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Interaction of glutathione depletion and psychotropic drug treatment in prepulse inhibition in rats and mice

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ABSTRACT

Oxidative stress has been implicated in several psychiatric illnesses, including schizophrenia. Glutathione is the brain's primary antioxidant and decreased levels of brain glutathione are reported in schizophrenia. Prepulse inhibition (PPI) is a measure of sensory gating, and PPI is reduced in schizophrenia. This study aimed to investigate the effects of brain glutathione depletion on PPI regulation.

Rats and mice were treated with the glutathione-depleting agent, 2-cyclohexene-1-one (CHX), and tested for baseline PPI and its disruption by treatment with amphetamine and MK-801.

Treatment with CHX caused significant depletion of GSH in frontal cortex and striatum of rats and mice. Baseline PPI and startle were not altered. However, the disruption of PPI after treatment with amphetamine was absent in CHX-treated rats. In contrast, the effect of MK-801 was not altered by CHX-treatment, nor was there any effect of CHX treatment in mice.

These data show an interaction of glutathione depletion with the effects of amphetamine treatment on PPI in rats. This effect could reflect loss of plasticity in PPI regulation caused by the additive effects of CHX-induced glutathione depletion and additional oxidative stress caused by amphetamine-induced dopamine release. The significance of these results for schizophrenia is discussed.

1. Introduction

Increasingly, oxidative stress is implicated in the pathophysiology of neurological and psychiatric disorders. Levels of antioxidants have been shown to be altered and increased markers of oxidative stress have been reported in schizophrenia and bipolar disorder (Dean et al., 2009; Ng et al., 2008). Levels of glutathione (GSH), a ubiquitous antioxidant, are reduced in the dorsolateral prefrontal cortex and caudate nucleus in schizophrenia (Do et al., 2000; Yao et al., 2006). However, it is unclear how glutathione depletion is involved in this illness.

Prepulse inhibition (PPI) is a cross-species model of sensorimotor gating, which is the ability to filter out irrelevant information and protect against sensory 'overload' (Braff and Geyer, 1990). PPI is disrupted in schizophrenia and other mental illnesses, and after treatment with dopaminergic or glutamatergic drugs, such as D-amphetamine or the N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, in rats and mice (Dai et al., 2004; Geyer et al., 2001; van den Buuse et al., 2005). In addition to their effects on PPI, these psychotropic drugs have pro-oxidant effects that may result in cellular oxidative stress. Amphetamine treatment causes increased dopamine release, which mimics similar changes seen during psychosis. As a result of these increased levels of dopamine, amphetamine treatment indirectly increases production of reactive oxygen species (ROS) through auto-oxidation of dopamine and production of metabolites such as quinones (Fleckenstein et al., 2007; Graham et al., 1978). While changes in brain GSH levels have not been reported following a single dose of amphetamine, the changes in oxidative stress markers it causes (Valvassori et al., 2010; Frey et al. 2006) could interact with decreased GSH levels. MK-801 treatment in rats has been shown to decrease antioxidant levels, including GSH and superoxide dismutase (SOD), and induce apoptosis (Ozyurt et al., 2007; Bondy and Guo, 1996). Furthermore, GSH modulates glutamate receptors (Ogita et al., 1986; Varga et al., 1997). For example, in glutathione peroxidase knockout mice, NMDA receptor function was decreased (Jiang et al. 2000). Therefore, GSH depletion may lead to changes in the effects of NMDA receptor antagonists on behaviour (Jacobsen et al., 2005) including PPI.

In rats and mice, brain GSH levels can be reduced using pharmacological agents such as 2-cyclohexene1-one (CHX) which conjugates to GSH via glutathione transferase (Masukawa et al., 1989). We have previously shown that a CHX-induced reduction of

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brain GSH levels caused deficits in short-term spatial memory in the Y-maze (Dean et al., 2009a). In that study, GSH levels in the striatum and frontal cortex were depleted by approximately fifty percent, 90 min after CHX administration, a level of depletion similar to that seen in similar brain regions (dorsolateral prefrontal cortex and caudate nucleus) in schizophrenia. The aim of the present study was firstly to examine the effects of CHX-induced reduction of brain GSH levels on PPI and startle amplitude. Secondly, both the behavioural (PPI and startle amplitude) and biochemical effects (glutathione levels) of brain GSH depletion was examined after treatment with amphetamine and MK-801. We used both rats and mice as there are similarities as well as differences in PPI regulation between these two species (Gogos et al., 2008). Both rats and mice have been used successfully in amphetamine-induced PPI disruption (van den Buuse et al., 2005), however between species differences in the combined effects with glutathione depletion are unknown and hence, both species have been investigated in this study. As is often the case in psychiatric research, one species may be more appropriately suited to modelling a particular aspect or aspects of a disorder than another.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats with an average body weight of 338 g or C57Bl/6 mice with an average body weight of 27 g were obtained from the University of Melbourne, Australia, or the Animal Resources Centre, Perth, Australia. Animals were maintained in standard housing with a 12-hour light/dark cycle with food and water *ad libitum*. All drugs were dissolved in saline and injected intraperitoneally at an injection volume of 1 ml/kg in rats and 10 ml/kg in mice. All experiments were approved by the University of Melbourne Animal Ethics Committee.

2.2. Drug treatment and prepulse inhibition testing schedule

The animals were assigned to four parallel treatment groups as outlined in Table 1. All drugs used in these experiments were purchased from Sigma-Aldrich (Missouri, USA). Each animal underwent one PPI habituation session to allow acclimation to the testing procedure (no data obtained), followed by four experimental PPI sessions (Table 1). The first experimental session was to assess baseline PPI, for which all animals received a saline injection and were tested 15 min later. One to three days later, animals were administered with D-amphetamine sulphate (amphetamine, rats: 2.5 mg/kg, mice: 25 mg/kg), MK-801 (rats: 0.1 mg/kg, mice: 0.25 mg/kg) or saline and 15 min later tested for the effect of these treatments on PPI (session 2). These doses are based on previous literature and preliminary experiments in our laboratory (Choy et al., 2009; Kusljic et al., 2005; Chavez et al., 2009). Three or four days after this session, the same animals were administered CHX (rats: 75 mg/kg, mice: 120 mg/kg (Dean et al., 2009a)) or saline and returned to their home cage for 90 min. GSH depletion remains relatively stable for up to 9 h following CHX injection (Benzi et al., 1991). The animals were then tested for effects on baseline PPI (session 3, no treatments) after which they were returned to their home cage for a further 135 min. Animals were then again treated with amphetamine, MK-801 or saline, with each animal receiving the same treatment as they had for session 2, and 15 min later tested for PPI (session 4). The 'saline-alone' group was included as control for the effect of CHX on GSH levels in other groups and to investigate potential time-related effects of the multiple, sequential PPI testings. Each animal served as its own control and hence no amphetamine/MK-801-only groups were included. This increased the statistical power and reduced the total number of animals used in the experiments. Repeated treatment with amphetamine or MK-801 in this experimental protocol was not expected to result in sensitisation, which could affect the PPI results (Druhan et al., 1998; Murphy et al., 2001). Similar repeated treatment protocols have been used routinely by our laboratory (van den Buuse, 2003) and elsewhere (Varty et al., 2001; Ralph-Williams et al., 2002). However, future studies could be done including a treatment group involving only one dose of amphetamine or MK-801 subsequent to CHX treatment, to eliminate any possibility of an influence of (de) sensitisation in the present results.

Prepulse inhibition testing was conducted using eight automated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA) as outlined previously (van den Buuse and Eikelis, 2001). Briefly, animals were placed in a clear Plexiglas cylinder attached to a platform with a piezoelectric accelerometer unit attached underneath. Whole-body responses to acoustic stimuli led to vibrations of the cylinder, which were measured by the accelerometer unit. A microcomputer and interface assembly controlled the delivery of the acoustic stimuli, and digitized and recorded the behavioural responses.

Each PPI session ran for approximately 40 min. The first 5 min of the session served as an acclimation period during which a 70 dB constant background noise was delivered. Following this, a total of 80 trials were delivered at varying intervals (range: 10–37 ms). The sessions commenced and ended with a block of ten 115 dB startle pulses which, together with 20 startle pulses in the main middle part of the session, allowed for calculation of startle habituation. Three prepulse–pulse trial types were included and consisted of a prepulse (PP) of 4, 8, or 16 dB above the 70 dB background 100 ms before the 115 dB startle pulse. Ten of each of these prepulse–pulse trials were delivered in a pseudo-randomized fashion. The PPI session also included 10 measurements where no auditory pulses ('no stimulation') were delivered to assess the extent of non-specific body movements.

PPI was calculated as the percentage that the prepulse inhibited the startle response: $(100 \times [(\text{pulse-alone trials}) - (\text{prepulse–pulse trials})]/(\text{pulse-alone trials}))$. Startle amplitude was calculated as the average of the 20 pulse-alone trials during the PPI session.

2.3. Determination of brain GSH levels in rats and mice

At the completion of the last PPI session, the animals were decapitated and striatum and frontal cortex samples were dissected, weighed and immediately sonicated in 5% sulfosalicylic acid in EDTA/phosphate buffer. Samples were then assayed for total GSH as previously described (Baker et al., 1990). This assay is based on the reaction of GSH with DTNB [5,5′-dithio-bis(2-nitrobenzoic acid)] to form TNB (2-nitrobenzoic acid) and GS-TNB (glutathione adduct). GS-TNB is then reduced by glutathione reductase to GSH and the cycle continues. The rate of production of TNB is directly proportional to the quantity of GSH in the sample. Production of TNB was measured at 414 nm, using a spectrophotometer, over 2 min. Samples were assayed in triplicate at a 1:200 dilution. Glutathione levels were expressed as mean μmol/g wet weight total GSH levels.

Table 1
Treatment groups — rats and mice.

Species/group	Treatment/session				n
	1 Saline	2 Drug	3 CHX	4 CHX/Drug	
Rat 1: Control	Saline	Saline	Saline	Saline	11
Rat 2: CHX	Saline	Saline	CHX	Saline	11
Rat 3: Amph[a]/CHX	Saline	Amph	CHX	Amph	8
Rat 4: MK-801/CHX	Saline	MK-801	CHX	MK-801	8
Mouse 1: Control	Saline	Saline	Saline	Saline	13
Mouse 2: CHX	Saline	Saline	CHX	Saline	13
Mouse 3: Amph/CHX	Saline	Amph	CHX	Amph	11
Mouse 4: MK-801/CHX	Saline	MK-801	CHX	MK-801	12

[a] Amph — D-amphetamine sulphate.

2.4. Statistical analysis

Data from each rat/mouse group (Table 1) were analysed separately. Repeated measures analysis of variance (ANOVA) was used to evaluate differences in startle and PPI between saline, 'drug', CHX and CHX/'drug' PPI sessions. For the analysis of startle data, repeated measures ANOVA included each startle block, with CHX and 'drug' as variables in a 2×2 design. For the analysis of PPI data, individual prepulse intensities were included and again, CHX and 'drug' were used as variables. Significant main effects and interactions were further analysed based on the results of the initial ANOVAs. When analysing PPI, a significant main effect of prepulse intensity was consistently found, reflecting greater PPI with increased prepulse intensity. These statistical results are not shown here in detail unless interactions occurred between prepulse intensity and other factors. Paired-sample t-tests were used to determine differences between sessions.

For GSH data, groups were compared using one-way ANOVA and Tukey's post-hoc analysis where appropriate. The total GSH data set was analysed together to compare levels of GSH depletion between individual psychotropic drug treatments and also compared to saline.

3. Results

Because no consistent interactive effects of CHX and psychotropic drug treatments were observed on startle habituation, these data will not be reported here.

3.1. Effect of CHX and psychotropic drug treatment on PPI in rats

There was no significant effect of saline treatment ($n=11$) or CHX treatment ($n=11$) on PPI in rats (Fig. 1A and B).

Amphetamine-treated rats ($N=8$; Fig. 1C) showed a main effect of CHX [$F_{1,7}=8.0$, $p=0.025$] and amphetamine [$F_{1,7}=34.7$, $p=0.001$] treatments, as well as an interaction between CHX and amphetamine treatments [$F_{1,7}=10.1$, $p=0.016$]. Further analysis showed that amphetamine treatment on its own induced a disruption of PPI [$F_{1,7}=39.0$, $p<0.001$]. The amphetamine-induced disruption was no longer found in the presence of CHX treatment (Fig. 1C). This was demonstrated by a significantly lower PPI after amphetamine-alone compared to CHX/amphetamine treatment [$F_{1,7}=28.6$, $p=0.001$] and the lack of a difference between CHX-alone and CHX/amphetamine treatments. CHX treatment alone had no effect on PPI in this group of rats.

Analysis of the effect of MK-801 (Fig. 1D) revealed a main effect of treatment [$F_{1,6}=9.1$, $p=0.023$] but no other effects or interactions. PPI was significantly reduced by MK-801 treatment ($n=8$) compared to saline [$F_{1,6}=29.8$, $p=0.002$] and a significant interaction with prepulse intensity was also found [$F_{2,12}=6.2$, $p=0.014$]. While the effect of MK-801 appeared greatest at lower prepulse intensities, pairwise comparisons showed a significant difference between saline and MK-801-alone sessions at PP4 ($p=0.004$), PP8 ($p=0.007$) and PP16 ($p=0.016$) (Fig. 1D).

3.2. Effect of CHX and psychotropic drug treatment on PPI in mice

In saline-treated control mice ($n=13$) there was a significant main effect of session [$F_{3,36}=3.5$, $p=0.025$], which was caused by significantly higher PPI during the last session compared to the first session [$F_{1,12}=10.7$, $p=0.007$], second session [$F_{1,12}=6.4$, $p=0.027$], and third session [$F_{1,12}=7.1$, $p=0.021$], (Fig. 1E). In CHX control mice ($n=13$; Fig. 1F), overall analysis showed no significant main effect of session although there was some interaction between CHX and prepulse intensity [$F_{6,72}=2.3$, $p=0.047$]. This was mainly caused by lower PPI at PP4 in the first (saline) session compared to the third (CHX) session ($p=0.03$). There were no other significant differences.

Analysis of the effect of CHX and amphetamine on PPI in mice ($n=11$) showed a main effect of amphetamine treatment [$F_{1,10}=9.1$, $p=0.013$], and interactions between amphetamine treatment and prepulse intensity [$F_{2,20}=3.5$, $p=0.049$], and between CHX and prepulse intensity [$F_{2,20}=5.4$, $p=0.013$]. Further analysis showed no significant interaction between prepulse intensity and either CHX or drug at any individual session. Importantly, there was no interaction between CHX treatment and amphetamine treatment, showing that in mice the effect of amphetamine was not influenced by prior CHX treatment. Indeed, there was no difference between amphetamine-alone and CHX/amphetamine sessions (Fig. 1G).

MK-801-treated mice ($n=12$) data showed a main effect of MK-801 [$F_{1,11}=24.9$, $p<0.001$], and a three-way interaction between prepulse intensity, CHX and MK-801 [$F_{2,22}=5.4$, $p=0.012$]. There was significant PPI disruption by MK-801 treatment both after saline pre-treatment [$F_{1,11}=28.3$, $p<0.001$] and after CHX pre-treatment [$F_{1,11}=8.9$, $p=0.012$]. Thus, the effect of MK-801 was similar on its own or in the presence of CHX. As with amphetamine, comparison of data after MK-801-alone and CHX/MK801 revealed no significant differences or interactions (Fig. 1H).

3.3. Effect of CHX and psychotropic drug treatment on startle amplitude in rats

In saline-treated control rats analysis of startle amplitude revealed a significant effect of session [$F_{3,30}=4.3$, $p=0.013$]. Further analysis revealed a minor increase in average startle amplitude in the third session, leading to a significant difference between sessions 2 and 3 ($p=0.015$), and 3 and 4 ($p=0.017$) (Fig. 2A).

A similar pattern was observed in CHX-treated rats, where analysis of startle amplitude showed a main effect of session [$F_{3,30}=5.6$, $p=0.004$] and pair-wise comparisons showed higher startle amplitudes in the third session (CHX) compared to the first (saline, $p=0.014$), second (saline, $p=0.018$) and fourth session (CHX, $p=0.036$) (Fig. 2B).

In amphetamine-treated rats, although startle again tended to be increased in the third session, there were no significant main effects or interactions (Fig. 2C).

In MK801-treated rats, there was a main effect of MK-801 [$F_{1,6}=9.3$, $p=0.022$] but no effect of CHX or interactions (Fig. 2D).

3.4. Effect of CHX and psychotropic drug treatment on startle amplitude in mice

There were no changes in startle amplitude in saline-treated mice (Fig. 2E). In CHX-treated mice, analysis of startle amplitude showed a session effect [$F_{3,36}=3.2$, $p=0.033$]. This effect was found in the third session, where CHX treatment had been administered and a slight reduction in startle amplitude was found compared with the second saline session ($p=0.044$) but not the first saline session (see Fig. 2F).

Amphetamine treatment had no effect on startle amplitude in mice (Fig. 2G). Similar to rats, MK-801 treatment significantly increased startle amplitude in mice [$F_{1,11}=18.0$, $p=0.001$] and this effect was not altered by CHX treatment (Fig. 2H).

3.5. Brain GSH depletion in rats

There were significant differences [$F_{3,33}=10.3$, $p<0.001$] between treatment groups in the frontal cortex (Fig. 3A). Tukey's post hoc analysis showed significant depletion of cortical GSH levels in CHX (32% depletion, $p<0.001$), CHX/amphetamine (32% depletion, $p=0.001$), and CHX/MK-801-treated (32% depletion, $p=0.001$) rats when compared with saline-treated animals. There were no significant differences in GSH levels in the frontal cortex between

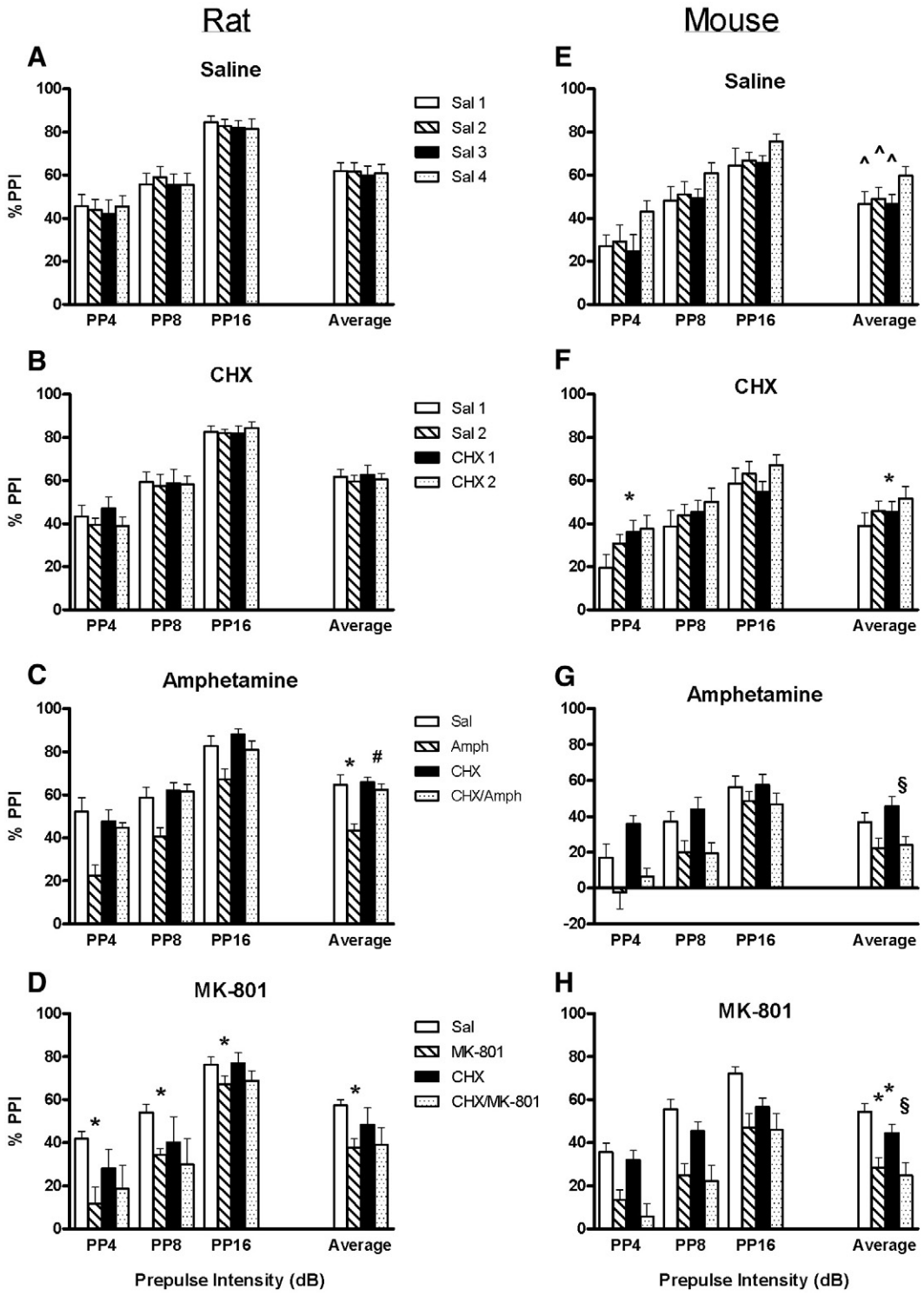

Fig. 1. Prepulse inhibition in rats (A–D) and mice (E–H) in the saline (sal), CHX, amphetamine (amph) and MK-801 treatment groups. * denotes significant difference from saline PPI session (session 1). # denotes significant difference from the drug-alone session (session 2). § denotes significant difference from the CHX-alone session (or saline session 3). ^ denotes significant difference from CHX/drug session (session 4).

CHX-treated groups. In the striatum (Fig. 3B) there was again an overall effect of treatment on GSH levels [$F_{3,32} = 12.1$, $p<0.001$]. Similar to the results from the frontal cortex, striatal GSH levels were significantly reduced in the CHX (34% depletion, $p<0.001$), CHX/amphetamine (35% depletion, $p<0.001$), and CHX/MK-801-treated (30% depletion, $p = 0.002$) rats when compared with saline controls. Again, there were no differences in GSH levels between CHX-treated groups.

3.6. Brain GSH depletion in mice

Investigation of the GSH levels in mice showed similar results to those seen in the rats. In the frontal cortex (Fig. 3C) there was a main effect of treatment on GSH levels [$F_{3,44} = 74.4$, $p<0.001$] and significant reductions in cortical GSH levels in CHX (54% depletion, $p<0.001$), CHX/amphetamine (50% depletion, $p<0.001$), and CHX/MK-801-treated (53% depletion, $p<0.001$) mice when compared to

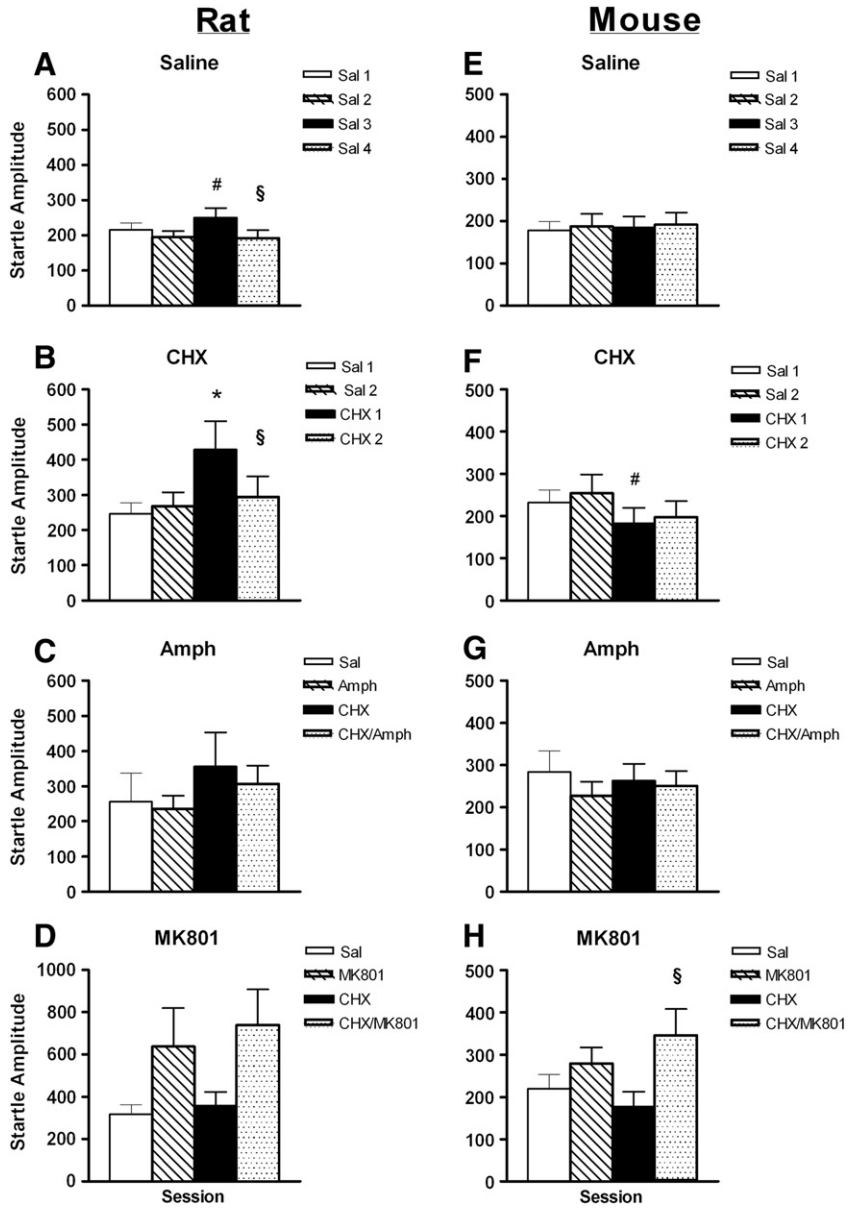

Fig. 2. Startle amplitude in rats (A–D) and mice (E–H) in the saline (sal), CHX, amphetamine (amph) and MK-801 treatment groups. * denotes significant difference from saline PPI session (session 1). # denotes significant difference from the second saline session (session 2). § denotes significant difference from the CHX-alone session (or saline session 3).

saline-treated animals. Also in the striatum (Fig. 3D), there was a main effect of treatment [$F_{3,45} = 114.4$, $p<0.001$] with significant depletion of GSH levels in all CHX-treated groups when compared to saline: CHX-treated animals were depleted by 58%, CHX/amphetamine animals by 54%, and CHX/MK-801-treated animals were depleted by 56% (all $p<0.001$). Similar to the rats, there were no significant differences in GSH levels between CHX-treated mouse groups in either the frontal cortex or striatum.

4. Discussion

Changes in brain antioxidant levels have been implicated in the pathophysiology of several psychiatric illnesses. Glutathione is the primary antioxidant in the brain and therefore decreased levels, as seen in schizophrenia, may result in cellular dysfunction due to oxidative damage (Dringen and Hirrlinger, 2003). The present study revealed that GSH depletion in rats and mice to an extent similar to that reported in schizophrenia (Do et al., 2000; Yao et al., 2006) did not model the disruptions of baseline PPI seen in this illness. Therefore, acutely reduced glutathione levels may not be directly involved in the disruption of PPI in schizophrenia. Startle amplitudes (and startle habituation, data not shown) were also not consistently affected by GSH depletion.

In the present study, GSH depletion caused a reduction of the effect of amphetamine, but not MK-801, on PPI in rats. Amphetamine treatment has been used extensively to induce a PPI deficit in both rats and mice and its critically dependent on intact dopaminergic innervation of the nucleus accumbens (Wan and Swerdlow, 1996). While treatment with both amphetamine and MK-801 may lead to changes in oxidative status (Ozyurt et al., 2007), particularly amphetamine causes excessive dopamine release and decreases antioxidant levels through auto-oxidation of dopamine and the production of hydrogen peroxide (Graham et al., 1978; Fleckenstein et al., 2007). Acute treatment with amphetamine (2 mg/kg) has been shown to reduce catalase levels in the rat striatum but not the prefrontal cortex or hippocampus (Frey et al., 2006). Furthermore, amphetamine treatment increased protein carbonyl and SOD levels in the striatum but not cortex or cerebellum, although thiobarbituric

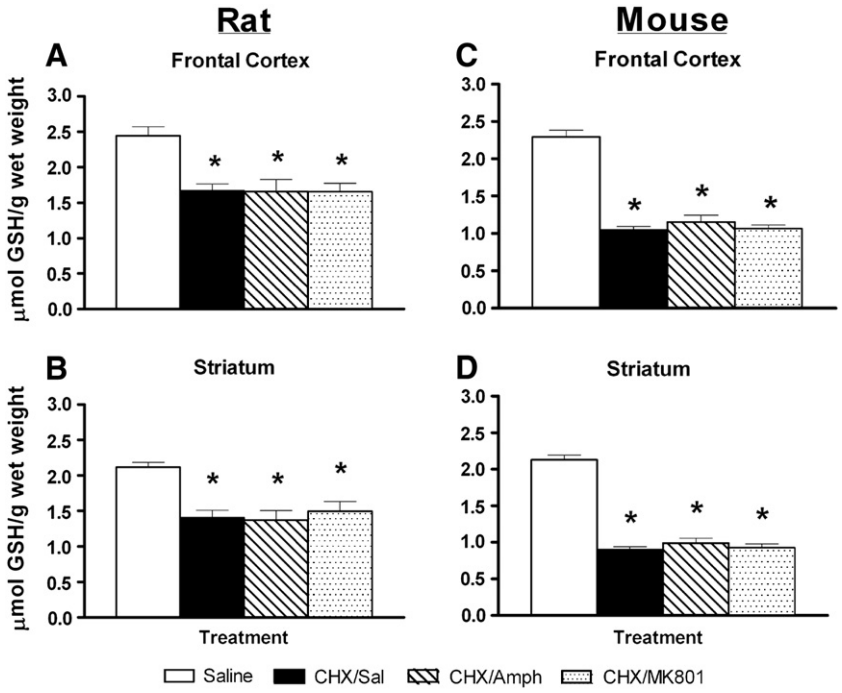

Fig. 3. GSH levels in rats (A and B) and mice (C and D) in the frontal cortex and striatum. * denotes significant difference from saline.

acid (TBARS) and catalase (CAT) were unaffected (Castro et al., 2009). A higher dose of amphetamine (5 mg/kg) increased nitric oxide and TBARS levels in the frontal cortex and striatum (Bashkatova et al., 2002). On the other hand, acute amphetamine or MK-801 treatment does not appear to directly impact on brain GSH levels. Indeed, the current study found no depletion of brain GSH levels in amphetamine- or MK-801-treated rats or mice over that of CHX alone. However, measurements of other antioxidants such as SOD, CAT and glutathione peroxidase, and markers of oxidative stress such as lipid peroxidation and protein carbonylation may reveal other effects of combined GSH depletion and increased dopamine release or NMDA receptor antagonism and should be considered in future studies. Similarly, models of chronic GSH depletion may also be considered as a future direction to ascertain the effects of long-term decreases in GSH levels and its effect on behaviour and biochemistry.

Large and repeated doses of amphetamine and methamphetamine lead to neuronal terminal damage (Wan et al., 2000). While these studies generally use upwards of two times the current dose of amphetamine, and repeated exposure, it may be speculated that in the current model, changes in oxidative stress caused by amphetamine-induced dopamine release were exacerbated by the CHX-induced depletion of GSH. Specifically, GSH depletion may lead to vulnerability where an amphetamine challenge, causing increased dopamine in the synapse, leads to a much faster or larger accrual of reactive oxygen species over a short time period than in the absence of CHX treatment. This may result in the auto-oxidation of dopamine in the synapse and accrual of dopamine quinones causing nerve terminal damage and/or damage to the dopamine transporter. Because the effect of amphetamine relies on intact nerve terminal function and the dopamine transporter, it may become ineffective if combined with CHX pre-treatment. Additionally, glutathione depletion has been shown to modulate dopamine release. For example, buthionine sulfoxamine (BSO)-treated mice had decreased dopamine levels in the nucleus accumbens following 5 mg/kg of amphetamine (Jacobsen et al., 2005). Further studies on dopamine release after combined CHX and amphetamine treatment are necessary to explore these possible presynaptic mechanisms.

Apart from presynaptic mechanisms, the effects of GSH depletion may be post-synaptic where the accumulation of ROS is causing changes in the post-synaptic receptor signalling. This may lead to a dysfunction of post-synaptic firing responses ultimately causing a loss of the effect of amphetamine treatment. Indeed, high levels of intrastriatal dopamine have led to terminal damage in rats, although the mechanisms by which this occurred were not explored (Filloux and Townsend, 1993). However, alterations in dopamine receptor-mediated, NMDA-evoked calcium responses in mouse embryonic cortical neuronal cultures have also been reported to be altered by both increased dopamine and depleted glutathione. In control conditions, dopamine administration increased NMDA-evoked calcium responses. Conversely, after GSH depletion by BSO, dopamine treatment decreased these evoked calcium responses. This may be attributable to a shift in dopamine receptor activation from D1 (shown to increase the function of L-type calcium channels) to D2 receptors (shown to attenuate function of L-type calcium channels) (Steullet et al., 2006). These effects appear to be mediated by redox-sensitive ryanodine receptors, indicating the involvement of oxidative factors in these changes. Loss of amphetamine-induced PPI reduction reflects loss of plasticity of PPI regulatory mechanisms and is, in itself, a form of PPI disruption. Sensory gating mechanisms may become insensitive to exogenous (amphetamine in our study) or endogenous (dopaminergic) modulation and may be incapable of adjusting to environmental demands. The nucleus accumbens is under constant regulatory control by limbic regions such as the prefrontal cortex, hippocampus and amygdala (Moore et al., 1999) and its threshold settings and functional output are altered where necessary. The present results may indicate a situation of reduced antioxidant defence and increased dopamine release where this complex regulatory network is dysfunctional and unable to adjust sensory gating (and PPI) when needed. However, at this point, this explanation is speculative and further experiment should be done, for example to assess if rats with GSH depletion and amphetamine treatment are less able to alter PPI in response to environmental stimuli such as stress. Interestingly, neurodevelopmental stress may lead to similar loss of plasticity of PPI regulation. We developed a two-hit neurodevelopmental stress model, including neonatal maternal

deprivation followed by young-adult glucocorticoid treatment, where amphetamine effects on PPI were lost in a way very similar to the present results (Choy and van den Buuse, 2008).

The loss of an effect of amphetamine on PPI when combined with GSH depletion may be further explored using compounds which enhance/restore the levels of GSH. Previously our research group has shown beneficial effects on symptomatology using the glutathione precursor, N-acetyl cysteine (NAC), as a treatment for schizophrenia (Berk et al., 2008). In rodent models where brain GSH depletion is induced, NAC has been shown to successfully restore GSH levels (Agarwal and Shukla, 1999; Khan et al. 2004). NAC provides the rate-limiting amino acid (cysteine) for glutathione production and has been shown to protect against dopamine-induced cell death (Hoyt et al., 1997). The effects of NAC treatment on PPI following CHX/amphetamine treatment could be explored in future studies.

The reversal of the amphetamine-induced PPI deficit in the presence of decreased brain GSH levels was not observed after treatment with the NMDA receptor antagonist, MK-801. This indicates a potential specificity of this finding to the dopaminergic pathway, potentially due to an increased level of dopamine release and ROS production following amphetamine treatment but not following MK-801. This is in line with previous studies showing that PPI disruption by MK-801 treatment is independent of dopamine release, i.e. was not blocked by dopamine receptor antagonists (Bast et al., 2000; Keith et al., 1991; Bubenikova et al., 2005).

In contrast to rats, there was no alteration in the pattern of PPI disruption following combined CHX and either amphetamine or MK-801 treatment in mice. This is even in the presence of increased depletion of brain GSH in mice than in rats following CHX treatment. The difference in species metabolism of CHX may account for the differences seen; however this requires further investigation. It has been reported that mice have a more robust antioxidant defence system (potentially explaining the requirement of higher doses of CHX to achieve significant depletion). Studies have shown compensatory mechanisms by antioxidants such as ascorbic acid in mice and this may contribute to the lack of effect following GSH depletion and increased dopamine release in mice (Meister, 1994).

5. Conclusion

In conclusion, this study has shown that brain GSH depletion alone does not affect sensorimotor gating in Sprague-Dawley rats or C57Bl/6 mice. However, in rats the effect of amphetamine to disrupt PPI was reversed, suggesting a decrease in pre-synaptic dopamine release or dopamine receptor function. This interaction was not observed with MK-801 in rats or with either amphetamine or MK-801 in mice. These data add to a growing body of literature exploring the behavioural consequences of brain GSH depletion (Dean et al., 2009) and may assist in understanding the impact of oxidative stress on illnesses where GSH depletion and altered dopamine release are features.

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