Brain Research 1666 (2017) 1–10

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Subchronic glucocorticoids, glutathione depletion and a postpartum model elevate monoamine oxidase a activity in the prefrontal cortex of rats

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ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form 27 March 2017
Accepted 29 March 2017
Available online 20 April 2017

Keywords:
Monoamine oxidase
Rat prefrontal cortex
Dexamethasone
Glutathione depletion
Postpartum model
Major depressive disorder

ABSTRACT

Recent human brain imaging studies implicate dysregulation of monoamine oxidase-A (MAO-A), in particular in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC), in the development of major depressive disorder (MDD). This study investigates the influence of four alterations underlying important pathologies of MDD, namely, chronic elevation of glucocorticoid levels, glutathione depletion, changes in female gonadal sex hormones and serotonin concentration fluctuation, on MAO-A and MAO-B activities in rats. Young adult rats exposed chronically to the synthetic glucocorticoid dexamethasone at 0, 0.05, 0.5, and 2.0 mg/kg/day (osmotic minipumps) for eight days showed significant dose-dependent increases in activities of MAO-A in PFC (+17%, $p < 0.001$) and ACC (+9%, $p < 0.01$) and MAO-B in PFC (+14%, $p < 0.001$) and increased serotonin turnover in the PFC (+31%, $p < 0.01$), not accounted for by dexamethasone-induced changes in serotonin levels, since neither serotonin depletion nor supplementation affected MAO-A activity. Sub-acute depletion of the major antioxidant glutathione by diethyl maleate (5 mmol/kg, i.p.) for three days, which resulted in a 36% loss of glutathione in PFC ($p = 0.0005$), modestly, but significantly, elevated activities of MAO-A in PFC and MAO-B in PFC, ACC and hippocampus (+6–9%, $p < 0.05$). Changes in estrogen and progesterone representing pseudopregnancy were associated with significantly elevated MAO-A activity in the ACC day 4–7 postpartum (10–18%, $p < 0.05$ to $p < 0.0001$) but not the PFC or hippocampus. Hence, our study provides data in support of strategies targeting glucocorticoid and glutathione systems, as well as changes in female sex hormones for normalization of MAO-A activities and thus treatment of mood disorders.

1. Introduction

Monoamine oxidase A (MAO-A) is a high density enzyme that participates in the metabolism of major monoamines, creates oxidative stress and participates in apoptosis (Youdim and Bakhle, 2006). Greater MAO-A activity and/or levels, particularly in the prefrontal (PFC) and anterior cingulate cortex (ACC), are associated with major depressive episodes (MDE), dysphoric states, and high risk states for MDE, such as alcohol dependence, withdrawal from cigarette smoking, early postpartum and in perimenopause and borderline personality disorder (Bacher et al., 2011; Chiuccariello et al., 2014; Johnson et al., 2011; Kolla et al., 2016; Matthews et al., 2014; Meyer et al., 2006, 2009; Rekkas et al., 2014; Sacher et al., 2010, 2014). Given that major depressive disorder (MDD) is a leading cause of death and disability in moderate to high income nations, affecting 4% of the general population

Abbreviations: ACC, anterior cingulate cortex; DEM, diethyl maleate; DEX, dexamethasone; GR, glucocorticoid receptor; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; MDD, major depressive disorder; MDE, major depressive episode; PFC, prefrontal cortex.

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http://dx.doi.org/10.1016/j.brainres.2017.03.032
0006-8993/

(World Health Organization, 2008), dysregulation of MAO-A activity is highly relevant for a strongly impactful psychiatric illness. Despite the importance of MAO-A dysregulation in relation to societal psychiatric burden, there are still gaps in our knowledge as to whether certain fundamental processes affect brain MAO-A activity during early adulthood when MDD frequently emerges.

Stressful events are implicated in the development of MDEs (Kendler and Gardner, 2016). While a substantial amount of information has accumulated with regard to the relationship between glucocorticoid agonism and MAO-A in cell lines, there is a lack of knowledge regarding this relationship during young adult age in brain regions implicated in affect, such as the PFC and ACC. Administration of the glucocorticoid receptor (GR) agonist dexamethasone (DEX) to neuroblastoma and glioblastoma cell lines for 2–4 days increases MAO-A and B activities and gene expression through direct binding to the promotor and influencing additional transcription factors, R1 and TIEG2 (Chen et al., 2011; Grunewald et al., 2012; Ou et al., 2006; Tazik et al., 2009). Consistent with this, Slotkin et al. found that chronic DEX treatment at brain-penetrant doses increased MAO-A activity in the PFC of aged rats, concluding that this relationship had important implications for geriatric depression (Slotkin et al., 1998). Subsequent groups also found that chronic DEX treatment increased MAO-A and B levels in the substantia nigra and MAO-A mRNA in the dorsal raphe nucleus of young rats (Arguelles et al., 2010; Jahng et al., 2008). Interestingly, chronic unpredictable mild stress is associated with elevated MAO-A activity in whole rat brain, but the more specific phenomenon of elevated glucocorticoid agonism was not investigated (Grunewald et al., 2012; Harris et al., 2015; Lin et al., 2005). Hence, while there is a substantial body of work evaluating the relationship between glucocorticoid agonism and MAO-A activity, it has not been selectively investigated in the PFC and ACC of young to moderate-aged adult mammalian brain.

It has been demonstrated that glutathione (GSH) levels in postmortem PFC are deficient in several psychiatric illnesses including MDD and bipolar disorder (Gawryluk et al., 2011). GSH is a key antioxidant in the mitochondria, where it neutralizes reactive oxygen species including hydrogen peroxide produced by MAO-A (Mari et al., 2009; Youdim and Bakhle, 2006). GSH levels have also been shown to be decreased *in vivo* in the occipital cortex in MDD, as measured with magnetic resonance spectroscopy (Godlewska et al., 2014; Shungu et al., 2012). Some oxidative stressors, such as the toxin rotenone, increase MAO-A mRNA, protein and activity levels in human neuroblastoma cells and MAO-A mRNA in cultured mouse dopamine neurons (Fitzgerald et al., 2014; Tao et al., 2012), but it is unknown whether the oxidative stress of GSH depletion influences MAO-A activity in the brain.

MDD rates are higher in women than men (Kuehner, 2003), and one highly impactful sex-specific factor is pregnancy. Postpartum depression (PPD) is the most common complication of child bearing, affecting 13% of mothers (O'Hara and Swain, 1996). Postpartum blues, a syndrome of sadness, is a healthy range prodromal state, affecting 70% of new mothers, that, when severe, is associated with PPD (Adewuya, 2006; O'Hara et al., 1991). Elevated MAO-A density is the strongest postpartum brain marker with the highest magnitude of change in early postpartum, being increased, on average, by 43% throughout cortical and subcortical brain regions (Sacher et al., 2010). MAO-A density is also increased in the PFC and ACC of women with first-onset PPD (Sacher et al., 2014). Changes in gonadal steroids may have a causal role in contributing to elevations in MAO-A. Estrogen levels drop by 100–1000 fold in women in the first 3–4 days postpartum (Nott et al., 1976; O'Hara et al., 1991). Furthermore, an inverse relationship between estrogen levels and MAO-A activity has been reported in cell lines and the rat brain. Estrogen administration to the human neuroblastoma cell line was shown to decrease MAO-A activity (Ma et al., 1993, 1995). Consistently, estrogen administration decreased brain MAO-A activity in ovariectomized and intact rats (Holschneider et al., 1998; Leung et al., 1980; Luine and McEwen, 1977). Conversely, MAO-A activity was increased following discontinuation of estrogen treatment in the brain of ovariectomized rats (Jones and Naftolin, 1990). However, brain MAO-A activity has never been investigated in any animal postpartum model. In the present study, we investigated MAO-A activity in the simulated pseudopregnancy rat model of PPD (Galea et al., 2001), applying a modified version of the model that administers estrogen and progesterone for 21 days at levels simulating human pregnancy (Suda et al., 2008).

In the present study, we wished to address the gaps of knowledge in the literature regarding the effects of specific influences, namely glucocorticoid agonism, oxidative stress induced by GSH depletion and changes in gonadal hormones early postpartum on MAO-A activity in the PFC, ACC and hippocampus. For the DEX and GSH experiments, the PFC was the primary region of interest given the previously observed effects of DEX treatment on PFC MAO-A activity/level in another model (Slotkin et al., 1998) and the finding of decreased GSH levels in the PFC in MDD (Gawryluk et al., 2011). The ACC and hippocampus were investigated as secondary regions as they are also important affect-modulating regions with altered MAO-A levels in MDD (Meyer et al., 2006, 2009). We hypothesized that DEX treatment and GSH depletion would increase MAO-A and B activities in the PFC of young rats. For the early postpartum experiment, the PFC and ACC were the primary regions of interest as MAO-A density is elevated in these regions in women with PPD (Sacher et al., 2010). We hypothesized that estrogen and progesterone withdrawal would increase MAO-A activity.

2. Results

2.1. DEX treatment

2.1.1. Dose-response DEX treatment

To examine the effect of GR agonism on MAO-A and B activities, rats were treated with a range of doses of DEX subchronically. One-way ANOVAs revealed that DEX treatment induced a significant increase in MAO-A activity in the PFC [$F(3,31) = 13.43$, $p < 0.0001$] and the ACC [$F(3,31) = 4.28$, $p = 0.01$], and showed a trend in the hippocampus [$F(3,31) = 2.72$, $p = 0.06$] (Fig. 1A). In the PFC, MAO-A activity was increased by 17% in the DEX 2.0 mg/kg/d and 7% in the DEX 0.5 mg/kg/d groups relative to controls. In the ACC, MAO-A activity was increased by 9%. Linear regression analyses also demonstrated that DEX plasma concentrations significantly predicted MAO-A activity in the PFC [$R^2 = 0.54$, $p < 0.0001$], the ACC [$R^2 = 0.32$, $p = 0.0004$], and the hippocampus [$R^2 = 0.19$, $p = 0.009$] (Fig. 1B).

One-way ANOVAs revealed that DEX treatment induced a significant increase in MAO-B activity in the PFC [$F(3,31) = 6.23$, $p = 0.002$], but not in the ACC [$F(3,31) = 0.45$, $p = 0.72$], nor the hippocampus [$F(3,31) = 1.84$, $p = 0.16$] (Fig. 2A). MAO-B activity was increased by 14% in the DEX 2.0 mg/kg/d group in the PFC. Linear regression analyses demonstrated that DEX plasma concentrations significantly predicted MAO-B activity in the PFC [$R^2 = 0.35$, $p = 0.0002$], but not in the ACC [$R^2 = 0.04$, $p = 0.24$], and with a trend in the hippocampus [$R^2 = 0.12$, $p = 0.054$] (Fig. 2B).

One-way ANOVAs revealed that, in the PFC, DEX treatment significantly increased 5-HIAA levels [$F(3,30) = 3.42$, $p = 0.03$] and serotonin turnover [$F(3,30) = 4.21$, $p = 0.01$], decreased L-tryptophan [$F(3,30) = 5.81$, $p = 0.003$] and tyrosine [$F(3,30) = 45.13$, $p < 0.001$], but did not alter 5-HT [$F(3,30) = 1.18$, $p = 0.34$] and NE [$F(3,30) = 1.74$, $p = 0.18$] levels (Supplementary Table 1).

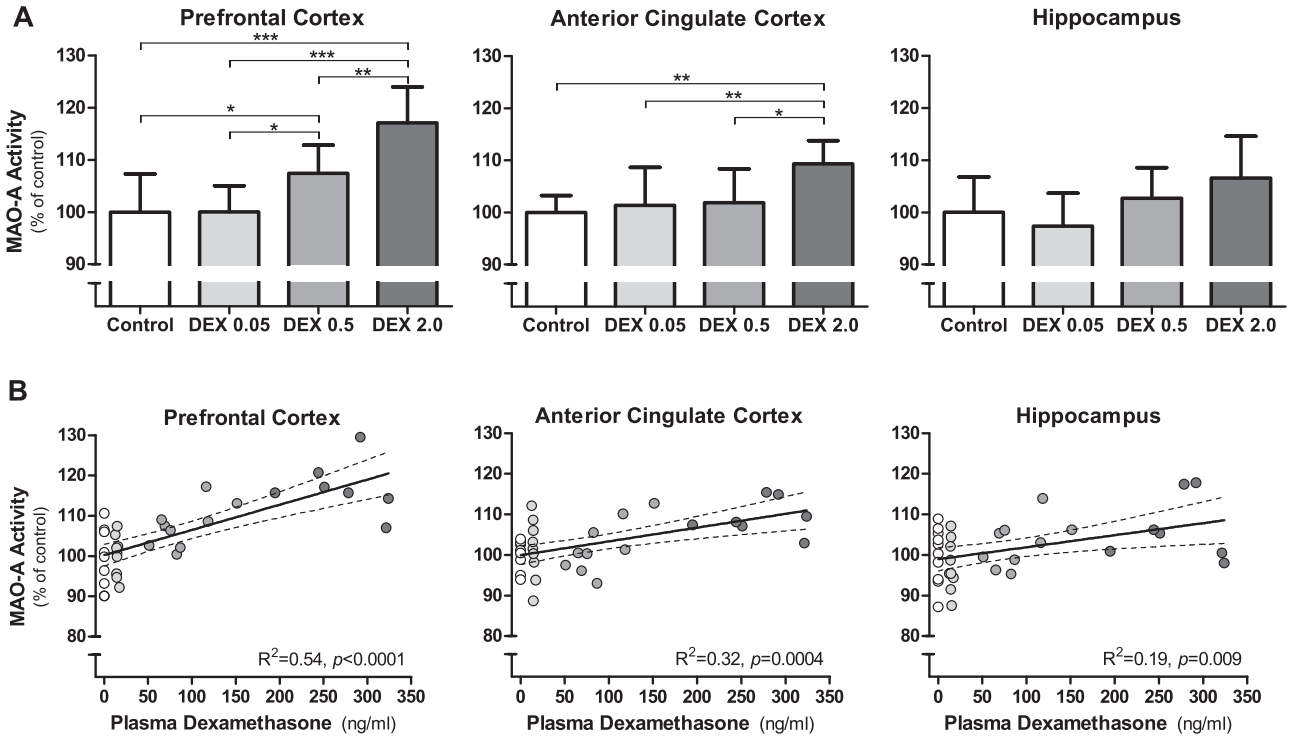

Fig. 1. A. Effect of 8-day dexamethasone (DEX) treatment on MAO-A activity. DEX delivered by s.c. minipump at doses of 0.05 mg/kg/d, 0.5 mg/kg/d and 2.0 mg/kg/d. Data analysis for each brain region was performed using one-way ANOVA with the least significant difference (LSD) test for the pairwise comparisons: prefrontal cortex ($p < 0.0001$), anterior cingulate cortex ($p = 0.01$), hippocampus ($p = 0.06$). Results are Mean ± SD relative to control, n = 7–10/group. $^{*}p < 0.05$, $^{***}p < 0.001$. B. Prediction of MAO-A activity from plasma DEX concentration. Results are fitted with linear regression plus the 95% confidence interval shown as a dotted line.

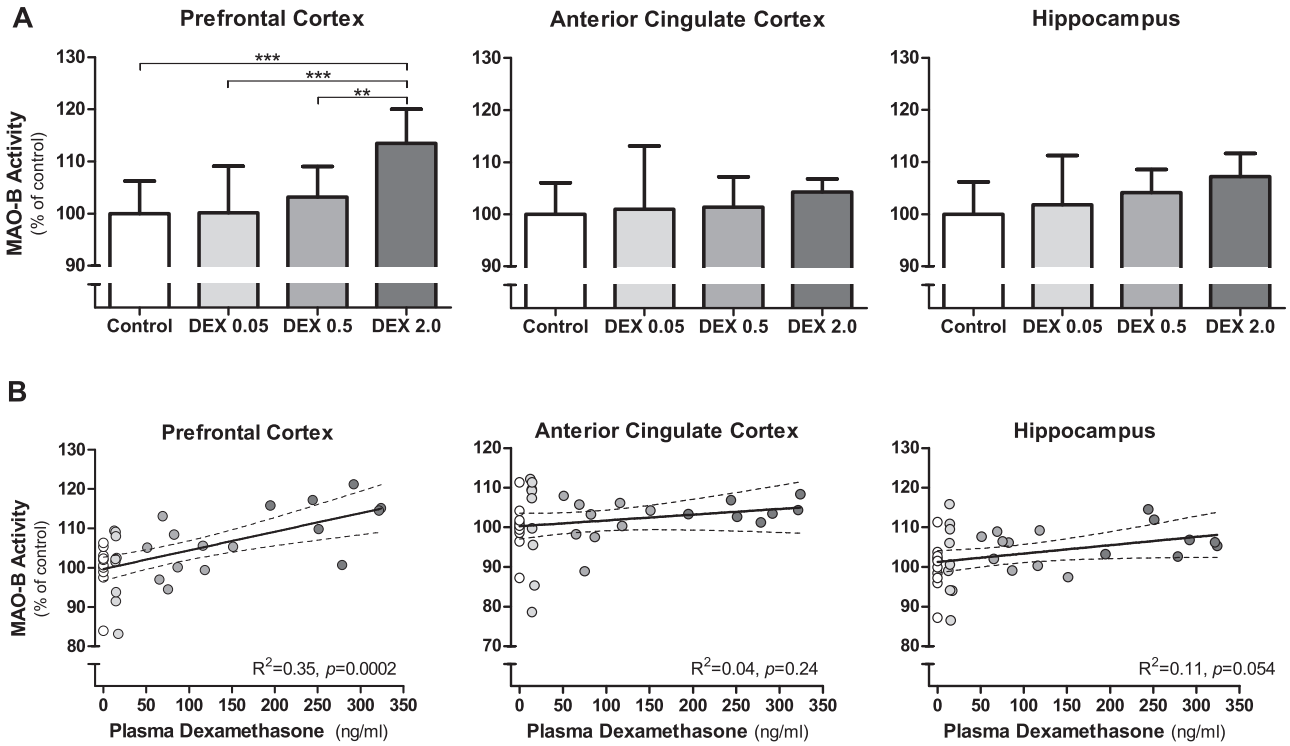

Fig. 2. A. Effect of 8-day dexamethasone (DEX) treatment on MAO-B activity. DEX delivered by s.c. minipump at doses of 0.05 mg/kg/d, 0.5 mg/kg/d and 2.0 mg/kg/d. Data analysis for each brain region was performed using one-way ANOVA with the least significant difference (LSD) test for the pairwise comparisons: prefrontal cortex ($p = 0.002$), anterior cingulate cortex ($p = 0.72$), hippocampus ($p = 0.16$). Results are Mean ± SD relative to control, n = 7–10/group. $^{*}p < 0.05$, $^{**}p < 0.01$. B. Prediction of MAO-B activity from plasma DEX concentration. Results are fitted with linear regression plus the 95% confidence interval shown as the dotted line.

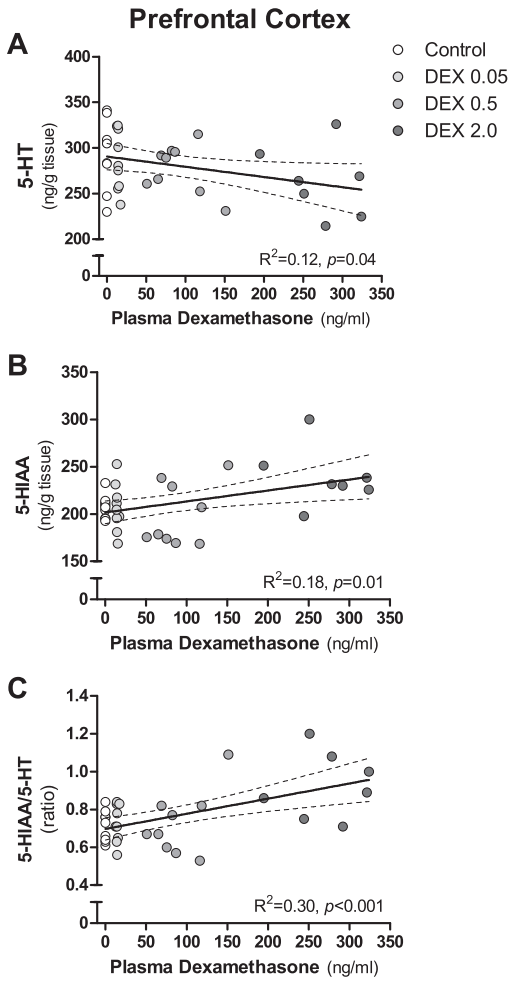

Fig. 3. Linear regression of plasma dexamethasone with A. serotonin level, B. 5-HIAA level and C. serotonin turnover rate (5-HIAA/5-HT ratio) in the prefrontal cortex. Results are fitted with linear regression and the 95% confidence interval shown as a dotted line. 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid.

However, linear regression showed that plasma DEX levels significantly predicted 5-HT [$R^2 = 0.12$, $p = 0.04$], as well as 5-HIAA levels [$R^2 = 0.18$, $p = 0.01$] and 5-HTturnover rate [$R^2 = 0.30$, $p < 0.001$] (Fig. 3). Importantly, the effect of DEX on MAO-A activity was not due to changes in serotonin levels, as neither serotonin supplementation nor depletion had an effect on MAO-A activity on their own (see Supplementary Material part 3).

We further examined whether serotonin turnover rate was related to MAO-A activity in the PFC. Linear regression analysis showed that MAO-A activity did not significantly predict 5-HIAA/5-HT ratio [$R^2 = 0.05$, $p = 0.22$] (Supplementary Fig. 1). However, we considered the possibility that modest MAO-A activity increases may be compensated for by other mechanisms, and so changes in 5-HT turnover rate may occur only once a threshold increase is reached. To explore that, we did a post-hoc comparison between those rats whose MAO-A activity was elevated beyond the upper limit of MAO-A activity (110.6% of mean control) observed in the control group (n = 8) versus all rats within the limit (n = 26). Relative to controls, the mean MAO-A activity of the normal group was 101.8% and that of the high group was 117.9%. Due to large differences in sample sizes and violation of homogeneity of variance, we applied the nonparametric Mann-Whitney *U* test. The high MAO-A activity group was found to have significantly higher 5-HT turnover rate (Median = 0.93) than the normal MAO-A activity group (Median = 0.72) [U = 53.00, $p = 0.04$] (Fig. 4).

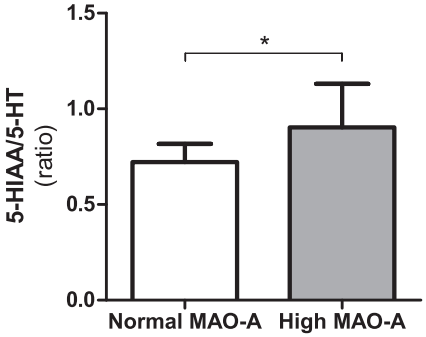

Fig. 4. Serotonin turnover rate (5-HIAA/5-HT ratio) in relation to MAO-A activity. Mann-Whitney *U* test revealed that the high MAO-A activity group had significantly higher serotonin turnover rate than the normal MAO-A activity group ($p = 0.04$). High MAO-A = MAO-A activity beyond the upper limit of the control group (110.6% of mean control); Normal MAO-A = MAO-A activity within the limit. 5-HT = serotonin, 5-HIAA = 5-hydroxyindoleacetic acid.

Additional analyses showing that DEX treatment decreased rat body weight can be found in the supplementary materials.

2.1.2. Prolonged DEX treatment

We also tested whether the low dose of DEX may have an effect on MAO-A and B activities with longer exposure. Independent-samples *t*-tests showed that 0.05 mg/kg/day of DEX for 28 days had no effect on MAO-A activity in any of the regions examined: PFC [t(18) = 0.21, $p = 0.83$], ACC [t(18) = 1.30, $p = 0.21$], and hippocampus [t(18) = 0.61, $p = 0.55$] (Fig. 5A). Independent-samples *t*-tests also showed that the DEX treatment had no effect on MAO-B activity in any of the regions examined: PFC [t(18) = 1.10,

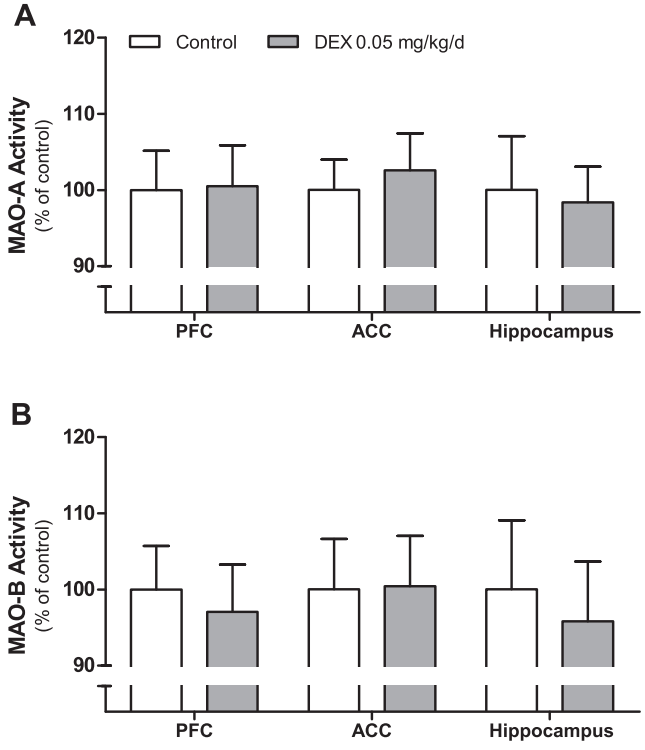

Fig. 5. A. Effect of 28-day treatment with 0.05 mg/kg/d dexamethasone (DEX) delivered by s.c. minipump on A. MAO-A activity and B. MAO-B activity. For both enzymes, data analysis for each brain region was performed using independent samples *t*-test. Results are Mean ± SD relative to control, n = 10/group. PFC = prefrontal cortex, ACC = anterior cingulate cortex.

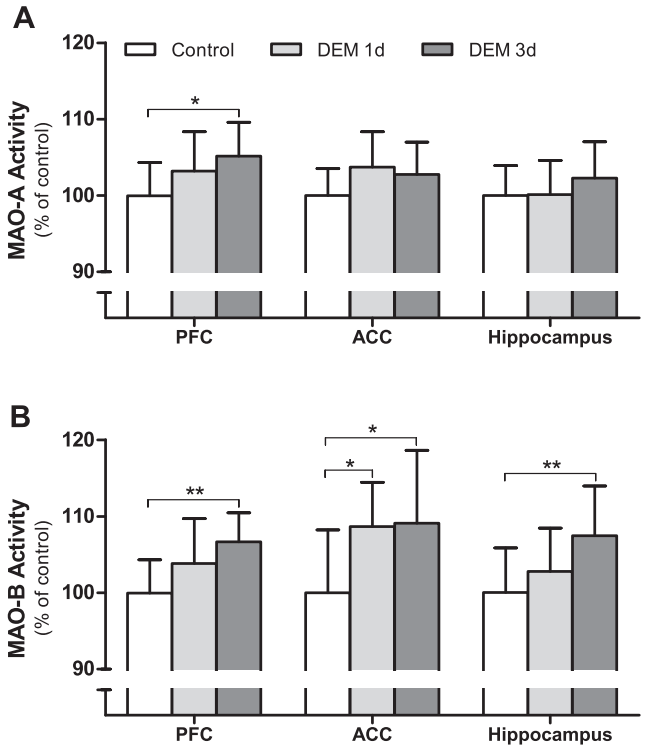

Fig. 6. Effect of glutathione depletion with diethyl maleate (DEM) on A. MAO-A activity: PFC ($p = 0.04$), ACC ($p = 0.10$), hippocampus ($p = 0.40$), and B. MAO-B activity: PFC ($p = 0.01$), ACC ($p = 0.01$), hippocampus ($p = 0.02$). For both enzymes, data analysis for each brain region was performed using one-way ANOVA with the least significant difference (LSD) test for the pairwise comparisons. Results are Mean ± SD relative to control, n = 11–12/group. $^{*}p < 0.05$, $^{**}p < 0.01$. Control = i.p. saline × 3 days; DEM 1d = i.p. saline × 2 days then 5 mmol/kg DEM × 1 day; DEM 3d = i.p. 5 mmol/kg DEM × 3 days; PFC = prefrontal cortex; ACC = anterior cingulate cortex.

$p = 0.28$], ACC [$t(18) = 0.13$, $p = 0.90$], and hippocampus [$t(18) = 1.10$, $p = 0.29$] (Fig. 5B).

Analysis demonstrating that DEX treatment decreased rat body weight can be found in the supplementary material.

2.2. Glutathione depletion

To examine the effect of GSH depletion on MAO-A and B activities, rats were treated with the GSH-conjugating agent diethyl maleate (DEM) for 1 or 3 days. One-way ANOVAs revealed that DEM treatment significantly increased MAO-A activity in the PFC [$F(2,31) = 3.49$, $p = 0.04$], showed a trend in the ACC [$F(2,31) = 2.44$, $p = 0.10$], and had no effect in the hippocampus [$F(2,31) = 0.94$, $p = 0.40$] (Fig. 6A). One-way ANOVAs also showed that DEM treatment significantly increased MAO-B activity in the PFC [$F(2,31) = 5.42$, $p = 0.01$], the ACC [$F(2,31) = 4.91$, $p = 0.01$], and the hippocampus [$F(2,31) = 4.46$, $p = 0.02$] (Fig. 6B). DEM treatment significantly decreased total GSH levels in the PFC [$F(2,31) = 9.905$, $p = 0.0005$] (Fig. 7).

DEM was associated with decreased body weight (see supplemental material).

2.3. Estrogen and progesterone withdrawal

The simulated pseudopregnancy rat model of PPD (Galea et al., 2001; Suda et al., 2008) was applied to evaluate the effect of abrupt declines in estrogen and progesterone levels in the first few days postpartum on MAO-A and B activities.

One-way ANOVA revealed that estrogen withdrawal increased MAO-A activity in the ACC [$F(4,49) = 7.72$, $p < 0.0001$], but not in

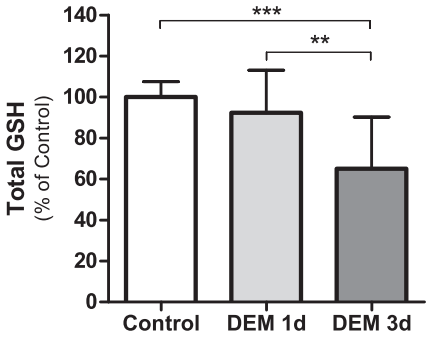

Fig. 7. Effect of diethyl maleate (DEM) treatment on total glutathione (GSH) levels in the prefrontal cortex ($p = 0.0005$). Data analysis was performed using one-way ANOVA with the least significant difference (LSD) test for the pairwise comparisons. Results are Mean ± SD relative to control, n = 11–12/group. $^{**}p < 0.01$, $^{***}p < 0.001$. Control = i.p. saline × 3 days; DEM 1d = i.p. saline × 2 days then 5 mmol/kg DEM × 1 day; DEM 3d = i.p. 5 mmol/kg DEM × 3 days.

the PFC [$F(4,52) = 1.15$, $p = 0.25$] or the hippocampus [$F(4,50) = 1.35$, $p = 0.27$]. Specifically, in the ACC, MAO-A activity was significantly increased following 7 days of withdrawal as compared with the control group and 0 days withdrawal (Fig. 8A).

One-way ANOVA revealed that estrogen withdrawal showed a trend increase in MAO-B activity in the ACC [$F(4,54) = 2.51$, $p = 0.052$], but had no effect in the PFC [$F(4,55) = 1.28$, $p = 0.29$] or the hippocampus [$F(4,54) = 1.01$, $p = 0.41$] (Fig. 8B).

3. Discussion

The present study investigated the effect of several different neural stimuli frequently altered in psychiatric disease on MAO-A activity in the PFC of young adult rats. The larger influences were exerted by GR agonism, in which DEX dose-dependently increased MAO-A and B activities in the PFC, and the pseudopregnancy model in which MAO-A activity was elevated over postpartum days 4–7. Subacute GSH depletion also increased MAO-A and B activities in the PFC. These findings extend our understanding of MAO-A regulation in affect-modulating areas of the brain, which has important implications for the treatment of MDEs given recent human imaging evidence implicating MAO-A in the development of MDEs (Meyer, 2012).

The results from the DEX experiment provide strong support that the previous models linking glucocorticoid effects to increased activity and synthesis in cell lines are relevant to the PFC and ACC in young adult mammalian brain (Grunewald et al., 2012; Ou et al., 2006). This is an important extension beyond the geriatric age as proposed by Slotkin et al., given that young adult age is associated with high risk for developing mood disorders, alcohol dependence, impulse dyscontrol and antisocial personality disorder, conditions associated with dysregulated MAO-A activity and/or density in the PFC (Alia-Klein et al., 2008; Johnson et al., 2011; Kolla et al., 2015; Matthews et al., 2014; Meyer et al., 2006, 2009; Soliman et al., 2011). This raises an interesting question of whether modulating glucocorticoid agonism could be useful therapeutically to stabilize or normalize MAO-A levels during high risk states for onset of MDD. This is an important issue because the ability to influence available MAO-A or MAO-A activity through other means is limited in clinical settings as the use of MAO-A inhibitors is limited by interactions with other medications and tyramine.

It was interesting that elevations in MAO-A activity through DEX treatment were associated with increased 5-HT turnover rate. Early environmental stress has been previously shown to both reduce 5-HT levels and increase MAO-A expression in the striatum and brainstem of mice (Wong et al., 2015). However, while it has

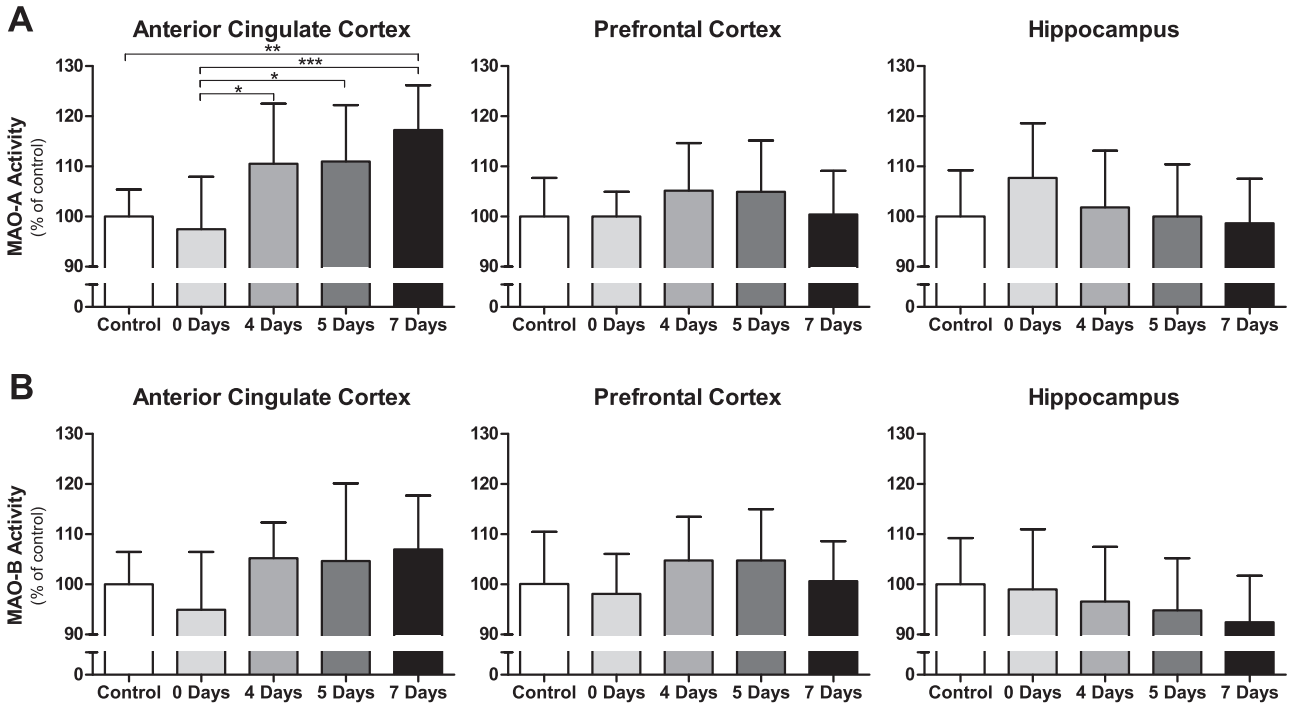

Fig. 8. Effect of estrogen and progesterone withdrawal on A. MAO-A activity: anterior cingulate cortex ($p < 0.0001$), prefrontal cortex ($p < 0.25$), hippocampus ($p = 0.27$), and B. MAO-B activity: anterior cingulate cortex ($p = 0.052$), prefrontal cortex ($p < 0.29$), hippocampus ($p = 0.41$). Nulliparous, ovariectomized rats were implanted with s.c. 21-day release pellets of 0.5 mg 17β-estradiol and 50 mg progesterone to simulate pregnancy. Rats experienced either 0, 4, 5 or 7 days of withdrawal following pellet removal. The control group was implanted with placebo and received sham removal surgery 5 days prior to sacrifice. Data analysis for each brain region was performed using one-way analysis of variance (ANOVA). Results are Mean ± SD relative to control, n = 10–12/group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

been demonstrated that raising MAO-A activity under chronic stress also lowers 5-HT levels (Grunewald et al., 2012), to our knowledge, this is the first study to directly link a threshold of elevated MAO-A activity itself to 5-HT turnover rate in the brain. This has implications for interpreting sequelae of elevated MAO-A activity in health and disease, as it would be expected that 5-HT turnover is also increased in such states.

We also showed that GSH depletion using DEM increased MAO-A activity in the PFC. To our knowledge, this is the first demonstration of an oxidative stressor increasing MAO-A activity *in vivo*. This raises the possibility that decreased GSH levels (Gawryluk et al., 2011) and increased MAO-A activity in the PFC in MDD are related pathologies, since the magnitude of GSH depletion in the PFC after 3-day DEM treatment in the rodents was approximately 35%, which is close to the 50% GSH reduction reported in MDD. This may address in part an issue as to why elevated MAO-A activity (and density) persist in MDD during remission and recovery when stress levels have attenuated (Johnson et al., 2011; Meyer et al., 2009).

Similarly to the effect on MAO-A activity, glucocorticoids and GSH depletion significantly increased MAO-B activity in the PFC (and ACC after GSH depletion). This suggests that the relationship between increased glucocorticoid agonism and elevated MAO-B activity reported in older rodents by Slotkin et al. (1998), and of the effect of DEX on increasing MAO-B transcription in cell lines, is also applicable towards young adult mammals (Chen et al., 2011; Slotkin et al., 1998; Tazik et al., 2009). The effect in the present study on MAO-A and MAO-B is comparable, whereas Slotkin et al. (1998) had a greater magnitude effect on MAO-A, although it is possible that since MAO-B levels increase with age, there may have been physiological ceiling effects in older adult mammals not seen in the present study. Our study argues for greater investigation of MAO-B in the PFC and ACC of psychiatric disorders of early adult age associated with either greater glucocorticoid activity or oxidative stress. It is interesting that to date, there have been no investigations of MAO-B in the PFC or ACC of MDD, even though EMSAM, a patch formulation of selegiline, a moderately selective MAO-B inhibitor, is an approved therapeutic for MDD (Bied et al., 2015).

We also found that estrogen and progesterone withdrawal simulating the early postpartum phase increased MAO-A activity in the ACC. This is the first investigation of MAO-A in an animal postpartum model, providing an important bridging of markers studied in humans and animals. While multiple factors have been implicated in PPD, our knowledge is incomplete. One area with gaps in knowledge involves the lack of overlap in markers studied across humans and animals. For example, in humans, reported changes include reduced occipital cortex GABA levels, reduced D_2 receptor binding in the ventral striatum, reduced 5-HT_{1A} receptor binding in the orbitofrontal, subgenual and mesiotemporal cortex, and increased MAO-A density throughout the brain (Epperson et al., 2006; Moses-Kolko et al., 2008, 2012; Sacher et al., 2014). On the other hand, animal studies focused on factors such as aberrant signal transduction, reduced neurogenesis and abnormalities of GABA receptors (Galea, 2008; Maguire and Mody, 2008; Suda et al., 2008). Such bridging of markers is important for cross-validation, improving understanding of underlying molecular mechanisms, and for developing clinical relevance. Our findings suggest that the rapid hormonal changes in the first several days postpartum may contribute to the elevated MAO-A density observed in women early postpartum and in PPD. Of note, the effect in our study was confined to the ACC, in contrast to the widespread elevations in MAO-A density seen in the human studies. While species differences may contribute to the discrepancy, it could also be that hormonal changes are one of several factors that contribute to elevation in MAO-A density early postpartum, with the ACC being the most sensitive region to such effects.

The main limitation of our study is the short durations of the DEX and DEM treatments, which were limited by toxicity. Both DEX and DEM may have a stronger effect on the periphery than the brain. DEX is a substrate of P-glycoprotein in the blood-brain barrier (Gruol and Bourgeois, 1994; Schinkel et al., 1995), so it is actively removed from the brain, resulting in a lower concentration of DEX in the brain as compared with the periphery. The brain has also been shown to be more resistant to GSH depletion by DEM – the same dose as used in our study was shown to deplete GSH in the brain by ~50%, while depleting it by 75–85% in the liver, kidney and heart of the same rats (Gerard-Monnier et al., 1992). The effects of DEX and GSH may have been more robust if given over a longer duration, hence the effects we observed may be underestimated. Another limitation is that the elevated MAO-A activity after DEX treatment does not necessarily apply to converse conditions. Under usual conditions of health without high levels of stress, it is possible that glucocorticoids are less influential, hence experimental conditions involving adrenalectomy need not result in reductions of MAO-A activity (Lindley et al., 2005). In addition, this study is not intended to be interpreted in relation to acute stress, since short term exposure to stress and glucocorticoids over the first 12 h regulates the MAO-A transcription repressor R1 in an opposite direction and is associated with reductions in MAO-A level and activity after 4 h (Ou et al., 2006; Soliman et al., 2012). Of note, the DEX and DEM treatments were only investigated in male rats, so future studies would need to verify whether the results extend to females. A limitation of the postpartum experiment is that estrogen and progesterone were investigated in conjunction, so future studies are necessary to tease apart their individual roles in influencing MAO-A activity.

In conclusion, we showed that both DEX treatment and GSH depletion increased MAO-A activity in young adult mammalian PFC. Collectively, the findings underscore the importance of considering certain fundamental processes in understanding the development of diseases with dysregulated MAO-A activity and show that glucocorticoid agonism and oxidative stress are two mechanisms of MAO-A regulation that can be studied *in vivo* in rats. The results also raise the interesting questions of whether modulating glucocorticoid agonism and GSH levels may be two therapeutic strategies for stabilizing or normalizing MAO-A in prodromal or high risk states for MDD and/or other conditions with dysregulated MAO-A activity/density.

4. Methods

For all experiments, male Sprague-Dawley rats were purchased from Charles River. All animals were sacrificed by decapitation without anesthesia. Brains were immediately removed, and rinsed in ice-cold phosphate saline buffer. PFC, ACC and hippocampus were dissected out on ice. Brain samples were homogenized in 2x HEPES-buffered saline (100 mM HEPES-NaOH, pH 7.4, 235 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2.8 mM $MgCl_2$) and stored at −80 °C until analysis. All experimental procedures were approved by the Animal Care Committee at the Centre for Addiction and Mental Health and complied with Canadian Council on Animal Care (CCAC) standards and guidelines.

4.1. MAO-A and B activities

MAO activities were determined by a spectrophotometric peroxidase-linked assay using amplex red as the chromogen, adapting a fluorometric method based on detection of H_2O_2 produced by MAO activity in a horseradish peroxidase-coupled reaction (Zhou and Panchuk-Voloshina, 1997). MAO-A and B activities were determined in tissue homogenates (final protein concentration at ~30 μg/ml in 1x HEPES buffered saline) at 37 °C for 1 h with tyramine (0.75 mM) as the substrate and amplex red (100 μM) plus horseradish peroxidase (1 unit/ml) as the chromogen. MAO-A activity was determined in the presence of 100 nM of deprenyl to inhibit MAO-B. MAO-B activity was determined in the presence of 50 nM clorgyline to inhibit MAO-A. Absorption at 571 nm was obtained, subtracting the background determined with 10 μM clorgyline to inhibit both MAOs, and converted to activities in nmol/hour/mg protein by using the extinction coefficient of resorufin (54,000 cm^{-1} M^{-1}) (Zhou et al., 1997). Protein concentration was determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA) with bovine plasma albumin as the standard. The assay was linear for at least 2 h and up to a protein concentration of at least 100 μg/ml. Results were then expressed as percent of the mean control group value.

Given that the most replicated evidence of elevated MAO-A activity and/or levels during MDE is in the PFC, this structure was the *a priori* region for analysis in all experiments. However, exploratory analyses were also performed on the ACC and hippocampus, as MAO-A density is elevated in MDD in these regions as well (Chiuccariello et al., 2014; Meyer et al., 2006, 2009).

4.2. Dexamethasone treatment

4.2.1. DEX administration

Male Sprague Dawley rats (325–375 g) were pair-housed on a 12 h–12 h light/dark cycle. They were fed standard rat chow and given access to water *ad libitum*. The animals were allowed to acclimate for two weeks, during which they were handled and weighed regularly. The animals were single-housed following osmotic minipump implantation. Animals were implanted with ALZET osmotic minipumps (Alzet model 2ML4, Durect Corp., Cupertino, CA, fill volume: 2 ml, pump rate: 2.5 μl/h, 28 days) subcutaneously (s.c.) on their backs through a mid-scapular incision. Surgery was performed under isoflurane anesthesia. Once anesthetized, animals were injected (s.c.) with the analgesics marcaine (0.5 ml/kg, single administration) prior to surgery and anafen (0.5 ml/kg single administration) after surgery.

Dexamethasone 21-phosphate disodium (Sigma-Aldrich Canada Co., Oakville, ON) was prepared in saline. To examine the effect of DEX dose, four groups of rats (n = 9–12/group) received either saline, or 0.05 mg/kg/day, 0.5 mg/kg/day, or 2.0 mg/kg/day DEX for 8 days delivered continuously by osmotic minipumps. To examine the effect of more prolonged DEX exposure, two groups of rats (n = 10/group) received either saline or 0.05 mg/kg/day DEX for 28 days delivered continuously by osmotic minipumps. This dosing regimen was associated with increased MAO-A activity in aged rats (Slotkin et al., 1998).

4.2.2. Plasma DEX analysis

For evaluations of the relationship between plasma DEX and MAO-A activity, in the dose-response experiment, plasma DEX levels were measured. Trunk blood was collected in EDTA tubes immediately following decapitation. Plasma was separated and stored at −80 °C until analysis. Plasma samples (200 μl) were spiked with 22 ng of internal standard (triamcinolone acetonide) and extracted twice with methyl tert-butyl ether (1:10, v/v). Organic layers were pooled and evaporated to dryness under N_2. Extracts were resuspended in 200 μl of HPLC-grade methanol and analyzed on a LC/MS/MS triple quadropole instrument (6410 QQQ, Agilent Technologies with an electrospray ionization source positive ion mode. Ten μl of sample was loaded and separated on a Zorbax XDB-C18 column (4.6 × 50 mm, 5 μm; Agilent) at 0.6 mL/min. The mobile phase consisted of HPLC grade water (A) and methanol (B) both containing 5 mM NH_4Ac. The following gradient was run: 0–1 min, 75% (B); 1–3.3 min, 75–100% (B);

3.3–7 min, 100% (B); 7–7.1 min, 100–75% (B); 7.1–12 min, 75% (B). MS parameters were as follows: nebulizer pressure 35 psi, drying gas (nitrogen) 10 L/min, VCap 4000 V, column temperature 40 °C and drying gas temperature 350 °C for all compounds. Positive ions $[M+H]^+$ for DEX (m/z 393 → 373, RT 5.7 min), and triamcinolone acetonide (m/z 435 → 415, RT 5.9 min) were monitored in multiple reaction monitoring mode. Fragmentor voltage was 105 V and 101 V for triamcinolone acetonide and DEX, respectively. Collision energy was 5 V and 1 V for triamcinolone acetonide and DEX, respectively.

4.2.3. Measurement of brain biogenic amines and related compounds

For the dose-response experiment, we also measured levels of biogenic amines in the PFC. Brains samples were frozen on dry ice immediately following decapitation and stored at −80 °C until analysis. Biogenic amines and their precursors (serotonin [5-HT], norepinephrine [NE], tryptophan and tyrosine) and the acid metabolite of 5-HT, i.e. 5-hydroxyindoleacetic acid (5-HIAA) were analyzed by HPLC with electrochemical detection (Baker et al., 1987; Parent et al., 2001). Frozen rat brains were homogenized in 0.1 N perchloric acid containing EDTA and ascorbic acid amounts. The mixture was centrifuged and the supernatant transferred to HPLC vial. The HPLC system consisted of a Waters Alliance 2695 XE Separations Module equipped with a Waters Atlantis dC18 (3 μm, 3.0 × 100 mm) analytical column and a Waters 2465 Electrochemical Detector.

4.2.4. Statistical analysis

For the primary analysis of the dose-response DEX treatment, MAO-A activity in the PFC was analyzed with one-way analysis of variance (ANOVA) with the least significant difference (LSD) test for the pairwise comparisons. For the secondary analysis, MAO-B activity in the PFC was analyzed with one-way ANOVA with the LSD test for the pairwise comparisons. Additionally, MAO-A and B activities in the ACC and hippocampus were analyzed with one-way ANOVAs with the LSD test for the pairwise comparisons. Biogenic amine levels and serotonin turnover rate (measured as the ratio of 5-HIAA to 5-HT) in the PFC were analyzed with one-way ANOVAs with the LSD test for the pairwise comparisons. Linear regressions were applied to test whether plasma DEX levels predicted MAO-A and B activities.

For the prolonged DEX treatment, independent samples t-tests were used to test the effect of treatment on MAO-A and B activities, respectfully, in each brain region.

Rat body weight was analyzed with two-way ANOVA (Day × Treatment) followed by independent-samples t-tests for between-group comparisons at each time point and repeated-measures t-tests for within-group pairwise comparisons of weight over time.

4.3. Glutathione depletion

4.3.1. Diethyl maleate treatment

Male Sprague Dawley rats (275–300 g) were pair-housed on a 12 h–12 h light/dark cycle. They were fed standard rat chow and given access to water *ad libitum*. The animals were allowed to acclimate for a week, during which they were handled and weighed regularly.

GSH was depleted with diethyl maleate (DEM), a GSH-conjugating agent (Gerard-Monnier et al., 1992). DEM (Sigma-Aldrich Canada Co., Oakville, ON) was dissolved in sunflower seed oil (Sigma-Aldrich Canada Co., Oakville, ON) as the vehicle. Three groups of rats (n = 12/group) received either (i) vehicle for 3 days, (ii) vehicle for 2 days and 5 mmol/kg DEM for 1 day, or (iii) 5 mmol/kg DEM for 3 days by i.p. injections. Animals were sacrificed 24 h after the last injection.

4.3.2. Measurement of GSH level in brain

Brain samples were frozen on dry ice immediately following decapitation and stored at −80 °C until analysis. Total GSH levels were determined in the PFC using the Glutathione Assay Kit (Cayman Chemical Company, Ann Arbor, MI, U.S.A.) using the kinetic method according to manufacturer's instructions. Total protein levels in each sample were analyzed using the Pierce™ BCA Protein Kit (Thermo Scientific, Rockford, IL, U.S.A.) according to manufacturer's instructions. GSH levels were normalized to the protein content of each sample.

4.3.3. Statistical analysis

For the primary analysis, MAO-A activity in the PFC was analyzed with one-way ANOVAs to assess the effect of group (each DEM treatment or control) with the LSD test for the pairwise comparisons. For the secondary analysis, MAO-B activity in the PFC was analyzed with one-way ANOVA with the LSD test for the pairwise comparisons. Additionally, MAO-A and B activities in the ACC and hippocampus were analyzed with one-way ANOVAs with the LSD test for the pairwise comparisons. GSH levels in the PFC were analyzed with one-way ANOVA with the LSD test for the pairwise comparisons. Rat body weight was analyzed with two-way ANOVA (Day × Treatment) followed by one-way ANOVAs for between-group comparisons at each time point and repeated-measures t-tests for within-group pairwise comparisons of weight over time.

4.4. Estrogen and progesterone withdrawal

4.4.1. Hormone administration for simulated pseudopregnancy

Adult female Sprague Dawley, nulliparous rats (200–250 g) ovariectomized by the vendor were single-housed on a 12 h–12 h light/dark cycle. They were fed standard rat chow and given access to water *ad libitum*. The animals were allowed to acclimate for at least 7 days, during which they were handled and weighed regularly. Animals received subcutaneous (s.c.), suprascapular implantations of 21-day release pellets (Innovative Research of America). Surgery was performed under isoflurane anesthesia.

Rats were separated into 5 groups (12/group). One group was implanted with placebo pellets. The other 4 groups were implanted with hormone pellets containing 0.5 mg 17β-estradiol (E2) and 50 mg progesterone (P4) for an estrogen and progesterone replacement-simulated pseudopregnancy (Galea et al., 2001; Suda et al., 2008). Following the pellet treatment, all groups underwent surgery for pellet removal using the same procedures as for implantation. The groups were sacrificed after varying treatment withdrawal durations. One E2-P4 group (Group II) had no treatment withdrawal and was given sham surgery after 16 days (5 days prior to sacrifice) to match the intermediate delay between the second surgery and sacrifice in the other groups. The placebo group (Group I) was sacrificed 5 days after the pellet removal to match the intermediate treatment withdrawal duration in the other groups. The treatment regimen for the 5 groups is detailed in Table 1.

17β-estradiol (E2, 0.5 mg) and 50 mg progesterone (P4, 50 mg) or placebo were delivered by a 21-day release s.c. pellet. Group II (no withdrawal) received sham surgery after 16 days with the pellet not being removed. For all other groups, the pellet was removed after 21 days. The groups received varying treatment withdrawal periods prior to sacrifice.

4.4.2. Statistical analysis

Given that MAO-A level is elevated in the ACC, PFC and hippocampus in early postpartum, postpartum depression and during major depressive episodes, all three regions were chosen for analysis (Meyer et al., 2006, 2009; Sacher et al., 2010, 2014)

Table 1
Simulated pseudopregnancy treatment regimen.

Experimental Group	Treatment	Pellet Removal (d)	Withdrawal (d)	Total Time (d)
I	Placebo	21	(5)	26
II	E2 & P4	16 – sham surgery	0	21
III	E2 & P4	21	4	25
IV	E2 & P4	21	5	26
V	E2 & P4	21	7	28

The primary analysis evaluated MAO-A activity in the ACC, PFC and hippocampus with one-way analysis of variance (ANOVA), followed by pairwise comparisons for regions where the ANOVA was significant. Secondary analysis evaluated MAO-B activity in the same brain regions with one-way ANOVA followed by pairwise comparisons for significant results.

Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research (Dr. Meyer, Canada Research Chair), and the Ontario Ministry of Training, Colleges and Universities (Ms. Raitsin, Ontario Graduate Scholarship).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.brainres.2017.03.032.

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