

Behavioural, neurochemical, inflammatory and  
mitochondrial markers following social isolation rearing  
in rats before and after selected drug intervention

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**Fil 4:13**

“Ek is tot alles instaat deur Hom wat my krag gee.”

**Phil 4:13**

“I can do everything through Him who gives me strength.”

# Abstract

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## **Purpose:**

Schizophrenia is a progressive degenerative illness that has been causally linked to mitochondrial dysfunction, oxidative stress and a pro-inflammatory state. Social isolation rearing (SIR) in rats models the neurodevelopmental aspects of schizophrenia. The antioxidant and glutamate modulator, N-acetyl cysteine (NAC), has demonstrated therapeutic potential in schizophrenia as adjunctive treatment, although this has not been tested in the SIR model. The purpose of this study was to assess whether SIR induces changes in mitochondrial function (adenosine triphosphate (ATP)), pro- vs. anti-inflammatory cytokine balance, tryptophan metabolism, a disturbance in cortico-striatal monoamines and related metabolites, and associated alterations in behaviors akin to schizophrenia, viz. social interaction, object recognition memory and prepulse inhibition (PPI). Moreover, I evaluated whether these bio-behavioral alterations could be reversed with sub-chronic clozapine, or NAC, and whether NAC may bolster the response to clozapine treatment.

## **Methods:**

The objectives of the study were pursued through separately conducted studies. Male Sprague-Dawley (SD) rats (10 rats/group) were used in this study (Ethics number: NWU-0035-08-S5). Rats were randomly allocated to either social rearing or SIR for 8 weeks receiving either no treatment, vehicle, NAC (150 mg/kg/day), clozapine (5 mg/kg/day) or a combination of clozapine + NAC (CLZ + NAC) during the last 11 or 14 days of social rearing or SIR. After the 8 weeks, rats were tested for social interactive behaviors, object recognition memory and prepulse inhibition (PPI). Peripheral tryptophan metabolites (determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS)) and pro- and anti-inflammatory cytokines (IL-4, IL-6, TNF- $\alpha$ , IFN- $\gamma$ ) (enzyme-linked immunosorbent assay (ELISA)) were determined. Cortico-striatal ATP (bioluminescence assay) and monoamines (high performance liquid chromatography (HPLC)) were also determined.

## **Results:**

SIR-induced significant deficits in social interactive behaviours, object recognition memory and PPI, associated with increased peripheral kynurenine, quinolinic acid (QA), and pro-inflammatory cytokines, as well as a decrease in kynurenic acid (KYNA), neuroprotective ratio and anti-inflammatory cytokines. I also observed an increase in striatal, but reduced frontal cortical ATP, dopamine, serotonin as well as their metabolites and noradrenaline's metabolite, with noradrenaline increased in both brain regions in SIR rats. A separate dose-response study of NAC

(50, 150, 250 mg/kg/day) found 150 mg/kg to be the most appropriate dose for the NAC and CLZ + NAC studies. Clozapine, NAC as well as CLZ + NAC reversed all these changes, with NAC being less effective than CLZ alone. CLZ + NAC was found to be more effective than clozapine alone in reversing certain bio-behavioral alterations induced by SIR. In addition NAC alone dose dependently reversed most of the SIR induced alterations.

**Conclusion:**

SIR induces behavioral alterations, a pro-inflammatory state, mitochondrial dysfunction and cortico-striatal monoamine alterations, closely resembling evidence in schizophrenia. Importantly, all these bio-behavioral alterations were reversed with clozapine, NAC and CLZ + NAC treatment. However, CLZ + NAC was more effective than clozapine alone in reversing some bio-behavioral alterations, supporting the therapeutic application of NAC as adjunctive treatment in schizophrenia. In addition, NAC dose dependently reversed SIR-induced cortico-striatal serotonin, noradrenaline and metabolites, emphasizing NAC's potential use in other anxiety and stress-related disorders.

**Keywords:** Social isolation, schizophrenia, N-acetyl cysteine, clozapine, social interaction, object recognition, prepulse inhibition, pro-inflammatory state, neuroprotective ratio, LC-MS/MS.

# Opsomming

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## **Doel:**

Skisofrenie is 'n progressiewe degeneratiewe siekte, wat moontlik veroorsaak word deur mitochondriale disfunksie, oksidatiewe stres en 'n pro-inflammatoriese toestand. 'n Model wat die sensuwee-ontwikkelingsaspekte van skisofrenie naboots, is sosiale isolasiegeïnduseerde stres (SSI) by rotte. N-asetielsisteien (NAC) het terapeutiese potensiaal in die behandeling van skisofrenie, maar dis nog nooit in 'n sensuwee-ontwikkelingsdieremodel van skisofrenie getoets nie. Die doel van die studie was dus om vas te stel of SSI die volgende faktore kan beïnvloed, nl. mitochondriale funksie (adenosientrifosfaat (ATF)), die balans tussen pro- en anti-inflammatoriese sitokiene, triptofaan metabolisme, frontale kortikostriatale monoamiene en verwante metaboliete; asook gedragsveranderinge eie aan skisofrenie soos sosiale interaksie, voorwerpherkenning en prepuls inhibisie. Ek het ook die effek van sub-kroniese behandeling met klosapien en NAC, sowel as die moontlikheid dat NAC die effek van klosapien moontlik kan versterk, m.b.v al bogenoemde parameters ondersoek.

## **Metodes:**

Verskillende studies het afsonderlik bygedra ten einde die doelwitte van die projek te bereik. Manlike Sprague-Dawley (SD) rotte is gebruik (10 rotte / groep) (Etiek nommer: NWU-0035-08-S5) en is met spening onwillekeurig verdeel in 'n groep wat vir 8 weke blootgestel is aan of SSI of groeps-(sosiale) behuising. Tydens die laaste 11 of 14 dae is hulle behandel met een van die volgende regimens, soutoplossing, NAC (150 mg/kg/dag), klosapien (5 mg/kg/dag) of 'n kombinasie van NAC + klosapien (CLZ + NAC). Na afloop van die 8 weke, is die rotte onderwerp aan die volgende gedragstoetse: sosiale interaksie, voorwerpherkenning en prepuls inhibisie (PPI). Perifere triptofaan metaboliete (deur middel van 'n vloeistofchromatografie-tandem massa-spektrometrie (LC-MS/MS) metode), pro- en anti-inflammatoriese sitokiene (IL-4, IL-6, TNF- $\alpha$ , IFN- $\gamma$ ) (m.b.v ensiem gekoppelde immuun metode (ELISA)), sowel as kortiko-striatale adenosientrifosfaat (ATF) (bioluminessensie metode) en monoamiene (hoë-doeltreffendheid vloeistofchromatografie (HDVC) metode) is bepaal.

**Resultate:**

SSI het betekenisvolle veranderinge in sosiale interaksie, voorwerpherkenning en PPI veroorsaak in samehang met verhoogde kynurenien, quinoliensuur (QS), pro-inflammatoriese sitokiene en verlaagde kynuriensuur (KS), neuro-beskerende balans en anti-inflammatoriese sitokiene. ATF, dopamien, serotonien sowel as hulle metaboliete en noradrenalien se metaboliet was onderskeidelik laag in die frontale korteks, maar verhoog in die striatum, met verhoogde noradrenalien in beide die breinareas van SSI rotte. 'n Aparte dosis-respons studie met NAC (50, 150, 250 mg/kg/dag) het aangedui dat 150 mg/kg die optimale dosis is om vir die NAC en CLZ + NAC behandelings te gebruik. Behandeling met klosapien, NAC sowel as CLZ + NAC het al die bogenoemde neurochemiese en gedragsveranderinge omgekeer, terwyl die CLZ + NAC kombinasie meer effektief was as klosapien alleen om van die genoemde veranderinge om te keer. NAC alleen was in staat om op 'n dosis-afhanklike wyse die meeste SSI-geinduseerde veranderinge om te keer.

**Gevolgtrekking:**

SSI induseer gedragsveranderinge, 'n pro-inflammatoriese toestand, mitochondriale disfunksie en kortiko-striatale monoamienveranderinge wat in noue ooreenstemming is met bevindings in skisofrenie. Van belang is dat al die bogenoemde bio-gedragsveranderinge omgekeer kon word met sub-chroniese klosapien-, NAC- sowel as met CLZ + NAC behandeling. CLZ + NAC in kombinasie was egter meer effektief as klosapien alleen om van die bio-gedragsveranderinge om te keer, 'n bevinding wat die terapeutiese potensiaal van NAC as addisionele middel by die behandeling van skisofrenie ondersteun. Die feit dat NAC ook SIS-geinduseerde veranderinge in kortiko-striatale serotonien, noradrenalien en metaboliete omgekeer het, beklemtoon die moontlike terapeutiese potensiaal daarvan ook in ander ang- en stresverwante siektetoestande.

**Sleutelwoorde:** Sosiale isolasie, skisofrenie, N-asetielsisteien, klosapien, sosiale interaksie, voorwerpherkenning, prepuls inhibisie, pro-inflammatoriese toestand, neurobeskerende balans, LC-MS/MS.

# Awards obtained

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- YOUNG SCIENTIST BEST POSTER AWARD IN BASIC PHARMACOLOGY *at the* International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS), South Africa, KZN, Durban. (25 - 27 September 2011).
- WHITEHEAD SCIENTIFIC INTERNATIONAL TRAVEL AWARD *for* best postgraduate proposal to be presented at an international congress (April 2012).
- BEST PUBLICATION IN PHARMACOLOGY *at the* Pharmaceutical Society of South Africa (PSSA) congress, South Africa, Western Cape, Grahamstown, (12 - 15 September 2012)
- YOUNG SCIENTIST AWARD IN BASIC PHARMACOLOGY *at the* South African Society for Basic and Clinical Pharmacology (SASBCP) congress, South Africa, Gauteng, Pretoria (29 September – 2 October 2012).

# Congress Proceedings

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*The results obtained in this study were presented at four national congresses (podium presentations and one poster presentation) as well as two international congresses (poster presentations).*

- (a) MARISA MÖLLER, ROBIN EMSLEY, BRIAN H. HARVEY (2011). Neurochemical, peripheral and behavioural effects of social isolation rearing in rats: Relevance for schizophrenia (*podium presentation*). International Biological Psychiatry Congress, South Africa, Western Cape, Stellenbosch (22 - 25 September 2011).
- (b) MARISA MÖLLER, JAN L. DU PREEZ, BRIAN H. HARVEY (2011). Social Isolation Rearing in Rats Alters Plasma Tryptophan Metabolism and is Reversed by Sub-chronic Clozapine Treatment (*podium presentation*). International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS), South Africa, KZN, Durban (25 - 27 September 2011).
- (c) MARISA MÖLLER, JAN L. DU PREEZ, BRIAN H. HARVEY (2011). Development and Validation of a Single Analytical Method for the Determination of Tryptophan and Five of its Metabolites in Rat Plasma (*poster presentation*). International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS), South Africa, KZN, Durban (25 - 27 September 2011).
- (d) BRIAN H. HARVEY, MARISA MÖLLER, JAN L. DU PREEZ (2011). Isolation Rearing Alters Plasma Tryptophan Metabolism in Rats and Decreases the Neuroprotective Ratio: Reversal by Clozapine (*poster presentation*). Anxiety and Depression: 21<sup>st</sup> Neuropharmacology Conference. United States, Virginia, Washington DC (10 - 11 November 2011).

- (e) MARISA MÖLLER, JAN L. DU PREEZ, BRIAN H. HARVEY (2012). Social Isolation Rearing in Rats Alters Plasma Tryptophan Metabolism and is Reversed by Sub-chronic Clozapine Treatment (*poster presentation*). 3<sup>rd</sup> Schizophrenia International Research Society Conference, Schizophrenia: The Globalization of Research. Italy, Florence (14 - 19 April 2012).
  
- (f) MARISA MÖLLER, ROBIN EMSLEY, BRIAN H. HARVEY (2012). Social isolation rearing in rats induces schizophrenia-like behaviours that are reversed by sub-chronic treatment with clozapine or N-acetyl cysteine (*poster presentation*). 3<sup>rd</sup> Schizophrenia International Research Society Conference, Schizophrenia: The Globalization of Research. Italy, Florence (14 - 19 April 2012).
  
- (g) MARISA MÖLLER, JAN L. DU PREEZ, ROBIN EMSLEY, BRIAN H. HARVEY (2012). Social Isolation Rearing Induces a Pro-inflammatory State in Rats with Associated Deficits in Sensorimotor Gating, and is reversed by Clozapine or N- acetylcysteine (*podium presentation*). South African Society for Basic and Clinical Pharmacology (SASBCP) congress, South Africa, Gauteng, Pretoria (29 September – 2 October 2012).

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