Environmental Research 185 (2020) 109483

Contents lists available at ScienceDirect

Environmental Research

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

Ozone increases plasma kynurenine-tryptophan ratio and impacts hippocampal serotonin receptor and neurotrophic factor expression: Role of stress hormones

Mercedes Rose[a,b], Alain Filiatreault[a], Josée Guénette[a], Andrew Williams[a], Errol M. Thomson[a,b,*]

[a] Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, K1A 0K9, Canada
[b] Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, K1H 8M5, Canada

ARTICLE INFO

Keywords:
Air pollution
Glucocorticoids
Brain
Rat
Neurotoxicity

ABSTRACT

Air pollution is associated with adverse impacts on the brain, including cognitive decline and increased incidence of dementia, depression and anxiety; however, underlying mechanisms remain unclear. We have shown that both ozone and particulate matter activate the hypothalamic-pituitary-adrenal (HPA) axis, increasing plasma glucocorticoids and altering mRNA profiles in multiple tissues including the brain. HPA axis dysregulation has been associated with central nervous system impacts, including key effects in the hippocampus; accordingly, we hypothesized that pollutant-dependent increases in glucocorticoid levels impact biological pathways relevant to brain health. Fischer-344 rats were treated with metyrapone (0 or 50 mg/kg), a glucocorticoid synthesis inhibitor, and exposed to ozone (0 or 0.8 ppm) for 4 h (n = 5/group) to investigate the role of glucocorticoids in ozone-dependent effects on tryptophan metabolism and expression of serotonin receptors and neurotrophic factors. Ozone increased plasma levels of the tryptophan metabolite kynurenine (~2-fold) and decreased tryptophan levels (~1.2 fold). Hippocampal expression of serotonin receptors exhibited differential regulation following exposure, and expression of key neurotrophic factors (brain-derived neurotrophic factor, vascular endothelial growth factor A, insulin-like growth factor-1, tyrosine kinase receptor B, b-cell lymphoma 2) was decreased. Some, but not all effects were abrogated by metyrapone treatment, suggesting both glucocorticoid-dependent and -independent regulation. Exposure to exogenous corticosterone (10 mg/kg) followed by clean air reproduced the ozone effects that were blocked with metyrapone, confirming the specificity of effects to glucocorticoids. These results indicate that ozone can modify pathways relevant to brain health and establish a role for the HPA axis in mediating these effects.

1. Introduction

Air pollution has widespread health impacts, with an estimated 4.2 million deaths attributed to ambient air pollution worldwide (Cohen et al., 2017). While these estimates are based upon the relationship between air pollution and cardiovascular and respiratory disease, a growing literature indicates a link between air pollution and central nervous system (CNS) disorders, including cognitive decline, neurodegenerative and neuropsychiatric disorders such as Alzheimer's, dementia, depression, anxiety, bi-polar disorder and autism, as summarized by recent reviews (Allen et al., 2017; Power et al., 2016). Given the substantial and growing prevalence of these conditions (Whiteford et al., 2015), and ubiquitous exposure of the population to pollutants, even small pollutant-dependent changes in relative risk represent a significant public health burden.

While particulate matter has been the main target of investigation for neurological impacts, with exposure associated with increased inflammation (Bai et al., 2019; Ehsanifar et al., 2019), impaired neurogenesis (Woodward et al., 2018) and behavioural alterations (Fonken et al., 2011), gaseous components of air pollution can also impact the brain. Due to its highly reactive nature, ozone gas is entirely consumed in the lungs upon inhalation, but can impact tissues and processes beyond the lungs through processes such as systemic increases in oxidative stress (Kelly, 2003), inflammation (González-Guevara et al., 2014) and activation of the hypothalamic-pituitary-adrenal (HPA) axis (Thomson et al., 2016, 2013). Epidemiological studies report associations between exposure to ozone and incidence of neuropsychiatric and neurodegenerative disorders (Lim et al., 2012; Kioumourtzoglou et al.,

* Corresponding author. Inhalation Toxicology Laboratory, Hazard Identification Division, Environmental Health Science and Research Bureau, Health Canada, 2203B Tunney's Pasture, Ottawa, ON, K1A 0K9, Canada.
E-mail address: errol.thomson@canada.ca (E.M. Thomson).

https://doi.org/10.1016/j.envres.2020.109483
Received 4 March 2020; Received in revised form 31 March 2020; Accepted 1 April 2020
Available online 05 April 2020

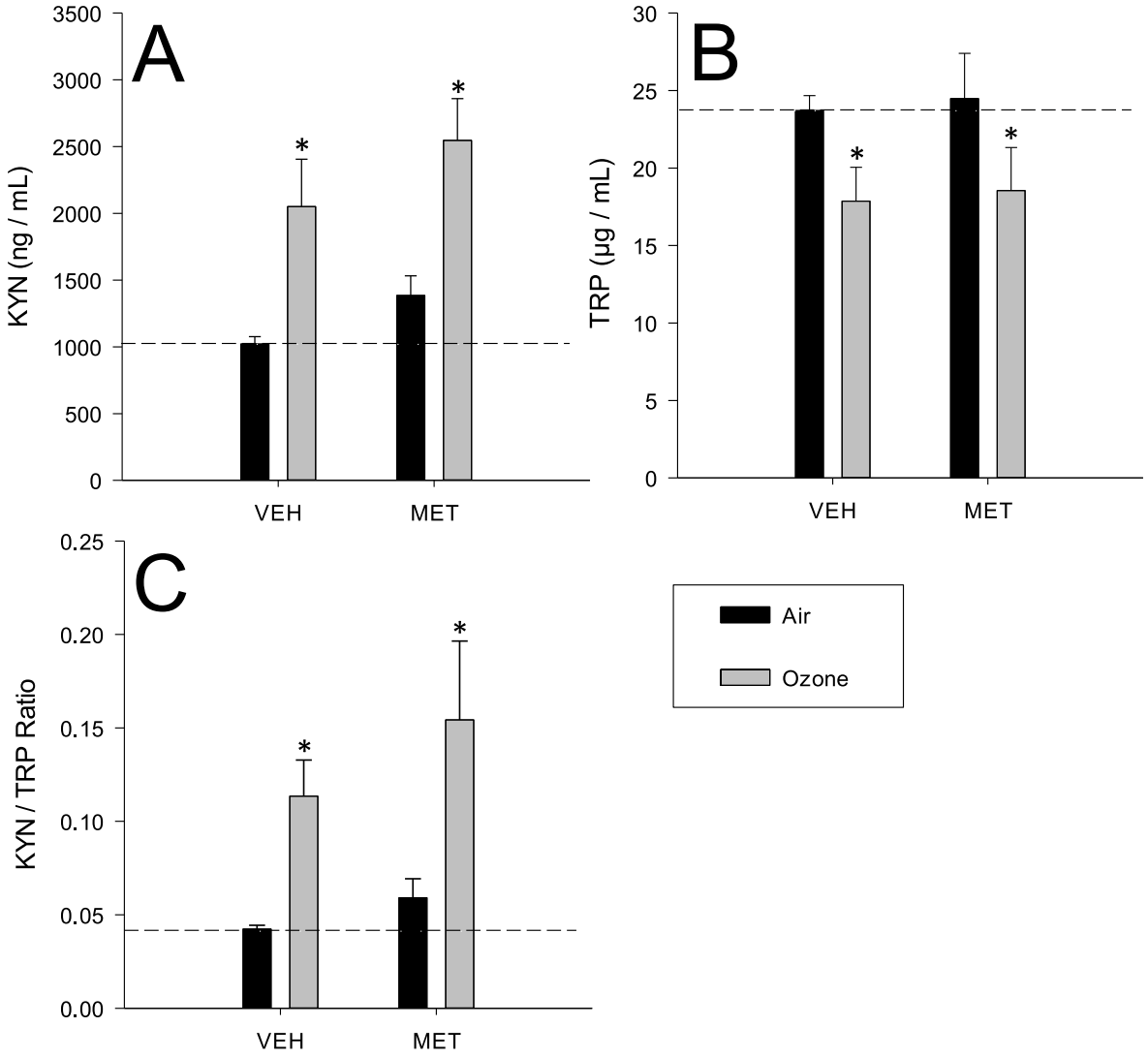

Fig. 1. Plasma kynurenine-tryptophan ratio following ozone exposure. Plasma concentrations of kynurenine and tryptophan were measured in Fischer-344 rats exposed to ozone (0.8 ppm) or air for 4 h following treatment with metyrapone (MET) or vehicle (VEH). Two-way ANOVA was used to evaluate significant *Ozone* (ozone vs. air) and *Metyrapone* (50 mg/kg metyrapone vs. VEH) effects, followed by Holm-Sidak procedure to elucidate pattern of significant effects. **A)** Kynurenine (KYN); **B)** Tryptophan (TRP); **C)** Kynurenine-Tryptophan ratio. * Ozone vs. air within VEH or MET, $p < 0.05$; error bars = 95% CI; n = 5/group.

2017; reviewed by Zhao et al., 2018) and decreased cognitive performance in middle aged adults (Chen and Schwartz, 2009; Gatto et al., 2014). Several rodent studies demonstrate that ozone exposure is capable of causing neurophysiological changes such as decreased short and long term memory retention (Dorado-Martínez et al., 2001; Rivas-Arancibia et al., 2009), reduced hippocampal cell numbers (Rivas-Arancibia et al., 2009), increased apoptotic indicators and endoplasmic-reticulum stress (Rodríguez-Martínez et al., 2016), increased depression and anxiety-like behaviour and decreased serotonin levels in the frontal cortex and hippocampus (Mokoena et al., 2015). However, the mechanisms through which ozone could result in such impacts on the CNS are not fully understood.

As extrapulmonary effects of ozone, unlike particulate matter, are attributable solely to secondary effects rather than having the potential to also be caused by direct effects of the pollutant, ozone provides a useful model to study indirect mechanisms that could underlie systemic effects that may be common to other pollutants. We have shown previously that both ozone and particulate matter activate the HPA axis and increase plasma levels of the stress hormone corticosterone, resulting in glucocorticoid-dependent effects in multiple tissues including the brain (Thomson et al., 2013, 2016, 2019). Glucocorticoid dysregulation is considered an underlying mechanism contributing to the development of a number of CNS disorders (reviewed in Lucassen et al., 2014). For instance, patients with depression (Peeters et al., 2004; Sachar et al., 1973; Sepehr et al., 2005) and dementia (reviewed in Ouanes and Popp, 2019) have been shown to have disrupted secretion of cortisol suggesting impaired regulation of the HPA axis. Glucocorticoids can alter processes associated with CNS disorders such as tryptophan metabolism (Badawy, 2017; Comings et al., 1995), serotonin signaling (reviewed in Lanfumey et al., 2008) and neurogenesis (reviewed in Kino, 2015), and chronic elevation of glucocorticoid levels can lead to impairment of HPA axis regulation as well as glucocorticoid resistance (Cohen et al., 2012; Zhu et al., 2014). Given that HPA axis dysregulation impacts brain processes and that exposure to air pollutants activates the HPA axis, we hypothesized that the HPA axis may serve as an important mediator of pollutant effects on biological pathways relevant to brain health.

The hippocampus is a stress-sensitive, serotonin-receptor rich region of the brain impacted by a number of neurological disease processes (reviewed in Sala et al., 2004). Adult neurogenesis occurs primarily in the hippocampus (reviewed in Ming and Song, 2011), and hippocampal atrophy is often observed in dementia (Chow et al., 2012; Evans et al.,

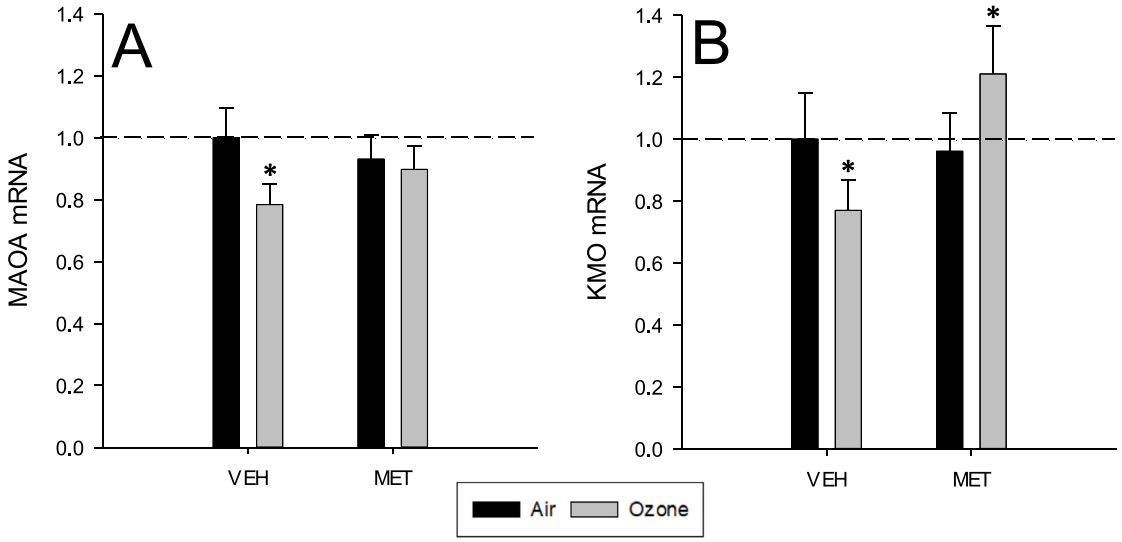

Fig. 2. Hippocampal gene expression of MAOA and KMO following ozone exposure. Hippocampal expression of MAOA and KMO enzymes was measured in Fischer-344 rats pre-treated with metyrapone (MET) or vehicle (VEH) and exposed to ozone (0.8 ppm) or air for 4 h. Expression represented as fold change (FC) relative to VEH/air group. Statistical analysis conducted using two-way ANOVA (factors: *Ozone* and *Metyrapone)* followed by Holm-Sidak multiple comparison procedure to elucidate pattern of significant effects. **A)** MAOA; **B)** KMO. *Ozone vs air within VEH or MET, $p < 0.05$; error bars = 95% CI; n = 4 or 5/group.

2018) and depression (Bremner et al., 2000; Macqueen et al., 2003). Chronic elevation of glucocorticoids has been associated with decreases in memory, a primary function of the hippocampus, and loss of hippocampal volume (Lupien et al., 1998). Here, we use an acute ozone exposure model to facilitate assessment of early impacts in the hippocampus and plasma that can be linked directly to exposure, and that are less likely to be obscured by the significant cellular and tissue changes that can arise as a result of disease progression in chronic exposure models. Specifically, we examined impacts on tryptophan metabolism and serotonin signaling, as well as effects on genes involved in neuronal cell death and hippocampal neurogenesis, mechanisms thought to contribute to neurotoxicity and brain health. Metyrapone, an 11-β-hydroxylase inhibitor that blocks the ozone-induced increase in glucocorticoid synthesis (Thomson et al., 2016), and exogenous corticosterone were used to investigate whether observed effects occurred in a glucocorticoid-dependent or independent manner.

2. Materials and methods

2.1. Animals

Male Fischer-344 rats weighing 200–250 g (specific pathogen free; Charles River, St. Constant, Québec, Canada) maintained on a 12 h light/dark cycle were provided with food and water ad libitum. Housing consisted of HEPA-filtered individual plexiglass cages with wood-chip bedding. All experimental protocols were reviewed and approved by the Animal Care Committee of Health Canada.

2.2. Inhalation exposure

Inhalation exposure methods were previously described in detail (Thomson et al., 2016). Briefly, rats were subcutaneously injected with either metyrapone (50 mg/kg; Sigma-Aldrich Canada Co., Oakville, Ontario, Canada) or vehicle (40% propylene glycol in buffered saline) 1 h prior to nose-only inhalation exposure to either clean air or 0.8 ppm ozone (n = 5/group) for 4 h. Previously conducted dosimetric calculations (Thomson et al., 2005) estimated that exposure to 0.8 ppm ozone produced a centriacinar dose on the order of 3 to 4-fold higher than the internal dose resulting from a plausible exposure scenario in humans (0.12 ppm ozone for 12 h followed by exposure to 0.06 ppm ozone for 12 h). For ethical reasons, nose-only exposures were restricted to 4 h, and so the dose rate was clearly higher in the experimental model compared to an environmental exposure spread over a 24 h period. An additional group (n = 5) was subcutaneously injected with corticosterone (10 mg/kg; Sigma-Aldrich) 1 h prior to exposure to clean air (4 h).

2.3. Sample collection

Rats were anesthetised with isoflurane (5% at 1.5 L of O2/min) and euthanised via exsanguination 0 or 24 h post exposure. Blood was collected from the abdominal aorta and stored in vacutainer tubes containing EDTA at 10 mg/ml and phenyl methyl sulfonyl fluoride at 1.7 mg/ml. Blood was centrifuged at 1448 × *g* for 10 min to separate plasma. Brain tissue was dissected, and the hippocampus was collected and added to RNAlater (Sigma-Aldrich Canada Co.). All samples were snap-frozen in liquid nitrogen and stored at −80 °C.

2.4. Tryptophan and Kynurenine concentrations

Plasma concentrations of tryptophan and kynurenine were measured using ELISA kits (Immusol, France) according to manufacturer's directions.

2.5. RNA isolation

Hippocampal samples (frozen) were ground using a mortar and pestle over dry ice and sonicated briefly in TRIzol reagent (Invitrogen Canada Inc., Burlington, Ontario, Canada) for homogenization. Total RNA was isolated following manufacturer's instructions. Quantification of RNA was performed using RiboGreen RNA Quantitation Reagent and Kit (Molecular Probes, Eugene, Oregon) and RNA quality was assessed using Experion™ RNA STdSens Analysis Kit (Bio-Rad, Mississauga, Ontario, Canada). cDNA was generated according to manufacturer's directions with MuLV reverse transcriptase and random hexamers (Applied Biosystems, Mississauga, Ontario, Canada). Additionally, negative control samples were created by incubating RNA samples with all reagent components except reverse transcriptase to assess genomic

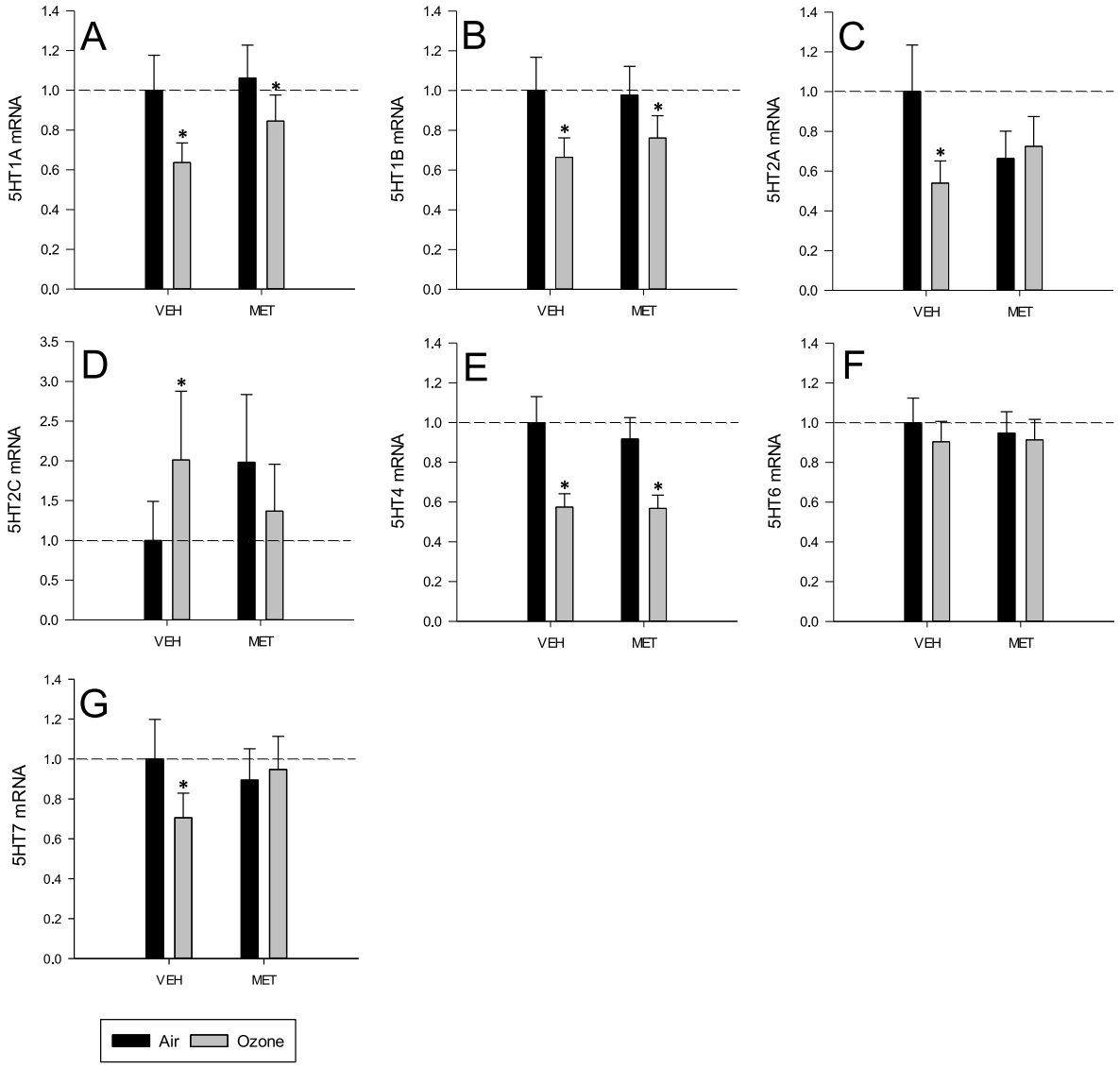

Fig. 3. Ozone-induced gene expression changes of serotonin receptors within the hippocampus. Hippocampal mRNA of serotonin receptors was measured in Fischer-344 rats treated with metyrapone (MET) or vehicle (VEH) prior to exposure to ozone (0.8 ppm) vs. air for 4 h. Expression is represented as fold change (FC) relative to VEH/air exposure group. Significant *Ozone* (air vs. ozone) and *Metyrapone* (50 mg/kg metyrapone vs. VEH) effects were determined using two-way ANOVA and Holm-Sidak procedure to elucidate pattern of significant effects. **A)** 5-HT1A; **B)** 5-HT1B; **C)** 5-HT2A; **D)** 5-HT2C; **E)** 5-HT4; **F)** 5-HT6; **G)** 5-HT7. *Ozone vs air within VEH or MET, $p < 0.05$; error bars = 95% CI; n = 4 or 5/group.

DNA contamination.

2.6. RT-qPCR

Universal Probe Library design software (Roche Diagnostics Canada, Laval, Québec, Canada) and Primer-BLAST software (National Center for Biotechnology Information, Bethesda, Maryland) were used to generate primers (Supplementary Data) with an optimal annealing temperature of 60 °C. A serial dilution of rat brain cDNA was used to validate primers and confirm high-efficiency reactions. Duplicate reactions containing BRIGHTGreen 2x qPCR Mastermix (Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada), primers (200 nM) and cDNA were incubated (10 µl/well; 384-well plate) in a spectrofluorometric thermal cycler (Lightcycler 480, Roche Diagnostic Canada). The following PCR cycle was utilized: 95 °C for 3 min to activate the iTAQ polymerase followed by 50 cycles of denaturation at 95 °C, annealing at 60 °C, and elongation at 72 °C, each for 10 s. Fluorescence was monitored at every cycle during the elongation step. Melt curves were conducted for every run to confirm unique product. Expression calculations were conducted relative to β-actin (verified to be invariant with treatment) using the delta-delta Ct method (Livak and Schmittgen, 2001).

2.7. Statistical analyses

Data were assessed by two-way ANOVA using factors *Ozone* (0, 0.8 ppm) and *Metyrapone* (0, 50 mg/kg), followed by Holm-Sidak multiple comparison procedure to determine significant effects ($\alpha = 0.05$). Pearson correlations were conducted to compare responses to ozone and corticosterone. All statistical analyses were performed using Sigma-Plot 13.0 (Systat Software Inc., San Jose, California). For simplicity, only factor interactions and main effects that included *Ozone* are presented in text and figures. Full statistical data are presented in Supplementary Data.

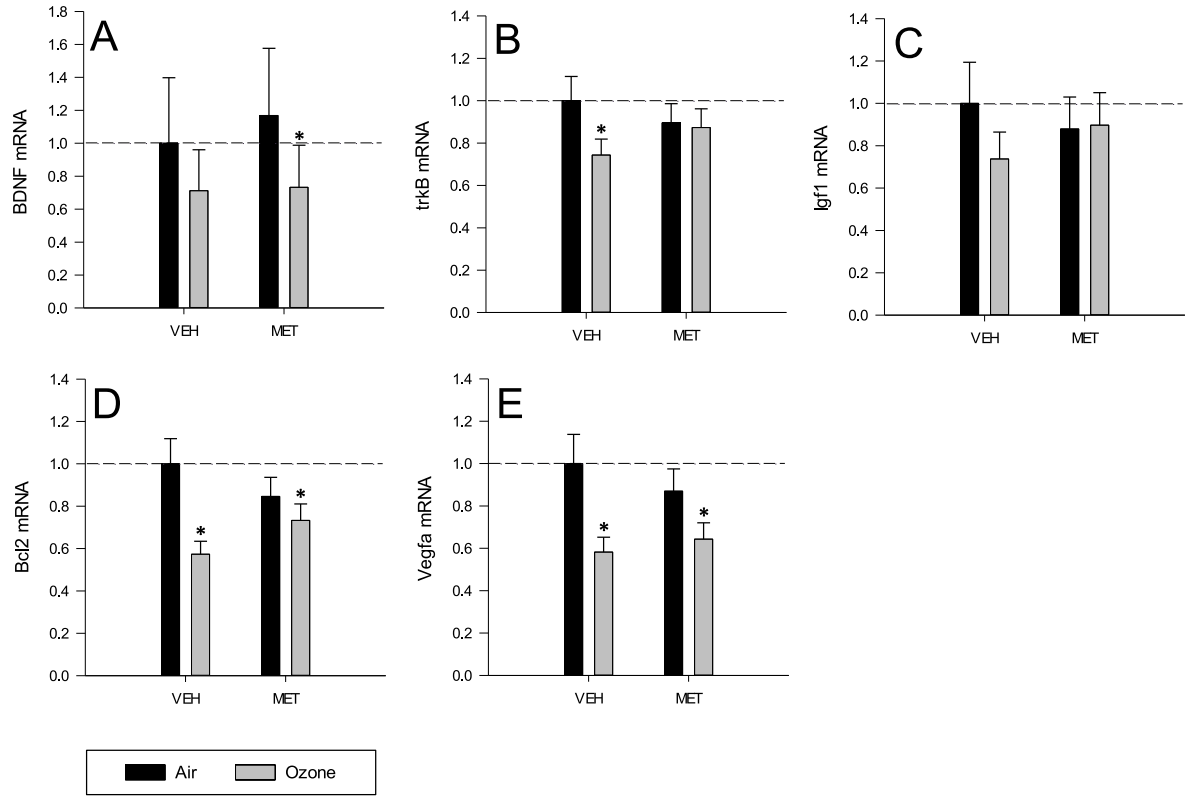

Fig. 4. Hippocampal expression of neurotrophic genes following ozone exposure. Fischer-344 rats were treated with metyrapone (MET) or vehicle (VEH) before 4 h exposure to either ozone (0.8 ppm) or air. Hippocampal expression of genes was measured with RT-qPCR and is represented as fold change (FC) relative to VEH/air exposure group. Two-way ANOVA with Holm-Sidak multiple comparisons procedure was used to evaluate significant effects (factors: *Ozone, Metyrapone*). **A)** BDNF; **B)** trkB; **C)** Igf1; **D)** Bcl2; **E)** Vegfa. *Ozone vs air within VEH or MET, $p < 0.05$; error bars = 95% CI; n = 4 or 5/group.

3. Results

3.1. Kynurenine and tryptophan concentration in plasma

Increases in the ratio of kynurenine/tryptophan are associated with a number of conditions, including various CNS disorders (reviewed in Lovelace et al., 2017). To determine whether ozone could affect this ratio, plasma concentrations of kynurenine and tryptophan were measured immediately after exposure (Fig. 1). Ozone increased plasma kynurenine (approximately 2-fold; *Ozone* main effect, $p < 0.001$) and decreased tryptophan (*Ozone* main effect, $p = 0.023$). As a result, the ratio of kynurenine to tryptophan increased significantly with ozone exposure (*Ozone* main effect, $p < 0.001$). Metyrapone treatment did not significant modify these effects.

3.2. MAOA and KMO expression

Monoamine oxidase A (MAOA) and kynurenine monooxygenase (KMO) are both enzymes which can alter concentrations of different end products of tryptophan metabolism. MAOA oxidizes serotonin, reducing serotonin availability. KMO catalyzes the conversion of kynurenine into 3-hydroxykynurenine, a precursor for quinolinic acid which is thought to have neurotoxic effects (reviewed in Lugo-Huitrón et al., 2013). Ozone decreased hippocampal MAOA mRNA expression immediately after exposure, an effect prevented by metyrapone (*Ozone* × *Metyrapone* interaction, $p = 0.018$; Fig. 2A). KMO mRNA was also decreased by ozone, and this effect was prevented with metyrapone treatment (*Ozone* × *Metyrapone* interaction, $p < 0.001$; Fig. 2B).

3.3. Serotonin receptor expression in the hippocampus

Serotonin (5-HT) receptors mediate many serotonergic functions within the brain. Analysis of hippocampal serotonin receptor mRNA expression immediately after exposure revealed that ozone exposure resulted in differential expression of many of these receptors (Fig. 3). Significant decreases in 5-HT1A, 5-HT1B and 5-HT4 following ozone exposure were all metyrapone independent (for all: *Ozone* main effect, $p < 0.001$). In contrast, metyrapone abrogated ozone-dependent decreases of 5-HT2A (*Ozone* × *Metyrapone* interaction, $p < 0.001$) and 5-HT7 (*Ozone* × *Metyrapone* interaction, $p = 0.02$), as well as the ozone-dependent increase in 5-HT2C (*Ozone* × *Metyrapone* interaction, $p = 0.009$). No significant change was observed in 5-HT6 expression.

3.4. mRNA expression of neurotrophic genes

Both serotonin and glucocorticoids regulate expression of many genes involved in neurogenesis and survival within the hippocampus (Banasr et al., 2004; Odaka et al., 2017). Accordingly, we investigated whether ozone affected the expression of genes involved in neurogenic and neurotrophic processes in a corticosterone-dependent manner (Fig. 4). Ozone decreased brain-derived neurotrophic factor (BDNF; *Ozone* main effect, $p = 0.015$) and vascular endothelial growth factor A (Vegfa; *Ozone* main effect, $p < 0.001$), effects that were both metyrapone-independent. In contrast, ozone-dependent decreases in tyrosine kinase receptor B (trkB; *Ozone* × *Metyrapone* interaction, $p = 0.011$), B-cell lymphoma 2 (Bcl2; *Ozone* × *Metyrapone* interaction, $p < 0.001$) and insulin-like growth factor-1 (Igf1; *Ozone* × *Metyrapone* interaction, $p = 0.051$) tended to be sensitive to metyrapone.

3.5. mRNA expression after 24 h

To determine whether effects of short-term exposure to ozone were transient or sustained, mRNA expression was measured 24 h after ozone exposure and compared to effects detected immediately after exposure.

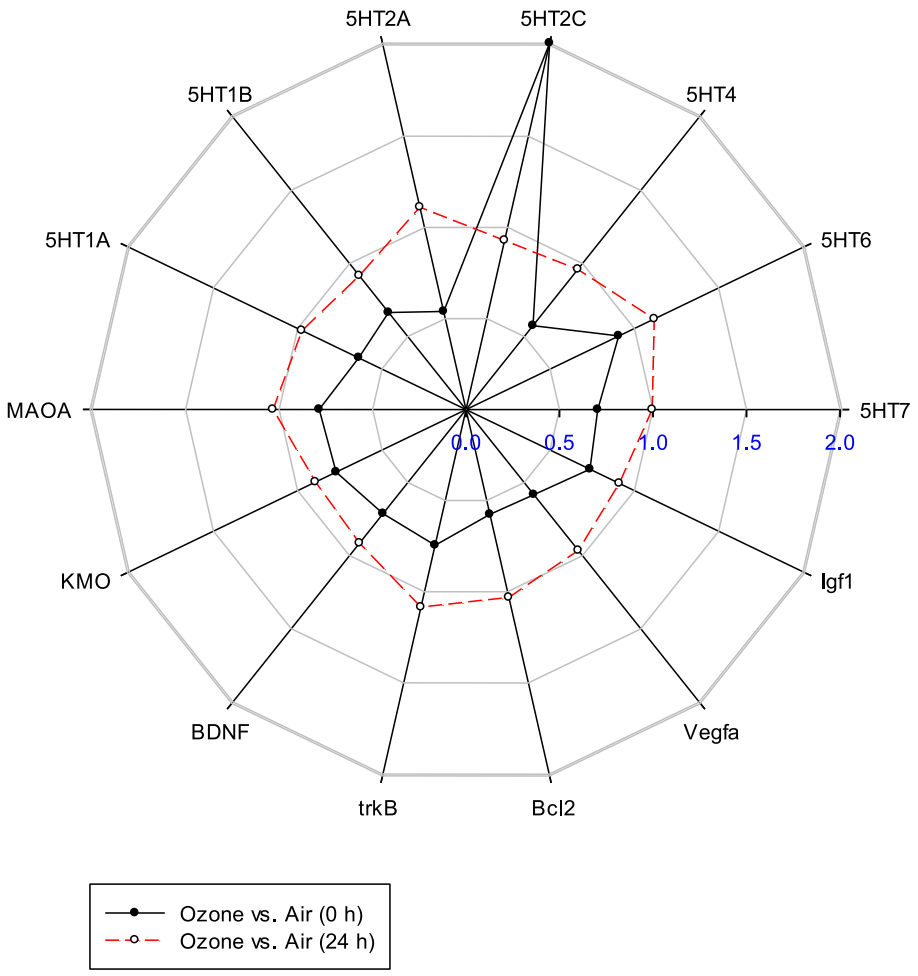

Fig. 5. Gene expression changes 0 and 24 h post-ozone exposure. Data from Fisher-344 rats treated with vehicle (VEH) and exposed to ozone (0.8 ppm) for 4 h. Samples collected 0 h or 24 h after exposure. Gene expression is represented as fold change relative to VEH/air exposure group corresponding to appropriate time point. n = 5/group.

For all genes, mRNA expression returned to or approached control levels 24 h after exposure (Fig. 5; complete statistical assessment presented in Supplementary Data).

3.6. Reproduction of ozone effects with exogenous corticosterone treatment

To independently confirm the glucocorticoid-specificity of ozone effects, rats were administered corticosterone and effects were compared for genes that had exhibited a significant *Ozone* × *Metyrapone* interaction. Corticosterone treatment reproduced effects of ozone exposure ($r = 0.83$, $p = 0.01$; Fig. 6).

4. Discussion

To gain insight into mechanisms that could underlie associations between air pollutants and CNS impacts, we measured key factors in plasma and the hippocampus relevant to serotonergic/neurotrophic pathways, and evaluated the role of HPA axis activation as a potential mediator. Our findings reveal that 1) acute ozone exposure resulted in a significant increase in the plasma kynurenine/tryptophan ratio, 2) ozone exposure resulted in differential expression of MAOA and KMO, many serotonin receptors, and neurotrophic genes, 3) blockade of ozone-induced glucocorticoid release abrogated some but not all of these effects, and 4) treatment with exogenous corticosterone reproduced ozone-induced effects that had been prevented by metyrapone, confirming the specificity of effects to glucocorticoids. Our data show that exposure to ozone can affect serotonin and neurotrophic pathways in both glucocorticoid-dependent and independent manners.

Altered metabolism of tryptophan, a serotonin and kynurenine precursor, has implications in brain health. It has been hypothesized that a shift in tryptophan metabolism from the production of serotonin towards the production of kynurenine may lead to reduced serotonin availability (Lapin and Oxenkrug, 1969) as well as to increases in neurotoxic kynurenine metabolites (Saito et al., 1993), both of which have been associated with CNS disorders (Lugo-Huitrón et al., 2013). Additionally, increases in circulating kynurenine/tryptophan ratios are associated with a variety of diseases, including lung cancer (Suzuki et al., 2010), coronary disease (Sulo et al., 2013), glioma pathophysiology (Adams et al., 2014), Alzheimer's disease (Widner et al., 2000), chronic kidney disease (Schefold et al., 2009), relapses of multiple sclerosis (Mancuso et al., 2015), and suicide (Bradley et al., 2015; Messaoud et al., 2018; Sublette et al., 2011). Elevated glucocorticoids activate tryptophan-2,3-dioxygenase (TDO), one of two main enzymes responsible for kynurenine metabolism, and treatment with dexamethasone and hydrocortisone can decrease plasma tryptophan levels and may alter availability of tryptophan in the brain (reviewed in Miura et al., 2008). The increase in the kynurenine/tryptophan ratio we observed following ozone exposure was not diminished with metyrapone

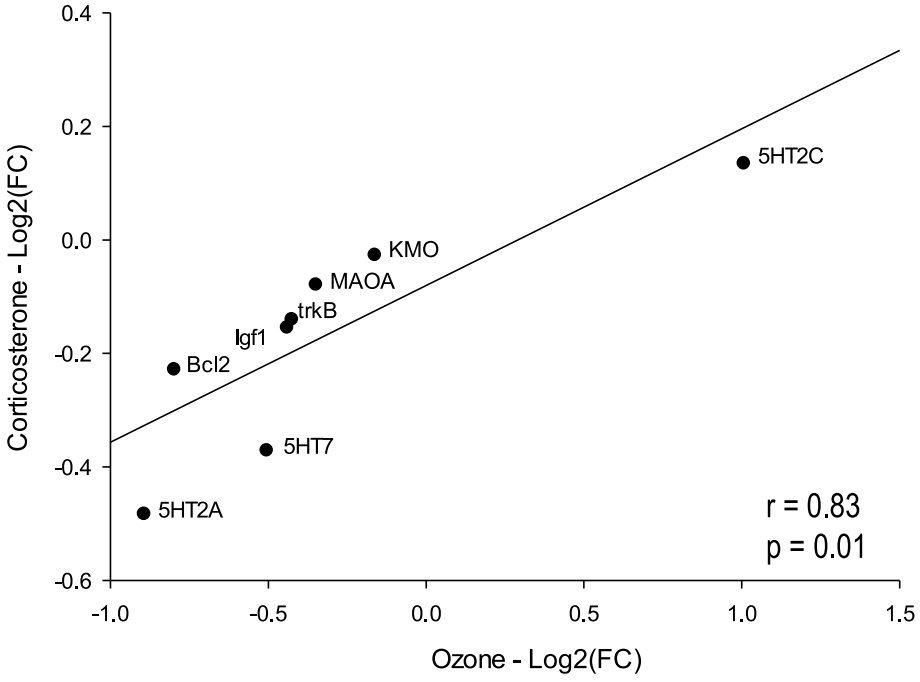

Fig. 6. Reproducing ozone-induced changes of stress-regulated genes by treatment with exogenous corticosterone. Data from Fischer-344 rats treated with corticosterone (CORT) and exposed to clean air for 4 h, or treated with vehicle (VEH) and exposed to ozone (0.8 ppm) for 4 h. Hippocampal gene expression is represented as fold change (FC; Log2) relative to VEH/air. Expression of genes with a significant *Ozone* × *Metyrapone* interaction (Two-way ANOVA) was compared to expression of genes from VEH/ozone group using Pearson correlation ($r = 0.83$; $p = 0.01$). n = 5/group.

treatment, suggesting involvement of a glucocorticoid-independent pathway. Indoleamine 2,3-dioxygenase (IDO), the other enzyme responsible for kynurenine metabolism, is activated by cytokines (reviewed in Miura et al., 2008); however, there is little evidence of increased plasma cytokines immediately after ozone exposure in this model (Thomson et al., 2016).

In addition to altered tryptophan metabolism, ozone exerted differential glucocorticoid-dependent and independent effects on the expression of genes implicated in serotonin and kynurenine metabolism, and of serotonin receptors, in the hippocampus. Decreases in MAOA, KMO, 5-HT1A, 5-HT1B, 5-HT2A, 5-HT4, 5-HT7 and an increase in 5-HT2C were found following ozone exposure, with MAOA, KMO, 5-HT2A, 5-HT2C and 5-HT7 regulated via glucocorticoid-dependent mechanisms. The glucocorticoid-dependency of these effects is supported by previous studies showing glucocorticoid regulation of these genes (Dwivedi et al., 2005; Holmes et al., 1997; Le Corre et al., 1997; Lee et al., 2009). While glucocorticoids have been shown to downregulate 5-HT1A (Le Corre et al., 1997; Meijer and de Kloet, 1994; Neumaier et al., 2000; Vázquez et al., 2012), the lack of *Ozone* × *Metyrapone* interactions in 5-HT1A, 5-HT1B, and 5-HT4 suggest that these genes are regulated primarily via glucocorticoid-independent mechanisms in our ozone model. The physiological consequences of altered serotonin receptor activity and expression have been the focus of many studies seeking to understand the role of these receptors in neuropsychiatric disorders, a number of which show that alterations in receptor activity are associated with and can lead to either anti-depressant or pro-depressant effects (Bonaventure et al., 2007; Hedlund et al., 2005; Licht et al., 2009; Lucas et al., 2007; Martin et al., 2014; Nautiyal et al., 2016; Nautiyal and Hen, 2017; Sibille et al., 1997; Wesołowska et al., 2006). Our results are broadly consistent with prior studies that have shown alterations in serotonin content in the brain after ozone exposure (Skillen et al., 1961; González-Piña and Paz, 1997; Huitrón-Reséndiz et al., 1994; Mokoena et al., 2015; Paz and Huitrón-Reséndiz, 1996).

In addition to tryptophan metabolism and serotonin system dysregulation, interference with hippocampal neurogenesis as well as neuronal survival has been suggested to contribute to a variety of CNS disorders (reviewed in Toda et al., 2019). Stress has been shown to play a significant role in neurogenesis, as both acute and chronic stress, as well as exogenous corticosterone, have been demonstrated to impair neurogenic processes (Coe et al., 2003; Gould et al., 1997; Kino, 2015; Tanapat et al., 2001). Additionally, it has been suggested that adult hippocampal neurogenesis is critical in modulating the ability of the hippocampus to exert negative feedback control over the HPA axis, and thus decreases in neurogenesis could put the individual more at risk of stress-induced neurotoxicity (Mirescu et al., 2004; Toda et al., 2019). In the present study, the role of both glucocorticoid-dependent and independent mechanisms in mediating ozone effects is evident in the reduced expression of key neurotrophic and neuroprotective genes BDNF, Igf1, Vegfa, Bcl2 and trkB. Ozone-dependent decreases of Igf1, Bcl2 and trkB were blocked by metyrapone, consistent with known regulation of these genes by glucocorticoids (Basta-Kaim et al., 2014; Lembessis et al., 2004). In contrast, while glucocorticoid regulation of BDNF and Vegfa has been previously demonstrated (Barbany and Persson, 1992; Smith et al., 1995), our results suggest regulation by other factors. Decreases in the expression of these neurotrophic factors could reduce their neuroprotective effect, leading to a decrease in neurogenesis and increase in neuronal cell death (Anilkumar and Prehn, 2014; Bathina and Das, 2015; Lange et al., 2016). Thus, we provide evidence that air pollution may influence neurogenesis and neuronal cell death pathways, a hypothesis established by past studies (Hedges et al., 2019; Rivas-Arancibia et al., 2015; Rodríguez-Martínez et al., 2016; Woodward et al., 2018), and that this can occur through glucocorticoid-dependent and independent pathways.

The physiological ramifications of the observed effects likely depend upon innate differences in sensitivity, duration of exposure, and the context in which the stress hormone-dependent effects occur. Ozone-exposure can activate many systemic pathways in addition to the HPA axis, such as oxidative stress and inflammation which have been suggested to mediate air-pollution induced neurological effects previously (Mokoena et al., 2015; Rivas-Arancibia et al., 2009). While these various pathways may act independently from each other, it is important to recognize that these pathways can also interact (Thomson, 2019). For instance, inflammatory processes can activate the HPA axis (reviewed in Bellavance and Rivest, 2014), and the HPA axis can also increase sensitivity to the immune response through priming of microglia (Frank et al., 2012). Glucocorticoids may also increase oxidative stress (You et al., 2009), and these pathways may act to regulate the same downstream targets. Additionally, humans are exposed to variety of different pollutants and other environmental factors, which may act on these pathways to induce synergistic or additive effects that can

impact health outcomes (reviewed in Mauderly and Samet, 2009; Thomson, 2019). Genetic and epigenetic factors as well may also influence how pollutant-induced changes occur and manifest among the human population.

Several factors warrant consideration when interpreting these results. Despite training in nose-only exposure tubes, nose-only exposure itself is stressful (Thomson et al., 2009), and thus ozone effects are occurring in the context of existing stress. As equivalent conditions were used for all treatment groups, this should not confound interpretation of results. Secondly, this experiment was conducted using Fischer rats, known to have a hyper-responsive stress axis (Dhabhar et al., 1993). We have shown that rats that differ in HPA axis function have differential sensitivity to impacts of ozone on lung injury and inflammation, providing insight into a potential susceptibility factor (Thomas et al., 2018); such innate differences may also be relevant to CNS effects of pollutant exposure. Third, metyrapone could impose off-target effects independent of glucocorticoid-synthesis blockade. However, reproduction of ozone-induced effects with exogenous corticosterone independently confirmed that effects observed are glucocorticoid-dependent. Finally, the present study was based on analyses conducted in the whole hippocampus, and was therefore insensitive to potential regional specific effects of glucocorticoids on gene expression (e.g. Le Corre et al., 1997). Previous work examining ozone-dependent glucocorticoid signaling demonstrated widespread actions across brain regions, with some evidence of regional differences (Thomson et al., 2019).

5. Conclusions

In conclusion, we show for the first time that exposure to the common air pollutant ozone alters the ratio of tryptophan and kynurenine in blood, an effect associated with several disease states. We also provide evidence supporting a role for glucocorticoids in mediating the link between pollutant exposure and neurotoxicity by demonstrating that exposure to ozone can impact serotonin and neurogenic systems through glucocorticoid-dependent and -independent mechanisms. Our findings, alongside results from previous studies showing how ozone exposure activated stress-responsive regions of the brain (Gackière et al., 2011) and can induce stress hormone-dependent transcriptional responses within the brain (Henriquez et al., 2019; Thomson et al., 2019, 2013), are consistent with a role for the HPA axis in contributing to the neurophysiological abnormalities instigated by air pollution exposure. Elucidation of underlying mechanisms through which air pollutants can impact the CNS should provide support for epidemiological associations and insight into those subpopulations that may be most at risk.

Funding sources

Funding for this study was provided by the Clean Air Regulatory Agenda and the Addressing Air Pollution Horizontal Initiative.

Ethics statement

All experimental protocols were reviewed and approved by the Animal Care Committee of Health Canada.

CRediT authorship contribution statement

Mercedes Rose: Investigation, Formal analysis, Writing - original draft, Visualization. **Alain Filiatreault:** Investigation, Visualization. **Josée Guénette:** Methodology. **Andrew Williams:** Formal analysis. **Errol M. Thomson:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to acknowledge the assistance of Kevin Curtin, Kevin Kittle, Karine Chamberland, Meghan Kavanagh, Dr. Santokh Gill, Julie Todd, Amy Richardson, Marjolaine Godbout-Cheliak, and Dr. Mike Wade. Funding for this study was provided by the Clean Air Regulatory Agenda and the Addressing Air Pollution Horizontal Initiative.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.109483.

References

Adams, S., Teo, C., McDonald, K.L., Zinger, A., Bustamante, S., Lim, C.K., Sundaram, G., Braidy, N., Brew, B.J., Guillemin, G.J., 2014. Involvement of the kynurenine pathway in human glioma pathophysiology. PLoS One 9, 1–28. https://doi.org/10.1371/journal.pone.0112945.

Allen, J.L., Klocke, C., Conrad, K., Sobolewski, M., Cory-Slechta, D., 2017. Cognitive effects of air pollution exposures and potential mechanistic underpinnings. Curr. Environ. Heal. Reports 4, 180–191. https://doi.org/10.1007/s40572-017-0134-3.

Anilkumar, U., Prehn, J.H.M., 2014. Anti-apoptotic BCL-2 family proteins in acute neural injury. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2014.00281.

Badawy, A.A.B., 2017. Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. Int. J. Tryptophan Res. 10. https://doi.org/10.1177/1178646917691938.

Bai, K.-J., Chuang, K.-J., Chen, C.-L., Jhan, M.-K., Hsiao, T.-C., Cheng, T.-J., Chang, L.-T., Chang, T.-Y., Chuang, H.-C., 2019. Microglial activation and inflammation caused by traffic-related particulate matter. Chem. Biol. Interact. 311, 108762. https://doi.org/10.1016/j.cbi.2019.108762.

Banasr, M., Hery, M., Printemps, R., Daszuta, A., 2004. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology 29, 450–460. https://doi.org/10.1038/sj.npp.1300320.

Barbany, G., Persson, H., 1992. Regulation of neurotrophin mRNA expression in the rat brain by glucocorticoids. Eur. J. Neurosci. 4, 396–403. https://doi.org/10.1111/j.1460-9568.1992.tb00888.x.

Basta-Kaim, A., Szczesny, E., Glombik, K., Stachowicz, K., Slusarczyk, J., Nalepa, I., Zelek-Molik, A., Rafa- Zablocka, K., Budziszewska, B., Kubera, M., Leskiewicz, M., Lason, W., 2014. Prenatal stress affects insulin-like growth factor-1 (IGF-1) level and IGF-1 receptor phosphorylation in the brain of adult rats. Eur. Neuropsychopharmacol 24, 1546–1556. https://doi.org/10.1016/j.euroneuro.2014.07.002.

Bathina, S., Das, U.N., 2015. Brain-derived neurotrophic factor and its clinical implications. Arch. Med. Sci. https://doi.org/10.5114/aoms.2015.56342.

Bellavance, M.A., Rivest, S., 2014. The HPA - immune axis and the immunomodulatory actions of glucocorticoids in the brain. Front. Immunol. https://doi.org/10.3389/fimmu.2014.00136.

Bonaventure, P., Kelly, L., Aluisio, L., ... 2007. 5-hydroxytryptamine (5-HT) 7 receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram. Pharmacol. 321, 690–698. https://doi.org/10.1124/jpet.107.119404.5-HT.

Bradley, K.A.L., Case, J.A.C., Khan, O., Ricart, T., Hanna, A., Alonso, C.M., Gabbay, V., 2015. The role of the kynurenine pathway in suicidality in adolescent major depressive disorder. Psychiatr. Res. 227, 206–212. https://doi.org/10.1016/j.psychres.2015.03.031.

Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L., Charney, D.S., 2000. Hippocampal volume reduction in major depression. Am. J. Psychiatr. 157, 115–117. https://doi.org/10.1176/ajp.157.1.115.

Chen, J.C., Schwartz, J., 2009. Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology 30, 231–239. https://doi.org/10.1016/j.neuro.2008.12.011.

Chow, N., Aarsland, D., Honarpisheh, H., Beyer, M.K., Somme, J.H., Elashoff, D., Rongve, A., Tysnes, O.B., Thompson, P.M., Apostolova, L.G., 2012. Comparing hippocampal atrophy in alzheimer's dementia and dementia with lewy bodies. Dement. Geriatr. Cognit. Disord. 34, 44–50. https://doi.org/10.1159/000339727.

Coe, C.L., Kramer, M., Czéh, B., Gould, E., Reeves, A.J., Kirschbaum, C., Fuchs, E., 2003. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile Rhesus monkeys. Biol. Psychiatr. 54, 1025–1034. https://doi.org/10.1016/S0006-3223(03)00698-X.

Cohen, A.J., Brauer, M., Burnett, R., Anderson, H.R., Frostad, J., Estep, K., Balakrishnan, K., Brunekreef, B., Dandona, L., Dandona, R., Feigin, V., Freedman, G., Hubbell, B., Jobling, A., Kan, H., Knibbs, L., Liu, Y., Martin, R., Morawska, L., Pope, C.A., Shin, H.,

Straif, K., Shaddick, G., Thomas, M., van Dingenen, R., van Donkelaar, A., Vos, T., Murray, C.J.L., Forouzanfar, M.H., 2017. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet 389, 1907–1918. https://doi.org/10.1016/S0140-6736(17)30505-6.

Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., Turner, R.B., 2012. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proc. Natl. Acad. Sci. U. S. A. 109, 5995–5999. https://doi.org/10.1073/pnas.1118355109.

Comings, D.E., Muhleman, D., Dietz, G., Sherman, M., Forest, G.L., 1995. Sequence of human tryptophan 2, 3-dioxygenase (TDO2): presence of a glucocorticoid response-like element composed of a GTT repeat and an intronic CCCCT repeat. Genomics 29, 390–396. https://doi.org/10.1006/geno.1995.9990.

Dhabhar, F.S., Mcewen, B.S., Spencer, R.L., 1993. Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels-a comparison between Sprague-Dawley, Fischer 344 and Lewis rats. Brain Res. 616, 98.

Dorado-Martínez, C., Paredes-Carbajal, C., Mascher, D., Borgonio, G., Rivas-Arancibia, S., 2001. Effects of different ozone doses on memory , motor activity and lipid peroxidation levels , in rats. Int. J. Neurosci. 108, 149–161. https://doi.org/10.3109/00207450108986511.

Dwivedi, Y., Mondal, A.C., Payappagoudar, G.V., Rizavi, H.S., 2005. Differential regulation of serotonin (5HT)2A receptor mRNA and protein levels after single and repeated stress in rat brain: role in learned helplessness behavior. Neuropharmacology 48, 204–214. https://doi.org/10.1016/j.neuropharm.2004.10.004.

Ehsanifar, M., Jafari, A.J., Nikzad, H., Zavareh, M.S., Atlasi, M.A., Mohammadi, H., Tameh, A.A., 2019. Prenatal exposure to diesel exhaust particles causes anxiety, spatial memory disorders with alters expression of hippocampal pro-inflammatory cytokines and NMDA receptor subunits in adult male mice offspring. Ecotoxicol. Environ. Saf. 176, 34–41. https://doi.org/10.1016/J.ECOENV.2019.03.090.

Evans, T.E., Adams, H.H.H., Licher, S., Wolters, F.J., van der Lugt, A., Ikram, M.K., O'Sullivan, M.J., Vernooij, M.W., Ikram, M.A., 2018. Subregional volumes of the hippocampus in relation to cognitive function and risk of dementia. Neuroimage 178, 129–135. https://doi.org/10.1016/j.neuroimage.2018.05.041.

Fonken, L.K., Xu, X., Weil, Z.M., Chen, G., Sun, Q., Rajagopalan, S., Nelson, R.J., 2011. Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. Mol. Psychiatr. 16, 987–995. https://doi.org/10.1038/mp.2011.76.

Frank, M.G., Thompson, B.M., Watkins, L.R., Maier, S.F., 2012. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. Brain Behav. Immun. 26, 337–345. https://doi.org/10.1016/j.bbi.2011.10.005.

Gackière, F., Saliba, L., Baude, A., Bosler, O., Strube, C., 2011. Ozone inhalation activates stress-responsive regions of the CNS. J. Neurochem. 117, 961–972. https://doi.org/10.1111/j.1471-4159.2011.07267.x.

Gatto, N.M., Henderson, V.W., Hodis, H.N., St John, J.A., Lurmann, F., Chen, J.C., Mack, W.J., 2014. Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. Neurotoxicology 40, 1–7. https://doi.org/10.1016/j.neuro.2013.09.004.

González-Guevara, E., Carlos Martínez-Lazcano, J., Custodio, V., Hernández-Cerón, M., Rubio, C., Paz, C., nica Custodio, V., Hernández-Ceró, M., 2014. Exposure to ozone induces a systemic inflammatory response: possible source of the neurological alterations induced by this gas Exposure to ozone induces a systemic inflammatory response: possible source of the neurological alterations induced by this gas. Inhal. Toxicol. 26, 485–491. https://doi.org/10.3109/08958378.2014.922648.

González-Piña, R., Paz, C., 1997. Brain monoamine changes in rats after short periods of ozone exposure. Neurochem. Res. 22, 63–66. https://doi.org/10.1023/A:1027329405112.

Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A.M., Fuchs, E., 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17, 2492–2498. https://doi.org/10.1523/jneurosci.17-07-02492.1997.

Hedges, D.W., Erickson, L.D., Kunzelman, J., Brown, B.L., Gale, S.D., 2019. Association between exposure to air pollution and hippocampal volume in adults in the UK Biobank. Neurotoxicology 74, 108–120. https://doi.org/10.1016/J.NEURO.2019.06.005.

Hedlund, P.B., Huitron-Resendiz, S., Henriksen, S.J., Sutcliffe, J.G., 2005. 5-HT7 receptor inhibition and inactivation induce antidepressantlike behavior and sleep pattern. Biol. Psychiatr. 58, 831–837. https://doi.org/10.1016/j.biopsych.2005.05.012.

Henriquez, A.R., House, J.S., Snow, S.J., Miller, C.N., Schladweiler, M.C., Fisher, A., Ren, H., Valdez, M., Kodavanti, P.R., Kodavanti, U.P., 2019. Ozone-induced dysregulation of neuroendocrine axes requires adrenal-derived stress hormones. Toxicol. Sci. 172, 38–50. https://doi.org/10.1093/toxsci/kfz182.

Holmes, M.C., French, K.L., Seckl, J.R., 1997. Dysregulation of diurnal rhythms of serotonin 5-HT. Situ 17, 4056–4065.

Huitrón-Reséndiz, S., Custodio-Ramírez, V., Escalante-Membrillo, C., González-Piña, R., Paz, C., 1994. Sleep alterations and brain regional changes of serotonin and its metabolite in rats exposed to ozone. Neurosci. Lett. 177, 119–122. https://doi.org/10.1016/0304-3940(94)90059-0.

Kelly, F.J., 2003. Oxidative stress: its role in air pollution and adverse health effects. Occup. Environ. Med. 60, 612–616. https://doi.org/10.1136/oem.60.8.612.

Kino, T., 2015. Stress, glucocorticoid hormones, and hippocampal neural progenitor cells: implications to mood disorders. Front. Physiol. 6. https://doi.org/10.3389/fphys.2015.00230.

Kioumourtzoglou, M.A., Power, M.C., Hart, J.E., Okereke, O.I., Coull, B.A., Laden, F., Weisskopf, M.G., 2017. The association between air pollution and onset of depression among middle-aged and older women. Am. J. Epidemiol. 185, 801–809. https://doi.org/10.1093/aje/kww163.

Lanfumey, L., Mongeau, R., Cohen-Salmon, C., Hamon, M., 2008. Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2008.04.006.

Lange, C., Storkebaum, E., De Almodóvar, C.R., Dewerchin, M., Carmeliet, P., 2016. Vascular endothelial growth factor: a neurovascular target in neurological diseases. Nat. Rev. Neurol. 12, 439–454. https://doi.org/10.1038/nrneurol.2016.88.

Lapin, I., Oxenkrug, G., 1969. Intensification of the central serotonergic processes as A possible determinant of the thymoleptic effect. Lancet 293, 132–136.

Le Corre, S., Sharp, T., Young, A., Harrison, P., 1997. Increase of 5-HT7 (serotonin-7) and 5-HT1A receptor mRNA expression in rat hippocampus after adrenalectomy. Psychopharmacology (Berl) 130, 368–374.

Lee, T.T.Y., Redila, V.A., Hill, M.N., Gorzalka, B.B., 2009. 5-HT2A receptor mediated neuronal activation within the paraventricular nucleus of the hypothalamus is desensitized following prolonged glucocorticoid treatment. Eur. J. Pharmacol. 602, 54–57. https://doi.org/10.1016/j.ejphar.2008.11.035.

Lembessis, P., Kalariti, N., Koutsilieris, M., 2004. Glucocorticoid receptor function suppresses insulin-like growth factor 1 activity in human KLE endometrial-like cells. Vivo (Brooklyn) 18, 43–48.

Licht, C.L., Marcussen, A.B., Wegener, G., Overstreet, D.H., Aznar, S., Knudsen, G.M., 2009. The brain 5-HT4 receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. J. Neurochem. 109, 1363–1374. https://doi.org/10.1111/j.1471-4159.2009.06050.x.

Lim, Y., Kim, H., Kim, J.H., Bae, S., Park, H.Y., Hong, Y., 2012. Air pollution and symptoms of depression in elderly adults. Environ. Health Perspect. 120, 1023–1028. https://doi.org/10.1289/ehp.1104100.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 C T method. METHODS 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Lovelace, M.D., Varney, B., Sundaram, G., Lennon, M.J., Lim, C.K., Jacobs, K., Guillemin, G.J., Brew, B.J., 2017. Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. Neuropharmacology. https://doi.org/10.1016/j.neuropharm.2016.03.024.

Lucas, G., Rymar, V.V., Du, J., Mnie-Filali, O., Bisgaard, C., Manta, S., Lambas-Senas, L., Wiborg, O., Haddjeri, N., Piñeyro, G., Sadikot, A.F., Debonnel, G., 2007. Serotonin4 (5-HT4) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 55, 712–725. https://doi.org/10.1016/j.neuron.2007.07.041.

Lucassen, P.J., Pruessner, J., Sousa, N., Almeida, O.F.X., Van Dam, A.M., Rajkowska, G., Swaab, D.F., Czéh, B., 2014. Neuropathology of stress. Acta Neuropathol. 127, 109–135. https://doi.org/10.1007/s00401-013-1223-5.

Lugo-Huitrón, R., Ugalde Muñiz, P., Pineda, B., Pedraza-Chaverrí, J., Ríos, C., Pérez-De La Cruz, V., 2013. Quinolinic acid: an endogenous neurotoxin with multiple targets. Oxid. Med. Cell. Longev. 2013. https://doi.org/10.1155/2013/104024.

Lupien, S.J., De Leon, M., De Santi, S., Convit, A., Tarshish, C., Nair, N.P.V., Thakur, M., McEwen, B.S., Hauger, R.L., Meaney, M.J., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69–73. https://doi.org/10.1038/271.

Macqueen, G.M., Campbell, S., McEwen, B.S., Macdonald, K., Amano, S., Joffe, R.T., Nahmias, C., Young, L.T., 2003. Course of illness, hippocampal function, and hippocampal volume in major depression. Proc. Natl. Acad. Sci. Unit. States Am. 100, 1387–1392. https://doi.org/10.1073/pnas.0337481100.

Mancuso, R., Hernis, A., Agostini, S., Rovaris, M., Caputo, D., Fuchs, D., Clerici, M., 2015. Indoleamine 2,3 dioxygenase (Ido) expression and activity in relapsing-remitting multiple sclerosis. PLoS One 10, 1–9. https://doi.org/10.1371/journal.pone.0130715.

Martin, C.B.P., Hamon, M., Lanfumey, L., Mongeau, R., 2014. Controversies on the role of 5-HT2Creceptors in the mechanisms of action of antidepressant drugs. Neurosci. Biobehav. Rev. 42, 208–223. https://doi.org/10.1016/j.neubiorev.2014.03.001.

Mauderly, J.L., Samet, J.M., 2009. Is there evidence for synergy among Air pollutants in causing health effects? Environ. Health Perspect. 117, 1–6. https://doi.org/10.1289/ehp.11654.

Meijer, O.C., de Kloet, E.R., 1994. Corticosterone suppresses the expression of 5-HT1Areceptor mRNA in rat dentate gyrus. Eur. J. Pharmacol. Mol. Pharmacol. 266, 255–261. https://doi.org/10.1016/0922-4106(94)90134-1.

Messaoud, A., Mensi, R., Douki, W., Neffati, F., Najjar, M.F., Gobbi, G., Valtorta, F., Gaha, L., Comai, S., 2018. Reduced peripheral availability of tryptophan and increased activation of the kynurenine pathway and cortisol correlate with major depression and suicide. World J. Biol. Psychiatr. 1–9. https://doi.org/10.1080/15622975.2018.1468031. 0.

Ming, G. li, Song, H., 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70, 687–702. https://doi.org/10.1016/j.neuron.2011.05.001.

Mirescu, C., Peters, J.D., Gould, E., 2004. Early life experience alters response of adult neurogenesis to stress. Nat. Neurosci. 7, 841–846. https://doi.org/10.1038/nn1290.

Miura, H., Ozaki, N., Sawada, M., Isobe, K., Ohta, T., Nagatsu, T., 2008. A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. Stress 11, 198–209. https://doi.org/10.1080/10253890701754068.

Mokoena, M.L., Harvey, B.H., Viljoen, F., Ellis, S.M., Brink, C.B., 2015. Ozone exposure of Flinders Sensitive Line rats is a rodent translational model of neurobiological oxidative stress with relevance for depression and antidepressant response. Psychopharmacology (Berl) 232, 2921–2938. https://doi.org/10.1007/s00213-015-3928-8.

Nautiyal, K.M., Hen, R., 2017. Serotonin receptors in depression: from A to B. F1000Research 6, 123. https://doi.org/10.12688/f1000research.9736.1.

Nautiyal, K.M., Tritschler, L., Ahmari, S.E., David, D.J., Gardier, A.M., Hen, R., 2016. A lack of serotonin 1B autoreceptors results in decreased anxiety and depression-related

behaviors. Neuropsychopharmacology 41, 2941–2950. https://doi.org/10.1038/npp.2016.109.
Neumaier, J.F., Sexton, T.J., Hamblin, M.W., Beck, S.G., 2000. Corticosteroids regulate 5-HT1A but not 5-HT1B receptor mRNA in rat hippocampus. Brain Reseach Mol. Brain Res. 82, 65–73. https://doi.org/10.1038/jid.2014.371.
Odaka, H., Adachi, N., Numakawa, T., 2017. Impact of glucocorticoid on neurogenesis. Neural Regen. Res. 12, 1028–1035. https://doi.org/10.4103/1673-5374.211174.
Ouanes, S., Popp, J., 2019. High cortisol and the risk of dementia and alzheimer's disease: a review of the literature. Front. Aging Neurosci. 11. https://doi.org/10.3389/fnagi.2019.00043.
Paz, C., Huitrón-Reséndiz, S., 1996. The effects of ozone exposure on the sleep-wake cycle and serotonin contents in the pons of the rat. Neurosci. Lett. 204, 49–52. https://doi.org/10.1016/0304-3940(96)12313-2.
Peeters, F., Nicolson, N.A., Berkhof, J., 2004. Levels and variability of daily life cortisol secretion in major depression. Psychiatr. Res. 126, 1–13. https://doi.org/10.1016/j.psychres.2003.12.010.
Power, M.C., Adar, S.D., Yanosky, J.D., Weuve, J., 2016. Exposure to air pollution as a potential contributor to cognitive function, cognitive decline, brain imaging, and dementia: a systematic review of epidemiologic research. Neurotoxicology 56, 235–253. https://doi.org/10.1016/j.neuro.2016.06.004.
Rivas-Arancibia, S., Guevara-Guzmán, R., López-Vidal, Y., Rodríguez-Martínez, E., Zanardo-Gomes, M., Angoa-Pérez, M., Raisman-Vozari, R., 2009. Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. Toxicol. Sci. 113, 187–197. https://doi.org/10.1093/toxsci/kfp252.
Rivas-Arancibia, S., Zimbrón, L.F.H., Rodríguez-Martínez, E., Maldonado, P.D., Borgonio Pérez, G., Sepúlveda-Parada, M., 2015. Oxidative stress-dependent changes in immune responses and cell death in the substantia nigra after ozone exposure in rat. Front. Aging Neurosci. 7, 65. https://doi.org/10.3389/fnagi.2015.00065.
Rodríguez-Martínez, E., Nava-Ruiz, C., Escamilla-Chimal, E., Borgonio-Perez, G., Rivas-Arancibia, S., 2016. The effect of chronic ozone exposure on the activation of endoplasmic reticulum stress and apoptosis in rat hippocampus. Front. Aging Neurosci. 8. https://doi.org/10.3389/fnagi.2016.00245.
Sachar, E.J., Hellman, L., Roffwarg, H.P., Halpern, F.S., Fukushima, D.K., Gallagher, T.F., 1973. Disrupted 24-hour patterns of cortisol secretion in psychotic depression. Arch. Gen. Psychiatr. 28, 19–24. https://doi.org/10.1001/archpsyc.1973.01750310011002.
Saito, K., Crowley, J.S., Markey, S.P., Heyes, M.P., 1993. A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. J. Biol. Chem. 268, 15496–15503.
Sala, M., Perez, J., Soloff, P., Ucelli Di Nemi, S., Caverzasi, E., Soares, J.C., Brambilla, P., 2004. Stress and hippocampal abnormalities in psychiatric disorders. Eur. Neuropsychopharmacol. https://doi.org/10.1016/j.euroneuro.2003.12.005.
Schefold, J.C., Zeden, J.P., Fotopoulou, C., Von Haehling, S., Pschowski, R., Hasper, D., Volk, H.D., Schuett, C., Reinke, P., 2009. Increased indoleamine 2,3-dioxygenase (Ido) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: a possible link between chronic inflammation and uraemic symptoms. Nephrol. Dial. Transplant. 24, 1901–1908. https://doi.org/10.1093/ndt/gfn739.
Sepehr, Z.B., Philip, H., Cowen, J., 2005. Increased salivary cortisol after waking in depression. Psychopharmacology (Berl) 182, 54–57. https://doi.org/10.1007/s00213-005-0062-z.
Sibille, E., Sarnyai, Z., Benjamin, D., Gal, J., Baker, H., Toth, M., 1997. Antisense inhibition of 5-hydroxytryptamine 2a receptor induces an antidepressant-like effect in mice. Mol. Pharmacol. 52, 1056–1063. https://doi.org/10.1124/mol.52.6.1056.
Skillen, Ralph, Thienes, Clinton, Cangelosi, Joseph, Strain, Lorrene, 1961. Brain 5-Hydroxytryptamine in Ozone-Exposed Rats. Experimental Biology and Medicine 108, 121–122. https://doi.org/10.3181/00379727-108-26866.
Smith, M.A., Makino, S., Kvetnansky, R., Post, R.M., 1995. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J. Neurosci. 15, 1768–1777. https://doi.org/10.1523/jneurosci.15-03-01768.1995.
Sublette, M.E., Galfalvy, H.C., Fuchs, D., Lapidus, M., Grunebaum, M.F., Oquendo, M.A., John Mann, J., Postolache, T.T., 2011. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. Brain Behav. Immun. 25, 1272–1278. https://doi.org/10.1016/j.bbi.2011.05.002.
Sulo, G., Vollset, S.E., Nygard, O., Midttun, O., Ueland, P.M., Eussen, S.J.P.M., Pedersen, E.R., Tell, G.S., 2013. Neopterin and kynurenine-tryptophan ratio as predictors of coronary events in older adults, the Hordaland Health Study. Int. J. Cardiol. 168, 1435–1440. https://doi.org/10.1016/j.ijcard.2012.12.090.
Suzuki, Y., Suda, T., Furuhashi, K., Suzuki, M., Fujie, M., Hahimoto, D., Nakamura, Y., Inui, N., Nakamura, H., Chida, K., 2010. Increased serum kynurenine/tryptophan ratio correlates with disease progression in lung cancer. Lung Canc. 67, 361–365. https://doi.org/10.1016/j.lungcan.2009.05.001.
Tanapat, P., Hastings, N.B., Rydel, T.A., Galea, L.A.M., Gould, E., 2001. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. J. Comp. Neurol. 437, 496–504. https://doi.org/10.1002/cne.1297.
Thomas, J., Guénette, J., Thomson, E.M., 2018. Stress axis variability is associated with differential ozone-induced lung inflammatory signaling and injury biomarker response. Environ. Res. 167, 751–758. https://doi.org/10.1016/j.envres.2018.09.007.
Thomson, E., Kumarathasan, P., Goegan, P., Aubin, R.A., Vincent, R., 2005. Differential regulation of the lung endothelin system by urban particulate matter and ozone. Toxicol. Sci. 88, 103–113. https://doi.org/10.1093/toxsci/kfi272.
Thomson, E.M., 2019. Air pollution, stress, and allostatic load: linking systemic and central nervous system impacts. J. Alzheim. Dis. 69, 597–614. https://doi.org/10.3233/JAD-190015.
Thomson, E.M., Filiatreault, A., Guénette, J., 2019. Stress hormones as potential mediators of air pollutant effects on the brain: rapid induction of glucocorticoid-responsive genes. Environ. Res. 178, 108717. https://doi.org/10.1016/j.envres.2019.108717.
Thomson, E.M., Pal, S., Guénette, J., Wade, M.G., Atlas, E., Holloway, A.C., Williams, A., Vincent, R., 2016. Ozone inhalation provokes glucocorticoid-dependent and -independent effects on inflammatory and metabolic pathways. Toxicol. Sci. 152, 17–28. https://doi.org/10.1093/toxsci/kfw061.
Thomson, E.M., Vladisavljevic, D., Mohottalage, S., Kumarathasan, P., Vincent, R., 2013. Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicol. Sci. 135, 169–181. https://doi.org/10.1093/toxsci/kft137.
Thomson, E.M., Williams, A., Yauk, C.L., Vincent, R., 2009. Impact of nose-only exposure system on pulmonary gene expression. Inhal. Toxicol. 21, 74–82. https://doi.org/10.1080/08958370902962309.
Toda, T., Parylak, S.L., Linker, S.B., Gage, F.H., 2019. The role of adult hippocampal neurogenesis in brain health and disease. Mol. Psychiatr. 24, 67–87. https://doi.org/10.1038/s41380-018-0036-2.
Vázquez, D.M., Neal, C.R., Patel, P.D., Kaciroti, N., López, J.F., 2012. Regulation of corticoid and serotonin receptor brain system following early life exposure of glucocorticoids: long term implications for the neurobiology of mood. Psychoneuroendocrinology 37, 421–437. https://doi.org/10.1016/j.psyneuen.2011.07.012.
Wesołowska, A., Nikiforuk, A., Stachowicz, K., 2006. Potential anxiolytic and antidepressant effects of the selective 5-HT7receptor antagonist SB 269970 after intrahippocampal administration to rats. Eur. J. Pharmacol. 553, 185–190. https://doi.org/10.1016/j.ejphar.2006.09.064.
Whiteford, H.A., Ferrari, A.J., Degenhardt, L., Feigin, V., Vos, T., 2015. The global burden of mental, neurological and substance use disorders: an analysis from the global burden of disease study 2010. PLoS One 10. https://doi.org/10.1371/journal.pone.0116820.
Widner, B., Leblhuber, F., Walli, J., Tilz, G.P., Demel, U., Fuchs, D., 2000. Tryptophan degradation and immune activation in Alzheimer's disease. J. Neural. Transm. 107, 343–353. https://doi.org/10.1007/s007020050029.
Woodward, N.C., Haghani, A., Johnson, R.G., Hsu, T.M., Saffari, A., Sioutas, C., Kanoski, S.E., Finch, C.E., Morgan, T.E., 2018. Prenatal and early life exposure to air pollution induced hippocampal vascular leakage and impaired neurogenesis in association with behavioral deficits. Transl. Psychiatry 8. https://doi.org/10.1038/s41398-018-0317-1.
You, J.-M., Yun, S.-J., Nam, Kyong Nyon, Kang, Chulhun, Won, R., Lee, Eunjoo H., You, J.M., Yun, S.J., Nam, K.N., Kang, C., Lee, E.H., 2009. Mechanism of glucocorticoid-induced oxidative stress in rat hippocampal slice cultures. Can. J. Physiol. Pharmacol. 87, 440–447. https://doi.org/10.1139/Y09-027.
Zhao, T., Markevych, I., Romanos, M., Nowak, D., Heinrich, J., 2018. Ambient ozone exposure and mental health: a systematic review of epidemiological studies. Environ. Res. 165, 459–472. https://doi.org/10.1016/j.envres.2018.04.015.
Zhu, L.J., Liu, M.Y., Li, H., Liu, X., Chen, C., Han, Z., Wu, H.Y., Jing, X., Zhou, H.H., Suh, H., Zhu, D.Y., Zhou, Q.G., 2014. The different roles of glucocorticoids in the hippocampus and hypothalamus in chronic stress-induced HPA axis hyperactivity. PLoS One 9. https://doi.org/10.1371/journal.pone.0097689.