
Chapter 6

Manuscript D

“Ready for submission” article in *Metabolic Brain Disease*, entitled:

“N-acetylcysteine treatment reverses changes in cortico-striatal monoamines induced by social isolation rearing in rats.”

Introduction

This chapter presents the “ready for submission” manuscript for publication in *Metabolic Brain Disease*, published by Springer. The manuscript is presented in the required format prescribed by *Instructions to the Authors*, and as outlined on the journal website:

<http://www.springer.com/biomed/neuroscience/journal/11011>

The manuscript will begin with the title, contributing authors and affiliations on a separate page, followed by an Abstract also on a single page. Thereafter will follow the main body of the manuscript, including: Introduction, Materials and methods, Results, Discussion, Conclusion, Author disclosures, Acknowledgements, References, Figure Legends and Figures. As per the journal submission format, all figures are separate, and placed at the end of the manuscript.

In order to allow more meaningful evaluation and interpretation of this manuscript, dopamine data presented in Chapter 5, Manuscript C, were included in this manuscript as well and was deemed necessary to allow for greater clarity and interpretation of findings described in this last manuscript. However, this is deemed a “ready for submission” manuscript and if submitted in the future, a license for the re-use of data will be obtained from Manuscript C’s publishers (Elsevier).

Authors' contributions

- *M. Möller* designed the study along with *BH Harvey*, undertook the entire analytical laboratory and statistical analyses as well as the animal study, wrote the first draught of the manuscript, and edited the manuscript after receiving comments from co-authors for publication.
- *JL du Preez* supervised all aspects of the laboratory analysis.
- *F Viljoen* supervised the monoamine laboratory analysis.
- *M Berk* advised on the study design, the use of N-acetyl cysteine and proof read the final manuscript.
- *BH Harvey* supervised the study design and assisted in the interpretation of the study data, as well as finalized the manuscript for publication.

All co-authors provided permission to use this manuscript as part of M Möller's Phd thesis.

N-acetyl cysteine reverses social isolation rearing induced changes in corticostriatal monoamines in rats.

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Abstract

Schizophrenia, depression and anxiety related disorders are associated with early-life environmental stressors, with evidence implicating oxidative stress in the pathophysiology of these disorders. N-acetyl cysteine (NAC), a glutathione precursor and antioxidant, is emerging as a useful agent in the adjunctive treatment of these disorders. However, it is not known how these actions impact on brain monoamine metabolism, the principal target for current treatment of these disorders. Social isolation rearing (SIR) is a valid model of schizophrenia, depression and anxiety-related disorders in animals. This study evaluated the dose-dependent effects of NAC (50, 150 and 250 mg/kg/day) on SIR induced changes in cortico-striatal levels of dopamine (DA), serotonin (5-HT) noradrenaline (NA) and their metabolites. SIR induced significant deficits in frontal cortical DA, 3,4-dihydroxyphenylacetic acid (Dopac), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), with significant elevations in frontal cortical NA and striatal DA, Dopac, HVA, 5-HT, 5-HIAA, NA and MHPG. NAC 150 and 250 mg/kg significantly reversed all cortico-striatal DA, Dopac, HVA, 5-HT and 5-HIAA alterations as well as striatal NA elevation in SIR animals, with NAC 250 mg/kg also reversing elevations in MHPG and deficits in frontal cortical MHPG. In conclusion, as an early-life stressor SIR profoundly alters cortico-striatal DA, 5-HT and NA pathways that is reversed or abrogated by sub-chronic NAC treatment in a dose-dependent manner. A modulatory action on cortico-striatal monoamines may explain the therapeutic utility of NAC in psychiatric illnesses where redox dysfunction or oxidative stress is a causal factor.

Keywords: N-acetyl cysteine, monoamines, social isolation rearing, anxiety disorder, depression, schizophrenia.

Introduction

The exact aetiology of schizophrenia, depression and anxiety disorders remains incompletely understood, although disturbances in frontal cortical and striatal dopamine (DA), serotonin (5-HT) and/or noradrenaline (NA) release, metabolism and sub-cellular signalling are causally involved (reviewed in (Carlsson et al., 2001; Krishnan and Nestler, 2008). Importantly, the majority of drugs used clinically to treat these disorders target monoamine receptors, reuptake transporters and monoamine metabolism (McIntyre et al., 2007; Lieberman, 1993; Papakostas, 2008).

The frontal cortex and striatum plays a central role in the neuropathology of schizophrenia (Meyer-Lindenberg et al., 2002), depression (Abercrombie et al., 2006) and obsessive compulsive disorder (OCD) (Graybiel and Rauch, 2000), the latter an anxiety disorder of relevance for this paper. Thus for example, schizophrenia is associated with reduced prefrontal cortex DA, elevated ventral striatum DA (nucleus accumbens), (Bertolino et al., 1996; Meyer-Lindenberg et al., 2002) and elevated frontal cortical 5-HT (Sumiyoshi et al., 1996). Depression is associated with an overall reduction in DA, NA and 5-HT in the frontal cortex and striatum (reviewed in Donald and Robinson, 2007). Indeed, functional imaging studies in patients with depression show decreased metabolism of monoamines in the prefrontal cortex (Mayberg, 1994). Positron emission tomography (PET) studies demonstrate abnormally elevated monoamine activity in frontal cortex and caudate regions in patients with OCD (Baxter, 1992). Although a serotonergic dysfunction only explains up to 50% of the variability in OCD (Pauls et al., 2002), most studies have confirmed the involvement of 5-HT in its neuropathology, based on evidence such as altered 5-HT transporters (Stengler-Wenzke et al., 2004) and 5-HT receptors (Simpson et al., 2011), while the select response of the disorder to 5-HT reuptake inhibitors (SRI) (Pigott and Seay, 1999) provides the most convincing evidence. This conclusion is supported by work in animals (e.g. Korff et al., 2008).

Interestingly, schizophrenia, depression, bipolar disorder and OCD all present with a disturbance in cortico-striatal redox balance and oxidative stress (Gawryluk et al., 2011; Michel et al., 2007; Prabakaran et al., 2004; Wang et al., 2009). This has also been verified in translational animal models, for example in the social isolation rearing (SIR) model of schizophrenia (Möller et al., 2011), the deer mouse model of OCD (Güldenpfennig et al., 2011) and the chronic mild stress model of depression (Lucca et al., 2009). Importantly, factors involved in cellular oxidative stress are known to evoke monoaminergic changes, possibly mediating psychiatric manifestations (Garcia-Cazorla et al., 2008; Ng et al., 2008).

In many instances, oxidative stress can be causally related to increased glutamate activity evident in both the striatum and frontal cortex (reviewed in Amadio et al., 2004; Haroutunian et al., 2003), while schizophrenia (Prabakaran et al., 2004), depression (Paul and Scholnick, 2003) and OCD (Pittenger et al., 2006, 2011) are closely linked to altered glutamatergic activity. 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) (Hashimoto, 2009; Carlsson et al., 2001) but can induce structural damage to neurons that in turn impact on cellular redox balance (Smythies, 1999). NMDA receptor dysfunction in the frontal cortex and striatum has recently become a focus of attention in attempts to explain the neurobiology of schizophrenia, depression and OCD (Krivoy et al., 2008; Hashimoto, 2009; Pittenger et al., 2006). In fact NMDA receptor modulators and antagonists have opened new therapeutic horizons for the treatment of these disorders, such as ketamine for depression (Zarate et al., 2006), D-cycloserine for schizophrenia (Heresco-Levy et al., 2002) and riluzole or ketamine for OCD (Bloch et al., 2012; Grant et al., 2007).

The glutathione precursor, antioxidant (Kerksick and Willoughby, 2005) and glutamate modulator (Grant et al., 2009), N-acetyl cysteine (NAC), is a promising therapeutic agent in disorders where glutamate dysfunction and/or oxidative stress are evident, in this case schizophrenia, depression and anxiety related disorders (Dean et al., 2011). Recent studies

have highlighted its efficacy in bipolar disorder, schizophrenia (Berk et al., 2008a, b; Berk et al., 2011; Magalhaes et al., 2011) as well as OCD (Lafleur et al., 2006). Although these disorders present with altered glutamate and redox function (reviewed in Krishnan and Nestler, 2008; Hovatta et al., 2010) as alluded to earlier, the mainstay of treatment for all these disorders are aimed primarily at monoamine transmission. By virtue of its action on redox and glutamate systems in the frontal cortex and striatum (Arent et al., 2012; Baker et al., 2002; Dean et al., 2011), it can nevertheless be argued that the therapeutic effects of NAC in depression, schizophrenia and OCD may involve secondary actions on DA, 5-HT and NA pathways. However, this supposition has never been formerly tested or confirmed.

Early adverse experiences, including early life stress, may 'shape' 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, anxiety and/or depressive disorders (Kendler et al., 2002; Matheson et al., 2011). Post-natal SIR of rats is a relevant model of early-life chronic stress that displays parallel symptoms validated to represent core symptoms of anxiety (Evans et al., 2012; Kuramochi and Nakamura, 2009), of schizophrenia (e.g. deficits in reversal learning and prepulse inhibition) (Bianchi et al., 2006; Li et al., 2007; Möller et al., 2011), and depressive-like behaviours (Brenes et al., 2008). 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, mood and anxiety disorders, with the latter focus on OCD. We hypothesize that SIR will significantly alter cortico-striatal monoamine accumulation and metabolism compared to socially reared controls and that most of these alterations will be reversed with NAC treatment in a dose-dependent manner.

Material and methods

Animals

A total of 100 male Sprague-Dawley rats (160-190 g; Animal Research Centre, North West University) were used. The rats were reared under identical conditions: cages (230(h) x 380(w) x 380(l) mm) with sawdust (Möller et al., 2011), 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 ethical 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/0.5ml/day). The doses for NAC were selected based on earlier studies in rodents (Smaga et al., 2012; Fukami et al., 2004; Möller et al., 2012). 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. At weaning (post-natal day 21), rats were randomly allocated to 5 groups (10 rats/ group) of either SIR (1 animal/cage) or 5 groups (10 rats/ group) of social rearing (3-4 rats/cage) for 8 weeks. After the 8 weeks, the animals were sacrificed, frontal cortex and striatum rapidly dissected (Toua

et al., 2010; Möller et al., 2011), snap frozen in liquid nitrogen and stored at -80°C until the day of monoamine analyses.

Cortico-striatal monoamine analyses

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 high performance liquid chromatography (HPLC) system with electrochemical detection (HPLC-EC), as previously described (Harvey et al., 2006; Harvey et al., 2010). 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

Three-way ANOVA revealed significant treatment-group interactions 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). Post hoc Bonferroni testing indicated no significant alterations between DA, Dopac or HVA in the frontal cortex and striatum of the socially reared groups respectively (Fig. 1a-f). However, Bonferroni post hoc testing indicated 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 in SIR animals receiving either no treatment or vehicle treatment, compared to their socially reared controls.

In the frontal cortex, the SIR-induced decrease in DA (Fig. 1a) was partially reversed by NAC 150 mg/kg ($p = 0.04$) and 250 mg/kg ($p = 0.008$). The decrease in Dopac (Fig. 1b) was also partially reversed by 150 mg/kg NAC ($p < 0.05$) and 250 mg/kg NAC ($p = 0.01$). Similarly, the SIR-induced decrease in HVA (Fig. 1c) was partially reversed by 150 mg/kg NAC ($p = 0.009$), and fully reversed by 250 mg/kg NAC ($p < 0.0001$). In the striatum, SIR-induced increases in DA (Fig. 1d) was partially reversed by 150 mg/kg NAC ($p = 0.009$), and fully reversed by 250 mg/kg NAC ($p < 0.0001$). 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 partially reversed by 150 mg/kg NAC ($p = 0.001$), but fully reversed by 250 mg/kg NAC ($p < 0.0001$).

Cortico-striatal serotonin and 5-HIAA

Three-way ANOVA indicated significant cross-group interactions 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). Post hoc testing with Bonferroni indicated no significant changes with regards to cortico-striatal 5-HT (Fig. 2a & c) and 5-HIAA (Fig. 2b & d) respectively in the socially reared animals. However, Bonferroni post hoc test indicated a significant decrease in frontal cortical 5-HT and 5-HIAA levels ($p < 0.0001$; Fig. 2a & b) and

a significant increase in the striatal 5-HT and 5-HIAA levels ($p < 0.0001$; Fig. 2c & d) in SIR animals receiving no treatment or vehicle treatment compared to their socially reared controls.

Bonferroni post hoc test indicated that in the frontal cortex the SIR-induced 5-HT deficit was partially but significantly reversed with NAC 150 mg/kg ($p = 0.03$) and more completely reversed by NAC 250 mg/kg ($p < 0.0001$; Fig. 2a). Similarly, the 5-HIAA deficit was partially but significantly reversed with NAC 150 mg/kg ($p = 0.03$) but more fully reversed by NAC 250 mg/kg ($p < 0.0001$) in SIR animals (Fig. 2b). In the striatum, SIR-induced elevated 5-HT was partially and fully reversed with NAC 150 and 250 mg/kg respectively, ($p = 0.03$ and $p < 0.0001$, respectively) (Fig. 2C). Similarly SIR-induced elevation in 5-HIAA was also partially and fully reversed with NAC 150 and 250 mg/kg respectively, ($p = 0.018$ and $p < 0.0001$, respectively) in the SIR animals (Fig. 2d).

Cortico-striatal noradrenaline and MHPG

Three-way ANOVA indicated significant cross group interaction 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). 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, as indicated with Bonferroni post hoc test. 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.

In the frontal cortex, NAC 250 mg/kg was the only treatment that partially reversed the MHPG deficit in SIR animals ($p = 0.02$) (Fig. 3b). In the striatum the SIR-induced elevation in NA was partially and fully reversed with NAC 150 and 250 mg/kg respectively, ($p = 0.02$ and

$p < 0.0001$, respectively) (Fig. 3c), while the elevated MHPG was also partially but significantly reversed with NAC 250 mg/kg ($p = 0.04$) in the SIR animals (Fig. 3d).

Discussion

Key observations from this study are that SIR induces significant changes in frontal cortical and striatal monoamines compared to their socially reared controls. In the frontal cortex such changes include *decrements* in 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 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 elevations in MHPG and deficits in frontal cortical MHPG. Various forms of stress are known to provoke the release of glutamate (Musazzi et al., 2011; Swanson et al., 2005) which in turn stimulates the release of 5HT, NA and DA (Pittaluga et al., 2001; Harvey et al., 2006; Stahl, 2007). Such changes underlie the neurobiology of stress-related conditions and how SIR may evoke similar changes. Glutamate-mediated changes in monoamines, redox balance and other events may have relevance in explaining the clinical effects of NAC in schizophrenia, depression and OCD.

Dopamine changes and correlation with psychiatric illness

Excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA) neurones in the prefrontal cortex and hippocampus modulate cortical and sub-cortical DA activity (Lewis and González-Burgos, 2008; Stahl, 2007). If descending inhibitory glutamate-GABA pathways from the prefrontal cortex are ineffective, striatal (nucleus accumbens) DA activity is increased, and *vice versa* (Stahl, 2007), while cortical glutamate-GABA insufficiencies may also evoke frontal cortical decrements in DA (Stahl, 2007). A functional link between frontal

cortical D₂ receptor over-expression and GABAergic inhibition suggests that GABAergic hypo-function is related to altered striatal DA activity (Li et al., 2011; Stahl, 2007).

SIR increases striatal D₂ receptors (King et al., 2009), reduces frontal cortical D₁ receptor density (Toua et al., 2010) and increases or decreases frontal cortical DA, with unchanged striatal DA levels (Trabace et al., 2012). Considering these studies, neurotransmitter-dependent changes in receptor expression predict that elevated or reduced DA levels will down-regulate or up-regulate DA receptors, respectively. However, our data suggests reduced frontal cortical and elevated striatal DA, and that SIR adversely affects both DA synthesis *and* metabolism in the striatum and frontal cortex.

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), in line with the SIR data described here. Such changes are proposed to occur following a loss of ventral mesencephalon DA neurons projecting to the cortex during neurodevelopment, leading to prefrontal DA hypoactivity and mesolimbic DA hyperactivity (reviewed in Howes and Kapur, 2009). This closely parallels the SIR-induced changes in frontal-cortical DA described in this study. Depressive symptoms (eg. avolition, guilt, suicidality, social withdrawal) are ascribed to frontal cortical hypo-dopaminergia (Krishnan and Nestler, 2008). However, striatal DA levels in depression are more often reduced (Nestler and Carlezon, 2006), being linked to symptoms such as anhedonia, reduced motivation and decreased energy levels. Thus, while SIR closely emulates the DA'ergic profile of schizophrenia, this profile is similar but not exactly as one would predict for depression, explaining why these disorders are phenotypically different, but also why patients with schizophrenia so often present with symptoms of depression (Hafner et al., 2005).

OCD presents with a hyper-dopaminergic state in the basal ganglia (striatum, thalamus and amygdala) (Kim et al., 2003). This neurochemical condition correlates with the profile

observed here following SIR, and confirms the efficacy of D₂ receptor antagonists in the treatment of OCD (reviewed in Graybiel and Rauch, 2000). Considering that OCD is an anxiety disorder, it is interesting that animal studies have indicated that chronic stress depletes DA within the rat prefrontal cortex (Mizoguchi et al. 2000; Harvey et al., 2006). Our data in SIR rats is thus also in line with typical DA changes following a chronic adverse experience.

Serotonin changes and correlation with psychiatric illness

Deficits in prefrontal 5-HT following SIR is hypothesized to contribute to the behavioural impairments associated with schizophrenia (Meltzer et al., 2003), depression (Meltzer, 1989) and OCD (Blier and de Montigny, 1998). 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). Moreover, post-mortem studies in schizophrenia indicate reduced frontal cortex 5-HT_{2A} and increased 5-HT_{1A} receptor density (Burnet et al., 1996, 1997) in psychosis (Rasmussen et al., 2010; Dean, 2003) and frontal cortical hypo-dopaminergia, respectively (Rollema et al., 2000). Another study indicated increased striatal but diminished frontal cortical 5-HT uptake sites in schizophrenia patients (Joyce et al., 1993).

Clinical studies in depression also observed reduced 5-HT_{2A} receptor density in the frontal cortex (Hurlemann et al., 2008; Ngan et al., 2000), while post-mortem studies in suicidal depressed patients indicated increased limbic and decreased frontal cortical 5-HT_{1A} receptors (reviewed in Savitz et al., 2009). Of note is that SIR-induced elevations in striatal 5-HT and 5-HIAA correlates with behavioural deficits observed in Flinders sensitive line (FSL) rats, a genetic model of depression (Zangen et al., 1997).

Data on cortico-striatal 5-HT metabolism in OCD is limited. However, only drugs that target the 5-HT₂ergic system are successful in treating OCD (Fineberg and Craig, 2007; Grados and Riddle, 2001). Moreover, 5-HT is implicated in a number of behavioural phenomena

related to OCD, including impulse control abnormalities, obsessions and anxiety (Barnes and Sharp, 1999; Cools et al., 2008), all confirming the central role of 5HT in OCD. Similarly various studies have demonstrated a decrease in midbrain levels of the 5HT transporter (SERT) in patients with OCD (Hesse et al., 2005; Reimold et al., 2007; Zitterl et al., 2008). Indeed, spontaneous stereotypy in deer mice is attenuated by 5-HT_{2A/C} receptor agonists (Korff et al., 2008). These findings do not exactly fit the 5-HT profile observed here following SIR, where we have found elevated striatal and decreased frontal cortical 5-HT levels. As with DA, explaining neurotransmitter changes by directly extrapolating 5-HT levels with 5-HT receptor expression is not straight forward. Indeed, tianeptine works opposite to an SRI to *reduce* synaptic 5HT levels yet is an effective antidepressant (Brink et al., 2006).

SIR decreases 5-HT in the frontal cortex (Trabace et al., 2012; Bickerdike et al., 1993; Jaffe et al., 1991), decreases 5-HT/5-HIAA in the frontal cortex and striatum (Rilke et al., 2001) and increases 5-HT in the nucleus accumbens (Brenes and Fornaguera, 2009). Thus SIR impairs not only 5-HT turnover but also its biosynthesis in both frontal cortex and striatum. In fact, our data on 5-HT and 5-HIAA concurs with this, with similarly 5-HT/5-HIAA changes in both brain regions, except being reduced in the frontal cortex but elevated in the striatum.

The deficit in frontal cortical 5HT and the elevation in striatal 5-HT and 5-HIAA in SIR rats could be explained by a hypo-glutamatergic state. As for DA explained earlier, deficits in cortical glutamate-GABA inhibitory pathways projecting to the striatum will increase striatal 5-HT release (Carlsson et al., 2001; Stahl, 2007), as noted here. Elevated 5-HT in the striatum in turn activates feedback pathways with a subsequent decrease in 5-HT release in the frontal cortex (Carlsson et al., 2001; Stahl, 2007), a response also noted here.

Noradrenaline changes and correlation with psychiatric illness

Post-mortem studies describe elevated brain NA levels in schizophrenia, and can be associated with the positive symptoms of the illness (Yamamoto and Hornykiewicz, 2004). This correlates well with increased striatal NA levels described here in SIR rats. On the other

hand, several lines of evidence suggest that NA is of major importance in depression (reviewed in Moret and Briley, 2011), including reduced levels of NA transporters in the locus coeruleus (Klimek et al., 1997), altered density and sensitivity of frontal cortical α_{2A} -adrenoceptors (Ordway et al., 2003; Valdizan et al., 2010), and a reduction of NA levels in non-compliant depressed patients (Ruhe et al., 2007). This contrasts with SIR-induced elevations in cortico-striatal NA described here. It should however be noted that, although NA is not deemed to play a major role in the pathology and treatment of schizophrenia or OCD (reviewed in Delgado and Moreno, 1998; Reynolds, 1992), anxiety disorders invariably present with increased NA reactivity and/or tone (Garvey and Tuason, 1996; Pervanidou, 2008), which is indeed congruent with SIR-induced changes in frontal cortical and striatal NA. SRI's also reduce NA via the inhibitory effects of 5-HT on NA transmission purported to underlie the efficacy of SRI's in treating OCD (Blier and Mansari, 2007).

Studies on the effects of SIR on cortico-striatal NA levels are limited, although one study indicated reduced striatal NA following SIR (Brenes et al., 2008). Since SIR is a recognised chronic stressor, it is worthwhile interpreting our data within these confines. Previous animal studies indicated that chronic stress increases NA activity in the prefrontal cortex and limbic regions (reviewed in Goddard et al., 2010; Finlay et al. 1995, 1997; Miner et al. 2006), which is strongly supported by data described here following SIR. Unlike the other monoamines, frontal cortical NA and MHPG responded differently to SIR, with NA elevated but MHPG reduced, thus suggesting that NA levels increase as a result of reduced metabolism. NA and MHPG were altered in the same direction in the striatum confirming a broad effect on NA synthesis and metabolism in this brain region.

Effects on NA very likely involve a stress response mediated by cortisol. Since glucocorticoid release is responsible for inhibiting NA secretion from the sympathetic nerve terminals (Pacak et al. 1995), lower cortisol levels may prolong the availability of NA in adrenergic synapses resulting in failure in shutting off the stress response (Yehuda 1998). This would exacerbate NA release, as observed here in the frontal cortex and striatum of

SIR rats. Clinical studies indicate that repeated acute traumatic stress increases central NA (Kukolja et al., 2008), while this is also evident in hippocampus but not frontal cortex in a rodent model of post-traumatic stress disorder (PTSD) (Harvey et al., 2006). Interestingly, changes in monoamine levels are highly dependent on the type of stress applied, with acute stress and re-exposure having diverse effects on NA, 5HT and DA accumulation in limbic regions of the rat brain (Harvey et al., 2006).

Effect of NAC on monoamine levels and metabolism

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. In SIR animals, sub-chronic NAC treatment demonstrated dose-dependently reversed SIR-induced cortico-striatal changes in DA, Dopac, HVA, 5-HT and 5-HIAA (NAC 150 and 250 mg/kg), reversed elevated frontal cortical NA (NAC 150 mg/kg), as well as deficits in frontal cortical MHPG and elevations in striatal NA and MHPG (NAC 250 mg/kg). These findings are congruent with NAC's reported therapeutic potential as adjunctive treatment in schizophrenia (and probably bipolar disorder) (Berk et al., 2008a, b; Berk et al., 2011) as well as OCD (Lafleur et al., 2006). Indeed, our study confirms that this beneficial response is mediated via a correction of aberrant monoamine activity.

Considering schizophrenia, by blocking 5-HT and DA receptors new generation antipsychotics improve positive symptoms, although less effective for negative symptoms (Miyamoto et al., 2005). NAC's therapeutic potential (Berk et al., 2008b) could be mediated by increasing frontal cortical but decreasing striatal DA levels, as observe in this study. However, NAC also targets cortico-striatal NA release and metabolism, suggesting a therapeutic potential in disorders associated with elevated striatal NA and MHPG, as well as deficits in frontal cortical MHPG, such as panic attacks and PTSD (Garvey et al., 1987; Pervanidou et al., 2007).

In depression, all current antidepressants acutely increase 5-HT, NA and/or DA in the cortico-striatal pathways (Elhwuegi, 2004). By acting primarily on non-monoaminergic processes, e.g. glutamate, mitochondrial function and redox balance, NAC will indirectly increase frontal cortical DA and 5-HT and is so doing improve monoamine deficits in bipolar patients, or correct depressive symptoms (Berk et al., 2008a). Indeed, an action on 5-HT, NA and/or DA in depression and bipolar disorder only represents an early step in a complex cascade of events leading to treatment response (Piñeyro and Blier, 1999).

In OCD, the hypothesis is that SRI non-responders represent reduced 5-HT activity involving other systems within the cortico-striatal pathways, such as DA (Harsanyi et al., 2007). A useful treatment strategy is thus to increase 5-HT activity and to attenuate DA activity with a neuroleptic (reviewed in Korff et al., 2008). In this study NAC attenuated elevated striatal DA (acting like an antipsychotic) but also re-establishes 5-HT homeostasis (acting like a SRI). Ultimately such an action will benefit refractive OCD treatment (Lafleur et al., 2006).

A unifying hypothesis of NAC action

Monoamines aside, schizophrenia, depression, bipolar disorder and OCD also presents with cortico-striatal oxidative stress and dysfunctional glutamatergic activity, as evidenced in clinical (Krishnan and Nestler, 2008; Hovatta et al., 2010) and animal studies (Möller et al., 2011; Guldenpfenning et al., 2011; Lucca et al., 2009). Considering its effects on the GSH redox system (Kerksick and Willoughby, 2005) and on the cystine glutamate transporter (Wu et al., 2004), NAC may positively influence a number of these disorders. However, its therapeutic benefit is unlikely to involve a purely antioxidant action since other typical antioxidants (eg. Vit C & E) are ineffective in anxiety disorders, depression, OCD and schizophrenia (Dodd et al., 2008). Dysfunction in cortico-striatal GABA/glutamate transmission is significant to precipitate schizophrenia, depression and/or an anxiety disorder (Krystal et al., 2002). By modulating glutamate dependent and/or independent actions on redox homeostasis, NAC may reverse the aforementioned deficits to positively influence monoamine imbalances. Although the focus of this study was on the frontal cortex

and striatum, schizophrenia, depression and anxiety disorders (such as OCD) are not limited to disturbances in these brain regions. Evaluating NAC's effects on monoamine receptors in the amygdala, hippocampus and other brain regions is thus highly recommended.

Concluding, this study confirms that SIR modifies cortico-striatal monoamine levels congruent with monoaminergic theories of relevance for schizophrenia, depression and anxiety disorders, and has demonstrated the dose-dependent reversal of these changes with sub-chronic NAC treatment. The therapeutic effect of NAC in anxiety, mood and psychotic disorders therefore involves monoaminergic systems, probably secondary to effects on glutamate and redox signaling processes. Finally, the study re-affirms the therapeutic potential of NAC as adjunctive treatment in schizophrenia, depression and OCD.

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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/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. 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/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/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.

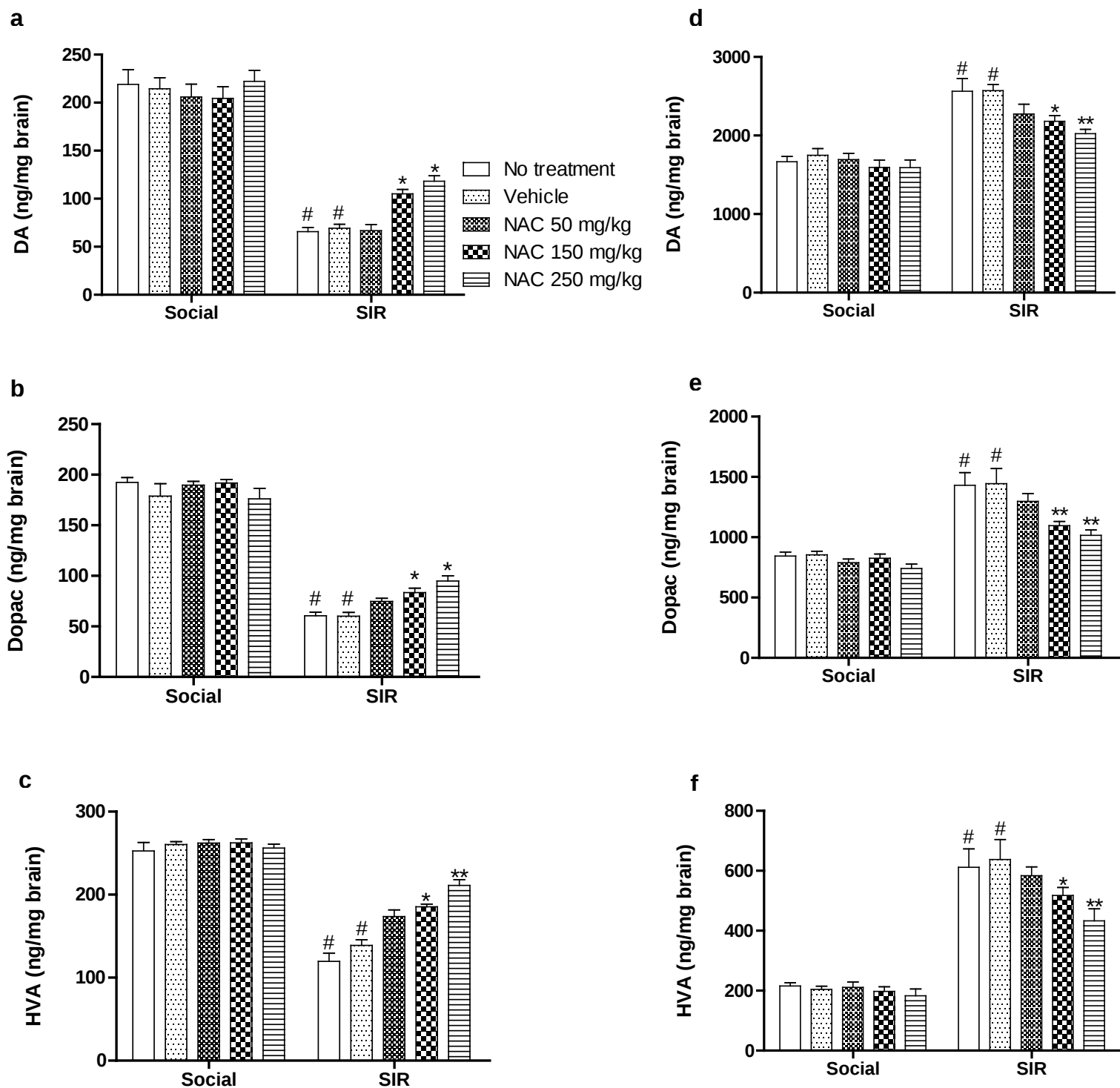


Fig. 1

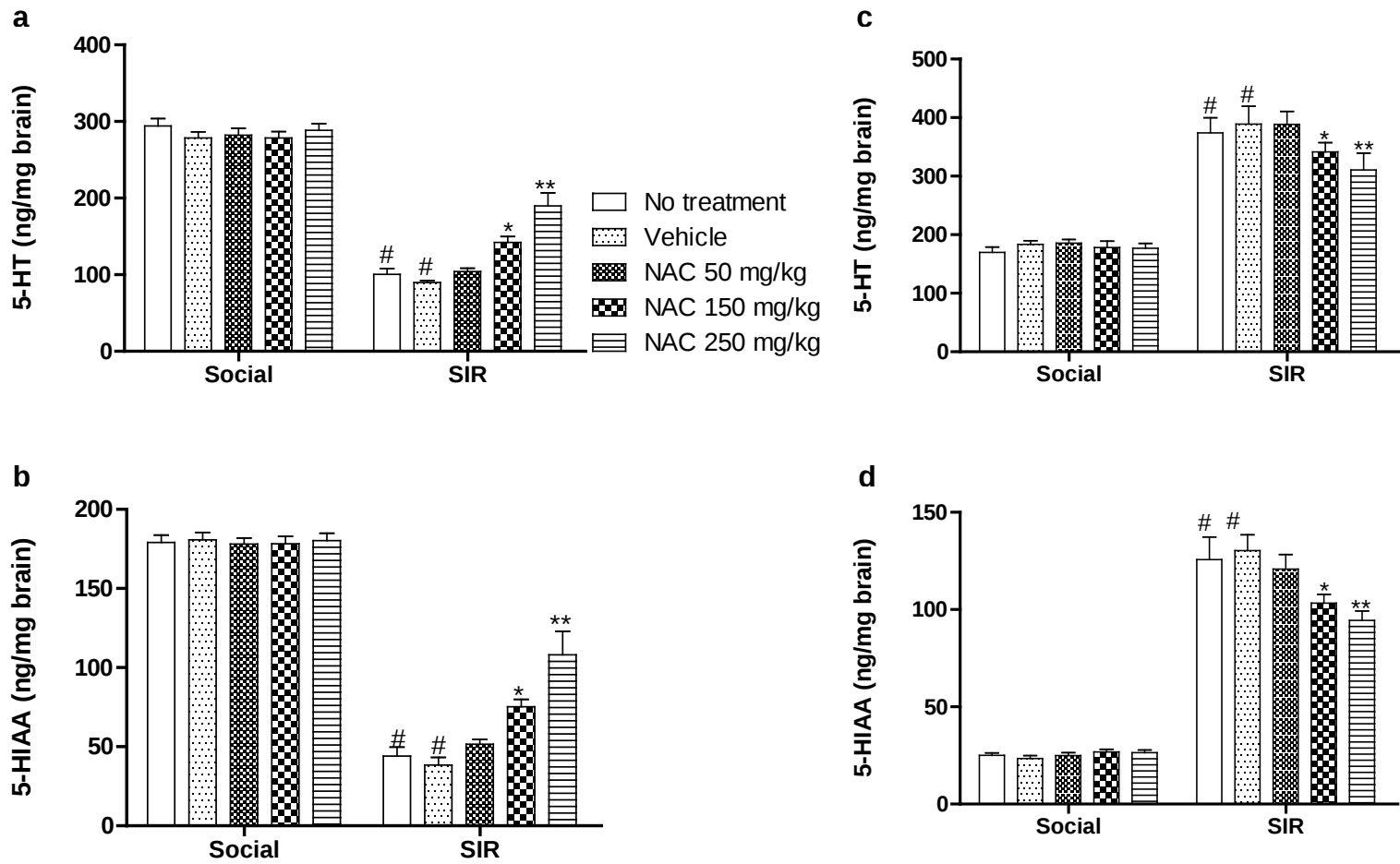


Fig. 2

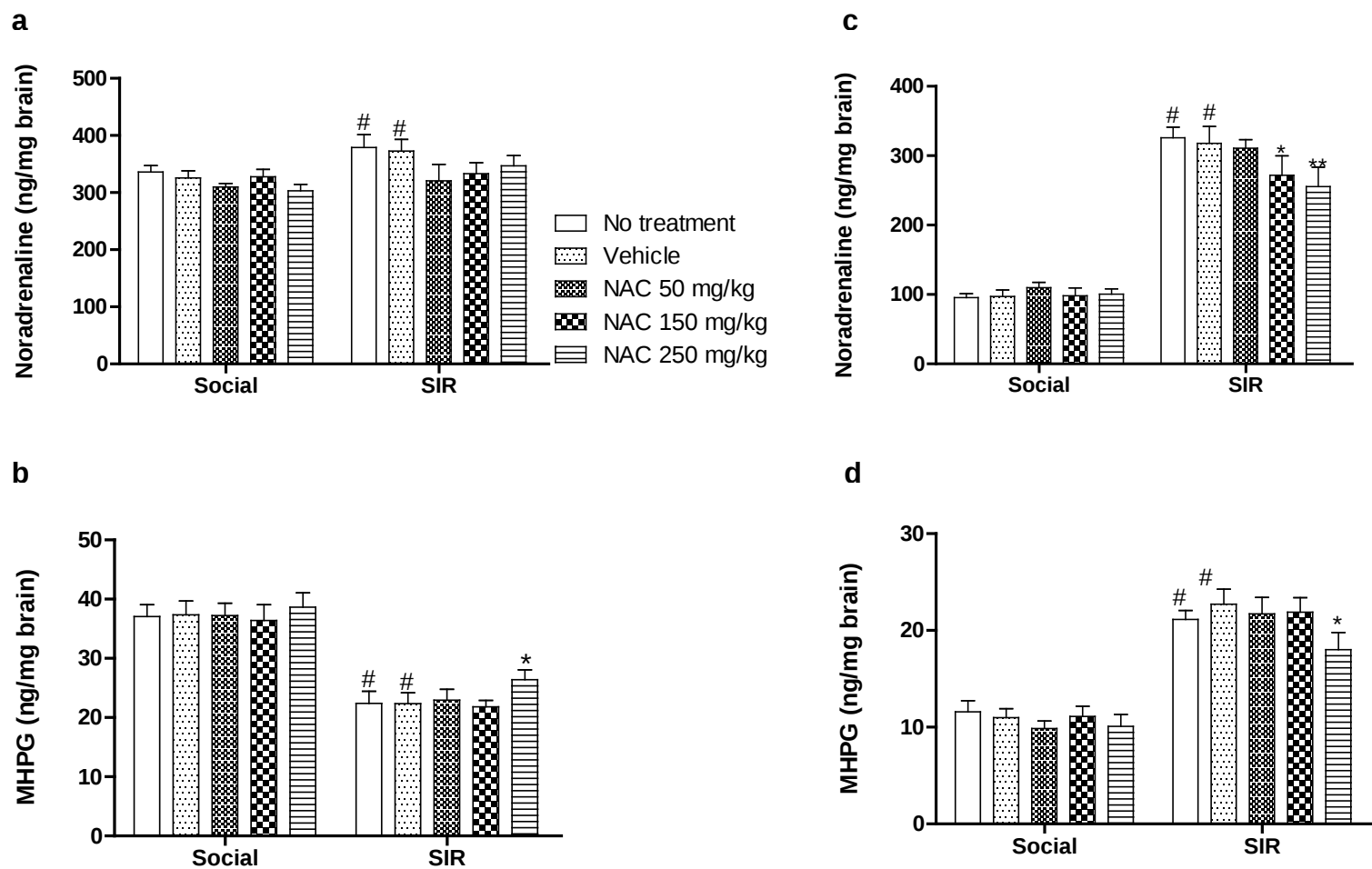


Fig. 3