some countries, it is also sold as a dietary supplement. The potential of NAC as a useful drug in the treatment of various psychiatric disorders has been supported by several clinical and pre-clinical studies (20–22). In the present study, we used our two-hit model to test whether NAC administration during peripubertal stress exposure could prevent the subsequent onset of behavioral dysfunctions in mice offspring exposed to a prenatal immune challenge.

## Methods and Materials

### Animals

C57BL/6J male mice were used throughout the study. Female and male breeders were obtained from our in-house specific pathogen free (SPF) colony at the age of 10-14 weeks. Breeding began after 2 weeks of acclimatization to the animal holding rooms, which were temperature- and humidity-controlled (21±1 °C, 55±5%) holding facilities under a reversed light-dark cycle (lights off: 8:00 A.M. to 8:00 P.M.). All animals had *ad libitum* access to standard rodent chow (Kliba 3430, Klibamuehlen, Kaiseraugst, Switzerland) and water unless specified otherwise. All procedures described in the present study had been previously approved by the Cantonal Veterinarian's Office of Zurich and are in agreement with the principles of laboratory animal care in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication, 2011). All efforts were made to minimize the number of animals used and their suffering.

**Prenatal Immune Activation**

Female mice were subjected to a timed-mating procedure fully described previously (23). Pregnant dams on gestational day 9 (GD9), which roughly corresponds to the first trimester of human pregnancy, received either a single injection of synthetic double-stranded RNA poly(I:C) (polyinosinic-polycytidylic acid potassium salt; Sigma-Aldrich) at a dose of 1 mg/kg or vehicle (sterile pyrogen-free 0.9% NaCl). Solutions were injected via the intravenous (i.v.) route at the tail vein under mild physical constraint. The dose of poly(I:C) was chosen based on our previous findings showing that this immunological manipulation leads to modest and transient cytokine elevations in the maternal host (15). All solutions were freshly prepared at the day of administration, and animals were returned to their home cages immediately after the injection procedure.

**Peripubertal Stress Exposure**

Offspring born to poly(I:C)-treated (POL) or vehicle-treated (CON) mothers were weaned on postnatal day (PND) 21 and caged as littermates of 2-3 animals per cage. They were left undisturbed (= no stressor; S-) or exposed to variable and unpredictable stress between PND 30 and 40 (S+). This time window was selected based on our previous findings showing that this is a critical period of vulnerability for stress-induced neuropathological changes in prenatally immune-challenged animals (15). Littermates were preferentially assigned to different postnatal conditions in order to minimize potential confounding factors due to litter effects. The stress procedure included exposure to the following five stressors applied on alternate days and described in detail elsewhere (15): 1. electric foot shock; 2. restraint stress; 3. swimming stress; 4. food deprivation; 5. repeated home cage changes.

**N-acetylcysteine Treatment**

N-acetylcysteine (NAC; Sigma-Aldrich, Switzerland) was dissolved in regular tap water (1 g/L) and provided via regular drinking bottles. Vehicle (VEH)-exposed animals received tap water only. NAC dosage was estimated at circa 300 mg/kg/day. No significant differences in liquid intake between NAC and VEH groups were observed. Fresh solutions were provided every other day. NAC was administered throughout the peripubertal stress exposure: treatment started 24 h before the first stressor on PND 30 and ended after the last stress procedure on PND 40.

**Behavioral Analyses**

Behavioral tests started 4 weeks after exposure to the last stressor, and were performed between PND 70 and PND 100. This age corresponds to the early adult stage of maturation when the combined effects of prenatal immune activation and peripubertal stress become behaviorally manifested (15). A schematic representation of the experimental design is shown in **Figure 1**.

**Elevated Plus Maze**

The elevated plus maze was used to evaluate innate anxiety-like behavior. The apparatus was made of Plexiglas and consisted of 4 equally spaced arms (5 × 30 cm) radiating from a square center (5 × 5 cm). One pair of opposing arms was enclosed with black walls (height: 15 cm) except for the side adjoining the central square (CZ). The remaining two arms were exposed with a parameter border (height: 3 mm) along the outer edges. The maze was elevated 70 cm above floor level and positioned in a testing room with diffused lighting (approximately 20 lux in open arm and 10 lux in closed arm). A digital camera mounted above the plus maze captured images at a rate of 5 Hz and transmitted them to a PC running the EthoVision tracking system (Noldus Technology, Wageningen, The Netherlands). A test session began by placing the animal in the CZ facing one of the closed arms, followed by 5 min of free exploration before the subject was returned to the home cage. After each trial, the apparatus was cleaned with water and dried. The relative (percent) open arm entries during the 5-min test period were analyzed in order to index anxiety-related behavior. The percent open arm frequency was calculated as [(open arm entries) / (total arm entries) × 100]. In addition, total distance moved in the entire maze was analyzed in order to assess general locomotor activity.

**Prepulse Inhibition of the Acoustic Startle Reflex**

Sensorimotor gating was assessed by the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex. The test refers to the reduced startle reaction in response to a startle-eliciting pulse stimulus when this is shortly preceded by a weak prepulse stimulus. The apparatus consisted of four startle chambers for mice (San Diego Instruments, San Diego, CA, USA) and has been fully described elsewhere (23). During a 45-min test session, subjects were presented with a series of discrete test trials comprising a mixture of four trial types: pulse-alone, prepulse-plus-pulse, prepulse-alone, and no-stimulus (no stimulus other than the constant 65 $dB_A$ background noise). The startle program consisted of three different intensities of a 40-ms white noise pulse (100, 110, and 120 $dB_A$) combined with three different intensities of a 20-ms prepulse (71, 77, and 83 $dB_A$, which corresponded to 6, 12, and 18 dB above background, respectively). The stimulus-onset asynchrony of the prepulse and pulse stimuli on all prepulse-plus-pulse trials was 100 ms (onset-to-onset). Each session began with a 2-min acclimatization period in the Plexiglas enclosure, followed by 6 consecutive pulse-alone trials in order to habituate and stabilize the startle response. Subsequently, each trial stimulus was presented 12 times in a pseudorandom order with an average interval between successive trials of 15 ± 5 s. The session was concluded with 6 consecutive pulse-alone trials. Boxes were cleaned with water and dried between sessions. For each subject PPI was indexed as percent inhibition of startle response obtained in the prepulse-plus-trials compared to pulse-alone trials by following the expression: [1-(mean reactivity on prepulse-plus-pulse trials / mean reactivity on pulse-alone trials) × 1/100]. The first and last six trials were not included in the calculation of percent PPI. In addition to PPI, reactivity to pulse-alone trials and prepulse-alone trials were also analyzed.

**Locomotor Sensitivity to Amphetamine Challenge**

Amphetamine-induced locomotor activity in an open field arena was used to assess the sensitivity to this indirect dopamine receptor agonist. The apparatus consisted of 4 identical square arenas (40 × 40 × 35 cm) made of white Plexiglas, located in a testing room under dim diffused lighting (approximately 35 lux as measured in the center of the arenas). A digital camera mounted directly above the 4 arenas captured images at a rate of 5 Hz. Videos were analyzed with Ethovision tracking system (Noldus, Wageningen, The Netherlands). Animals were injected intraperitoneally (i.p.) with vehicle solution (isotonic 0.9% NaCl) and immediately placed in the apparatus to measure basal locomotor activity for 30 min. Subsequently, the animals were removed from the apparatus and treated with amphetamine (AMPH, 2.5 mg/kg, i.p.; D-amphetamine sulfate, Sigma-Aldrich, Switzerland). They were then immediately returned to the same arena, and the locomotor response was recorded for 90 min. Solutions were injected in a volume of 5 mL/kg.

**Statistical Analyses**

All data were analyzed using analysis of variance (ANOVA) to identify the main effects of prenatal immune treatment, postnatal stress exposure, and preventive NAC treatment, as well as their interactions. The number of individual offspring in each treatment condition was used as the sample sizes in each ANOVA. Fisher's least significant difference (LSD) post-hoc tests were used whenever significant interactions were obtained by the initial ANOVAs. Statistical significance

was set at $p<0.05$. All statistical analyses were performed using the statistical software StatView (version 5.0).

## Results

### N-acetylcysteine Does Not Prevent Anxiety-Like Behavior Induced by Peripubertal Stress

In agreement with our previous findings (15), peripubertal stress increased anxiety-like behavior in the elevated plus maze test regardless of the prenatal immune history. As shown in **Figure 2A**, stressed offspring, regardless of prenatal condition, displayed a significant reduction in the frequency to enter the open arms compared to non-stressed animals (main effect of stress: $F_{1,60}=12.4$, $p<0.001$). NAC failed to prevent the stress-induced changes in anxiety-like behavior, since a comparable reduction in open arm frequencies was observed in stressed animals regardless of whether they received NAC or VEH (**Figure 2A**). The stress protocol also induced changes in locomotor activity, as the total distance moved on the elevated plus maze was increased in stressed animals (main effect of stress: $F_{1,60}=11.0$, $p<0.01$); NAC did not affect the distance moved during the test (**Figure 2B**).

### N-acetylcysteine Prevents the Emergence of Sensorimotor Gating Deficits Induced by Prenatal Immune Activation Combined with Peripubertal Stress

In a next series of experiments we tested whether NAC treatment could prevent the emergence of sensorimotor gating deficits in adult animals submitted to the two-hit model. Sensorimotor gating was evaluated using the paradigm of PPI of

the acoustic startle reflex. In line with our previous findings (15), we observed an interaction between the two environmental manipulations: neither prenatal immune activation alone, nor stress alone, was sufficient to significantly affect PPI in VEH-treated animals, but only the combination of the two insults resulted in a significant reduction of PPI (**Figure 3**). Peripubertal NAC administration was effective in preventing the disruption of PPI induced by combined exposure to immune activation and stress (prenatal × stress × treatment interaction: $F_{1,60}=5.7$, $p<0.05$).

The beneficial effects of NAC on PPI scores in stressed POL offspring are independent of possible influences on startle reactivity and prepulse-induced reactivity, since there were no prenatal × stress × treatment × pulse or prenatal × stress × treatment × prepulse interactions ($F_{2,120}=0.05$, $p>0.05$ and $F_{2,120}=0.04$, $p>0.05$, respectively).

**N-acetylcysteine Prevents the Emergence of Hypersensitivity to Amphetamine Induced by Prenatal Immune Activation Combined with Peripubertal Stress**

Another pathological consequence of combined exposure to prenatal immune activation and peripubertal stress is the adult onset of increased sensitivity to psychotomimetic drugs (15). We tested whether NAC treatment could prevent the interactive effects between the two environmental manipulations on the development of potentiated locomotor response to the indirect dopamine receptor agonist amphetamine (AMPH). Consistent with our previous report, stress exposure in POL offspring markedly increased AMPH-induced locomotor activity in the open field test compared to stress exposure in CON offspring (**Figure 4**). NAC treatment fully prevented this pathological phenotype in stressed POL offspring (prenatal × stress × treatment × bins interaction: $F_{11,660}=4.0$, $p<0.0001$). However, NAC increased

sensitivity to amphetamine in non-stressed POL offspring, as POL/S-/NAC group did not differ from POL/S+/VEH, while it differed from POL/S-/VEH offspring at individual bins (**Figure 4A**). ANOVA restricted to non-stressed groups revealed a significant treatment × bins interaction ($F_{11,330}$=3.4, p<0.0001). This suggests that NAC may have opposite effects depending on the prenatal and postnatal histories of the animals. Namely, NAC can blunt sensitivity to amphetamine in POL/S+ animals, and potentiate it in POL/S- animals.

NAC treatment did not affect basal locomotor activity as assessed during the initial vehicle exposure phase that preceded the subsequent AMPH challenge (main effect of treatment: $F_{1,60}$=0.22, p>0.05). This suggests that the beneficial effects of NAC against AMPH-induced hyperactivity emerge independently of possible influences on basal locomotor activity.

**Discussion**

In our environmental two-hit model, prenatal immune challenge interacted with peripubertal stress to induce pathological effects on adult behaviors, including PPI deficits and increased sensitivity to the locomotor effects of AMPH. NAC administration during the course of peripubertal stress prevented the emergence of such behavioral abnormalities in adult offspring exposed to prenatal immune activation. In contrast, it did not normalize the increased anxiety-like behavior induced by peripubertal stress regardless of exposure to the first environmental hit. These findings indicate that NAC does not protect against stress-induced behavioral abnormalities in general, but prevents the pathological consequences of the interaction between prenatal immune activation and peripubertal stress exposure.

The preventive effects of NAC on PPI deficits and hypersensitivity to amphetamine are consistent with the beneficial effects reported in other rodent models. We have previously showed that treatment with NAC (60 or 120 mg/kg/day) during a critical developmental period prevented the increased sensitivity to amphetamine in social isolation-reared mice (24). More recently, Cabungcal et al. showed that NAC treatment during adolescence prevents the emergence of electrophysiological, histological and behavioral abnormalities in the neonatal ventral hippocampal lesion rat model (25). The antioxidants apocynin, a NADPH oxidase inhibitor, and ebselen, a glutathione peroxidase mimic, were also effective in preventing the behavioral deficits in this lesion model (25).

Clinical trials have already established the positive effects of NAC as an adjunctive treatment for neuropsychiatric disorders when initiated after the onset of overt psychopathological symptoms (22). The preventive potential of NAC when given during prodromal stages, however, still awaits examination. In view of the converging findings suggesting that dysregulation in redox/inflammatory processes may be relevant especially in prodromal and first-episode subjects (26–28), early intervention with NAC may be effective.

Even though it is known that different pathophysiological processes are relevant at different stages (29; 30), unfortunately current treatment does not match the course of the disorder. Ideally, prevention and intervention strategies should be tailored according to the stage of the disorder. It is important to emphasize, however, that the accurate identification of at-risk subjects is critical to avoid intervention in individuals who might turn out to be false-positives (31–33). This matter is even more relevant considering that drugs can have opposite effects depending on the biological context. In the case of our study, NAC somewhat potentiated the effects of

amphetamine in non-stressed poly(I:C) offspring, even though it fully prevented the hypersensitivity in animals exposed to both hits. In the work of Cabungcal et al. (25), NAC seemed to increase the levels of the oxidative stress marker 8-oxo-dG in the prefrontal cortex of adult sham animals. It is hard to predict what would be the consequences of NAC administration during a critical developmental period in subjects wrongly identified as at-risk for psychosis. Hopefully, the analysis of neuroimaging and genetic data now available will help advance the development of biomarkers that will result in much improved selection criteria in the near future.

A limitation of our study is that we cannot at this point define the exact mechanism(s) underlying the preventive effects of NAC in our model. Investigating whether oxidative stress is present in relevant brain areas of animals submitted to the two-hit model is an important next step. However, considering that NAC targets antioxidant, inflammatory, glutamatergic and neurotrophic pathways (34–36), it is possible that multiple actions are involved in its overall mechanism of action. We believe that the ability of NAC to modulate glutamate is an advantage rather than a caveat. For instance, in rats prenatally exposed to valproate, the effects of NAC in preventing autistic-like behavior were blocked by an antagonist of metabotropic glutamate receptors 2/3 (37).

In this study we restricted NAC treatment to the time of peribubertal stress exposure, mainly because: first, this maturational period was associated with microglia activation and increased expression of inflammatory cytokines (15); and second, it is hypothesized that therapeutic interventions are most effective if they are administered during critical periods of vulnerability early in the course of the illness (30; 38; 39). In the interest of translational value, it is also necessary to probe whether NAC can prevent the onset of behavioral abnormalities when administered

after the stress exposure. The effects of NAC treatment in adult animals after the onset of behavioral symptoms also remain to be investigated.

The peripubertal period is a critical developmental stage in which pathophysiological conditions can negatively affect the developing brain, and also represents a window of opportunity for therapeutic intervention. Our findings provide novel evidence that NAC has preventive effects against adult behavioral abnormalities in a two-hit model that incorporates two environmental risk factors implicated in neuropsychiatric disorders, especially in schizophrenia. In conclusion, our preclinical data support attempts to explore the use of NAC as a tool for prophylactic psychiatry, and encourages the implementation of clinical trials to evaluate the effects of NAC in prodromal and first-episode subjects.

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**Financial Disclosures**

All authors declare that they have no conflicts of interest to disclose.

**References**

1. Global Burden of Disease Study 2013 Collaborators (2015): Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. . doi: 10.1016/S0140-6736(15)60692-4.

2. Insel TR (2010): Rethinking schizophrenia. *Nature*. 468: 187–193.

3. Jacka FN, Berk M (2014): Prevention of Schizophrenia—Will a Broader Prevention Agenda Support This Aim? *Schizophr Bull*. 40: 237–239.

4. McGorry PD (2015): Early Intervention in Psychosis. *J Nerv Ment Dis*. 203: 310–318.

5. McGorry PD, Purcell R, Goldstone S, Amminger GP (2011): Age of onset and timing of treatment for mental and substance use disorders: implications for preventive intervention strategies and models of care. *Curr Opin Psychiatry*. 24: 301–306.

6. Marenco S, Weinberger DR (2000): The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. *Dev Psychopathol*. 12: 501–527.

7. Rapoport JL, Addington AM, Frangou S, Psych MRC (2005): The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry*. 10: 434–449.

8. Brown AS (2012): Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. *Dev Neurobiol*. 72: 1272–1276.

9. Brown AS, Derkits EJ (2010): Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry*. 167: 261–280.

10. Mednick SA, Machon RA, Huttunen MO, Bonett D (1988): Adult schizophrenia following prenatal exposure to an influenza epidemic. *Arch Gen Psychiatry*. 45: 189–192.

11. Selten J-P, Frissen A, Lensvelt-Mulders G, Morgan VA (2010): Schizophrenia and 1957 pandemic of influenza: meta-analysis. *Schizophr Bull*. 36: 219–228.

12. Bayer TA, Falkai P, Maier W (1999): Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the “two hit hypothesis.” *J Psychiatr Res*. 33: 543–548.

13. Clarke MC, Tanskanen A, Huttunen M, Whittaker JC, Cannon M (2009): Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. *Am J Psychiatry*. 166: 1025–1030.

14. Maynard TM, Sikich L, Lieberman JA, LaMantia AS (2001): Neural development, cell-cell signaling, and the “two-hit” hypothesis of schizophrenia. *Schizophr Bull*. 27: 457–476.

15. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, *et al.* (2013): Stress in Puberty Unmasks Latent Neuropathological Consequences of Prenatal Immune Activation in Mice. *Science*. 339: 1095–1099.

16. Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M (2009): Redox dysregulation, neurodevelopment, and schizophrenia. *Curr Opin Neurobiol*, Development. 19: 220–230.

17. Kulak A, Steullet P, Cabungcal J-H, Werge T, Ingason A, Cuenod M, Do KQ (2012): Redox Dysregulation in the Pathophysiology of Schizophrenia and Bipolar Disorder: Insights from Animal Models. *Antioxid Redox Signal*. 18: 1428–1443.

18. Baker DA, McFarland K, Lake RW, Shen H, Toda S, Kalivas PW (2003): N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. *Ann N Y Acad Sci*. 1003: 349–351.

19. Baker DA, Xi Z-X, Shen H, Swanson CJ, Kalivas PW (2002): The origin and neuronal function of in vivo nonsynaptic glutamate. *J Neurosci Off J Soc Neurosci*. 22: 9134–41.

20. Berk M, Malhi GS, Gray LJ, Dean OM (2013): The promise of N-acetylcysteine in neuropsychiatry. *Trends Pharmacol Sci*. 34: 167–177.

21. Dean O, Giorlando F, Berk M (2011): N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. *J Psychiatry Neurosci JPN*. 36: 78–86.

22. Deepmala null, Slattery J, Kumar N, Delhey L, Berk M, Dean O, *et al.* (2015): Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. *Neurosci Biobehav Rev*. 55: 294–321.

23. Meyer U, Feldon J, Schedlowski M, Yee BK (2005): Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev*. 29: 913–947.

24. Herrmann AP, Benvenutti R, Pilz LK, Elisabetsky E (2014): N-acetylcysteine prevents increased amphetamine sensitivity in social isolation-reared mice. *Schizophr Res*. 155: 109–111.

25. Cabungcal J-H, Counotte DS, Lewis EM, Tejeda HA, Piantadosi P, Pollock C, *et al.* (2014): Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. *Neuron*. 83: 1073–1084.

26. Cannon TD, Chung Y, He G, Sun D, Jacobson A, van Erp TGM, *et al.* (2015): Progressive Reduction in Cortical Thickness as Psychosis Develops: A Multisite Longitudinal Neuroimaging Study of Youth at Elevated Clinical Risk. *Biol Psychiatry*, Schizophrenia and Neurodevelopment. 77: 147–157.

27. Pasternak O, Westin C-F, Dahlben B, Bouix S, Kubicki M (2015): The extent of diffusion MRI markers of neuroinflammation and white matter deterioration in chronic schizophrenia. *Schizophr Res*. 161: 113–118.

28. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB (2014): Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. *JAMA Psychiatry*. 71: 1121–1128.

29. Pantelis C, Yücel M, Wood SJ, Velakoulis D, Sun D, Berger G, *et al.* (2005): Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. *Schizophr Bull*. 31: 672–696.

30. Tandon R, Nasrallah HA, Keshavan MS (2010): Schizophrenia, “just the facts” 5. Treatment and prevention. Past, present, and future. *Schizophr Res*. 122: 1–23.

31. Marshall M, Rathbone J (2011): Early intervention for psychosis. *Cochrane Database Syst Rev*. CD004718.

32. De Koning MB, Bloemen OJN, van Amelsvoort TAMJ, Becker HE, Nieman DH, van der Gaag M, Linszen DH (2009): Early intervention in patients at ultra high risk of psychosis: benefits and risks. *Acta Psychiatr Scand*. 119: 426–442.

33. Stafford MR, Jackson H, Mayo-Wilson E, Morrison AP, Kendall T (2013): Early interventions to prevent psychosis: systematic review and meta-analysis. *The BMJ*. 346: f185.

34. Dean O, Giorlando F, Berk M (2011): N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. *J Psychiatry Neurosci JPN*. 36: 78–86.

35. Oja SS, Janáky R, Varga V, Saransaari P (2000): Modulation of glutamate receptor functions by glutathione. *Neurochem Int*. 37: 299–306.

36. Palacio JR, Markert UR, Martínez P (2011): Anti-inflammatory properties of N-acetylcysteine on lipopolysaccharide-activated macrophages. *Inflamm Res Off J Eur Histamine Res Soc Al*. 60: 695–704.

37. Chen Y-W, Lin H-C, Ng M-C, Hsiao Y-H, Wang C-C, Gean P-W, Chen PS (2014): Activation of mGluR2/3 underlies the effects of N-acetylcystein on amygdala-associated autism-like phenotypes in a valproate-induced rat model of autism. *Front Behav Neurosci*. 8: 219.

38. Birchwood M, Todd P, Jackson C (1998): Early intervention in psychosis. The critical period hypothesis. *Br J Psychiatry Suppl*. 172: 53–59.

39. Francey SM, Nelson B, Thompson A, Parker AG, Kerr M, Macneil C, *et al.* (2010): Who needs antipsychotic medication in the earliest stages of psychosis? A reconsideration of benefits, risks, neurobiology and ethics in the era of early intervention. *Schizophr Res*. 119: 1–10.

## Figure Legends

**Figure 1.** Experimental design. First hit: prenatal viral-like immune challenge induced by the synthetic double-stranded RNA poly(I:C) (1 mg/kg, i.v.) on gestational day (GD) 9; control mothers received vehicle solution (0.9% NaCl, i.v.). Offspring born to poly(I:C)-treated (POL) or vehicle-treated (CON) mothers were weaned on postnatal day (PND) 21. Second hit: during PND 30-40 mice were left undisturbed (S-) or exposed to variable and unpredictable stress (S+). The stress protocol, applied on alternate days, included: 1. electric foot shock; 2. restraint stress; 3. swimming stress; 4. water deprivation; 5. repeated home cage change. Simultaneously with the second hit animals received N-acetylcysteine (NAC) dissolved in tap water and provided via drinking bottles (1 g/L, p.o.); vehicle (VEH)-exposed animals received tap water only. Treatment started 24 h before the first stressor and ended after the last stress procedure on PND 40. The long-term behavioral effects of NAC treatment on single or combined exposure to prenatal immune challenge and peripubertal stress were assessed between PND 70 and 100.

**Figure 2.** Effects of N-acetylcysteine on the elevated plus maze in mice exposed to the two-hit model. Pregnant mice were injected with control solution (CON) or poly(I:C) (POL), and the resulting offspring were left undisturbed (S-) or subjected to unpredictable stress (S+) during peripubertal development. Animals received regular tap water (VEH) or N-acetylcysteine (NAC) throughout the stress procedure. **(A)** The bar plot shows the percent entries in the open arm (%). $p$=0.0008 refers to the significant main effect of peripubertal stress. **(B)** The bar plot shows the total distance

moved (m) during the entire test period. $p$=0.001 refers to the significant main effect of peripubertal stress. $n$=8-9 per group. Mean + SEM.

**Figure 3.** Effects of N-acetylcysteine on the prepulse inhibition of the acoustic startle reflex in mice exposed to the two-hit model. Pregnant mice were injected with control (CON) solution or poly(I:C) (POL), and the resulting offspring were **(A)** left undisturbed (S-) or **(B)** subjected to unpredictable stress (S+) during peripubertal development. Animals received regular tap water (VEH) or N-acetylcysteine (NAC) throughout the stress procedure. The line plots depict the percent prepulse inhibition as a function of increasing prepulse intensities (dB above background of 65 dB). The bar plots show the mean percent prepulse inhibition across the 3 prepulse intensities. *$p$<0.01, reflecting the significant difference between POL/S+/VEH offspring and the other S+ groups. $n$=8-9 per group. Mean + SEM.

**Figure 4.** Effects of N-acetylcysteine on the locomotor response to acute amphetamine challenge in mice exposed to the two-hit model. Pregnant mice were injected with control (CON) solution or poly(I:C) (POL), and the resulting offspring were **(A)** left undisturbed (S-) or **(B)** subjected to unpredictable stress (S+) during peripubertal development. Animals received regular tap water (VEH) or N-acetylcysteine (NAC) throughout the stress procedure. Locomotor activity was measured in an open field arena after vehicle (saline, SAL) injection and subsequent treatment with the indirect dopamine receptor agonist amphetamine (AMPH, 2.5 mg/kg, i.p.). The line plots depict the distance moved as a function of successive 5-min bins. The bar plots depict the mean distance moved after AMPH treatment. #$p$<0.05, reflecting the significant differences between POL/S-/NAC and POL/S-/VEH

offspring at individual bins; $^{\S}p<0.05$, reflecting the significant differences between POL/S+/VEH and the other S+ groups at individual bins. *$p<0.05$, **$p<0.01$ and ***$p<0.001$, reflecting the indicated differences in the S+ groups. $n$=8-9 per group. Mean + SEM.

**Figure 1**

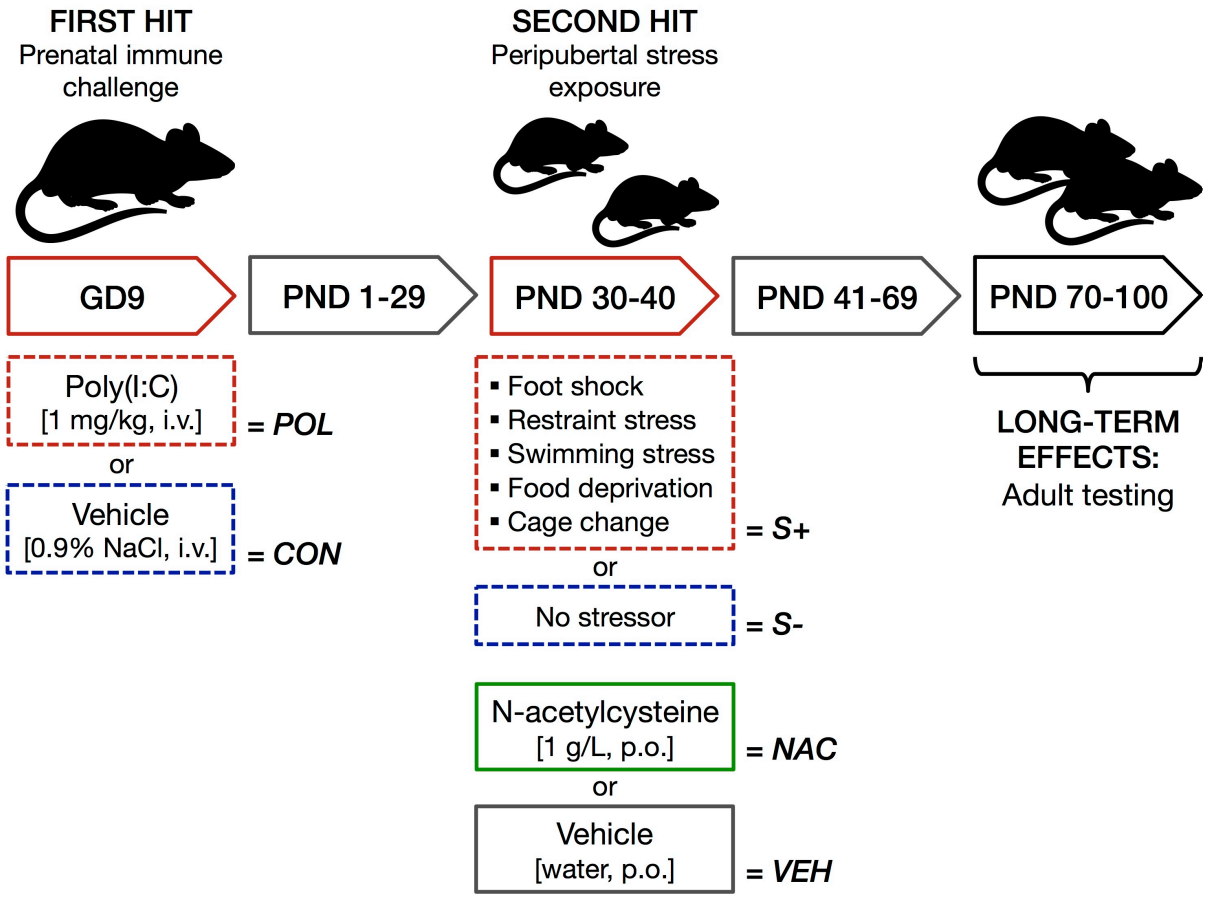

FIRST HIT
Prenatal immune challenge
SECOND HIT
Peripubertal stress exposure
GD9
PND 1-29
PND 30-40
PND 41-69
PND 70-100
Poly(I:C) [1 mg/kg, i.v.]
= POL
or
Vehicle [0.9% NaCl, i.v.]
= CON
▪ Foot shock
▪ Restraint stress
▪ Swimming stress
▪ Food deprivation
▪ Cage change
= S+
or
No stressor
= S-
N-acetylcysteine [1 g/L, p.o.]
= NAC
or
Vehicle [water, p.o.]
= VEH
LONG-TERM EFFECTS:
Adult testing

**Figure 2**

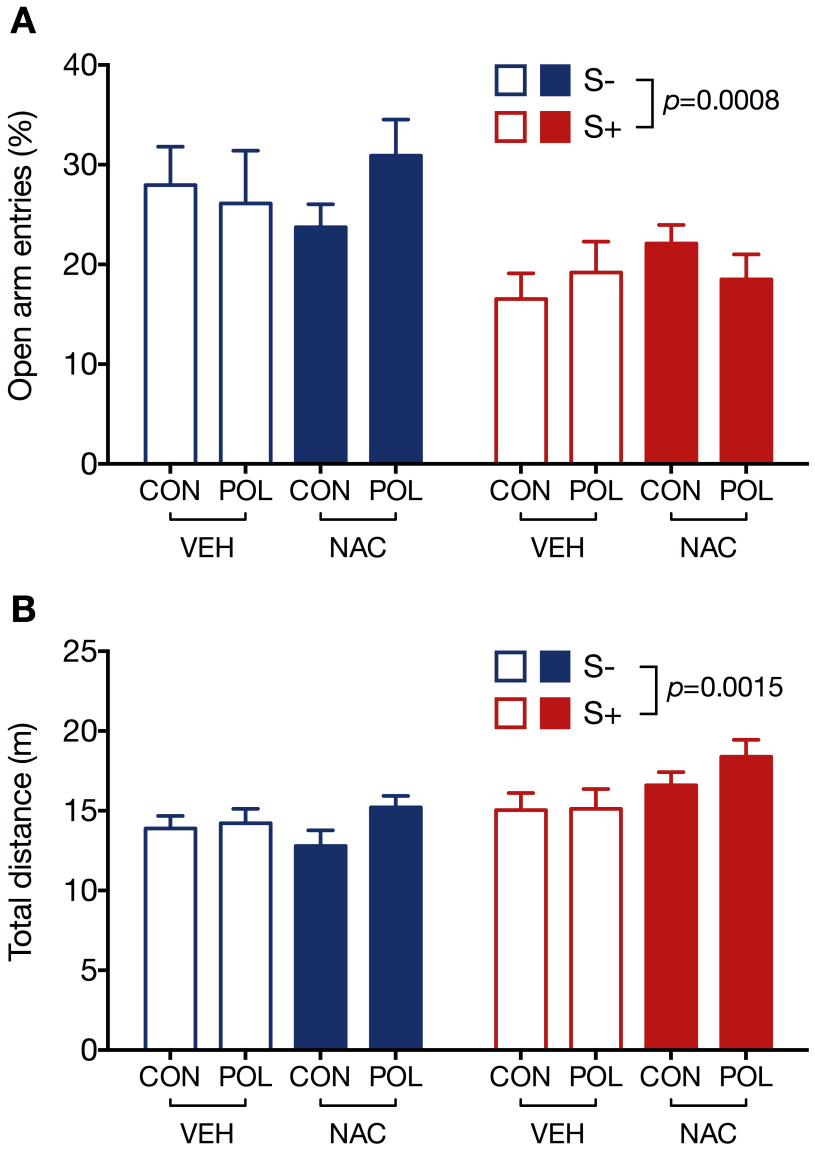

A
Open arm entries (%)
40
30
20
10
0
S-
S+
p=0.0008
CON POL CON POL CON POL CON POL
VEH NAC VEH NAC
B
Total distance (m)
25
20
15
10
5
0
S-
S+
p=0.0015
CON POL CON POL CON POL CON POL
VEH NAC VEH NAC

**Figure 3**

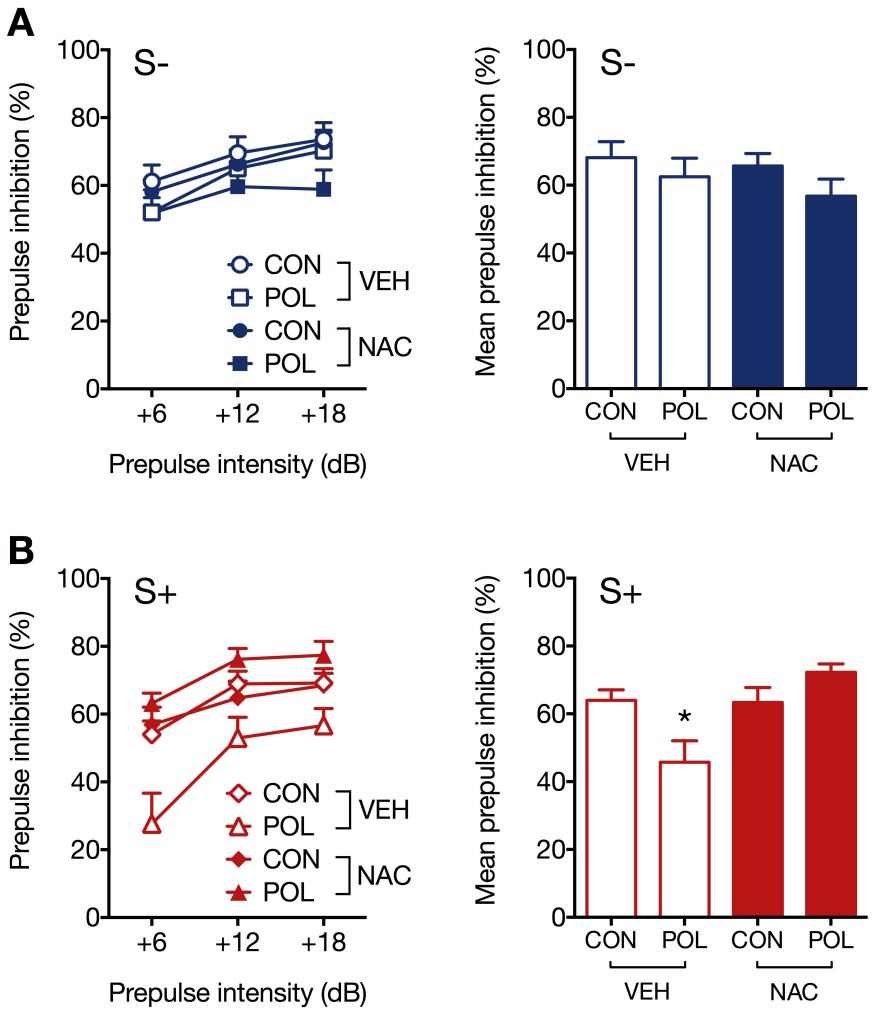
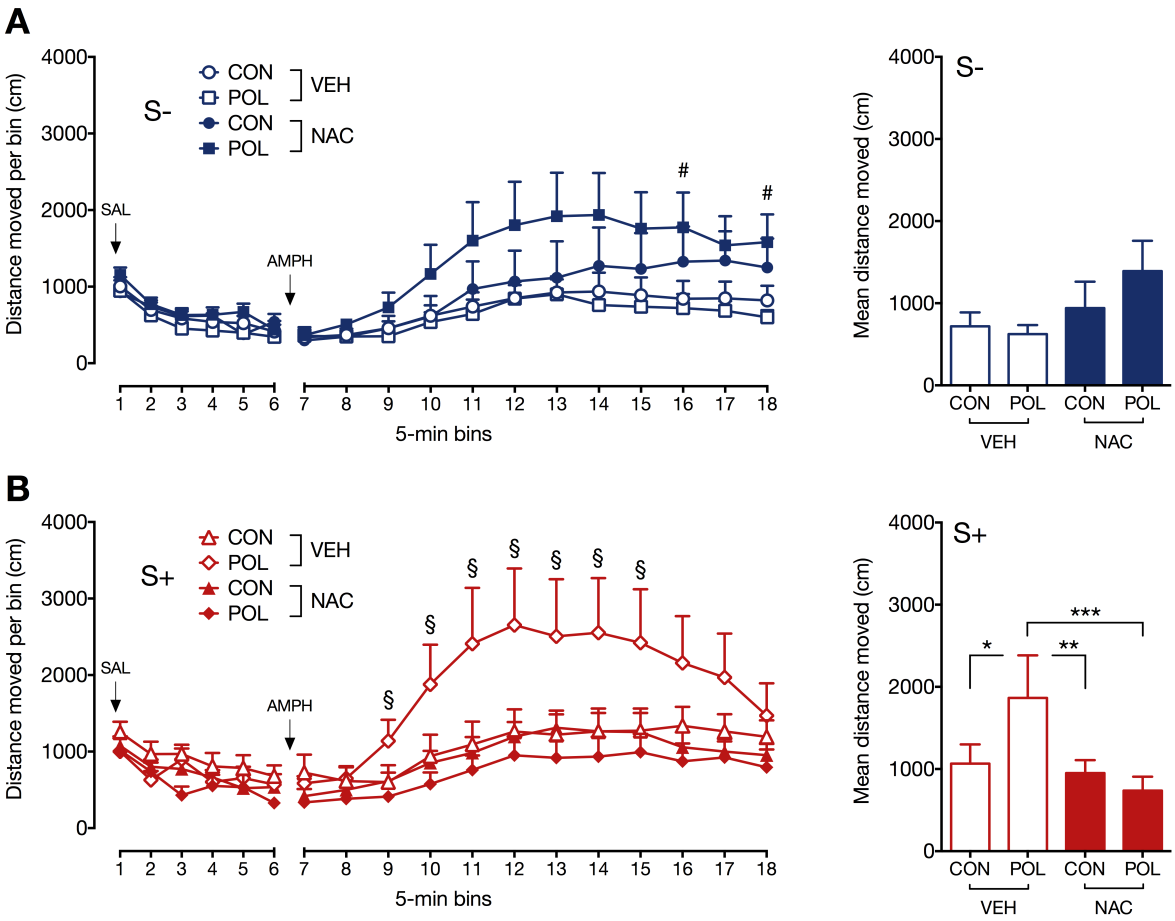

A
S-
Prepulse inhibition (%)
100
80
60
40
20
0
CON
POL
VEH
CON
POL
NAC
+6
+12
+18
Prepulse intensity (dB)
S-
Mean prepulse inhibition (%)
CON
POL
CON
POL
VEH
NAC
B
S+
Prepulse inhibition (%)
Prepulse intensity (dB)
S+
Mean prepulse inhibition (%)
*

**Figure 4**


A
S-
CON
POL
CON
POL
VEH
NAC
SAL
AMPH
Distance moved per bin (cm)
4000
3000
2000
1000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
5-min bins
#
#
Mean distance moved (cm)
CON POL CON POL
VEH NAC
B
S+
§
§
§
§
§
§
*
**
***

# PARTE III

*Onde os resultados da tese são discutidos*

## DISCUSSÃO

Nessa tese foram investigados os efeitos da N-acetilcisteína (NAC) em três modelos relevantes ao desenvolvimento da esquizofrenia. A hipótese de que NAC modula processos envolvidos na patofisiologia desse transtorno e, portanto, seria capaz de prevenir alterações comportamentais foi confirmada nesse trabalho. A figura abaixo (**figura 2**) ilustra os principais resultados obtidos nos modelos de isolamento social pós-desmame, sensibilização a anfetamina e ativação imune pré-natal combinada com estresse peripubertal variado.

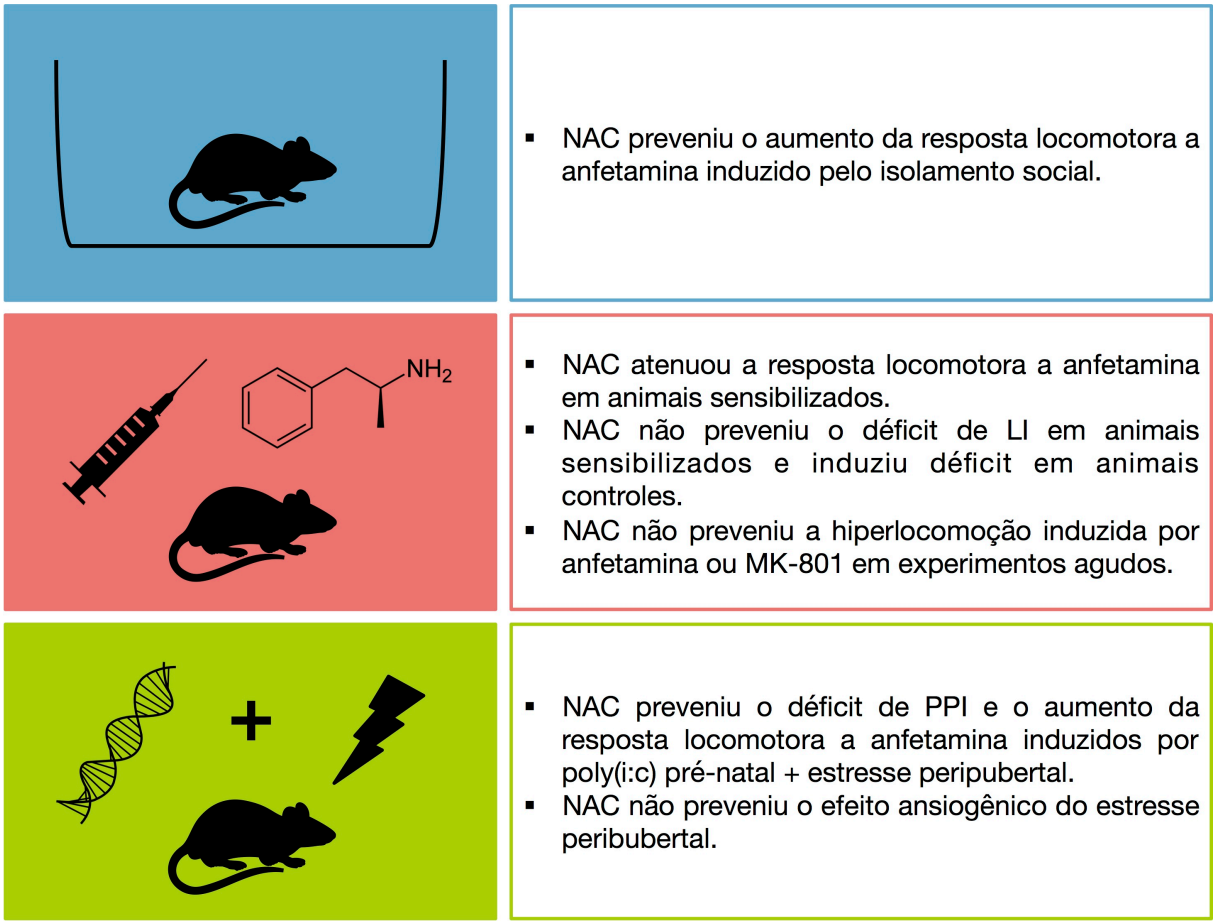



**Figura 2.** Principais resultados obtidos quanto aos efeitos da N-acetilcisteína (NAC) nos três modelos animais empregados nessa tese. LI: inibição latente, PPI: inibição por prepulso da resposta de sobressalto.

O conjunto de dados apresentados nessa tese deixa diversos questionamentos sem resposta, sendo que o principal deles se refere aos mecanismos que medeiam os efeitos de NAC. Não é possível nesse momento definir qual o mecanismo de ação de NAC responsável pelos efeitos observados, mas trabalhos já publicados na literatura indicam que a atividade antioxidante tem alta relevância nos efeitos preventivos de NAC no contexto da esquizofrenia. Cabungcal e colaboradores (2014), por exemplo, mostraram que os antioxidantes ebselen, um mímico da glutationa peroxidase, e apocinina, um inibidor da NADPH oxidase, apresentaram efeitos similares ao NAC ao prevenir o déficit de PPI induzido por lesão hipocampal neonatal em ratos. Além disso, Shirai e colaboradores (2015) investigaram os efeitos do antioxidante sulfurofano, um ativador do fator de transcrição Nrf2, no modelo de administração repetida de fenciclidina (um antagonista NMDA). Os autores observaram que o sulforano foi capaz de atenuar os déficits cognitivos, o dano oxidativo ao DNA e a diminuição no número de interneurônios que expressam parvalbumina no córtex pré-frontal e hipocampo de camundongos. Testar se outras substâncias com atividade exclusivamente antioxidante reproduzem os efeitos obtidos com NAC nos modelos aqui testados é uma possibilidade interessante para melhor definir o papel da ação antioxidante nesses modelos.

As propriedades neuroinflamatórias de NAC também devem ser relevantes nesse contexto, principalmente no modelo de “dois hits”, já que se observou ativação microglial em resposta ao estresse peripubertal em camundongos previamente expostos a ativação imune pré-natal (Giovanoli et al., 2013). Entretanto, a modulação glutamatérgica também pode estar envolvida nos efeitos de NAC. Tal hipótese poderia ser testada diretamente com a realização de experimentos com

antagonistas de receptores metabotrópicos de glutamato (mGluRs). Ao ativar o antiporter cistina-glutamato presente nos astrócitos, NAC aumenta a liberação extra-sináptica de glutamato, que por sua vez ativa mGluRs neuronais, inibindo a liberação sináptica de neurotransmissores. Baker e colaboradores (2008) observaram que esse mecanismo é responsável pelo efeito protetor de NAC contra os déficits cognitivos e de interação social induzidos por fenciclidina, e Lee e colaboradores (2014) mostraram que a ativação de mGluRs é necessária para o efeito de NAC contra os efeitos do agonista serotonérgico DOI. Essa abordagem farmacológica de utilizar antagonistas, porém, pode ser problemática, já que o bloqueio de mGluRs pode induzir efeitos *per se*, e o aumento no número de variáveis e grupos experimentais diminuiria o poder estatístico das análises em alguns dos modelos aqui testados.

Desregulação do estado redox, neuroinflamação e falhas na transmissão glutamatérgica são considerados um “hub central” na patofisiologia da esquizofrenia (Steullet et al., *in press*), e podem explicar as alterações funcionais e de conectividade desencadeadas por fatores ambientais e genéticos. Genes relevantes a esses três aspectos estão implicados nos “108 loci” associados a risco de esquizofrenia identificados no maior estudo de associação genômica em esquizofrenia publicado até agora (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Portanto, o mais provável é que os múltiplos mecanismos de ação de NAC possam contribuir para seus efeitos benéficos no contexto da esquizofrenia (**Figura 3**).

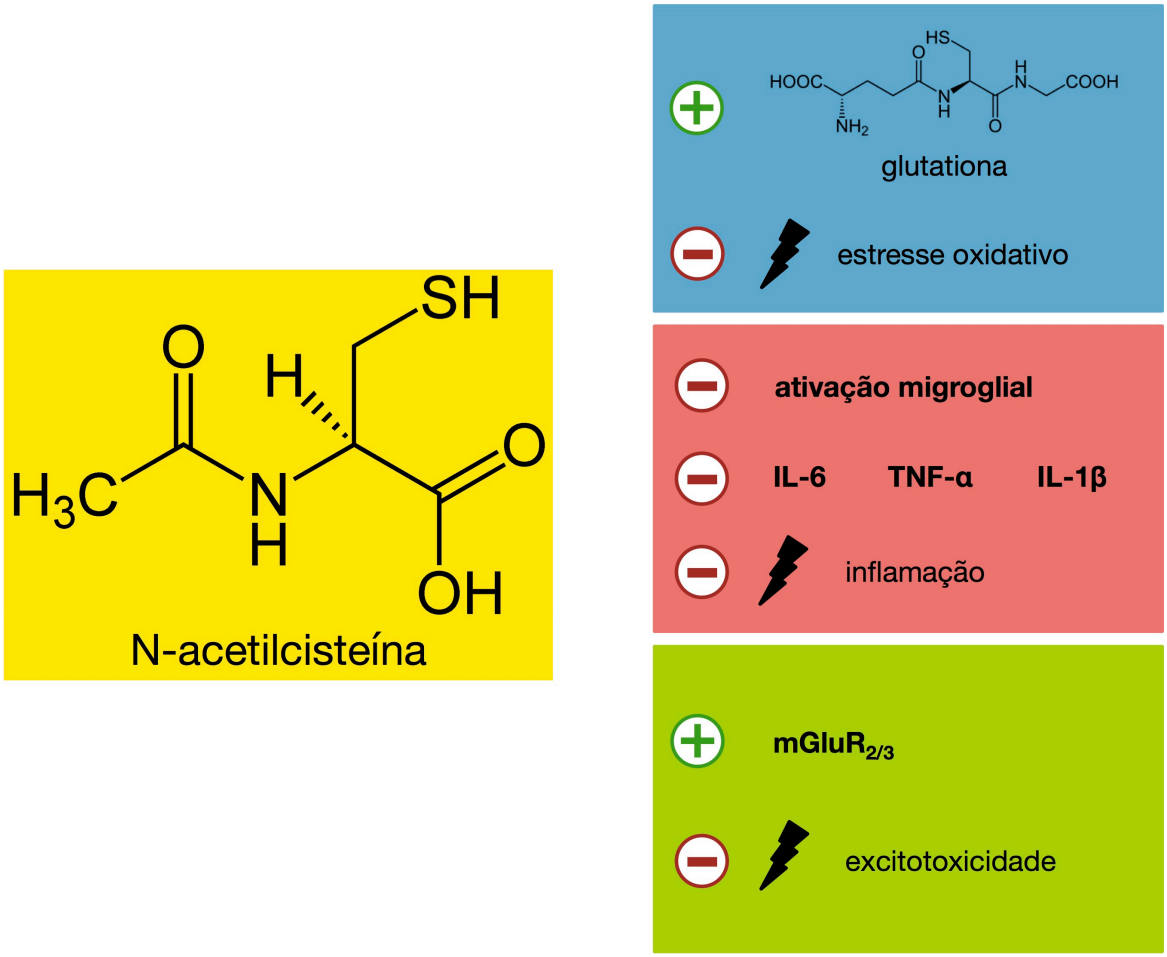



**Figura 3.** Mecanismos de ação da N-acetilcisteína com relevância para seus efeitos no contexto da esquizofrenia.

Algum esclarecimento sobre o mecanismo de ação de NAC será obtido com as análises dos encéfalos de uma segunda coorte de animais submetidos ao modelo de dois hits e tratados com NAC. 24 horas após o último estressor, os animais foram anestesiados e perfundidos com líquido cefalorraquidiano artificial; o hemisfério direito foi congelado e o esquerdo pós-fixado em paraformaldeído 4%. As amostras serão analisadas em cooperação com o Prof. Dr. Urs Meyer e serão verificados marcadores de estresse oxidativo e neuroinflamação através de análises histológicas e moleculares.

Os efeitos de NAC em animais controle também precisam ser melhor estudados. No modelo de sensibilização a anfetamina, por exemplo, NAC *per se*

causou um déficit de inibição latente. No modelo de dois hits, NAC aumentou a resposta a anfetamina em animais expostos a ativação imune pré-natal e não estressados na puberdade. É possível que o tratamento com NAC resulte em uma curva dose-efeito que pode ser explicada pelo fenômeno de hormese. De fato, pesquisas recentes têm demonstrado que níveis baixos de estresse oxidativo estão envolvidos em processos importantes de sinalização celular e produzem efeitos benéficos para a saúde (Ristow & Schmeisser, 2014; Ristow, 2014). Essas evidências colocam em dúvida a percepção de benefícios inquestionáveis dos antioxidantes que, dependendo do estado redox, podem ser contraproducentes ou inclusive prejudiciais. Em conjunto, esses dados reforçam a necessidade de selecionar com precisão os indivíduos que eventualmente serão submetidos ao tratamento com NAC com fins preventivos.

Nos capítulos apresentados nessa tese, NAC foi administrado tanto por via intraperitoneal quanto por via oral (diluído na água de beber). Sabe-se que a biodisponibilidade oral de NAC é baixa (Samuni et al., 2013), mas a administração repetida pode resultar em um aumento da biodisponibilidade. Apesar de não ser possível determinar com precisão a dose recebida por cada animal devido a variações no consumo de água, os efeitos obtidos com a administração oral foram robustos (capítulo 3), e uma importante vantagem é evitar a exposição dos animais a procedimentos estressantes em um período sensível do desenvolvimento.

Embora utilizamos apenas camundongos machos nos trabalhos dessa tese, sabe-se que existem diferenças de gênero no curso da esquizofrenia: em mulheres, o início dos sintomas ocorre em geral 4 a 5 anos mais tarde do que em homens, e há um segundo pico de início dos sintomas, em torno dos 45-50 anos de idade, o que coincide com a menopausa (Häfner, 2003; Häfner et al., 1991; Seeman and

Lang, 1990). Também foram documentadas diferenças de gênero quanto a resposta ao tratamento (Goldstein et al., 1998; Grigoriadis and Seeman, 2002) e vulnerabilidade a estressores (DeSantis et al., 2011). Apesar dessas diferenças, o modelo de dois-hits não revelou qualquer efeito sexo-dependente nos principais correlatos comportamentais, neuroanatômicos e neuroquímicos investigados (Giovanoli et al., 2013). O efeito preventivo de NAC nesse modelo também foi investigado em fêmeas, e não houve diferenças nos resultados em comparação com camundongos machos (dados não incluídos na tese). Isso sugere que os hormônios sexuais, apesar de suas propriedades neuromodulatórias, não interferem de forma significativa no estabelecimento das alterações comportamentais induzidas pelo modelo e na eficácia do tratamento com NAC.

Desde os tempos de Emil Kraepelin a esquizofrenia foi associada a um futuro sem esperanças. Apesar de ser um transtorno grave e de não haver, na época, tratamentos farmacológicos eficazes, em 1927 essa visão já havia sido desafiada pelo psiquiatra Harry Sullivan, autor do seguinte pensamento: "*I feel certain that many incipient cases might be arrested before the efficient contact with reality is suspended, and a long stay in institutions made necessary*" (Sullivan, 1927). Quase um século depois, os avanços da ciência e dos programas de intervenção precoce indicam que essa "impressão" pode estar mais perto de se tornar realidade.

## CONCLUSÃO

O estudo de possíveis estratégias de prevenção é particularmente importante para transtornos psiquiátricos como a esquizofrenia, cuja chance de recuperação funcional completa é muito baixa uma vez estabelecida a doença em sua forma plena. A adolescência é uma fase crítica de vulnerabilidade, mas também representa uma janela

de oportunidade para prevenção, e os dados apresentados nessa tese corroboram o uso de NAC como estratégia farmacológica com potencial para atenuar, adiar, ou mesmo prevenir o surgimento de alterações comportamentais características de transtornos psicóticos. Ensaios clínicos em indivíduos em risco de converter a psicose são necessários para avaliar a real eficácia e segurança desse fármaco.

# REFERÊNCIAS BIBLIGRÁFICAS

Achat-Mendes, C., Anderson, K.L., and Itzhak, Y. (2007). Impairment in consolidation of learned place preference following dopaminergic neurotoxicity in mice is ameliorated by N-acetylcysteine but not D1 and D2 dopamine receptor agonists. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *32*, 531–541.

Alaräisänen, A., Miettunen, J., Räsänen, P., Fenton, W., Koivumaa-Honkanen, H.-T.J., and Isohanni, M. (2009). Suicide rate in schizophrenia in the Northern Finland 1966 Birth Cohort. Soc. Psychiatry Psychiatr. Epidemiol. *44*, 1107–1110.

Amen, S.L., Piacentine, L.B., Ahmad, M.E., Li, S.-J., Mantsch, J.R., Risinger, R.C., and Baker, D.A. (2011). Repeated N-acetyl cysteine reduces cocaine seeking in rodents and craving in cocaine-dependent humans. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *36*, 871–878.

American Psychiatric Association (2014). Manual diagnóstico e Estatístico de Transtornos Mentais - 5.ed.: DSM-5 (Artmed Editora).

Amminger, G.P., Schäfer, M.R., Papageorgiou, K., Klier, C.M., Cotton, S.M., Harrigan, S.M., Mackinnon, A., McGorry, P.D., and Berger, G.E. (2010). Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch. Gen. Psychiatry *67*, 146–154.

Andreasen, N.C. (2008). The relationship between creativity and mood disorders. Dialogues Clin. Neurosci. *10*, 251–255.

Andréasson, S., Allebeck, P., Engström, A., and Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet Lond. Engl. *2*, 1483–1486.

Arendt, M., Rosenberg, R., Foldager, L., Perto, G., and Munk-Jørgensen, P. (2005). Cannabis-induced psychosis and subsequent schizophrenia-spectrum disorders: follow-up study of 535 incident cases. Br. J. Psychiatry J. Ment. Sci. *187*, 510–515.

Arent, C.O., Réus, G.Z., Abelaira, H.M., Ribeiro, K.F., Steckert, A.V., Mina, F., Dal-Pizzol, F., and Quevedo, J. (2012). Synergist effects of n-acetylcysteine and deferoxamine treatment on behavioral and oxidative parameters induced by chronic mild stress in rats. Neurochem. Int. *61*, 1072–1080.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., and Moffitt, T.E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ *325*, 1212–1213.

Arseneault, L., Cannon, M., Witton, J., and Murray, R.M. (2004). Causal association between cannabis and psychosis: examination of the evidence. Br. J. Psychiatry J. Ment. Sci. *184*, 110–117.

Awad, N., Khatib, N., Ginsberg, Y., Weiner, Z., Maravi, N., Thaler, I., Ross, M.G., Itsokovitz-Eldor, J., and Beloosesky, R. (2011). N-acetyl-cysteine (NAC) attenuates LPS-induced maternal and amniotic fluid oxidative stress and inflammatory responses in the preterm gestation. Am. J. Obstet. Gynecol. *204*, 450.e15–e20.

Babulas, V., Factor-Litvak, P., Goetz, R., Schaefer, C.A., and Brown, A.S. (2006). Prenatal exposure to maternal genital and reproductive infections and adult schizophrenia. Am. J. Psychiatry *163*, 927–929.

Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Toda, S., and Kalivas, P.W. (2003a). N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. Ann. N. Y. Acad. Sci. *1003*, 349–351.

Baker, D.A., McFarland, K., Lake, R.W., Shen, H., Tang, X.-C., Toda, S., and Kalivas, P.W. (2003b). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat. Neurosci. *6*, 743–749.

Baker, D.A., Madayag, A., Kristiansen, L.V., Meador-Woodruff, J.H., Haroutunian, V., and Raju, I. (2008). Contribution of cystine-glutamate antiporters to the psychotomimetic effects of phencyclidine. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *33*, 1760–1772.

Bauzo, R.M., Kimmel, H.L., and Howell, L.L. (2012). The cystine-glutamate transporter enhancer N-acetyl-L-cysteine attenuates cocaine-induced changes in striatal dopamine but not self-administration in squirrel monkeys. Pharmacol. Biochem. Behav. *101*, 288–296.

Bayer, T.A., Falkai, P., and Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the “two hit hypothesis.” J. Psychiatr. Res. *33*, 543–548.

Beloosesky, R., Gayle, D.A., Amidi, F., Nunez, S.E., Babu, J., Desai, M., and Ross, M.G. (2006). N-acetyl-cysteine suppresses amniotic fluid and placenta inflammatory cytokine responses to lipopolysaccharide in rats. Am. J. Obstet. Gynecol. *194*, 268–273.

Beloosesky, R., Weiner, Z., Khativ, N., Maravi, N., Mandel, R., Boles, J., Ross, M.G., and Itskovitz-Eldor, J. (2009). Prophylactic maternal n-acetylcysteine before lipopolysaccharide suppresses fetal inflammatory cytokine responses. Am. J. Obstet. Gynecol. *200*, 665.e1–e5.

Beloosesky, R., Weiner, Z., Ginsberg, Y., and Ross, M.G. (2012). Maternal N-acetyl-cysteine (NAC) protects the rat fetal brain from inflammatory cytokine responses to lipopolysaccharide (LPS). J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. *25*, 1324–1328.

Berk, M., Copolov, D., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I., Anderson-Hunt, M., Judd, F., Katz, F., Katz, P., et al. (2008). N-acetyl cysteine as a glutathione precursor for schizophrenia--a double-blind, randomized, placebo-controlled trial. Biol. Psychiatry *64*, 361–368.

Berk, M., Malhi, G.S., Gray, L.J., and Dean, O.M. (2013). The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol. Sci. *34*, 167–177.

Birley, J.L., and Brown, G.W. (1970). Crises and life changes preceding the onset or relapse of acute schizophrenia: clinical aspects. Br. J. Psychiatry J. Ment. Sci. *116*, 327–333.

Bois, C., Whalley, H.C., McIntosh, A.M., and Lawrie, S.M. (2015). Structural magnetic resonance imaging markers of susceptibility and transition to schizophrenia: a review of familial and clinical high risk population studies. J. Psychopharmacol. Oxf. Engl. *29*, 144–154.

Boksa, P. (2012). Abnormal synaptic pruning in schizophrenia: Urban myth or reality? J. Psychiatry Neurosci. JPN *37*, 75–77.

Borgwardt, S.J., McGuire, P.K., Aston, J., Gschwandtner, U., Pflüger, M.O., Stieglitz, R.-D., Radue, E.-W., and Riecher-Rössler, A. (2008). Reductions in frontal, temporal and parietal volume associated with the onset of psychosis. Schizophr. Res. *106*, 108–114.

Breggin, P.R. (2009). Medication Madness: The Role of Psychiatric Drugs in Cases of Violence, Suicide, and Crime (New York: St. Martin's Griffin).

Brown, A.S. (2012). Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Dev. Neurobiol. *72*, 1272–1276.

Brown, A.S., and Derkits, E.J. (2010a). Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry *167*, 261–280.

Brown, A.S., and Derkits, E.J. (2010b). Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry *167*, 261–280.

Brown, A.S., Cohen, P., Harkavy-Friedman, J., Babulas, V., Malaspina, D., Gorman, J.M., and Susser, E.S. (2001). Prenatal rubella, premorbid abnormalities, and adult schizophrenia. Biol. Psychiatry *49*, 473–486.

Brown, A.S., Schaefer, C.A., Wyatt, R.J., Begg, M.D., Goetz, R., Bresnahan, M.A., Harkavy-Friedman, J., Gorman, J.M., Malaspina, D., and Susser, E.S. (2002). Paternal age and risk of schizophrenia in adult offspring. Am. J. Psychiatry *159*, 1528–1533.

Brown, A.S., Begg, M.D., Gravenstein, S., Schaefer, C.A., Wyatt, R.J., Bresnahan, M., Babulas, V.P., and Susser, E.S. (2004). Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch. Gen. Psychiatry *61*, 774–780.

Brown, A.S., Schaefer, C.A., Quesenberry, C.P., Liu, L., Babulas, V.P., and Susser, E.S. (2005). Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. Am. J. Psychiatry *162*, 767–773.

Brown, A.S., Vinogradov, S., Kremen, W.S., Poole, J.H., Deicken, R.F., Penner, J.D., McKeague, I.W., Kochetkova, A., Kern, D., and Schaefer, C.A. (2009). Prenatal exposure to maternal infection and executive dysfunction in adult schizophrenia. Am. J. Psychiatry *166*, 683–690.

Buka, S.L., Tsuang, M.T., Torrey, E.F., Klebanoff, M.A., Bernstein, D., and Yolken, R.H. (2001). Maternal infections and subsequent psychosis among offspring. Arch. Gen. Psychiatry *58*, 1032–1037.

Bulut, M., Savas, H.A., Altindag, A., Virit, O., and Dalkilic, A. (2009). Beneficial effects of N-acetylcysteine in treatment resistant schizophrenia. World J. Biol. Psychiatry Off. J. World Fed. Soc. Biol. Psychiatry *10*, 626–628.

Cabungcal, J.-H., Counotte, D.S., Lewis, E.M., Tejeda, H.A., Piantadosi, P., Pollock, C., Calhoon, G.G., Sullivan, E.M., Presgraves, E., Kil, J., et al. (2014). Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. Neuron *83*, 1073–1084.

Cannon, T.D., Cadenhead, K., Cornblatt, B., Woods, S.W., Addington, J., Walker, E., Seidman, L.J., Perkins, D., Tsuang, M., McGlashan, T., et al. (2008). Prediction of psychosis in youth at high clinical risk: a multisite longitudinal study in North America. Arch. Gen. Psychiatry *65*, 28–37.

Cannon, T.D., Chung, Y., He, G., Sun, D., Jacobson, A., van Erp, T.G.M., McEwen, S., Addington, J., Bearden, C.E., Cadenhead, K., et al. (2015). Progressive Reduction in Cortical Thickness as Psychosis Develops: A Multisite Longitudinal Neuroimaging Study of Youth at Elevated Clinical Risk. Biol. Psychiatry *77*, 147–157.

Carlat, D. (2010). Unhinged: The Trouble with Psychiatry - A Doctor's Revelations about a Profession in Crisis (Simon and Schuster).

Chang, E.Y., Zhang, J., Sullivan, S., Newman, R., and Singh, I. (2011). N-acetylcysteine attenuates the maternal and fetal proinflammatory response to intrauterine LPS injection in an animal model for preterm birth and brain injury. J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. *24*, 732–740.

Chen, H.-H., Stoker, A., and Markou, A. (2010). The glutamatergic compounds sarcosine and N-acetylcysteine ameliorate prepulse inhibition deficits in metabotropic glutamate 5 receptor knockout mice. Psychopharmacology (Berl.) *209*, 343–350.

Clarke, M.C., Tanskanen, A., Huttunen, M., Whittaker, J.C., and Cannon, M. (2009). Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. Am. J. Psychiatry *166*, 1025–1030.

Corbit, L.H., Chieng, B.C., and Balleine, B.W. (2014). Effects of repeated cocaine exposure on habit learning and reversal by N-acetylcysteine. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *39*, 1893–1901.

Corcoran, C., Walker, E., Huot, R., Mittal, V., Tessner, K., Kestler, L., and Malaspina, D. (2003). The stress cascade and schizophrenia: etiology and onset. Schizophr. Bull. *29*, 671–692.

Cornblatt, B.A., Carrión, R.E., Addington, J., Seidman, L., Walker, E.F., Cannon, T.D., Cadenhead, K.S., McGlashan, T.H., Perkins, D.O., Tsuang, M.T., et al. (2012). Risk factors for psychosis: impaired social and role functioning. Schizophr. Bull. *38*, 1247–1257.

Correll, C.U., Hauser, M., Auther, A.M., and Cornblatt, B.A. (2010). Research in people with psychosis risk syndrome: a review of the current evidence and future directions. J. Child Psychol. Psychiatry *51*, 390–431.

Costa-Campos, L., Herrmann, A.P., Pilz, L.K., Michels, M., Noetzold, G., and Elisabetsky, E. (2013). Interactive effects of N-acetylcysteine and antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry *44*, 125–130.

Dalman, C., and Allebeck, P. (2002). Paternal age and schizophrenia: further support for an association. Am. J. Psychiatry *159*, 1591–1592.

Day, R., Nielsen, J.A., Korten, A., Ernberg, G., Dube, K.C., Gebhart, J., Jablensky, A., Leon, C., Marsella, A., and Olatawura, M. (1987). Stressful life events preceding the acute onset of schizophrenia: a cross-national study from the World Health Organization. Cult. Med. Psychiatry *11*, 123–205.

Deepmala, null, Slattery, J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., and Frye, R. (2015). Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. Neurosci. Biobehav. Rev. *55*, 294–321.

DeSantis, S.M., Baker, N.L., Back, S.E., Spratt, E., Ciolino, J.D., Moran-Santa Maria, M., Dipankar, B., and Brady, K.T. (2011). Gender differences in the effect of early life trauma on hypothalamic-pituitary-adrenal axis functioning. Depress. Anxiety *28*, 383–392.

D'Souza, D.C. (2007). Cannabinoids and psychosis. Int. Rev. Neurobiol. *78*, 289–326.

Ellenbroek, B.A. (2012). Psychopharmacological treatment of schizophrenia: what do we have, and what could we get? Neuropharmacology *62*, 1371–1380.

Faludi, G., and Mirnics, K. (2011). Synaptic changes in the brain of subjects with schizophrenia. Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci. *29*, 305–309.

Farokhnia, M., Azarkolah, A., Adinehfar, F., Khodaie-Ardakani, M.-R., Hosseini, S.-M.-R., Yekehtaz, H., Tabrizi, M., Rezaei, F., Salehi, B., Sadeghi, S.-M.-H., et al. (2013). N-acetylcysteine as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia: a randomized, double-blind, placebo-controlled study. Clin. Neuropharmacol. *36*, 185–192.

Featherstone, R.E., Rizos, Z., Kapur, S., and Fletcher, P.J. (2008). A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. Behav. Brain Res. *189*, 170–179.

Ferdinand, R.F., Sondeijker, F., van der Ende, J., Selten, J.-P., Huizink, A., and Verhulst, F.C. (2005). Cannabis use predicts future psychotic symptoms, and vice versa. Addict. Abingdon Engl. *100*, 612–618.

Fergusson, D.M., Horwood, L.J., and Swain-Campbell, N.R. (2003). Cannabis dependence and psychotic symptoms in young people. Psychol. Med. *33*, 15–21.

Ferreira, F.R., Biojone, C., Joca, S.R.L., and Guimarães, F.S. (2008). Antidepressant-like effects of N-acetyl-L-cysteine in rats. Behav. Pharmacol. *19*, 747–750.

Ferri, C., Chisholm, D., Van Ommeren, M., and Prince, M. (2004). Resource utilisation for neuropsychiatric disorders in developing countries: a multinational Delphi consensus study. Soc. Psychiatry Psychiatr. Epidemiol. *39*, 218–227.

Fone, K.C.F., and Porkess, M.V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci. Biobehav. Rev. *32*, 1087–1102.

Fornito, A., Yung, A.R., Wood, S.J., Phillips, L.J., Nelson, B., Cotton, S., Velakoulis, D., McGorry, P.D., Pantelis, C., and Yücel, M. (2008). Anatomic abnormalities of the anterior cingulate cortex before psychosis onset: an MRI study of ultra-high-risk individuals. Biol. Psychiatry *64*, 758–765.

Fournier, M., Ferrari, C., Baumann, P.S., Polari, A., Monin, A., Bellier-Teichmann, T., Wulff, J., Pappan, K.L., Cuenod, M., Conus, P., et al. (2014). Impaired metabolic reactivity to oxidative stress in early psychosis patients. Schizophr. Bull. *40*, 973–983.

Frankowska, M., Jastrzębska, J., Nowak, E., Białko, M., Przegaliński, E., and Filip, M. (2014). The effects of N-acetylcysteine on cocaine reward and seeking behaviors in a rat model of depression. Behav. Brain Res. *266*, 108–118.

Fukami, G., Hashimoto, K., Koike, K., Okamura, N., Shimizu, E., and Iyo, M. (2004). Effect of antioxidant N-acetyl-L-cysteine on behavioral changes and neurotoxicity in rats after administration of methamphetamine. Brain Res. *1016*, 90–95.

Fusar-Poli, P., Borgwardt, S., Crescini, A., Deste, G., Kempton, M.J., Lawrie, S., Mc Guire, P., and Sacchetti, E. (2011). Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. Neurosci. Biobehav. Rev. *35*, 1175–1185.

Fusar-Poli P, Bonoldi I, Yung AR, and et al (2012). Predicting psychosis: Meta-analysis of transition outcomes in individuals at high clinical risk. Arch. Gen. Psychiatry *69*, 220–229.

Gaite, L., Vázquez-Barquero, J.L., Borra, C., Ballesteros, J., Schene, A., Welcher, B., Thornicroft, G., Becker, T., Ruggeri, M., and Herrán, A., EPSILON Study Group (2002). Quality of life in patients with schizophrenia in five European countries: the EPSILON study. Acta Psychiatr. Scand. *105*, 283–292.

Gardner, D.M., Baldessarini, R.J., and Waraich, P. (2005). Modern antipsychotic drugs: a critical overview. Can. Med. Assoc. J. *172*, 1703–1711.

Geddes, J.R., Burgess, S., Hawton, K., Jamison, K., and Goodwin, G.M. (2004). Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomized controlled trials. Am. J. Psychiatry *161*, 217–222.

Gere-Pászti, E., and Jakus, J. (2009). The effect of N-acetylcysteine on amphetamine-mediated dopamine release in rat brain striatal slices by ion-pair reversed-phase high performance liquid chromatography. Biomed. Chromatogr. BMC *23*, 658–664.

Geyer, M.A., Wilkinson, L.S., Humby, T., and Robbins, T.W. (1993). Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biol. Psychiatry *34*, 361–372.

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Voget, M., Willi, R., Winter, C., Riva, M.A., Mortensen, P.B., Feldon, J., et al. (2013). Stress in Puberty Unmasks Latent Neuropathological Consequences of Prenatal Immune Activation in Mice. Science *339*, 1095–1099.

Goldstein, J.M., Seidman, L.J., Goodman, J.M., Koren, D., Lee, H., Weintraub, S., and Tsuang, M.T. (1998). Are there sex differences in neuropsychological functions among patients with schizophrenia? Am. J. Psychiatry *155*, 1358–1364.

Goriely, A., McGrath, J.J., Hultman, C.M., Wilkie, A.O.M., and Malaspina, D. (2013). “Selfish spermatogonial selection”: a novel mechanism for the association between advanced paternal age and neurodevelopmental disorders. Am. J. Psychiatry *170*, 599–608.

Green, M.F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? Am. J. Psychiatry *153*, 321–330.

Grigoriadis, S., and Seeman, M.V. (2002). The role of estrogen in schizophrenia: implications for schizophrenia practice guidelines for women. Can. J. Psychiatry Rev. Can. Psychiatr. *47*, 437–442.

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., et al., CDBE2010Study Group (2011). Cost of disorders of the brain in Europe 2010. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. *21*, 718–779.

Haber, M., Abdel Baki, S.G., Grin’kina, N.M., Irizarry, R., Ershova, A., Orsi, S., Grill, R.J., Dash, P., and Bergold, P.J. (2013). Minocycline plus N-acetylcysteine synergize to modulate inflammation and prevent cognitive and memory deficits in a rat model of mild traumatic brain injury. Exp. Neurol. *249*, 169–177.

Häfner, H. (2003). Gender differences in schizophrenia. Psychoneuroendocrinology *28 Suppl 2*, 17–54.

Häfner, H., and an der Heiden, W. (1999). The course of schizophrenia in the light of modern follow-up studies: the ABC and WHO studies. Eur. Arch. Psychiatry Clin. Neurosci. *249 Suppl 4*, 14–26.

Häfner, H., Behrens, S., De Vry, J., and Gattaz, W.F. (1991). Oestradiol enhances the vulnerability threshold for schizophrenia in women by an early effect on dopaminergic neurotransmission. Evidence from an epidemiological study and from animal experiments. Eur. Arch. Psychiatry Clin. Neurosci. *241*, 65–68.

Häfner, H., Löffler, W., Maurer, K., Hambrecht, M., and an der Heiden, W. (1999). Depression, negative symptoms, social stagnation and social decline in the early course of schizophrenia. Acta Psychiatr. Scand. *100*, 105–118.

Hall, F.S. (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. Crit. Rev. Neurobiol. *12*, 129–162.

Haller, C.S., Padmanabhan, J.L., Lizano, P., Torous, J., and Keshavan, M. (2014). Recent advances in understanding schizophrenia. F1000prime Rep. *6*, 57.

Hashimoto, K., Tsukada, H., Nishiyama, S., Fukumoto, D., Kakiuchi, T., Shimizu, E., and Iyo, M. (2004). Effects of N-acetyl-L-cysteine on the reduction of brain dopamine transporters in monkey treated with methamphetamine. Ann. N. Y. Acad. Sci. *1025*, 231–235.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H.-U., and Os, J. van (2004). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. BMJ *330*, 11.

Howes, O.D., Montgomery, A.J., Asselin, M.-C., Murray, R.M., Valli, I., Tabraham, P., Bramon-Bosch, E., Valmaggia, L., Johns, L., Broome, M., et al. (2009). Elevated striatal dopamine function linked to prodromal signs of schizophrenia. Arch. Gen. Psychiatry *66*, 13–20.

Howes, O.D., Bose, S.K., Turkheimer, F., Valli, I., Egerton, A., Valmaggia, L.R., Murray, R.M., and McGuire, P. (2011). Dopamine synthesis capacity before onset of psychosis: a prospective [18F]-DOPA PET imaging study. Am. J. Psychiatry *168*, 1311–1317.

Insel, T.R. (2010). Rethinking schizophrenia. Nature *468*, 187–193.

Jacka, F.N., and Berk, M. (2014). Prevention of Schizophrenia—Will a Broader Prevention Agenda Support This Aim? Schizophr. Bull. *40*, 237–239.

Jamison, K.R. (1989). Mood disorders and patterns of creativity in British writers and artists. Psychiatry *52*, 125–134.

Job, D.E., Whalley, H.C., Johnstone, E.C., and Lawrie, S.M. (2005). Grey matter changes over time in high risk subjects developing schizophrenia. NeuroImage *25*, 1023–1030.

Jones, C., Watson, D., and Fone, K. (2011). Animal models of schizophrenia. Br. J. Pharmacol. *164*, 1162–1194.

Jung, W.H., Kim, J.S., Jang, J.H., Choi, J.-S., Jung, M.H., Park, J.-Y., Han, J.Y., Choi, C.-H., Kang, D.-H., Chung, C.K., et al. (2011). Cortical thickness reduction in individuals at ultra-high-risk for psychosis. Schizophr. Bull. *37*, 839–849.

Kaduszkiewicz, H., Zimmermann, T., Beck-Bornholdt, H.-P., and Bussche, H. van den (2005). Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. BMJ *331*, 321–327.

Karalija, A., Novikova, L.N., Kingham, P.J., Wiberg, M., and Novikov, L.N. (2012). Neuroprotective effects of N-acetyl-cysteine and acetyl-L-carnitine after spinal cord injury in adult rats. PloS One *7*, e41086.

Karalija, A., Novikova, L.N., Kingham, P.J., Wiberg, M., and Novikov, L.N. (2014). The effects of N-acetyl-cysteine and acetyl-L-carnitine on neural survival, neuroinflammation and regeneration following spinal cord injury. Neuroscience *269*, 143–151.

Karksson, J.L. (1970). Genetic association of giftedness and creativity with schizophrenia. Hereditas *66*, 177–182.

Kau, K.S., Madayag, A., Mantsch, J.R., Grier, M.D., Abdulhameed, O., and Baker, D.A. (2008). Blunted cystine-glutamate antiporter function in the nucleus accumbens promotes cocaine-induced drug seeking. Neuroscience *155*, 530–537.

Kaufman, S.B., and Paul, E.S. (2014). Creativity and schizophrenia spectrum disorders across the arts and sciences. Front. Psychol. *5*, 1145.

Keefe, R.S.E., Bilder, R.M., Davis, S.M., Harvey, P.D., Palmer, B.W., Gold, J.M., Meltzer, H.Y., Green, M.F., Capuano, G., Stroup, T.S., et al. (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. Arch. Gen. Psychiatry *64*, 633–647.

Keshavan, M.S., Anderson, S., and Pettegrew, J.W. (1994). Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. J. Psychiatr. Res. *28*, 239–265.

Keshavan, M.S., Diwadkar, V.A., Montrose, D.M., Rajarethinam, R., and Sweeney, J.A. (2005). Premorbid indicators and risk for schizophrenia: a selective review and update. Schizophr. Res. *79*, 45–57.

Keshavan, M.S., Prasad, K.M., and Pearlson, G. (2007). Are brain structural abnormalities useful as endophenotypes in schizophrenia? Int. Rev. Psychiatry Abingdon Engl. *19*, 397–406.

Keshavan, M.S., Kulkarni, S.R., Bhojraj, T., Francis, A., Diwadkar, V., Montrose, D.M., Seidman, L., Sweeney, J., Keshavan, M.S., Kulkarni, S., et al. (2010). Premorbid cognitive deficits in young relatives of schizophrenia patients. Front. Hum. Neurosci. *3*, 62.

Khan, M., Sekhon, B., Jatana, M., Giri, S., Gilg, A.G., Sekhon, C., Singh, I., and Singh, A.K. (2004). Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke. J. Neurosci. Res. *76*, 519–527.

Khandaker, G.M., Pearson, R.M., Zammit, S., Lewis, G., and Jones, P.B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. JAMA Psychiatry *71*, 1121–1128.

Kim, J.H., and Vezina, P. (2002). The mGlu2/3 receptor agonist LY379268 blocks the expression of locomotor sensitization by amphetamine. Pharmacol. Biochem. Behav. *73*, 333–337.

Kirsch, I. (2010). Emperor's New Drugs: Exploding the Antidepressant Myth (Basic Books).

Kirsch, I., Deacon, B.J., Huedo-Medina, T.B., Scoboria, A., Moore, T.J., and Johnson, B.T. (2008). Initial Severity and Antidepressant Benefits: A Meta-Analysis of Data Submitted to the Food and Drug Administration. PLoS Med *5*, e45.

Klosterkötter, J., Schultze-Lutter, F., Bechdolf, A., and Ruhrmann, S. (2011). Prediction and prevention of schizophrenia: what has been achieved and where to go next? World Psychiatry Off. J. World Psychiatr. Assoc. WPA *10*, 165–174.

Kneeland, R.E., and Fatemi, S.H. (2013). Viral infection, inflammation and schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry *42*, 35–48.

Kohn, R., Saxena, S., Levav, I., and Saraceno, B. (2004). The treatment gap in mental health care. Bull. World Health Organ. *82*, 858–866.

Kong, A., Frigge, M.L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., Gudjonsson, S.A., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., et al. (2012). Rate of de novo mutations and the importance of father's age to disease risk. Nature *488*, 471–475.

De Koning, M.B., Bloemen, O.J.N., van Amelsvoort, T.A.M.J., Becker, H.E., Nieman, D.H., van der Gaag, M., and Linszen, D.H. (2009). Early intervention in patients at ultra high risk of psychosis: benefits and risks. Acta Psychiatr. Scand. *119*, 426–442.

Krezel, A., Wójcik, J., Maciejczyk, M., and Bal, W. (2003). May GSH and L-His contribute to intracellular binding of zinc? Thermodynamic and solution structural study of a ternary complex. Chem. Commun. Camb. Engl. 704–705.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H.-U., Höfler, M., and Henquet, C. (2011). Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. BMJ *342*, d738.

Kyaga, S., Lichtenstein, P., Boman, M., Hultman, C., Långström, N., and Landén, M. (2011). Creativity and mental disorder: family study of 300,000 people with severe mental disorder. Br. J. Psychiatry J. Ment. Sci. *199*, 373–379.

Lanté, F., Meunier, J., Guiramand, J., De Jesus Ferreira, M.-C., Cambonie, G., Aimar, R., Cohen-Solal, C., Maurice, T., Vignes, M., and Barbanel, G. (2008). Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. Hippocampus *18*, 602–609.

Laruelle, M. (2000). The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. Brain Res. Brain Res. Rev. *31*, 371–384.

Lavoie, S., Murray, M.M., Deppen, P., Knyazeva, M.G., Berk, M., Boulat, O., Bovet, P., Bush, A.I., Conus, P., Copolov, D., et al. (2008). Glutathione precursor, N-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *33*, 2187–2199.

Lawrie, S.M., Whalley, H., Kestelman, J.N., Abukmeil, S.S., Byrne, M., Hodges, A., Rimmington, J.E., Best, J.J., Owens, D.G., and Johnstone, E.C. (1999). Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. Lancet Lond. Engl. *353*, 30–33.

Lee, M.-Y., Chiang, C.-C., Chiu, H.-Y., Chan, M.-H., and Chen, H.-H. (2014). N-acetylcysteine modulates hallucinogenic 5-HT(2A) receptor agonist-mediated responses: behavioral, molecular, and electrophysiological studies. Neuropharmacology *81*, 215–223.

Lehrer, D.S., Pato, M.T., Nahhas, R.W., Miller, B.R., Malaspina, D., Buckley, P.F., Sobell, J.L., Walsh-Messinger, J., Cohort Consortium, G.P., and Pato, C.N. (2015). Paternal age effect: Replication in schizophrenia with intriguing dissociation between bipolar with and without psychosis. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet.

Leitão, R.J., Ferraz, M.B., Chaves, A.C., and Mari, J.J. (2006). Cost of schizophrenia: direct costs and use of resources in the State of São Paulo. Rev. Saúde Pública *40*, 304–309.

Leucht, S., Arbter, D., Engel, R.R., Kissling, W., and Davis, J.M. (2009). How effective are second-generation antipsychotic drugs? A meta-analysis of placebo-controlled trials. Mol. Psychiatry *14*, 429–447.

Leucht, S., Hierl, S., Kissling, W., Dold, M., and Davis, J.M. (2012). Putting the efficacy of psychiatric and general medicine medication into perspective: review of meta-analyses. Br. J. Psychiatry *200*, 97–106.

Linck, V.M., Costa-Campos, L., Pilz, L.K., Garcia, C.R.L., and Elisabetsky, E. (2012). AMPA glutamate receptors mediate the antidepressant-like effects of N-acetylcysteine in the mouse tail suspension test. Behav. Pharmacol. *23*, 171–177.

Ludwig, A.M. (1992). Creative achievement and psychopathology: comparison among professions. Am. J. Psychother. *46*, 330–356.

Ludwig, A.M. (1994). Mental illness and creative activity in female writers. Am. J. Psychiatry *151*, 1650–1656.

Lutgen, V., Qualmann, K., Resch, J., Kong, L., Choi, S., and Baker, D.A. (2013). Reduction in phencyclidine induced sensorimotor gating deficits in the rat following increased system $xc^-$ activity in the medial prefrontal cortex. Psychopharmacology (Berl.) *226*, 531–540.

Madayag, A., Lobner, D., Kau, K.S., Mantsch, J.R., Abdulhameed, O., Hearing, M., Grier, M.D., and Baker, D.A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. J. Neurosci. Off. J. Soc. Neurosci. *27*, 13968–13976.

Mahadik, S.P., Evans, D., and Lal, H. (2001). Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry *25*, 463–493.

Malaspina, D., Harlap, S., Fennig, S., Heiman, D., Nahon, D., Feldman, D., and Susser, E.S. (2001). Advancing paternal age and the risk of schizophrenia. Arch. Gen. Psychiatry *58*, 361–367.

Manrique-Garcia, E., Zammit, S., Dalman, C., Hemmingsson, T., Andreasson, S., and Allebeck, P. (2012). Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort. Psychol. Med. *42*, 1321–1328.

Marwaha, S., Johnson, S., Bebbington, P., Stafford, M., Angermeyer, M.C., Brugha, T., Azorin, J.-M., Kilian, R., Hansen, K., and Toumi, M. (2007). Rates and correlates of employment in people with schizophrenia in the UK, France and Germany. Br. J. Psychiatry *191*, 30–37.

Maynard, T.M., Sikich, L., Lieberman, J.A., and LaMantia, A.S. (2001). Neural development, cell-cell signaling, and the “two-hit” hypothesis of schizophrenia. Schizophr. Bull. *27*, 457–476.

McGorry, P. (2011). Transition to Adulthood: The Critical Period for Pre-emptive, Disease-modifying Care for Schizophrenia and Related Disorders. Schizophr. Bull. *37*, 524–530.

McGorry, P.D. (2015). Early Intervention in Psychosis. J. Nerv. Ment. Dis. *203*, 310–318.

McGorry, P.D., Nelson, B., Amminger, G.P., Bechdolf, A., Francey, S.M., Berger, G., Riecher-Rössler, A., Klosterkötter, J., Ruhrmann, S., Schultze-Lutter, F., et al. (2009). Intervention in individuals at ultra-high risk for psychosis: a review and future directions. J. Clin. Psychiatry *70*, 1206–1212.

McGrath, J.J., Petersen, L., Agerbo, E., Mors, O., Mortensen, P.B., and Pedersen, C.B. (2014). A comprehensive assessment of parental age and psychiatric disorders. JAMA Psychiatry *71*, 301–309.

Mechelli, A., Riecher-Rössler, A., Meisenzahl, E.M., Tognin, S., Wood, S.J., Borgwardt, S.J., Koutsouleris, N., Yung, A.R., Stone, J.M., Phillips, L.J., et al. (2011). Neuroanatomical abnormalities that predate the onset of psychosis: a multicenter study. Arch. Gen. Psychiatry *68*, 489–495.

Mednick, S.A., Machon, R.A., Huttunen, M.O., and Bonett, D. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. Arch. Gen. Psychiatry *45*, 189–192.

Menninger KA (1919). Psychoses associated with influenza: I. general data: statistical analysis. J. Am. Med. Assoc. *72*, 235–241.

Meyer, U. (2014). Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. Biol. Psychiatry *75*, 307–315.

Meyer, U., and Feldon, J. (2010). Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog. Neurobiol. *90*, 285–326.

Meyer, U., Feldon, J., Schedlowski, M., and Yee, B.K. (2005). Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. Neurosci. Biobehav. Rev. *29*, 913–947.

Meyer, U., Feldon, J., and Fatemi, S.H. (2009). In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. Neurosci. Biobehav. Rev. *33*, 1061–1079.

Miller, B., Suvisaari, J., Miettunen, J., Järvelin, M.-R., Haukka, J., Tanskanen, A., Lönnqvist, J., Isohanni, M., and Kirkpatrick, B. (2011a). Advanced paternal age and parental history of schizophrenia. Schizophr. Res. *133*, 125–132.

Miller, B., Messias, E., Miettunen, J., Alaräisänen, A., Järvelin, M.-R., Koponen, H., Räsänen, P., Isohanni, M., and Kirkpatrick, B. (2011b). Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. Schizophr. Bull. *37*, 1039–1047.

Möller, M., Du Preez, J.L., Viljoen, F.P., Berk, M., Emsley, R., and Harvey, B.H. (2013). Social isolation rearing induces mitochondrial, immunological, neurochemical and behavioural deficits in rats, and is reversed by clozapine or N-acetyl cysteine. Brain. Behav. Immun. *30*, 156–167.

Moncrieff, J. (1995). Lithium revisited. A re-examination of the placebo-controlled trials of lithium prophylaxis in manic-depressive disorder. Br. J. Psychiatry J. Ment. Sci. *167*, 569–573; discussion 573–574.

Moore, T.H.M., Zammit, S., Lingford-Hughes, A., Barnes, T.R.E., Jones, P.B., Burke, M., and Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet Lond. Engl. *370*, 319–328.

Morgan, C., and Fisher, H. (2007). Environment and Schizophrenia: Environmental Factors in Schizophrenia: Childhood Trauma—A Critical Review. Schizophr. Bull. *33*, 3–10.

Mortensen, P.B., Nørgaard-Pedersen, B., Waltoft, B.L., Sørensen, T.L., Hougaard, D., and Yolken, R.H. (2007). Early infections of Toxoplasma gondii and the later development of schizophrenia. Schizophr. Bull. *33*, 741–744.

Moussawi, K., Pacchioni, A., Moran, M., Olive, M.F., Gass, J.T., Lavin, A., and Kalivas, P.W. (2009). N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat. Neurosci. *12*, 182–189.

Moussawi, K., Zhou, W., Shen, H., Reichel, C.M., See, R.E., Carr, D.B., and Kalivas, P.W. (2011). Reversing cocaine-induced synaptic potentiation provides enduring protection from relapse. Proc. Natl. Acad. Sci. U. S. A. *108*, 385–390.

Murphy, B.P., Chung, Y.-C., Park, T.-W., and McGorry, P.D. (2006). Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. Schizophr. Res. *88*, 5–25.

Murray, J.E., Everitt, B.J., and Belin, D. (2012). N-Acetylcysteine reduces early- and late-stage cocaine seeking without affecting cocaine taking in rats. Addict. Biol. *17*, 437–440.

Niemi-Pynttäri, J.A., Sund, R., Putkonen, H., Vorma, H., Wahlbeck, K., and Pirkola, S.P. (2013). Substance-induced psychoses converting into schizophrenia: a register-based study of 18,478 Finnish inpatient cases. J. Clin. Psychiatry *74*, e94–e99.

Van Os, J., Bak, M., Hanssen, M., Bijl, R.V., de Graaf, R., and Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. Am. J. Epidemiol. *156*, 319–327.

Van Os, J., Kenis, G., and Rutten, B.P.F. (2010). The environment and schizophrenia. Nature *468*, 203–212.

Pantelis, C., Yücel, M., Wood, S.J., Velakoulis, D., Sun, D., Berger, G., Stuart, G.W., Yung, A., Phillips, L., and McGorry, P.D. (2005). Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. Schizophr. Bull. *31*, 672–696.

Pascual, R., Zamora-León, S.P., and Valero-Cabré, A. (2006). Effects of postweaning social isolation and re-socialization on the expression of vasoactive intestinal peptide (VIP) and dendritic development in the medial prefrontal cortex of the rat. Acta Neurobiol. Exp. (Warsz.) *66*, 7–14.

Pasternak, O., Westin, C.-F., Dahlben, B., Bouix, S., and Kubicki, M. (2015). The extent of diffusion MRI markers of neuroinflammation and white matter deterioration in chronic schizophrenia. Schizophr. Res. *161*, 113–118.

Peleg-Raibstein, D., Knuesel, I., and Feldon, J. (2008). Amphetamine sensitization in rats as an animal model of schizophrenia. Behav. Brain Res. *191*, 190–201.

Penzes, P., Cahill, M.E., Jones, K.A., VanLeeuwen, J.-E., and Woolfrey, K.M. (2011). Dendritic spine pathology in neuropsychiatric disorders. Nat. Neurosci. *14*, 285–293.

Perkins, D.O., Gu, H., Boteva, K., and Lieberman, J.A. (2005). Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. Am. J. Psychiatry *162*, 1785–1804.

Perkins, D.O., Jeffries, C.D., Addington, J., Bearden, C.E., Cadenhead, K.S., Cannon, T.D., Cornblatt, B.A., Mathalon, D.H., McGlashan, T.H., Seidman, L.J., et al. (2015). Towards a Psychosis Risk Blood Diagnostic for Persons Experiencing High-Risk Symptoms: Preliminary Results From the NAPLS Project. Schizophr. Bull. *41*, 419–428.

Perry, V.H., and O'Connor, V. (2010). The Role of Microglia in Synaptic Stripping and Synaptic Degeneration: A Revised Perspective. ASN Neuro *2*, 281–291.

Post, F. (1994). Creativity and psychopathology. A study of 291 world-famous men. Br. J. Psychiatry J. Ment. Sci. *165*, 22–34.

Powell, S.B. (2010). Models of neurodevelopmental abnormalities in schizophrenia. Curr. Top. Behav. Neurosci. *4*, 435–481.

Powell, S.B., and Geyer, M.A. (2002). Developmental markers of psychiatric disorders as identified by sensorimotor gating. Neurotox. Res. *4*, 489–502.

Powell, S.B., and Geyer, M.A. (2007). Overview of animal models of schizophrenia. Curr. Protoc. Neurosci. Editor. Board Jacqueline N Crawley Al *Chapter 9*, Unit 9.24.

Power, R.A., Steinberg, S., Bjornsdottir, G., Rietveld, C.A., Abdellaoui, A., Nivard, M.M., Johannesson, M., Galesloot, T.E., Hottenga, J.J., Willemsen, G., et al. (2015). Polygenic risk scores for schizophrenia and bipolar disorder predict creativity. Nat. Neurosci. *18*, 953–955.

Ramirez-Niño, A.M., D'Souza, M.S., and Markou, A. (2013). N-acetylcysteine decreased nicotine self-administration and cue-induced reinstatement of nicotine seeking in rats: comparison with the effects of N-acetylcysteine on food responding and food seeking. Psychopharmacology (Berl.) *225*, 473–482.

Reichel, C.M., Moussawi, K., Do, P.H., Kalivas, P.W., and See, R.E. (2011). Chronic N-acetylcysteine during abstinence or extinction after cocaine self-administration produces enduring reductions in drug seeking. J. Pharmacol. Exp. Ther. *337*, 487–493.

Ristow, M. (2014). Unraveling the truth about antioxidants: mitohormesis explains ROS-induced health benefits. Nat. Med. *20*, 709–11.

Ristow, M., Schmeisser, K. (2014). Mitohormesis: Promoting Health and Lifespan by Increased Levels of Reactive Oxygen Species (ROS). Dose Response. *12*, 288–341.

Robinson, T.E., and Becker, J.B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. *396*, 157–198.

Russig, H., Durrer, A., Yee, B.K., Murphy, C.A., and Feldon, J. (2003). The acquisition, retention and reversal of spatial learning in the morris water maze task following withdrawal from an escalating dosage schedule of amphetamine in wistar rats. Neuroscience *119*, 167–179.

Sado, M., Inagaki, A., Koreki, A., Knapp, M., Kissane, L.A., Mimura, M., and Yoshimura, K. (2013). The cost of schizophrenia in Japan. Neuropsychiatr. Dis. Treat. *9*, 787–798.

Samuni, Y., Goldstein, S., Dean, O.M., Berk, M. (2013). The chemistry and biological activities of N-acetylcysteine. Biochim. Biophys. Acta. *1830*, 4117–29.

Satel, S.L., and Edell, W.S. (1991). Cocaine-induced paranoia and psychosis proneness. Am. J. Psychiatry *148*, 1708–1711.

Sato, M., Chen, C.C., Akiyama, K., and Otsuki, S. (1983). Acute exacerbation of paranoid psychotic state after long-term abstinence in patients with previous methamphetamine psychosis. Biol. Psychiatry *18*, 429–440.

Sato, M., Numachi, Y., and Hamamura, T. (1992). Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. Schizophr. Bull. *18*, 115–122.

Schafer, D.P., Lehrman, E.K., and Stevens, B. (2013). The "quad-partite" synapse: Microglia-synapse interactions in the developing and mature CNS. Glia *61*, 24–36.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. Nature *511*, 421–427.

Schneider, R., Santos, C.F., Clarimundo, V., Dalmaz, C., Elisabetsky, E., and Gomez, R. (2015). N-acetylcysteine prevents behavioral and biochemical changes induced by alcohol cessation in rats. Alcohol Fayettev. N *49*, 259–263.

Seeman, M.V., and Lang, M. (1990). The role of estrogens in schizophrenia gender differences. Schizophr. Bull. *16*, 185–194.

Selten, J.-P., Frissen, A., Lensvelt-Mulders, G., and Morgan, V.A. (2010). Schizophrenia and 1957 pandemic of influenza: meta-analysis. Schizophr. Bull. *36*, 219–228.

Shi, L., Fatemi, S.H., Sidwell, R.W., and Patterson, P.H. (2003). Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J. Neurosci. Off. J. Soc. Neurosci. *23*, 297–302.

Shirai, Y., Fujita, Y., Hashimoto, R., Ohi, K., Yamamori, H., Yasuda, Y., Ishima, T., Suganuma, H., Ushida, Y., Takeda, M., et al. (2015). Dietary Intake of Sulforaphane-Rich Broccoli Sprout Extracts during Juvenile and Adolescence Can Prevent Phencyclidine-Induced Cognitive Deficits at Adulthood. PloS One *10*, e0127244.

Sipos, A., Rasmussen, F., Harrison, G., Tynelius, P., Lewis, G., Leon, D.A., and Gunnell, D. (2004). Paternal age and schizophrenia: a population based cohort study. BMJ *329*, 1070.

Smaga, I., Pomierny, B., Krzyżanowska, W., Pomierny-Chamioło, L., Miszkiel, J., Niedzielska, E., Ogórka, A., and Filip, M. (2012). N-acetylcysteine possesses antidepressant-like activity through reduction of oxidative stress: behavioral and biochemical analyses in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry *39*, 280–287.

Smieskova, R., Fusar-Poli, P., Allen, P., Bendfeldt, K., Stieglitz, R.D., Drewe, J., Radue, E.W., McGuire, P.K., Riecher-Rössler, A., and Borgwardt, S.J. (2010). Neuroimaging predictors of transition to psychosis--a systematic review and meta-analysis. Neurosci. Biobehav. Rev. *34*, 1207–1222.

Sørensen, H.J., Mortensen, E.L., Reinisch, J.M., and Mednick, S.A. (2009). Association Between Prenatal Exposure to Bacterial Infection and Risk of Schizophrenia. Schizophr. Bull. *35*, 631–637.

Steullet, P., Cabungcal, J.H., Monin, A., Dwir, D., O'Donnell, P., Cuenod, M., and Do, K.Q. Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: A "central hub" in schizophrenia pathophysiology? Schizophr. Res.

Sullivan, H.S. (1927). The onset of schizophrenia. Am. J. Psychiatry *6*, 105–134.

Sun, D., Phillips, L., Velakoulis, D., Yung, A., McGorry, P.D., Wood, S.J., van Erp, T.G.M., Thompson, P.M., Toga, A.W., Cannon, T.D., et al. (2009). Progressive Brain Structural Changes Mapped as Psychosis Develops in "At Risk" Individuals. Schizophr. Res. *108*, 85–92.

Suvisaari, J., Haukka, J., Tanskanen, A., Hovi, T., and Lönnqvist, J. (1999). Association between prenatal exposure to poliovirus infection and adult schizophrenia. Am. J. Psychiatry *156*, 1100–1102.

Swerdlow, N.R., Braff, D.L., Taaid, N., and Geyer, M.A. (1994). Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Arch. Gen. Psychiatry *51*, 139–154.

Takahashi, T., Wood, S.J., Yung, A.R., Phillips, L.J., Soulsby, B., McGorry, P.D., Tanino, R., Zhou, S.-Y., Suzuki, M., Velakoulis, D., et al. (2009). Insular cortex gray matter changes in individuals at ultra-high-risk of developing psychosis. Schizophr. Res. *111*, 94–102.

Tandon, R., Keshavan, M.S., and Nasrallah, H.A. (2008). Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. Schizophr. Res. *102*, 1–18.

Tandon, R., Nasrallah, H.A., and Keshavan, M.S. (2009). Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophr. Res. *110*, 1–23.

Taracha, E., Kaniuga, E., Chrapusta, S.J., Boguszewski, P.M., Lehner, M., Krząścik, P., and Płaźnik, A. (2015). N-acetyl cysteine does not modify the sensitization of the rewarding effect of amphetamine as assessed with frequency-modulated 50-kHz vocalization in the rat. Behav. Brain Res. *280*, 141–148.

Torrey, E.F., and Peterson, M.R. (1973). Slow and latent viruses in schizophrenia. Lancet Lond. Engl. *2*, 22–24.

Torrey, E.F., Rawlings, R., and Waldman, I.N. (1988). Schizophrenic births and viral diseases in two states. Schizophr. Res. *1*, 73–77.

Torrey, E.F., Bartko, J.J., and Yolken, R.H. (2012). Toxoplasma gondii and other risk factors for schizophrenia: an update. Schizophr. Bull. *38*, 642–647.

Tsai, G.Y., Cui, J.Z., Syed, H., Xia, Z., Ozerdem, U., McNeill, J.H., and Matsubara, J.A. (2009). Effect of N-acetylcysteine on the early expression of inflammatory markers in the retina and plasma of diabetic rats. Clin. Experiment. Ophthalmol. *37*, 223–231.

Ujike, H. (2002). Stimulant-induced psychosis and schizophrenia: the role of sensitization. Curr. Psychiatry Rep. *4*, 177–184.

Walter, A., Studerus, E., Smieskova, R., Kuster, P., Aston, J., Lang, U.E., Radue, E.-W., Riecher-Rössler, A., and Borgwardt, S. (2012). Hippocampal volume in subjects at high risk of psychosis: a longitudinal MRI study. Schizophr. Res. *142*, 217–222.

Weiland, A., Garcia, S., and Knackstedt, L.A. (2015). Ceftriaxone and cefazolin attenuate the cue-primed reinstatement of alcohol-seeking. Front. Pharmacol. *6*, 44.

Whitaker, R. (2010). Anatomy of an Epidemic: Magic Bullets, Psychiatric Drugs, and the Astonishing Rise of Mental Illness in America (Crown/Archetype).

Van Winkel, R., Stefanis, N.C., and Myin-Germeys, I. (2008). Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr. Bull. *34*, 1095–1105.

Woodberry, K.A., Seidman, L.J., Giuliano, A.J., Verdi, M.B., Cook, W.L., and McFarlane, W.R. (2010). Neuropsychological profiles in individuals at clinical high risk for psychosis: relationship to psychosis and intelligence. Schizophr. Res. *123*, 188–198.

World Health Organization (2008). Integrating Mental Health Into Primary Care: A Global Perspective (World Health Organization).

World Health Organization (2013). Investing in mental health: evidence for action (World Health Organization).

Wu, E.Q., Birnbaum, H.G., Shi, L., Ball, D.E., Kessler, R.C., Moulis, M., and Aggarwal, J. (2005). The economic burden of schizophrenia in the United States in 2002. J. Clin. Psychiatry *66*, 1122–1129.

Yung, A.R., and McGorry, P.D. (2007). Prediction of psychosis: setting the stage. Br. J. Psychiatry. Suppl. *51*, s1–s8.

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I., and Lewis, G. (2002). Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. BMJ *325*, 1199.

Zammit, S., Allebeck, P., Dalman, C., Lundberg, I., Hemmingson, T., Owen, M.J., and Lewis, G. (2003). Paternal age and risk for schizophrenia. Br. J. Psychiatry J. Ment. Sci. *183*, 405–408.

Ziermans, T.B., Schothorst, P.F., Schnack, H.G., Koolschijn, P.C.M.P., Kahn, R.S., van Engeland, H., and Durston, S. (2012). Progressive structural brain changes during development of psychosis. Schizophr. Bull. *38*, 519–530.