N-acetyl cysteine reverses social isolation rearing induced changes in cortico-striatal monoamines in rats

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Abstract

Schizophrenia is causally associated with early-life environmental stress, implicating oxidative stress in its pathophysiology. N-acetyl cysteine (NAC), a glutathione precursor and antioxidant, is emerging as a useful agent in the adjunctive treatment of schizophrenia and other psychiatric illnesses. However, its actions on brain monoamine metabolism are unknown. Social isolation rearing (SIR) in rats presents with face, predictive and construct validity for schizophrenia. This study evaluated the dose-dependent effects of NAC (50, 150 and 250 mg/kg/day x 14 days) on SIR- vs. socially reared induced changes in cortico-striatal levels of dopamine (DA), serotonin (5-HT) noradrenaline (NA) and their associated metabolites. SIR induced significant deficits in frontal cortical DA and its metabolites, 3,4-dihydroxyphenylacetic acid (Dopac) and homovanillic acid (HVA), reduced 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and reduced levels of the NA metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG). In addition, significant elevations in frontal cortical NA and striatal DA, Dopac, HVA, 5-HT, 5-HIAA, NA and MHPG were also observed in SIR rats. NAC at 150 and 250 mg/kg reversed all cortico-striatal DA, Dopac, HVA, 5-HT, 5-HIAA and striatal NA alterations in SIR animals, with 250 mg/kg of NAC also reversing alterations in cortico-striatal MHPG. In conclusion, SIR profoundly alters cortico-striatal DA, 5-HT and NA pathways that parallel observations in schizophrenia, while these changes are dose-dependently reversed or abrogated by sub-chronic NAC treatment. A modulatory action on cortico-striatal monoamines may explain NACs' therapeutic use in schizophrenia and possibly other psychiatric disorders, where redox dysfunction or oxidative stress is a causal factor.

Keywords: N-acetyl cysteine, monoamines, social isolation rearing, frontal cortex, striatum, schizophrenia.

Introduction

The exact aetiology of schizophrenia remains incompletely understood, although variable disturbances in frontal cortical and striatal dopamine (DA), serotonin (5-HT) and/or noradrenaline (NA) release, metabolism and subcellular signalling are causally involved (reviewed in Carlsson et al., 2001; Yamamoto and Hornykiewicz, 2004). Importantly, the majority of drugs used clinically to treat schizophrenia target monoamine receptors, re-uptake transporters and monoamine metabolism (Lieberman, 1993; Carlsson et al., 2001).

The frontal cortex and striatum play a central role in the neuropathology of schizophrenia (Meyer-Lindenberg et al., 2002). For example, schizophrenia is associated with reduced prefrontal cortex DA, elevated ventral striatum DA (nucleus accumbens) (Meyer-Lindenberg et al., 2002), and elevated frontal cortical 5-HT (Sumiyoshi et al., 1996). An earlier review also found consistent evidence that the positive and negative symptoms observed in schizophrenia are associated with over-activity and under-activity of central NA, respectively (Yamamoto and Hornykiewicz, 2004).

Interestingly, recent studies have demonstrated that schizophrenia presents with evidence of cortico-striatal redox imbalance and oxidative stress (Gawryluk et al., 2011; Prabakaran et al., 2004; Wang et al., 2009), including reduction in both reduced and oxidized glutathione, reduced superoxide dismutase (SOD), reduced catalase and glutathione peroxidase, as well as increased lipid peroxidation (Mukherjee et al., 1996; Kuloglu et al., 2002; Kunz et al., 2008; Ng et al., 2008). This has also been verified in translational animal models of schizophrenia (Möller et al., 2011; Radonjić et al. 2010; Dean et al. 2009).

Importantly, factors involved in cellular oxidative stress are known to evoke monoaminergic changes that in turn may mediate psychiatric manifestations (Garcia-Cazorla et al., 2008). In many instances, oxidative stress can be causally related to increased glutamate activity evident in both the striatum and frontal cortex (Haroutunian et al., 2003). In fact, schizophrenia is closely linked to altered glutamatergic activity (Prabakaran et al., 2004).

Here excessive release of glutamate and activation of N-methyl-D-aspartate (NMDA) receptors not only alters cortico-striatal monoamines (DA, 5-HT and NA) (Carlsson et al., 2001), but can induce structural damage to neurons that in turn will impact on cellular redox balance (Smythies, 1999). In line with these observations, pre-clinical models such as social isolation rearing (SIR) has confirmed the presence of altered redox balance and oxidative stress (Möller et al., 2011), as well as elevations in nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase 2 (Nox2) (Schiavone et al., 2009). Nox2 is not only a major source of oxidative stress but also controls glutamate release in the prefrontal cortex (Sorce et al., 2010; reviewed in Schiavone et al., 2012). Moreover, NMDA receptor dysfunction in the frontal cortex and striatum has recently become a focus of attention in attempts to explain the neurobiology of schizophrenia (Krivoy et al., 2008). In fact NMDA receptor modulators and antagonists, such as D-cycloserine have opened new therapeutic horizons for the treatment of schizophrenia (Heresco-Levy et al., 2002). These observations raise the interesting question whether an antioxidant and glutamate modulator may offer clinical utility in treating schizophrenia. In this regard N-acetyl cysteine (NAC), a glutathione precursor, antioxidant (Kerksick and Willoughby, 2005) and glutamate modulator (Grant et al., 2009), is a promising therapeutic agent (Dean et al., 2011). Recent clinical studies have highlighted its efficacy in bipolar disorder and schizophrenia (Berk et al., 2008a, b; Berk et al., 2011; Magalhaes et al., 2011). Despite schizophrenia presenting with altered glutamate and redox function (Hovatta et al., 2010), the backbone of treatment is aimed primarily at monoamine transmission. However since oxidative stress may mediate altered monoaminergic activity (Garcia-Cazorla et al., 2008), NAC by virtue of its action on redox and glutamate systems in the frontal cortex and striatum (Arent et al., 2012; Dean et al., 2011), may very likely exert its therapeutic effects via secondary actions on DA, 5-HT and NA pathways. However, this requires confirmation, especially using a neurodevelopmental model of schizophrenia.

Early adverse experiences, including early life stress, may compound a pre-existing genetic vulnerability to stress and disease (Chorpita and Barlow, 1998), and is recognized as a pre-

eminent factor in the development of schizophrenia (Matheson et al., 2011). Post-natal SIR of rats is a relevant neurodevelopmental model of early-life chronic stress that displays parallel symptoms validated to represent core symptoms of schizophrenia (e.g. deficits in cognition, social interaction and prepulse inhibition (PPI)) (Möller et al., 2011, 2013). Furthermore, SIR produces various alterations in cortico-striatal monoamine pathways in rats (reviewed in Trabace et al., 2012).

The aim of this study was to evaluate the dose-dependent effects of sub-chronic NAC treatment on SIR-induced cortico-striatal monoamine changes, and whether any such changes can explain its therapeutic profile in schizophrenia. We hypothesize that SIR significantly alters cortico-striatal monoamine accumulation and metabolism compared to socially reared controls and that these alterations are reversed with NAC treatment in a dose-dependent manner, and in keeping with current constructs of monoamine involvement in schizophrenia.

Material and methods

Animals

Weaned male Sprague-Dawley pups and their pregnant dams were bred in-house in the Animal Research Center of North-West University (NWU). The 21 day-old pups were obtained from an on-going breeding program. The litters in the breeding colony were not culled. According to previously described methods (Toua et al., 2010; Möller et al., 2011, 2013), the weaned animals were randomly assimilated on post-natal day (PND) 21 into socially reared (three rats/cage) or SIR (one rat/cage) groups. Social or isolation rearing was maintained for 8 weeks, the socially reared and SIR animals did not differ significantly with respect to weight. A total of 100 male rats (160-190 g) were used and formed part of a previous study undertaken in our laboratory (Möller et al., 2013). The rats were reared under identical conditions: cages (230(h) x 380(w) x 380(l) mm) with sawdust (Möller et al., 2011,

2013), temperature ($21 \pm 0.5^{\circ}\text{C}$), humidity ($50 \pm 10\%$), white light (350-400 lux), 12 h light/dark cycle and free access to food and water. SIR and socially reared animals experienced minimal handling and no environmental enrichment. Sawdust was changed weekly. The animals were handled according to the code of ethics in research, training and testing of drugs in South Africa, with ethical approval for the study obtained from the North-West University ethics committee (NWU-0035-08-S5). No distressful effects of NAC were observed at the dosages used in this study and the number of animals used was the minimum required to obtain scientifically valid data.

Drug preparation and dosing

NAC (Sigma-Aldrich, Johannesburg, South Africa), dissolved in saline and buffered with 1M glacial acetic acid and NaOH (pH = 6.0), was administered via intraperitoneal (i.p.) injection in a dose ranging study (50, 150, 250 mg/kg in 0.5ml/day). The doses for NAC were selected based on earlier studies in rodents (Smaga et al., 2012; Möller et al., 2013). Control groups received an equivalent volume of vehicle i.p., comprising saline and 1 M glacial acetic acid, buffered with sodium hydroxide (NaOH) (pH = 6.0). NAC and vehicle were administered in the last 14 days of social/SIR rearing.

Study design

This study consisted of a SIR cohort and a parallel socially reared cohort only focusing on the dose dependent effects of NAC on regional brain monoamine changes. This study formed part of a larger study undertaken in our laboratory that focused on the comparative effects of clozapine and NAC on mitochondrial, immunological, neurochemical and behavioral changes evoked by SIR in rats (Möller et al., 2013). This study therefore used the same rats as presented in the “behavioural and immune-neurochemistry study” described in Möller et al., 2013, although only the dose response of NAC and that of clozapine on DA was presented in that study. The aim of the current study is to present the dose-dependent effect of NAC on all cortico-striatal monoamines, including DA, as well as

their monoamine metabolites. The relevance of these responses to the behavioural and neurochemical data presented in the earlier paper will also be given due consideration in the Discussion. At weaning PND 21, rats were randomly allocated to 5 groups (10 rats/group) of either SIR or 5 groups (10 rats/ group) of social rearing for 8 weeks. After the 8 weeks, the animals were sacrificed by decapitation for brain dissection.

Brain dissection

After decapitation, the brain was dissected into the right and left cerebral hemispheres, the olfactory bulb was removed and the frontal cortex was dissected with the corpus callosum anterior tip being the external limit (Swanson, 2004; Toua et al., 2010). The remaining brain was then placed on its ventral side and the striatum dissected out using the external walls of the lateral ventricles as internal limits and the corpus callosum as external limits (Toua et al., 2010; Möller et al., 2011, 2013). These brain regions were immediately fixed in liquid nitrogen and stored at -80 °C. On the day of assay, the tissue was thawed on ice, weighed and a 10% tissue homogenate prepared in a Teflon homogenizer using phosphate buffered saline (PBS).

Cortico-striatal monoamine analyses

To ensure blindness throughout the monoamine analyses, all samples were assigned a different colour for the treatment groups and a different letter for SIR or social rearing respectively. The person that quantified the high performance liquid chromatography (HPLC) results was also blind to the different groups. Quantification of cortico-striatal DA, 5-HT, NA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (Dopac), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were performed by a HPLC system with electrochemical detection (HPLC-EC), as previously described (Harvey et al., 2006). Monoamine concentrations in the cortico-striatal samples were determined by comparing the area under the peak of each monoamine to that of the internal standard, isoprenaline (range 5–50 ng/ml; Chemstation Rev. A 06.02 data acquisition and analysis software). Linear

standard curves (regression coefficient greater than 0.99) were found in this particular range. Monoamine concentrations were expressed as ng/mg wet weight of frontal cortical and striatal tissue (mean $\pm$ SEM).

Statistical analyses

To model the cortico-striatal monoamines, a three-way factorial analysis of variance (ANOVA) and Bonferroni post-hoc tests was applied for the respective treatments (no treatment, vehicle, NAC 50 mg/kg, NAC 150 mg/kg and NAC 250 mg/kg), rearing conditions (social vs. SIR) and brain area (frontal cortex and striatum). Data are expressed as the mean $\pm$ standard error of the mean (SEM), with a p value of < 0.05 deemed statistically significant (Graphpad Prism 5; SAS/STAT® Software).

Results

Cortico-striatal dopamine, Dopac and HVA

Significant interactions were observed between the treatment groups, with respect to frontal cortical DA ($F(6, 26) = 15.66$, $p < 0.0001$; Fig. 1a), Dopac ($F(6, 26) = 12.88$, $p < 0.0001$; Fig. 1b) and HVA ($F(6, 26) = 27.82$, $p < 0.0001$; Fig. 1c), as well as striatal DA ($F(6, 26) = 5.22$, $p < 0.0001$; Fig. 1d), Dopac ($F(6, 26) = 7.78$, $p < 0.0001$; Fig. 1e) and HVA ($F(6, 26) = 9.41$, $p < 0.0001$; Fig. 1f) (Three-way ANOVA). No significant differences were observed between DA, Dopac or HVA in the frontal cortex and striatum of the socially reared groups respectively (Fig. 1a-f) (post-hoc Bonferroni). However, a significant decrease in frontal cortical ($p < 0.0001$; Fig. 1a-c) and an increase in striatal ($p < 0.0001$; Fig. 1d-f) DA, Dopac and HVA were observed in SIR animals receiving either no treatment or vehicle treatment, compared to their socially reared controls (post-hoc Bonferroni).

After post-hoc Bonferroni testing we observed the following: In the frontal cortex, the SIR-induced decrease in DA (Fig. 1a) was significantly but not fully reversed by 150 mg/kg ($p =$

0.04) and 250 mg/kg ($p = 0.008$) NAC. The decrease in Dopac (Fig. 1b) was also significantly but not fully reversed by 150 mg/kg ($p < 0.05$) and 250 mg/kg ($p = 0.01$) NAC. Similarly, the SIR-induced decrease in HVA (Fig. 1c) was significantly reversed by 150 mg/kg ($p = 0.009$), and fully reversed by 250 mg/kg ($p < 0.0001$) NAC. In the striatum, SIR-induced increases in DA (Fig. 1d) was significantly reversed by 150 mg/kg ($p = 0.009$), and fully reversed by 250 mg/kg ($p < 0.0001$) NAC. The increase in Dopac (Fig. 1e) was fully reversed by both 150 and 250 mg/kg NAC ($p < 0.0001$), while the increase in HVA (Fig. 1f) was significantly reversed by 150 mg/kg ($p = 0.001$), but fully reversed by 250 mg/kg ($p < 0.0001$) NAC. In all instances 50 mg/kg NAC was ineffective in reversing SIR induced DA and metabolite changes in either brain region.

Cortico-striatal serotonin and 5-HIAA

Significant interactions were observed between the treatment groups, with respect to frontal cortical 5-HT ($F(4, 9) = 10.16$, $p < 0.0001$; Fig. 2a) and 5-HIAA ($F(4, 9) = 10.04$; $p < 0.0001$, Fig. 2b), as well as striatal 5-HT ($F(4, 9) = 1.46$, $p < 0.0001$; Fig. 2c) and 5-HIAA ($F(4, 9) = 4.42$, $p < 0.0001$; Fig. 2d) (Three-way ANOVA). No significant changes were observed with regards to cortico-striatal 5-HT (Fig. 2a & c) and 5-HIAA (Fig. 2b & d) respectively in the socially reared animals (post-hoc Bonferroni). However, a significant decrease in frontal cortical 5-HT and 5-HIAA levels ($p < 0.0001$; Fig. 2a & b) and a significant increase in striatal 5-HT and 5-HIAA levels ($p < 0.0001$; Fig. 2c & d) were observed in SIR animals receiving no treatment or vehicle treatment compared to their socially reared controls (post-hoc Bonferroni).

After post-hoc Bonferroni testing we observed the following: In the frontal cortex the SIR-induced 5-HT deficit was significantly reversed with 150 mg/kg NAC ($p = 0.03$) and fully reversed by 250 mg/kg NAC ($p < 0.0001$; Fig. 2a). Similarly, the 5-HIAA deficit was significantly reversed with 150 mg/kg NAC ($p = 0.03$) but fully reversed by 250 mg/kg NAC ($p < 0.0001$; Fig. 2b) in SIR animals. In the striatum, SIR-induced elevated 5-HT was

significantly reversed with 150 mg/kg NAC and more comprehensively reversed with 250 mg/kg NAC ($p = 0.03$ and $p < 0.0001$, respectively; Fig. 2C). Similarly SIR-induced elevation in 5-HIAA was also significantly reversed with 150 mg/kg NAC and more comprehensively reversed with 250 mg/kg NAC ($p = 0.018$ and $p < 0.0001$, respectively; Fig. 2d). In all instances 50 mg/kg NAC was ineffective in reversing SIR induced 5-HT and 5-HIAA changes in either brain region.

Cortico-striatal noradrenaline and MHPG

Significant interactions were observed between the treatment groups, with respect to frontal cortical NA ($F(4, 9) = 0.66$, $p = 0.008$; Fig. 3a) and MHPG ($F(4, 9) = 0.14$, $p < 0.0001$; Fig. 3b) as well as striatal NA ($F(4, 9) = 1.69$, $p < 0.0001$; Fig. 3c) and MHPG ($F(4, 9) = 0.79$, $p < 0.0001$; Fig. 3d) (Three-way ANOVA). In the socially reared treatment groups there were no significant differences with regards to cortico-striatal NA (Fig. 3a & c) and MHPG (Fig. 3b & d) respectively (post-hoc Bonferroni). However, in the SIR animals receiving no treatment or vehicle treatment, a significant elevation in frontal cortical NA ($p < 0.0001$; Fig. 3a) and a significant decrease in frontal cortical MHPG ($p < 0.0001$; Fig. 3b) was observed, while striatal NA ($p < 0.0001$; Fig. 3c) and MHPG ($p < 0.0001$; Fig. 3d) was significantly elevated compared to their socially reared controls (post-hoc Bonferroni).

After post-hoc Bonferroni testing we observed the following: In the frontal cortex, 250 mg/kg NAC was the only treatment that significantly reversed the MHPG deficit in SIR animals ($p = 0.02$; Fig. 3b). In the striatum the SIR-induced elevation in NA was significantly reversed with 150 and 250 mg/kg NAC respectively, ($p = 0.02$ and $p < 0.0001$, respectively; Fig. 3c), while the elevated MHPG was also significantly reversed with 250 mg/kg NAC ($p = 0.04$; Fig. 3d) in the SIR animals. In all instances 50 mg/kg NAC was ineffective in reversing SIR induced NA and MHPG changes in either brain region.

Discussion

Key observations from this study are that SIR induces significant changes in frontal cortical and striatal monoamines compared to their socially reared controls, and that these changes are amenable to treatment with NAC. SIR induced a significant decrease in frontal cortical DA and its metabolites, Dopac, HVA, as well as of 5-HT and its metabolite, 5-HIAA, and the NA metabolite, MHPG. Simultaneously SIR engendered an *elevation* in frontal cortical NA. Regarding the striatum, DA, Dopac, HVA, 5-HT, 5-HIAA, NA and MHPG were elevated in SIR rats. NAC 150 and 250 mg/kg (but not 50 mg/kg) significantly reversed all cortico-striatal DA, Dopac, HVA, 5-HT and 5-HIAA alterations as well as the elevation in striatal NA. NAC 250 mg/kg also reversed deficits in frontal cortical MHPG and elevations in striatal MHPG.

Post-mortem studies in schizophrenia indicate frontal cortical hypo-dopaminergia (Rollema et al., 2000) along with increased striatal but diminished frontal cortical 5-HT uptake sites (Joyce et al., 1993). The DA hypothesis of schizophrenia proposes a hyper-dopaminergic state in the striatum, predicting positive symptom expression, and a hypo-dopaminergic state in the frontal cortex mediating cognitive and negative symptoms (Harvey et al., 1999; Guillin et al., 2007). Such changes are proposed to occur following a loss of ventral mesencephalon DA neurons projecting to the cortex during neurodevelopment, leading to prefrontal DA hypo-activity and mesolimbic DA hyperactivity (reviewed in Howes and Kapur, 2009).

On the other hand, chronic psychosocial stress provokes glutamate release (Musazzi et al., 2011), in turn stimulating the release of 5HT, NA and DA (Stahl, 2007) purported to underlie schizophrenia (Haroutunian et al., 2003). Furthermore, excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA) neurones in the prefrontal cortex and hippocampus modulate cortical and sub-cortical DA, 5-HT and NA activity (Lewis and González-Burgos, 2008; Stahl, 2007). Thus, a loss of descending cortical inhibitory glutamate-GABA pathways (i.e. hypo-glutamatergia) will provoke an increase in striatal (nucleus accumbens) DA, 5-HT and NA activity (Carlsson et al., 2001; Stahl, 2007). This pathological profile is consistent with that in schizophrenia (paragraph above), and has been faithfully reproduced here in SIR animals. Elevated striatal DA, 5-HT and NA would normally decrease DA, 5-HT and NA

release in the frontal cortex (Carlsson et al., 2001; Stahl, 2007), also reproduced in our cortical DA, Dopac, HVA, 5-HT, 5-HIAA and MHPG results. Interestingly SIR evokes a significant *increase* in cortical NMDA receptor density (Toua et al., 2010), suggesting that cortical hypo-glutamatergia may indeed be driving these events. Deficits in prefrontal 5-HT following SIR is also hypothesized to contribute to the behavioural impairments associated with schizophrenia (Meltzer et al., 2003). Moreover, since d-lysergic acid (LSD), a 5HT_{2A} receptor partial agonist, *mimics* the positive symptoms observed in schizophrenia, the 5-HT hypothesis of schizophrenia proposes an excess of 5-HT in the striatum (Aghajanian and Marek, 2000), as demonstrated here. Increased NA reactivity and/or tone has been linked to anxiety observed in schizophrenia (Lysaker and Salyers, 2007), which is congruent with SIR-induced changes in frontal cortical and striatal NA levels demonstrated here (Blier and El Mansari, 2007). Elevated brain NA levels are also associated with the positive symptoms of the illness (Yamamoto and Hornykiewicz, 2004), which correlates well with the aforementioned NA levels as well as PPI deficits observed in SIR rats (Möller et al., 2013). Our monoamine data also parallels earlier SIR studies, where increases or decreases in frontal cortical DA, unchanged striatal DA levels and decreased cortical (or striatal) 5-HT/5-HIAA have been described (Trabace et al., 2012; Jaffe et al., 1991; Rilke et al., 2001), as well as decreased frontal cortical and elevated nucleus accumbens DA, Dopac, HVA and 5-HT levels (Powell et al., 2003; Brenes and Fornaguera, 2009). Moreover, behavioural studies describing positive and negative symptomology in rats (Möller et al., 2011; 2013) can be closely linked to these aberrant monoamine changes.

Although speculative without supporting *in vivo* microdialysis release studies, the observed parallel reduction or increase of both the neurotransmitter and its principle metabolite suggests an overall reduction/stimulation of neurotransmitter levels, with a subsequent reactive decrease/increase in its metabolism. However, where neurotransmitters levels are elevated but its metabolite reduced (eg. NA/MHPG in striatum), it may imply that neurotransmitter levels are bolstered following reduced metabolism. In fact, altered

functional activity of monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) are altered in schizophrenia (Zumárraga et al., 2010), which will lead to diverse changes in synaptic monoamine levels.

Since oxidative stress plays a prominent role in both schizophrenia (Ng et al., 2008; see Introduction) and in appropriate translational animal models (Möller et al., 2011; Radonjić et al. 2010; Dean et al. 2009), along with the fact that oxidative stress can evoke monoaminergic changes (Garcia-Cazorla et al., 2008), may a link with neuro-inflammatory cascades explain our observations? The mitochondria are central in redox homeostasis (Berk et al., 2013). We have earlier proposed that the effects of SIR on cortico-striatal DA is secondary to mitochondrial and immune-inflammatory related abnormalities and the effect of the latter on the glutamate-modulating actions of the tryptophan-kynurenine pathway (Möller et al., 2013). DA release in the nucleus accumbens is associated with an increase in mitochondrial ATP (Krügel et al., 2001), while glutamate-dependent striatal DA release involves hydrogen peroxide (H_2O_2) activation of ATP-sensitive K^+ channels (Avshalumov and Rice, 2003). DA dysfunction and schizophrenia progression is related to cytokines via inflammation, oxidative stress and apoptosis (Meyer and Feldon, 2009). Moreover, frontal-cortical glutamate mediated regulation of DA release (Schwartz et al., 2012) is modulated by kynurenic acid (KYNA) (Okuno et al., 2011), a known negative regulator of the NMDA receptor (Möller et al., 2012).

Redox-inflammatory pathways may also modulate serotonergic and noradrenergic transmission. Nitric oxide, for example, is causally implicated in schizophrenia (Bernstein et al., 2011), possibly contributing via regulation of glutamate-mediated release of 5-HT and NA (Milusheva et al., 2003; Harsing et al., 2004). Furthermore, depletion of cortical 5-HT as depicted in this paper, may occur via activation of indoleamine 2,3-dioxygenase (IDO) by pro-inflammatory cytokines, thereby promoting tryptophan metabolism via the kynurenine pathway and compromising 5-HT synthesis (Dantzer et al., 2011), possibly contributing to depressive symptoms. Although a reciprocal effect on striatal 5-HT is evident, this

nonetheless pre-empt 5HT_{2A} receptor activation, as proposed by the 5-HT hypothesis (Aghajanian and Marek, 2000). NA release, on the other hand, can be engendered by oxidative stress (Milusheva et al., 2003), an action that may reflect a compensatory anti-inflammatory response (O'Sullivan et al., 2009; McNamee et al., 2010). Indeed, SIR-induced increase in cortico-striatal NA demonstrated here may reflect such a reactive compensatory mechanism. Thus the presence of oxidative stress, inflammation, dysregulation of the kynurenine pathway and mitochondrial dysfunction may provide a rational explanation for cortico-striatal monoamine changes described in this study.

Consequently, SIR closely emulates the dopaminergic and serotonergic profile of schizophrenia following a chronic early life adverse experience (Chorpita and Barlow, 1998), but also displays disorganized glutamatergic and redox homeostasis. Sub-chronic NAC treatment dose-dependently reversed SIR-induced cortico-striatal changes in DA, Dopac, HVA, 5-HT and 5-HIAA, as well as deficits in frontal cortical MHPG and elevations in striatal NA and MHPG. These findings are congruent with human (Berk et al., 2008b) and animal (Möller et al., 2013) studies demonstrating NAC's therapeutic potential as adjunctive treatment in schizophrenia, and now confirms that its beneficial response involves a correction of aberrant monoamine activity. Important to note, however, is that the bio-behavioral effects of NAC are unlikely to involve a purely antioxidant action since other typical antioxidants (eg. Vitamin C & E) are ineffective in schizophrenia (Dodd et al., 2008). However, targeting NOX2, as well as redox impaired parvalbumin interneurons (Cabungal et al., 2012), modulating the cystine–glutamate antiporter (Wu et al., 2004), and reversing mitochondrial toxicity (Singh et al., 2010), will endow NAC with a multi-targeted capacity to address the complex neurobiology of schizophrenia.

In schizophrenia, new generation antipsychotics purportedly improve positive symptoms by blocking striatal D₂ receptors, while inhibitory actions on cortical 5-HT_{2C} receptors and the associated facilitation of cortical DA activity provide relief for negative and cognitive symptoms (Miyamoto et al., 2005). This and our earlier work (Möller et al., 2011, 2012,

2013) confirms that despite NAC's apparent lack of a direct action on monoaminergic signaling, its multimodal action involving glutamate and redox-immune-inflammatory processes includes a prominent action on key monoamines, and in relevant brain regions, involved in schizophrenia. By increasing frontal cortical but decreasing striatal DA, 5-HT and metabolite levels, and by targeting changes in cortico-striatal NA, NAC may mediate important therapeutic benefits in the treatment of schizophrenia. It is interesting that all doses of NAC failed to evoke any notable effects on cortico-striatal monoamines in healthy, socially reared animals, suggesting that NAC may operate only under pathological conditions. These data also provide impetus for further research into the therapeutic application of NAC in disorders associated with elevated striatal NA and MHPG and deficits in frontal cortical MHPG, such as panic attacks and posttraumatic stress disorder (Garvey et al., 1996; Pervanidou et al., 2008), disorders characterized by hypo-frontality, such as bipolar disorder and depression (Galynker et al., 1998), and disorders of altered cortico-thalamo-cortical DA-5-HT signalling, such as obsessive compulsive disorder (Ting and Feng 2008).

In conclusion, SIR destabilizes cortico-striatal monoamines congruent with monoaminergic theories of schizophrenia, while NAC demonstrates dose-dependent reversal of these changes. The therapeutic effect of NAC in schizophrenia probably involves an indirect action on monoaminergic systems, secondary to effects on glutamate, mitochondrial and inflammatory-redox signaling processes. Finally, the study re-affirms the therapeutic potential of NAC in the treatment of schizophrenia.

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Conflict of interest: The authors declare that over the past three years. Brian Harvey has participated in speakers/advisory boards and received honoraria from Organon, Pfizer and Servier, and has received research funding from Lundbeck. The authors declare that, except for income from the primary employer and research funding to BHH from the South African Medical Research Council, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. Michael Berk has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, MBF, NHMRC, Beyond Blue, Rotary Health, Geelong Medical Research Foundation, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Organon, Novartis, Mayne Pharma and Servier, has been a speaker for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck, Merck, Pfizer, Sanofi Synthelabo, Servier, Solvay and Wyeth, and served as a consultant to Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck Merck and Servier. Dr Berk is a co-inventor of two provisional patents regarding the use of NAC and related compounds for psychiatric indications, which, while assigned to the Flory Institute of Neuroscience and Mental Health, could lead to personal remuneration upon a commercialization event.

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Figure legends

Fig. 1 DA, Dopac and HVA levels in the frontal cortex (a), (b), (c) and striatum (d), (e), (f) in socially reared and SIR rats following drug treatments, as indicated ($n = 10/\text{group}$). $\#p < 0.0001$ vs. social no treatment, $*p < 0.05$ vs. SIR no treatment and vehicle, $**p < 0.0001$ vs. SIR no treatment and vehicle (Bonferroni post-hoc test). The dopamine data presented here (but not the DA metabolites) have been presented in Möller M, et al. *Brain Behav Immun.* 2013 May, 30:156-67 (used with permission - Elsevier Inc. license 3147511105152, May 14, 2013). Refer to text for precise p values

Fig. 2 5-HT and 5-HIAA levels in the frontal cortex (a), (b) and striatum (c), (d) in socially reared and SIR rats following drug treatments, as indicated ($n = 10/\text{group}$). $\#p < 0.0001$ vs. social no treatment, $*p < 0.05$ vs. SIR no treatment and vehicle, $**p < 0.0001$ vs. SIR no treatment and vehicle (Bonferroni post-hoc test). Refer to text for precise p values

Fig. 3 NA and MHPG levels in the frontal cortex (a), (b) and striatum (c), (d) in socially reared and SIR rats following drug treatments, as indicated ($n = 10/\text{group}$). $\#p < 0.001$ vs. social no treatment, $*p < 0.05$ vs. SIR no treatment and vehicle, $**p < 0.001$ vs. SIR no treatment and vehicle (Bonferroni post-hoc test). Refer to text for precise p values.

Fig. 1

Fig. 2

Fig. 3