The Effects of ROS and DNA Repair on Methylmercury-Initiated Neurodevelopmental Deficits

by

Kyla Schwarz-Lam

A thesis submitted in conformity with the requirements
for the degree of Doctor of Philosophy

Department of Pharmacology and Toxicology
University of Toronto

The Effects of ROS and DNA Repair on Methylmercury-Initiated Neurodevelopmental Deficits

Kyla Schwarz-Lam

Doctor of Philosophy

Department of Pharmacology and Toxicology
University of Toronto

2014

Abstract

Methylmercury (**MeHg**) is an environmental toxin to which we are exposed through the consumption of seafood. Reactive oxygen species (**ROS**) have been implicated in the mechanism of toxicity, and *in vitro* studies in our laboratory have implicated DNA oxidation, particularly the DNA repair enzyme oxoguanine glycosylase 1 (**OGG1**). My studies determined the effects of *in utero* exposure to MeHg on fetal brain DNA oxidation and postnatal neurodevelopmental deficits, and the role of ROS-mediated oxidative DNA damage using the free radical spin trap, α-phenyl-N-*tert*-butylnitrone (**PBN**), and DNA repair-deficient *ogg1* knockout mice. While neither MeHg nor PBN altered DNA oxidation in fetal brain, MeHg caused cognitive deficits in passive avoidance and novel object recognition, the latter of which was blocked by PBN pretreatment, suggesting ROS involvement. Preliminary longevity studies following one litter from each treatment group to 16 months suggest that *in utero* MeHg treatment may shorten lifespan. Endogenous DNA oxidation was increased in the brains of *ogg1* knockout fetuses compared to wild-type littermates, although this was not enhanced by MeHg. However, OGG1-deficient animals exhibited cognitive deficits in passive avoidance after MeHg treatment, suggesting a role for DNA damage. Furthermore, *ogg1*

knockout female mice exhibited a passive avoidance deficit compared to wild-type females regardless of treatment, corroborating a role for oxidative DNA damage in neurodevelopmental deficits. MeHg increased apoptosis in the hippocampal region of fetal brain, and may cause DNA double-strand breaks (**DSBs**), evidenced by enhanced phosphorylation of histone 2AX (**γH2AX**). *Ogg1* knockout progeny exhibited increased cellular proliferation or migration in the developing hippocampal region, which was blocked by MeHg. My results provide the first evidence that: (1) MeHg may decrease lifespan; (2) PBN protects against some postnatal neurodevelopmental deficits caused by *in utero* exposure to MeHg; and (3) DNA repair-deficient progeny are more susceptible to postnatal cognitive deficits caused by *in utero* MeHg exposure, suggesting that ROS-mediated DNA oxidation plays a role in MeHg-initiated neurodevelopmental deficits.

Acknowledgments

I'd like to thank Dr. Wells for giving me the opportunity over the past few years to grow as a scientist; with his continued support, I have learned invaluable lessons in critical thinking, effectively speaking about my work, and having the patience to tease out frustrating data. Additionally, I would like to thank CIHR and NIEHS for funding my work.

Thank you to Dr. Wiley and Dr. McPherson for their advice and support as members of my advisory committee. I'm also grateful to Dr. McPherson, as my co-supervisor, for all of his generosity, both financially and with his expertise and equipment. I'd also like to thank Dr. Laurie Chan for acting as my external examiner, and Dr. Hampson and Dr. Sun for serving on my defence committee.

To all of my fellow lab members, past and present: Julia Abramov, Crystal Lee, Margaret Loniewska, Gord McCallum, Lutfiya Miller, Stephanie Ondovcik, Tom Preston, Annmarie Ramkissoon, Aaron Shapiro, Michelle Siu, and Nicole Sweeting, thanks for celebrating the good times and commiserating over the bad times! Michelle, thanks for keeping me sane during the seemingly endless DNA extractions and HPLC runs; and Nicole, thanks for getting me hooked on crocheting and knitting! (Puns!) Thanks also to the Phit Phriday club for reminding me that pipetting and wiping down testing chambers with 70% ethanol for eight straight hours doesn't actually constitute exercise.

I'm grateful to Dr. Henderson and his lab for all of their advice and technical help with the von Frey filaments and paraffin processing. I'd also like to thank Laura Tamblyn and Meghan Larin for their assistance and advice with my immunohistochemical studies and imaging.

I am forever indebted to my parents, Chuen and Yuvonne Lam, and my sister, Danica Lam, for keeping me well fed and watered, and for always acting as sounding boards for both my griping and my ideas.

Last, but certainly not least, I thank my husband, Peter Schwarz-Lam. I don't know how I would have managed without your constant love and support. Thanks for always patiently knowing whether I needed you to listen to me talk about work, when I needed you to talk to me about work, and when I needed to just stop thinking about work. I am constantly impressed by your scientific acumen, your enthusiasm, and your dedication – you've always been the grad student I wanted to be.

Table of Contents

List of Tables

Section 1: Introduction

Section 2: Methods

List of Figures

Section 1: Introduction

Section 2: Methods

Section 3: Results

List of Abbreviations

+/+	Wild-type
+/-	Heterozygous
-/-	Homozygous knockout
8-oxodG	8-oxo-2’-deoxyguanine
8-oxodGuo	8-oxo-2’-deoxyguanosine
µg/L	microgram/L
AD	Alzheimer’s Disease
AIF	Apoptosis-inducing factor
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ASK1	Apoptosis signal-regulating kinase 1
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasis and Rad3-related protein
BBB	Blood-brain barrier
BER	Base excision repair
BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	Breast cancer type 2 susceptibility protein
BrdU	Bromodeoxyuridine
CA1	Cornu Ammonis 1

CA3	Cornu Ammonis 3
CSA	Cockayne Syndrome A
CSB	Cockayne Syndrome B
CYP	Cytochrome P450
dGuo	2’-deoxyguanosine
DHA	Docosahexaenoic acid
Dvl	Dishevelled
DMPS	Dimercaptopropanesulfonate
DMSA	Dimercaptosuccinic acid
DSB	Double-strand break
EPA	Environmental Protection Agency
ERCC1	Excision repair cross-complementing protein 1
EXO1	Exonuclease 1
fapyG	2,6-diamino-4-hydroxy-5-formamidopyrimidine
FEN1	Flap endonuclease1
Fpg	Formamidopyrimidine DNA glycosylase
GABA	γ-aminobutyric acid
GAD	Glutamate decarboxylase
GD	Gestational day
GGR	Global genome repair

GLAST	Glutamate/aspartate transporter
GLT1	Glutamate transporter-1
GSH	Glutathione
GSH-Px	Glutathione peroxidase
GST	Glutathione S-transferase
H_2O_2	Hydrogen peroxide
Hg	Mercury
$HO^{\cdot}$	Hydroxyl radical
HD	Huntington's Disease
HR	Homologous recombination
i.p.	Intraperitoneally
IHg	Inorganic mercury
LAT1	L-type large neutral amino acid transporter 1
LCPUFA	Long-chain polyunsaturated fatty acids
LPO	Lipoxygenase
LTP	Long-term potentiation
MCI	Mild cognitive impairment
MeHg	Methylmercury
mg/kg	milligram/kilogram
MLH1	MutL homolog 1

MMR	Mismatch repair
Mre11	Meiotic recombination 11 homolog 1
MRM	Multiple reaction monitoring
MRP2	Multidrug-resistance protein 2
MS	Mass spectrometry
MSH2	MutS protein homolog 2
MSH6	MutS protein homolog 6
MT	Metallothionein
MTH1	MutT homolog 1
MYH	MutY homolog
NAC	N-acetylcysteine
Nbs1	Nijmegen breakage syndrome protein 1
NEIL1	Endonuclease VIII-like 1
NER	Nucleotide excision repair
NHEJ	Nonhomologous end joining
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
Nrf2	Nuclear factor-erythroid 2-related factor-2

NRX	Nucleoredoxin
$O_2^{\cdot-}$	Superoxide
OAT1	Organic anion transporter-1
OCT1	Octamer binding transcription factor 1
OGG1	Oxoguanine glycosylase 1
PCNA	Proliferating cell nuclear antigen
PD	Parkinson's Disease
PBN	α-phenyl-N-*tert*-butylnitrone
PBS	Phosphate-buffered saline
PHS	Prostaglandin H synthase
PMS1	Postmeiotic segregation increased 1 protein
ppm	Parts per million
RFC	Replication Factor C
RfD	Reference Dose
ROS	Reactive oxygen species
RPA	Replication protein A
SCID	Severe combined immunodefiency
SOD	Superoxide dismutase
Sp1	Specificity protein 1
TCR	Transcription coupled repair

TFIIH	Transcription factor II human
THg	Total mercury
TRX	Thioredoxin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
XLF	XRCC4-like factor
XPA	Xeroderma pigmentosum group A protein
XPF	Xeroderma pigmentosum group F protein
XPG	Xeroderma pigmentosum group G protein
XRCC1	X-ray repair cross-complementing protein 1
XRCC4	X-ray repair cross-complementing protein 4

List of Appendices

Section 1: Introduction

1.1 Rationale and Research Objectives

1.1.1 Statement of the problem

Methylmercury (**MeHg**) is a widespread environmental contaminant to which we are primarily exposed by seafood in our diets. Cases of widespread and severe MeHg poisoning in Iraq and Japan have shown us that children and developing infants are more susceptible to its toxic effects, resulting in neurodevelopmental deficits, and decreased cerebral and cerebellar size, as well as a decrease in neuron number. While reactive oxygen species (**ROS**) have been implicated in the mechanism of toxicity, there is little direct evidence. The likely role of ROS, however, suggests that specific measures of oxidative damage to cellular macromolecules, and particularly DNA, may be crucial to the toxicity of MeHg. Previously, our laboratory provided the first direct *in vivo* evidence that knockout mice deficient in the repair of the oxidative DNA lesion 8-oxo-2'-deoxyguanine (**8-oxodG**) by the enzyme oxoguanine glycosylase 1 (**OGG1**) are more susceptible to the neurotoxic effects of *in utero* exposure to the ROS-initiating teratogen methamphetamine. Therefore, if ROS are involved in the mechanism of toxicity of MeHg, animals with an impaired capacity to repair oxidatively damaged DNA may be at greater risk of postnatal neurodevelopmental deficits after *in utero* exposure to MeHg.

1.1.2 Purpose of the study and objective

The purpose of my project was to determine the role that ROS, and more specifically, oxidative DNA damage, play in the mechanism of developmental toxicity of MeHg. To do so, I used two major mechanistic approaches in examining the effects of *in utero*

exposure to MeHg at the molecular, cellular, and functional levels: in the first, outbred CD-1 mice were exposed to a single dose of MeHg with or without pretreatment with the free radical spin trap α-phenyl-N-*tert*-butylnitrone (**PBN**) late in gestation to determine whether blocking MeHg-initiated ROS in fetal brain would reduce the incidence or severity of postnatal cognitive deficits in the offspring; in the second, DNA repair-deficient *ogg1* knockout mice were exposed *in utero* to the same MeHg dosing regimen, without PBN pretreatment, to determine whether impaired DNA repair would increase the incidence or severity of cognitive deficits in the offspring. In the CD-1 mice exposed to MeHg with or without PBN pretreatment, I measured 8-oxo-2'-deoxyguanosine (**8-oxodGuo**) levels in fetal brain; postnatal cognitive and neurodevelopmental outcomes using novel object recognition, passive avoidance, and von Frey filaments; and lifespan (**fig 1.1**). In the *ogg1* knockout animals, I measured 8-oxodGuo levels in fetal brain; DNA double-strand breaks (**DSBs**), apoptosis, and cellular proliferation and migration in the developing hippocampal region of the fetal brain; and examined the progeny for postnatal cognitive and neurodevelopmental outcomes using novel object recognition, passive avoidance, and von Frey filaments (**fig 1.2**).

1.1.3 Statement of research hypothesis and rationale for hypothesis

MeHg increases ROS formation in the fetal brain, causing oxidative damage to DNA that alters brain cell development, resulting in postnatal neurodevelopmental deficits. If this hypothesis is correct, pretreatment with an antioxidant should be protective: both fetal ROS levels and postnatal neurodevelopmental deficits should be decreased by prior *in utero* exposure to the free radical spin trapping agent PBN.

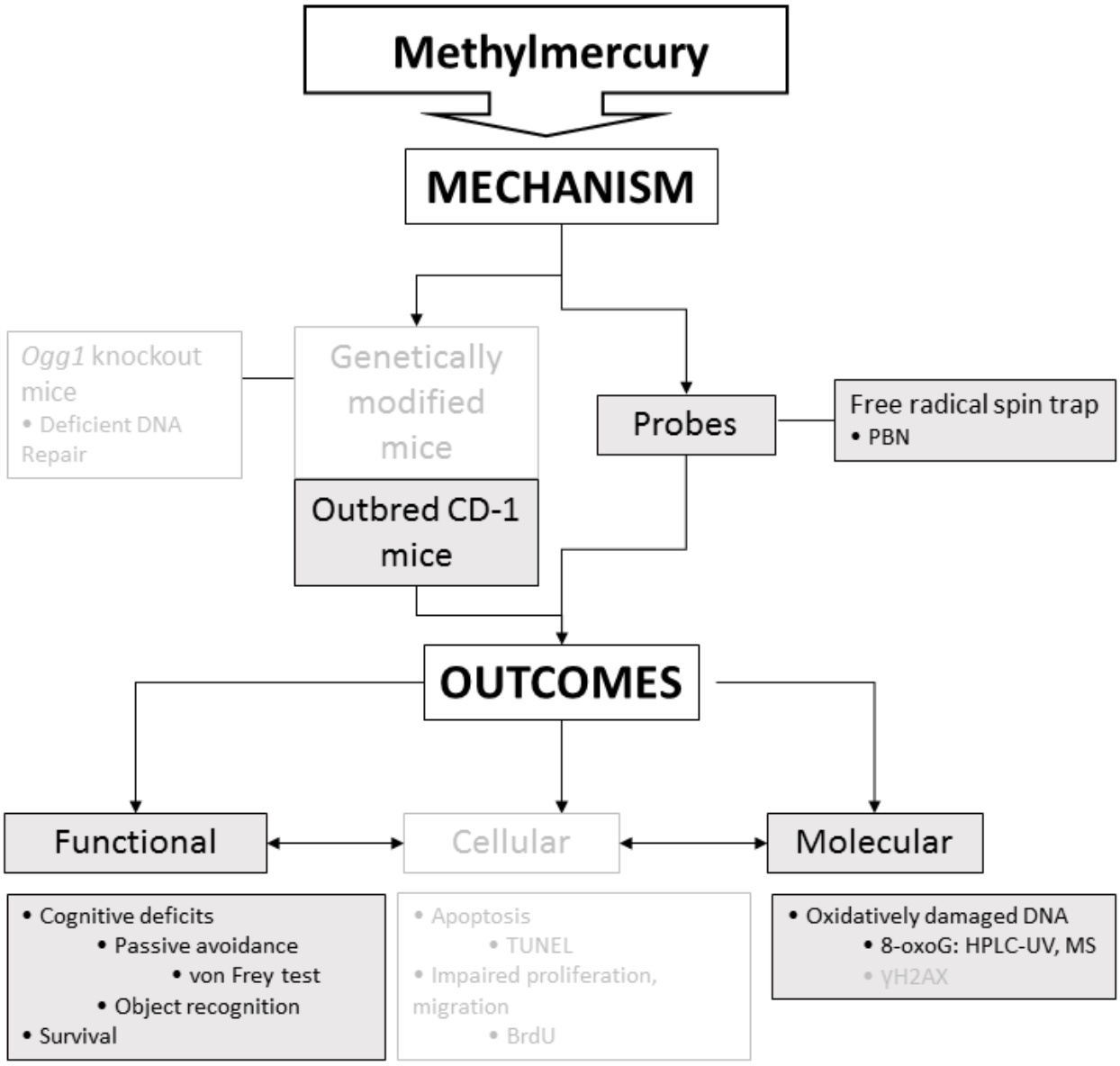

Figure 1.1: Measuring the effect of MeHg and PBN on neurodevelopment
To determine the effects of methylmercury (**MeHg**) and α-phenyl-N-*tert*-butylnitrone (**PBN**) on the developing brain, pregnant CD-1 dams were injected i.p. with 4 mg/kg MeHg, or an equivalent volume of phosphate-buffered saline (**PBS**), with or without pretreatment with 45 mg/kg PBN on gestational day (**GD**) 17. To quantify fetal levels of DNA oxidation in the brain, fetal brains were harvested 6 hr after treatment and 8-oxo-2'-deoxyguanosine (**8-oxodGuo**) levels were measured using high-performance liquid chromatography (**HPLC**) with tandem mass spectrometry (**MS**). To measure cognitive function, offspring were tested using the object recognition test at 6 weeks of age and the passive avoidance test at 4 months of age. Somatosensory function was confirmed using von Frey filaments. One litter from each treatment group was also followed to 16 months of age to measure effects on long-term survival.

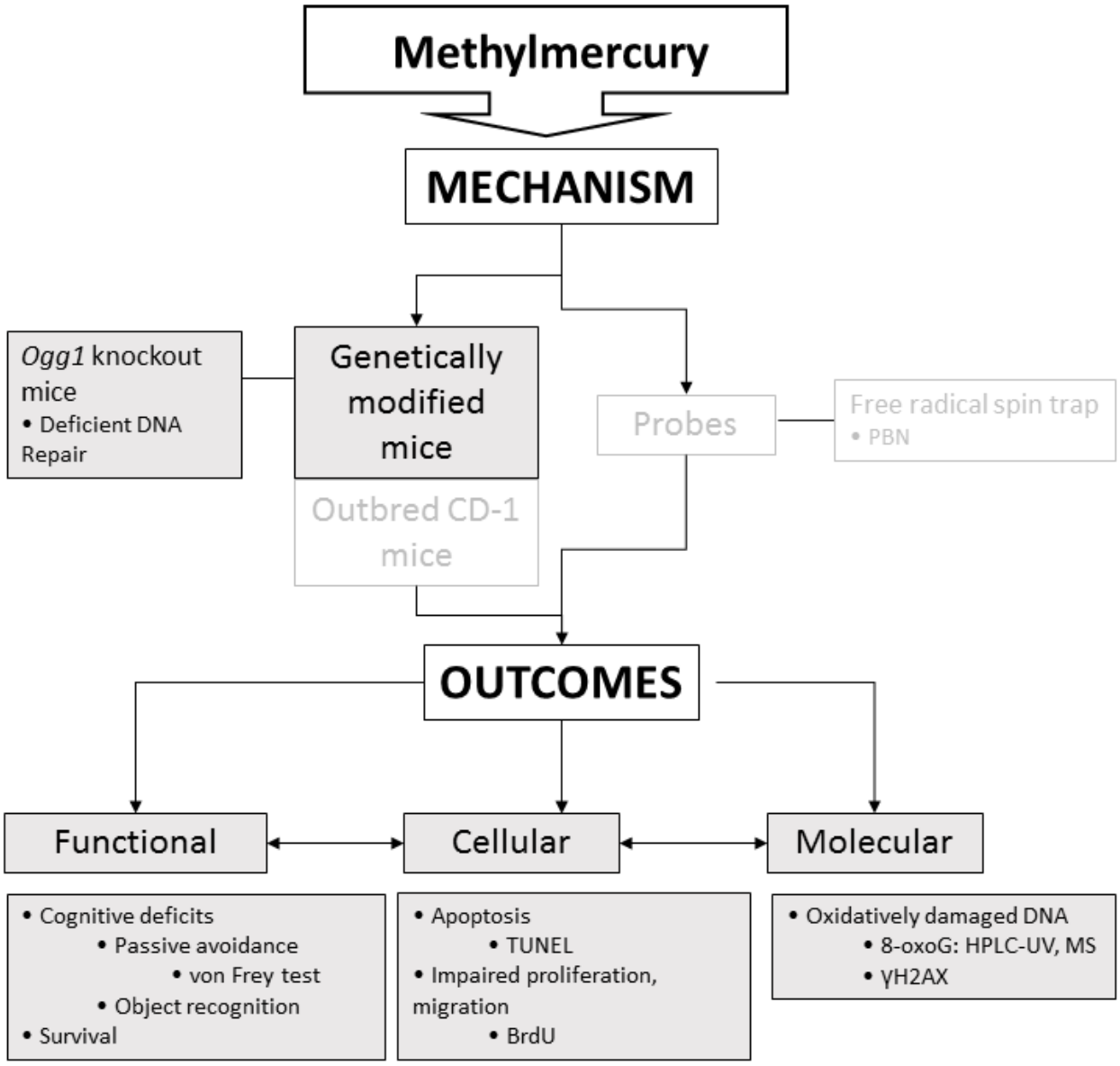

Figure 1.2: Measuring the effect of MeHg and OGG1 on neurodevelopment
To determine the effects of MeHg and impaired DNA repair on the developing brain, pregnant DNA repair-deficient *ogg1* heterozygous dams were injected i.p. with 4 mg/kg MeHg, or an equivalent volume of PBS on GD 17. To quantify fetal levels of DNA oxidation in the brain, fetal brains were harvested 6 hr after treatment and 8-oxodGuo levels were measured using HPLC-MS. DNA damage at this timepoint was also measured in fetuses from wild-type and *ogg1* knockout dams by counting the number of cells in the developing hippocampal region with DNA double-strand breaks (**DSB**) using immunohistochemical detection of γH2AX. Apoptosis and proliferation in the hippocampal region were also measured at this timepoint using immunohistochemical detection. To measure postnatal cognitive function, offspring from heterozygous dams were tested using the object recognition test at 6 weeks of age and the passive avoidance test at 4 months of age. Somatosensory function was confirmed using von Frey filaments.

Conversely, compromised levels of fetal DNA repair, in this study a deficiency in OGG1 in fetal brain, should increase the level of oxidatively damaged DNA in fetal brain and postnatal neurodevelopmental deficits. PBN has previously been shown to partially reduce MeHg-induced increases in ROS in astrocyte cell cultures (Shanker & Aschner, 2003). Other antioxidants, including catalase, Trolox, and ebselen have also been shown to reduce some toxic effects of MeHg (Shanker & Aschner, 2003; Yin *et al.*, 2011). Our laboratory has shown that *ogg1* knockout cell lines and cell lines overexpressing *hOgg1* are more susceptible to the cytotoxic effects of MeHg, suggesting that oxidative damage to DNA and DNA repair status may play crucial roles in modulating susceptibility to MeHg toxicity. The purpose of my project was to determine whether ROS, and more specifically DNA oxidation, are involved in the mechanism of developmental toxicity of MeHg.

1.2 Reactive Oxygen Species

Reactive oxygen species (**ROS**), comprised of superoxide (**$O_2^{\bullet-}$**), hydrogen peroxide (**H_2O_2**), and the hydroxyl radical (**$HO^{\bullet}$**), are highly reactive molecules that are capable of causing toxicity by oxidatively damaging cellular macromolecules, such as lipids, proteins, and DNA, and by disrupting signal transduction. However, they are also crucial regulators of signal transduction and the immune response.

1.2.1 Sources of ROS

1.2.1.1 Endogenous sources of ROS

ROS are formed as byproducts of mitochondrial respiration, when O_2 is converted to $O_2^{\bullet-}$ by Complexes I and III of the electron transport chain (**fig. 1.3**) (review: Balaban *et al.*, 2005). ROS are also an important component of the protective response of phagocytic leukocytes, which use the respiratory burst of NADPH oxidases to produce $O_2^{\bullet-}$ as a component of their antimicrobial activity (Bokoch & Knaus, 2003). NADPH oxidases convert NADPH and O_2 to NADP+, hydrogen, and $O_2^{\bullet-}$, and can also produce H_2O_2 to assist in signal transduction, discussed in section 1.2.3 (review: Serrander *et al.*, 2007; Nauseef, 2013). Other ROS-generating enzymes include xanthine oxidase, the cytochrome P450s (**CYPs**), and prostaglandin H synthases (**PHSs**). This endogenous production of ROS can be used to regulate cell signaling cascades, further discussed in section 1.2.3.

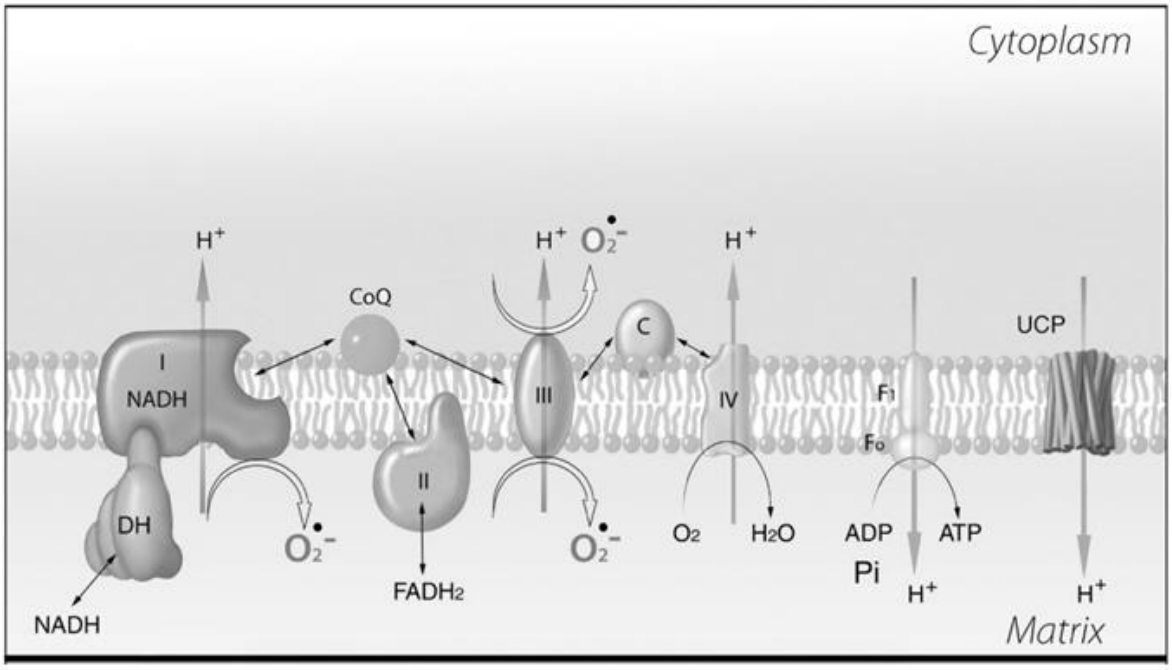

Figure 1.3 Generation of superoxide from the electron transport chain in mitochondria

Reprinted and modified from Cell, Vol. 120, Balaban et al, Mitochondria, oxidants, and aging, pp. 483 – 495, © 2005, with permission from Elsevier.

1.2.1.2 Exogenous sources of ROS

In addition to endogenous sources, ROS formation can also be enhanced in the body by xenobiotics. Bioactivation of xenobiotics by a number of enzymes, including PHSs, CYPs and lipoxygenases (**LPOs**), can result in free radical intermediates which react with oxygen to form ROS (**fig. 1.4**) (review: Wells *et al.*, 2010). As both PHS and LPO are expressed during development, they may be important contributors to the ROS-mediated developmental effects of xenobiotics including phenytoin and methamphetamine (Yu & Wells, 1995; Parman *et al.*, 1998; Jeng *et al.*, 2006). Xenobiotics that can be metabolized to quinones by PHS and CYPs can also form ROS by redox-cycling, whereby the quinone is reduced by NADPH P450 reductase to a semiquinone, which can be oxidized back to the quinone by reacting with O_2, in the process producing $O_2^{\cdot -}$ (**fig. 1.4**). Xenobiotics can also indirectly increase ROS levels by inducing enzymes like NADPH oxidases that produce ROS.

1.2.2 ROS-induced toxicity

In the body, ROS can attack cellular macromolecules, including proteins, lipids, and DNA. The oxidation of proteins can disrupt their function, as the oxidation of protein residues often leads to the activation or inactivation of the protein, further discussed in section 1.2.3. Lipid peroxidation in cell membranes can increase their permeability to ions, causing protein dysfunction, and can cause leakage of normally contained enzymes, including hydrolytic enzymes from lysosomes (Halliwell & Gutteridge, 2007). Oxidative DNA damage has been implicated in several forms of cancer (Audebert *et al.*, 2000; Goode *et al.*, 2002; Kunisada *et al.*, 2005; Singh *et al.*, 2013). $HO^{\cdot}$ can attack

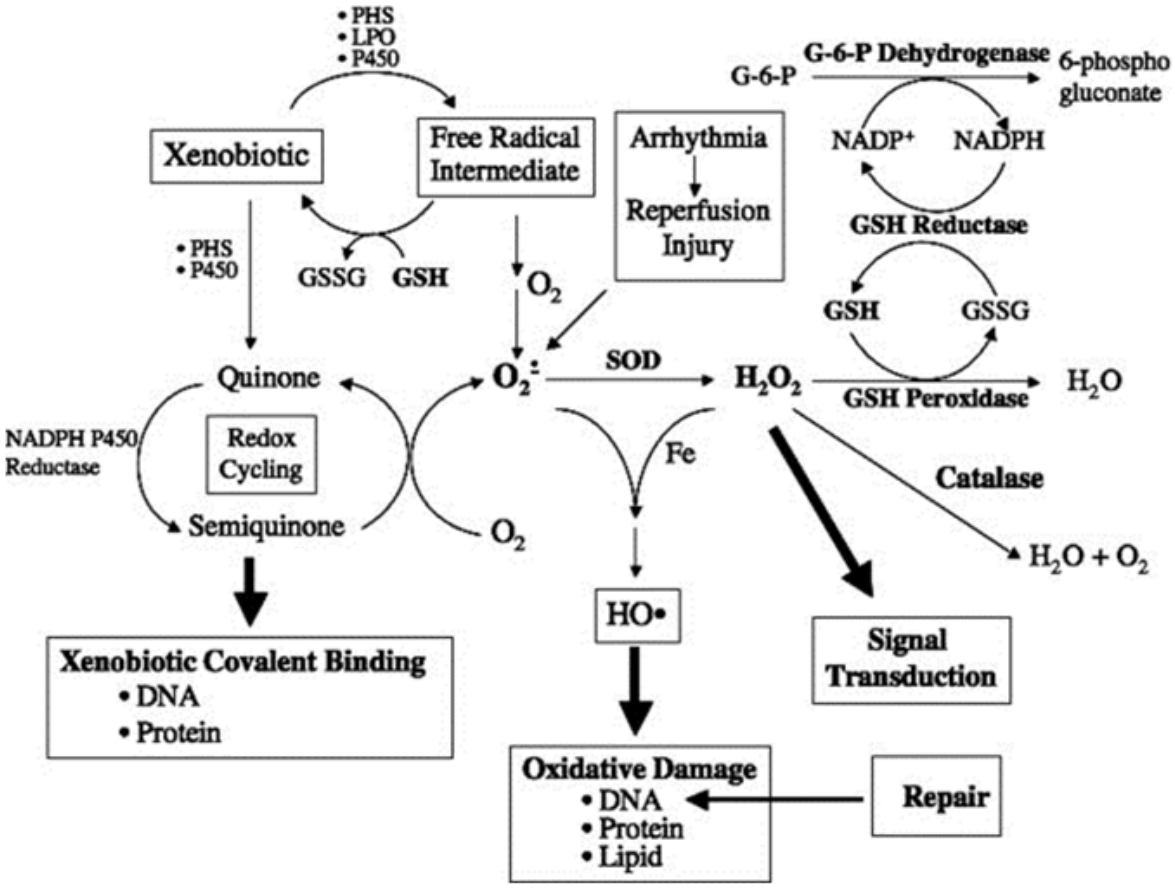

Figure 1.4 Formation, detoxification, and toxic effects of ROS

Abbreviations: **Fe**, iron; **G-6-P**, glucose-6-phosphate; **GSH**, glutathione; **GSSG**, glutathione disulfide; **HO$^{\cdot}$**, hydroxyl radical; **H_2O_2**, hydrogen peroxide; **H_2O**, water; **LPO**, lipoxygenase; **NADP+**, oxidized nicotinamide adenine dinucleotide phosphate; **NADPH**, reduced nicotinamide adenine dinucleotide phosphate; **O_2**, molecular oxygen; **$O_2^{\cdot-}$**, superoxide anion; **P450**, cytochrome P450; **PHS**, prostaglandin H synthase; **SOD**, superoxide dismutase

Reproduced from Wells et al., Oxidative DNA damage and repair in teratogenesis and neurodevelopmental deficits, Birth Defects Res C Embryo Today, 2010, with permission from John Wiley and Sons Inc.

DNA bases, resulting in DNA lesions that can cause mutagenesis, transcriptional mutagenesis, and transcriptional stalling (Xie *et al.*, 2004; Halliwell & Gutteridge, 2007; Pastoriza-Gallego *et al.*, 2007). Over 20 lesions have been identified, of which 8-oxodG is among the most common and most studied (Dizdaroglu, 2005; Hu *et al.*, 2005). 8-oxodG is further discussed in section 1.3.2.1. ROS can also attack the sugars of the DNA backbone, resulting in bulky cyclic lesions and single-strand breaks (Nakamura *et al.*, 2003; Halliwell & Gutteridge, 2007).

1.2.3 The role of ROS in cell signaling

While ROS are often thought of as toxic species, they also play an important role in signal transduction, through the regulation of protein activity by oxidation. Some proteins contain reactive cysteines that have pKas of approximately 4 or 5 because of the surrounding amino acids, instead of the typical pKas of 8 or 9 (Kim *et al.*, 2000b). These cysteines are easily oxidized, and this oxidation can regulate protein activation and inactivation (**fig. 1.5**) (review: Miki & Funato, 2012). While the initial oxidation of the thiol to a sulfenyl moiety is easily reduced back to the thiol, subsequent oxidations to sulfinyl and sulfonyl moieties cannot easily be reduced, potentially leading to permanent changes in protein activity. Oxidation of protein tyrosine phosphatases inactivates them, while oxidation of some tyrosine kinases activates them, allowing for sustained activation of other phosphorylated proteins.

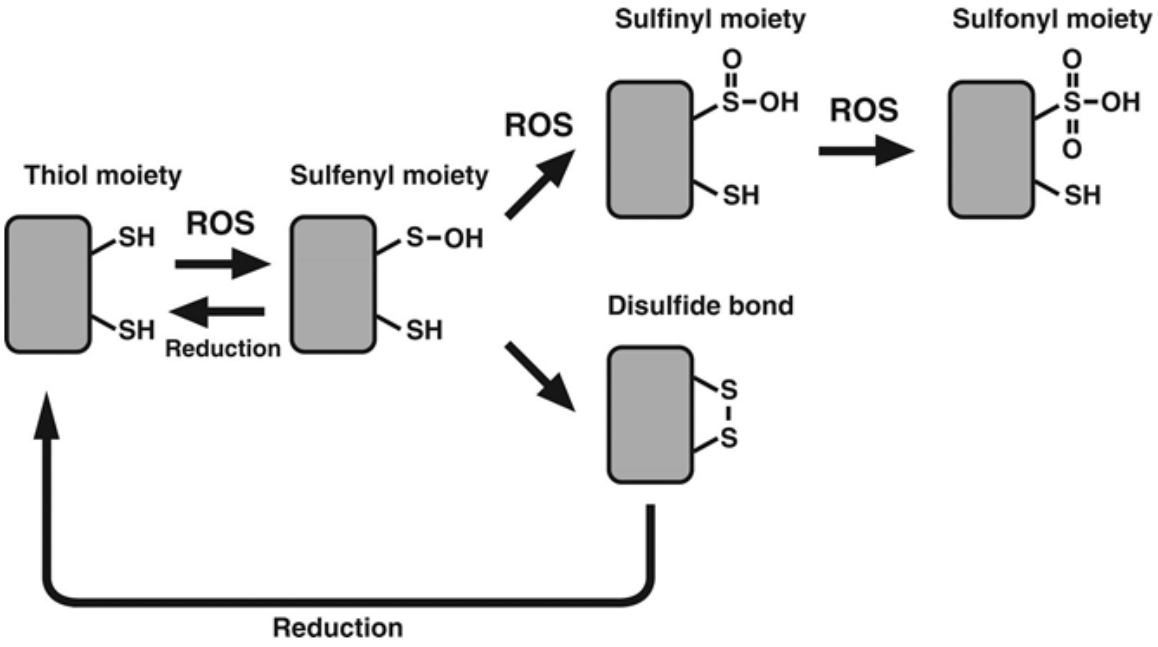

Figure 1.5 Regulation of protein activity by reversible oxidation of cysteine residues by ROS

from Miki and Funato, Regulation of intracellular signalling through cysteine oxidation by reactive oxygen species, J. Biochem, 2012, Vol. 151, Issue 3, pp. 255 – 261, by permission of Oxford University Press.

ROS can activate the Wnt/β-catenin pathway, which helps to guide differentiation, and maintain stem cell pluripotency (review: Funato & Miki, 2007). ROS-mediated oxidation of thiol groups in nucleoredoxin (**NRX**) triggers it to release dishevelled (**Dvl**), which is thereby activated (Funato *et al.*, 2006). Dvl prevents the degradation of β-catenin, allowing it to translocate to the nucleus and bind transcription factors from the TCF/LEF family, activating target gene transcription (Sokol, 2011). When an imbalance between ROS formation and detoxification exists, the normal functioning of ROS in regulating cell signaling can be disrupted.

1.2.4 Detoxification of ROS

1.2.4.1 Non-enzymatic ROS detoxifiers

Metallothioneins (**MTs**), which contain multiple thiol groups, protect the body against heavy metal toxicity by forming covalent adducts with the metals, but they can also reduce ROS-mediated toxicity via the preferential oxidation of their thiol groups (Jeong *et al.*, 2004). MT also protects against MeHg toxicity. MT-1 and -2 *null* mice exposed to MeHg throughout gestation and for 10 days after birth develop more severe cognitive deficits, revealed in passive avoidance tests and the Morris water maze, and decreased exploratory behaviour in the open field test compared to their wild-type littermates by 12 months of age (Yoshida *et al.*, 2008). MeHg has been shown to induce MT-1 and -2 in the cerebellum and liver, as well as MT-1 in the kidneys (Yasutake & Nakamura, 2011).

Glutathione (**GSH**) is used as a co-factor or substrate for ROS-detoxifying enzymes such as GSH peroxidase (**GSH-Px**) and GSH S-transferase (**GST**), respectively (reviews: Board & Menon, 2013; Brigelius-Flohé & Maiorino, 2013). The thiol group on the cysteine residue of GSH can be oxidized, resulting in its antioxidant properties. However, similar to many other antioxidants containing thiol groups, GSH may also protect against MeHg toxicity by binding directly to it, instead of detoxifying MeHg-enhanced ROS levels. Glutamate-cysteine ligase, which catalyzes the first and rate-limited step in the synthesis of GSH, is induced in the brains of female mice exposed subchronically to MeHg via drinking water (Thompson *et al.*, 1999). GSH is also an important facilitator of the excretion of MeHg from the body, discussed further in section 1.4.3.1 (Schläwicke Engström *et al.*, 2008; Lee *et al.*, 2010).

Similarly to MT and GSH, N-acetyl-cysteine (**NAC**) and other thiol-containing molecules can act as both MeHg and ROS detoxifiers through their thiol residues, which are easily oxidized and bind readily to MeHg. Detoxification of MeHg by NAC is further discussed in section 1.4.2.

Transcription factors also act as antioxidants by regulating the levels of other antioxidant proteins and enzymes. Nuclear factor-erythroid 2-related factor 2 (**Nrf2**) is normally bound to Keap1 in the cytoplasm (review: Surh, 2003). When Keap1 is oxidized, Nrf2 is released and travels to the nucleus, upregulating the transcription of a number of protective and antioxidant proteins, including GST, heme oxygenase 1, γ-

glutamate cysteine ligase, NADPH:quinone oxidoreductase, and DNA repair enzymes, including OGG1 (Itoh *et al.*, 1999; Dhénaut *et al.*, 2000; Hayes *et al.*, 2000; Shih *et al.*, 2003).

Fat-soluble vitamin E, the general name for a group of four tocopherols and four tocotrienols, can directly attack free radicals, becoming oxidized to a stable non-toxic radical (review: Niki & Traber, 2012). Vitamin E, generally administered as α-tocopherol, does appear to have some protective effects against MeHg toxicity, protecting cerebellar granule cells from lipid peroxidation, cell death and decreased migration after MeHg exposure (Shichiri *et al.*, 2007). After exposure to a chronic, relatively high dose of MeHg, improvements in postnatal rat survival, as well as some parameters of maternal health were observed with co-treatment with a combination of vitamin E and selenium (Beyrouty & Chan, 2006). Vitamin E can also counter some of the toxic effects of MeHg poisoning, reducing motor coordination deficits and mortality, as well as lipid peroxidation and protein oxidation (Yamashita *et al.*, 2004). Trolox, a water-soluble vitamin E derivative, has been shown to be partially protective against MeHg toxicity in adult rats, as well, restoring mitochondrial enzyme function in skeletal muscle and reducing MeHg-initiated apoptosis in the cerebellum (Usuki *et al.*, 2001).

Vitamin C, or ascorbic acid, can bind directly to ROS, but also assists in regenerating vitamin E by reducing oxidized vitamin E (Niki, 1987; Chan, 1993). There is limited evidence that vitamin C has a protective effect against MeHg toxicity. One study found

that vitamin C was protective against very low doses of MeHg in cerebellar granule cells (Farina *et al.*, 2009). However, an *in vitro* study on rabbit kidney cortical slices found that ascorbic acid increased the uptake of MeHg and lipid peroxidation (Fujimoto *et al.*, 1988). Studies in adult mice and Atlantic salmon have both shown that dietary MeHg can result in decreased vitamin C levels in the liver (de Freitas *et al.*, 2009; Olsvik *et al.*, 2011).

Ebselen is an organo-selenium compound that can act as a GSH-Px mimic, particularly phospholipid-hydroperoxide GSH-Px (Maiorino *et al.*, 1988). It has also been shown to protect against MeHg in astrocyte cultures. Pretreatment of ebselen in rat neonatal primary astrocyte cultures protected against MeHg-initiated glutamine uptake decreases and caspase-3 increases (Yin *et al.*, 2011). Similarly, *in vitro* studies in cortical slices from adult mice have demonstrated that ebselen blocked MeHg-initiated inhibition of glutamate uptake (Farina *et al.*, 2003). Since ebselen also appears to decrease MeHg-initiated ROS formation, it has been suggested that this may be the mechanism by which it blocks glutamate uptake inhibition (Farina *et al.*, 2003; Roos *et al.*, 2009). Ebselen, however, has no effect on glutamate uptake inhibition by IHg (Moretto *et al.*, 2005).

Selenium is an essential component for a number of enzymes, including antioxidant enzymes such as GSH-Px and thioredoxin reductases (Rotruck *et al.*, 1973; Zhong *et al.*, 2000). MeHg has been shown to inhibit both of these enzymes (Watanabe *et al.*,

1999; Usuki *et al.*, 2011). Selenoamino acids are capable of demethylating MeHg, and it appears that selenium-containing compounds are essential for binding and sequestering IHg (Khan & Wang, 2010; Korbas *et al.*, 2010). However, binding of selenium to MeHg also has the side effect of sequestering selenium, which can result in the inhibition of selenium-dependent enzymes, leading to increases in oxidative stress. Rodents exposed to low-selenium diets are more susceptible to the effects of MeHg than those with high-selenium diets (Ralston *et al.*, 2008). It has also been shown that diphenyl diselenide can decrease MeHg-initiated lipid peroxidation, reduce oxidative stress in the liver and cerebellum, and reduce Hg accumulation in the cerebrum, cerebellum, kidney, and liver (de Freitas *et al.*, 2009).

1.2.4.1.1 α-phenyl-N-tert-butylnitrone (PBN)

PBN is primarily known as a free radical spin trapping agent (**fig. 1.6**) (Floyd *et al.*, 2008). PBN traps radicals by reacting with them to form stable nitrone spin adducts.

In rats, PBN has a half-life of 2 – 3 hours, with levels in the brain peaking at 45 minutes after intraperitoneal (**i.p.**) injection, and at 15 minutes in the plasma (Chen *et al.*, 1990; Marklund *et al.*, 2002; Trudeau-Lame *et al.*, 2003). In mice, the half-life appears to be closer to an hour (Liu *et al.*, 1999). However, a previous study found that a dose of 265 mg of PBN i.p. trapped more radicals in mice when injected 3 hours prior to being γ irradiated than when it was injected 30 min prior to irradiation (Lai *et al.*, 1986). While there may be a number of factors contributing to this difference, including the free

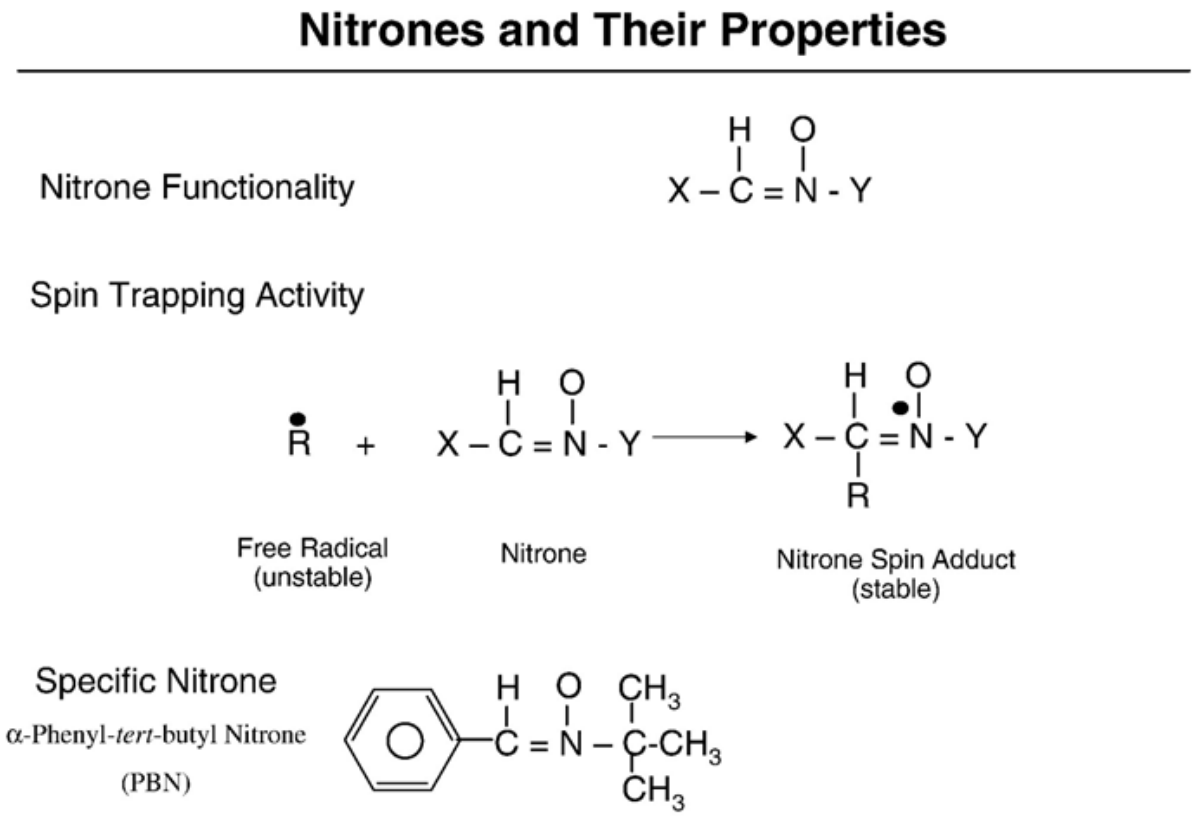

Figure 1.6 Structure and free radical trapping mechanism of PBN

Reprinted from Free Radic Biol Med, Vol. 45, Floyd et al, Nitrones as therapeutics, pp. 1361 – 1374, © 2008, with permission from Elsevier.

radical species and the timeframe in which they are formed, it is evident that PBN retains protective properties in the mouse for several hours. This is further supported by studies from our laboratory which demonstrated that PBN administered 2 hours prior to phenytoin in mice protected against increases in lipid peroxidation and protein oxidation (Liu & Wells, 1994). In monkeys, the half-life of PBN is approximately 0.75 hours, and similarly to the rat, the majority of PBN appears to be metabolized to α-hydroxy-phenyl-N-*tert*-butylnitrone in the liver (Trudeau-Lame *et al.*, 2003; Lame *et al.*, 2004). It is estimated that a dose of 150 mg/kg PBN will result in a brain concentration of 330 μM, and a blood concentration of 225 μM (Cheng *et al.*, 1993).

Nitrones have been studied for their effectiveness in treating stroke, cancer, and other diseases of aging. While the nitrones have as yet had little clinical success for the treatment of stroke, one PBN derivative, 2,4-disulfophenyl-*tert*-butylnitrone, has been shown to be safe clinically and potentially effective for the treatment of gliomas (Floyd *et al.*, 2011).

Our laboratory has repeatedly demonstrated that pretreatment with PBN can be protective against *in utero* exposure to ROS-initiating teratogens. In rabbits, PBN reduces DNA oxidation increases, structural deficits, increased fetal resorptions, and increased post-partum lethality caused by *in utero* exposure to thalidomide (Parman *et al.*, 1999); and in mice, PBN reduces cleft palate incidence and fetal resorptions (Wells

et al., 1989), as well as increased protein and lipid oxidation (Liu & Wells, 1994), caused by *in utero* exposure to phenytoin.

Although it is often assumed to exert its protective effects because of its spin trapping properties, there is little direct evidence to suggest that this is the primary mode of protective action for PBN. Additional possible protective mechanisms of PBN are summarized in **Table 1.1**. In models where no increases in free radicals, including oxygen radicals, were detected, PBN exposure blocked calcium channels and induced pulmonary artery relaxation (Anderson *et al.*, 1993); blocked the anticholinesterase effect of diisopropylphosphorofluoridate (Milatovic *et al.*, 2000b); decreased nitric oxide (**NO**) in a model of neuroinflammation (Endoh *et al.*, 2001); and decreased glutamate efflux in the ventral tegmental area after low-dose amphetamine exposure (Wolf *et al.*, 2000).

Additionally, PBN has been found to protect in an MPTP-induced Parkinson's Disease model without reducing the increase in $HO^{\cdot}$ observed in the substantia nigra (Ferger *et al.*, 2000). Similarly, in a model of β-amyloid injection-induced model of Alzheimer's Disease, PBN reduced dentate gyrus lesion size, but had little effect on lipid peroxidation (Montiel *et al.*, 2006).

Model	Dose/ concentration of PBN	Effect	Increase in ROS/free radicals?	Source
Rat pulmonary artery rings	1.0 mM	Relaxation of artery rings, inhibition of calcium influx	No	Anderson *et al.*1993
Lipopolysaccharide injection in adult mice	300 mg/kg	Decrease in inducible NOS protein levels, and nitric oxide levels	N/A	Miyajima and Kotake, 1995
MPTP-initiated Parkinson's Disease model in mice	200 mg/kg x 15 days	Protection against loss of body weight and mortality, partial protection of striatal dopamine depletion and reduced locomotor activity, but no decrease of hydroxyl radical levels	Yes, but no protection by PBN	Ferger *et al.* 2000
Adult rat	300 mg/kg	Protection of acetylcholinesterase activity	No	Milatovic *et al.*2000
In vitro: Purified acetylcholinesterase	1.0 mM	Reversible inhibition of acetylcholinesterase	N/A	Milatovic *et al.* 2000
5 mg/kg amphetamine in adult rat	60 mg/kg	Protected against glutamate efflux in ventral tegmental area	No	Wolf *et al.* 2000
Lipopolysaccharide injection in adult rats	250 mg/kg	Decrease in nitric oxide in the cerebrum, and in white blood cells in cerebrospinal fluid	No	Endoh *et al.* 2001
Iron-induced epilepsy rat model	100 mg/kg	Improved GLT-1 levels, decreased extracellular glutamate	N/A	Samuelsson *et al.* 2003
Intrahippocampal β-amyloid injection in adult rat	100 mg/kg	Reduced dentate gyrus lesion size, but only small decrease in lipid peroxidation	Yes, but only partial protection by PBN	Montiel *et al.* 2006
Cerebral hypoxia-ischemia in neonatal rats	100 mg/kg	Inhibition of interleukin-1β, TNF-α and iNOS mRNA	N/A	Lin *et al.* 2006
Neuroinflammation in neonatal rats	100 mg/kg	Rescue of neurological reflexes, passive avoidance learning, and CA1 neuronal loss	N/A	Fan *et al.*2010
My studies:				
4 mg/kg MeHg in developing mouse	45 mg/kg	Protection against novel object recognition deficit, possible prevention of MeHg-initiated decrease in lifespan	N/A	

Table 1.1: A comparison of the potentially ROS-independent effects of PBN in different *in vivo* and *in vitro* models

Other studies have identified other protective effects of PBN without measuring free radical formation, making it difficult to determine whether PBN exerts its protective effect in these models by free radical trapping, or by other mechanisms. It is important to note, then, that trapping of oxygen radicals by PBN may still be the primary protective mechanism in these models. It has been shown to decrease levels of inducible NO synthase (**NOS**) and protect against cognitive deficits in models of neuroinflammation (Lin *et al.*, 2006; Fan *et al.*, 2008); inhibit the activity of purified acetylcholinesterase (Milatovic *et al.*, 2000a); and restore glial glutamate transporter 1 (**GLT-1**) levels in a model of iron-induced epilepsy (Samuelsson *et al.*, 2003).

Of particular interest to my studies is that the decrease in extracellular glutamate may provide an alternative mechanism by which PBN can protect against MeHg toxicity, as MeHg is known to increase extracellular glutamate levels in the brain, potentially leading to excitotoxic damage (**fig. 1.12 and 1.13**) (Allen *et al.*, 2001; Juárez *et al.*, 2005; Mutkus *et al.*, 2005; Xu *et al.*, 2012). However, an important corollary is that each of the studies cited employed a higher PBN dose than mine, generally more than 2-fold higher. It is possible that at these higher doses, PBN has actions that do not occur at the lower dose I used. The 45 mg/kg dose of PBN that I employed was based on previous studies from our laboratory that showed that PBN pretreatment decreased protein and lipid oxidation levels caused by ROS-initiating teratogens at doses that had no other toxic effects (Liu & Wells, 1994).

1.2.4.2 Enzymatic pathways for detoxifying ROS

The levels of antioxidative enzymes are often low in the developing fetus compared to the adult, with notable exceptions including glucose-6-phosphate dehydrogenase, and the DNA repair enzyme OGG1 (Nicol *et al.*, 2000; Wong *et al.*, 2008; Wells *et al.*, 2009; Abramov & Wells, 2011). The fetus, therefore, can be especially susceptible to the toxic effects of ROS. Superoxide dismutase (**SOD**) converts $O_2^{\cdot-}$ to H_2O_2, which can be further detoxified by catalase to water and O_2, or by GSH-Px to water (**fig. 1.4**) (review: Wells *et al.*, 2010). GSH is oxidized during the detoxification of H_2O_2 by GSH-Px, but can be regenerated by GSH reductase, through the oxidation of NADPH to NADP+. NADPH is subsequently regenerated by G6PD. However, $O_2^{\cdot-}$ and H_2O_2 can also form $HO^{\cdot}$, which can oxidize cellular macromolecules, including lipids, proteins and DNA. The addition of exogenous catalase appears to be protective against MeHg-toxicity *in vitro*, restoring glutamate transport in cultured astrocytes and reducing MeHg-initiated DNA DSBs in mouse embryonic fibroblasts (Allen *et al.*, 2001; Ondovcik *et al.*, 2012). These studies implicate H_2O_2 in the mechanism of toxicity, and suggest that $HO^{\cdot}$, which is generated by the reduction of H_2O_2 and is responsible for oxidizing deoxyguanine to 8-oxodG, may be involved in the mechanism of toxicity.

1.3 Oxoguanine Glycosylase 1 (OGG1)

OGG1 is a base excision repair (**BER**) enzyme (Arai *et al.*, 1997; Tani *et al.*, 1998). BER is the major form of DNA repair that recognizes and removes single nucleotide lesions, including methylated and oxidatively damaged bases. OGG1 is involved primarily in short-patch BER, where only the damaged nucleotide is removed and replaced, as opposed to long-patch BER, where a short oligonucleotide strand is replaced (review: David *et al.*, 2007). Additional DNA repair pathways are discussed further in section 1.3.8.

OGG1 has been previously implicated by our laboratory in neurodevelopmental deficits caused by methamphetamine (Wong *et al.*, 2008). Studies in mouse embryonic fibroblasts in our laboratory have also shown that *ogg1* -/- cells are more susceptible to DNA DSBs, apoptosis, and cell cycle arrest after MeHg exposure than wild-type cells (Ondovcik *et al.*, 2012, 2013b). However, human embryonic kidney cells expressing *hOgg1* were also more sensitive to the cytotoxic effects of MeHg than their wild-type counterparts (Ondovcik *et al.*, 2013a). It is possible that this was caused by increased OGG1 activity in removing MeHg-induced DNA lesions without adequate activity from downstream enzymes to complete the DNA repair process. These studies suggest that OGG1 may be involved in the mechanism of toxicity of MeHg.

1.3.1 Structure and Localization

First identified in yeast but also found in eukaryotes, OGG1 is a functional homolog of the bacterial enzyme formamidopyrimidine DNA glycosylase (**fpg**) (van der Kemp *et al.*, 1996; Arai *et al.*, 1997; Tani *et al.*, 1998). *hOgg1* is found at 3p26 (Arai *et al.*, 1997; Ishida *et al.*, 1999a), while in the mouse, *ogg1* is found on chromosome 6 (Tani *et al.*, 1998). OGG1 belongs in the helix-hairpin-helix family of DNA glycosylases, and this domain appears to be conserved between the yeast, mouse and human OGG1 proteins (Arai *et al.*, 1997; Rosenquist *et al.*, 1997; Tani *et al.*, 1998). An additional zinc finger-like motif is relatively well-conserved among the three species (Tani *et al.*, 1998). Both the mouse and human genes consist of 7 exons (Tani *et al.*, 1998; Ishida *et al.*, 1999a). hOGG1 is 38% homologous with yeast OGG1, and approximately 84% homologous with mouse OGG1 (Arai *et al.*, 1997; Radicella *et al.*, 1997).

In humans, αOGG1 localizes to the nucleus while βOGG1 is found in the mitochondria (Nishioka *et al.*, 1999). Both enzymes are coded by the first six exons of the *Ogg1* gene, but the seventh exon that codes αOGG1 is approximately 9 kb before the last exon that codes βOGG1 (Ishida *et al.*, 1999a). Mitochondrial OGG1 activity has also been identified in rats and mice (Chen *et al.*, 2000; de Souza-Pinto *et al.*, 2001).

1.3.2 Lesions repaired

1.3.2.1 8-oxo-2'-deoxyguanine (8-oxodG)

8-oxodG (**fig. 1.7**) is formed when guanine is oxidized by a hydroxyl radical at the 8-carbon position, forming an oxidized guanine intermediate (review: Greenberg, 2012). This intermediate is further oxidized to form 8-oxodG. 8-oxodG can exist in both keto- and enol-forms, with the keto-form being favoured at physiological pH (Bjelland & Seeberg, 2003). 8-oxodG can mispair with adenine, resulting in mutagenesis in the form of G:T transversions (Shibutani *et al.*, 1991; Le Page *et al.*, 1998). It is one of the most common oxidative DNA lesions, and it is estimated that mammalian cells may form 10^3 – 10^5 8-oxodG lesions each day (Park *et al.*, 1992).

In an *ogg1* -/- mouse model, 2-fold increases in 8-oxodGuo have been observed in the liver, although not in the spleen or kidneys (Klungland *et al.*, 1999; Osterod *et al.*, 2001). Furthermore, 8-oxodGuo appears to accumulate with age in these mice (Osterod *et al.*, 2001). We have previously shown that *ogg1* -/- mice are more susceptible to the *in utero* effects of methamphetamine at exposures that increase 8-oxodGuo levels in the fetal brain, suggesting that it is a pathogenic lesion (Wong *et al.*, 2008).

While it is generally agreed that large, bulky lesions are capable of stalling transcription, whether or not 8-oxodG is capable of causing RNA polymerase to pause remains relatively controversial, as multiple studies have indicated that transcription bypass at 8-oxodG sites can be as high as 95% (Tornaletti *et al.*, 2004; Kuraoka *et al.*, 2007;

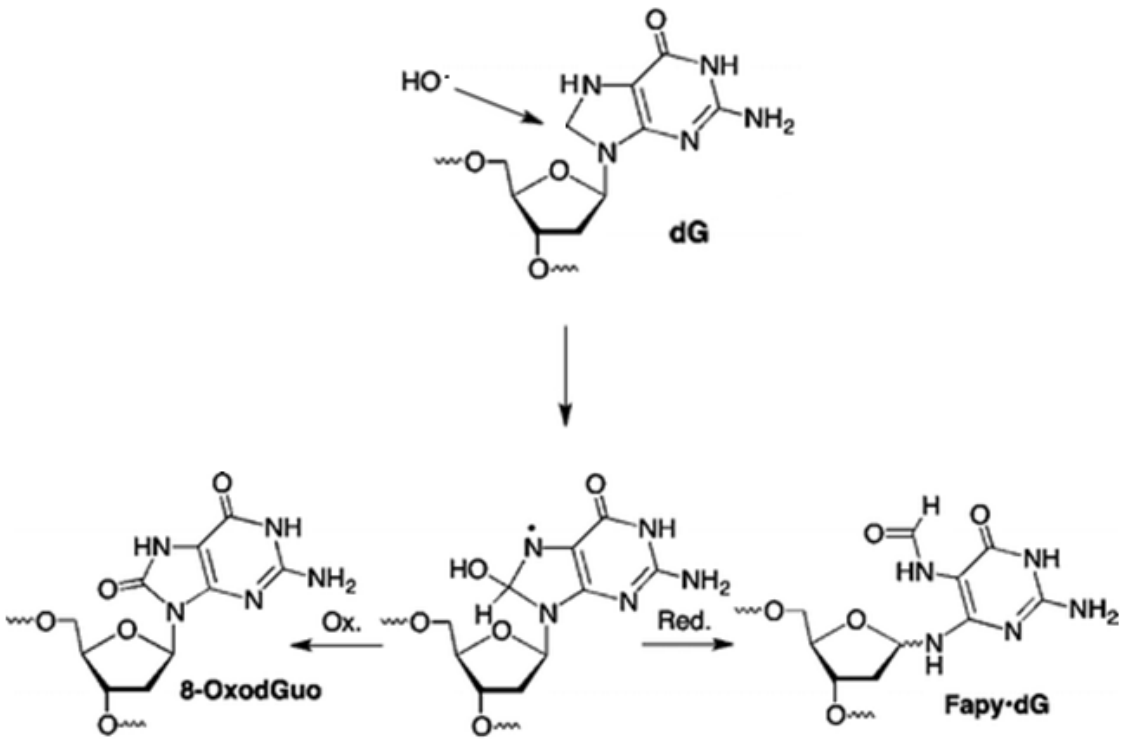

Figure 1.7: Formation of 8-oxodG and fapyG
deoxyGuanine (**dG**) can be oxidized by hydroxyl radicals at the 8-carbon position, resulting in a oxidized base intermediate that can be further oxidized to produce 8-oxo-2'-deoxyguanine (**8-oxodG**), or reduced to form 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**fapyG**). 8-oxodG can exist in both keto- and enol- forms.

Adapted with permission from Greenberg, The formamidopyrimidines: Purine lesions formed in competition with 8-oxopurines from oxidative stress, Acc Chem Res, Vol. 45, 2012. © 2013 American Chemical Society.

Saxowsky *et al.*, 2008). While oligonucleotides containing 8-oxodG appear to be able to partially stall RNA polymerase II, the presence of transcription elongation factors TFIIS, Cockayne Syndrome B (**CSB**), and elongin appear to facilitate the selective bypassing of 8-oxodG by RNA polymerase II (Kuraoka *et al.*, 2007). While this may appear protective, it is possible that this increased RNA polymerase bypass results in transcriptional mutagenesis, whereby a misincorporated adenine results in a mutant protein with impaired function. *Ogg1* -/- cells with 8-oxodG at codon 61 in the template strand of the Ras oncogene were more likely to form mutant Ras proteins, where a mispaired adenine in the transcribed RNA strand resulted in a glutamine→lysine mutation (Saxowsky *et al.*, 2008). This mutation resulted in a constitutively active Ras protein, greatly increasing the potential for carcinogenesis. Transcriptional mutagenesis and stalling may be particularly important for relatively non-replicating cell populations, such as in the brain, but also during critical windows of development.

There is evidence that under certain conditions, 8-oxodG is capable of stalling transcription, and this appears to be dependent on the context of the surrounding strand (Pastoriza-Gallego *et al.*, 2007). *Ogg1* -/- cells transfected with 8-oxodG-containing luciferase reporter plasmids were more likely to experience transcription arrest at the 8-oxodG sites, and this arrest was exacerbated by further loss of function mutations for the transcription coupled repair (**TCR**) protein CSB. Transcription arrest could be increased if the promoter region was not particularly strong, when 8-oxodG was inserted in GC-rich zones, and when 8-oxodG was farther from the promoter region. Moreover, it has also been shown that in the presence of OGG1, 8-oxodG can become more

effective at blocking transcription (Kitsera *et al.*, 2011). *Ogg1* -/- mouse embryonic fibroblasts transfected with 8-oxodG-containing EGFP reporter genes had approximately 1.2 fold greater EGFP expression than wild-type cells. In the wild-type cells, the presence of 8-oxodG in either the transcribed or non-transcribed strand caused transcription stalling. The authors suggested that BER intermediates containing single strand breaks might be responsible for the transcription arrest.

Previously, studies have shown that MeHg causes 2-fold increases in 8-oxodGuo levels in C6 glioma cells, with peak levels being observed 6 hr after exposure (Belletti *et al.*, 2002). Studies from a laboratory at the China Medical University in Liaoning have consistently found increases in 8-oxodG levels in the cerebral cortices of adult rats exposed chronically to MeHg (Xu *et al.*, 2012; Liu *et al.*, 2013). Similarly, a study examining the effect of MeHg exposure on the livers and kidneys of adult rats found increased 8-oxodG levels in the kidneys (Jin *et al.*, 2008). Industrial workers exposed to Hg also have elevated urinary 8-oxodGuo levels, although the relative contributions of Hg and MeHg exposure are difficult to interpret (Chen *et al.*, 2005). However, a study in human lymphocytes suggests that MeHg may be approximately twice as effective as IHg at inducing 8-oxodG formation (Ogura *et al.*, 1996).

1.3.2.2 2,6-diamino-4-hydroxy-5-formamidopyrimidine

While 8-oxodG is considered the primary lesion repaired by OGG1, other oxidative DNA lesions are also substrates. 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**fapyG**) is a ring-opened lesion which appears at similar or greater frequencies than 8-oxodG (**fig. 1.7**) (Proteggente *et al.*, 2001; Hu *et al.*, 2005; Greenberg, 2012). fapyG appears to be formed from the same intermediate as 8-oxodG: when guanine is oxidized by a hydroxyl radical, the resulting molecule can be further oxidized to form 8-oxodG, or it can be reduced to form fapyG (Munk *et al.*, 2007).

Unfortunately, there is currently no information on the role that fapyG might play in causing neurodevelopmental deficits. This is likely due to the fact that fapyG remains relatively unstudied. However, since we have previously shown that *ogg1* -/- female mice are more susceptible to the effects of ROS-generating xenobiotics like methamphetamine, and that *ogg1* -/- mouse embryonic fibroblasts are more susceptible to MeHg, it may well be that fapyG, as a common oxidative DNA lesion repaired by OGG1, is involved in the mechanism of toxicity.

1.3.2.3 Other lesions repaired

OGG1 can also repair a number of cytosine mispairings, including urea, 5-hydroxyuracil, 5,6-dihydrothymine, and thymine glycol (Ide & Kotera, 2004). Incision activity at 8-oxo-2'-deoxyadenine mispaired with cytosine, and at methyl-fapyG has also been detected (Girard *et al.*, 1998).

1.3.3 Expression

In mice, OGG1 activity is detectable in the fetal brain and liver on GD 17 at levels 2- to 6-fold higher than in the adult brain and liver (**fig. 1.8**), although they are comparable in other organs (Larsen *et al.*, 2006; Wong *et al.*, 2008). *Ogg1* -/- fetuses did not appear capable of repairing 8-oxodG lesions, suggesting that at this developmental stage, OGG1 is the sole source of protection against this lesion (Wong *et al.*, 2008). OGG1 mRNA levels appear to be at their highest during gestation and decrease with age (Riis *et al.*, 2002). Peak mitochondrial OGG1 activity in the rat also occurs late in gestation, and declines with age (Chen *et al.*, 2002a).

mOGG1 has been identified in the brain, liver, kidneys, spleen, heart, lung, skeletal muscle and testes, suggesting that its expression is relatively ubiquitous (Tani *et al.*, 1998). Expression of hOGG1 does not appear to change during the cell cycle (Dhénaut *et al.*, 2000).

1.3.4 Regulation

As the 5'-upstream region of the gene does not contain a TATA-sequence and is GC-rich, *Ogg1* appears to be a housekeeping gene, but there is also evidence that it can be induced (Ishida *et al.*, 1999a; Dhénaut *et al.*, 2000; Ramkissoon & Wells, 2013). The *Ogg1* promoter region contains a binding site for the transcription factor Nrf2 (Piao *et al.*, 2011; Singh *et al.*, 2013). Nrf2 is an important component of the cell's antioxidant defence, discussed in section 1.2.4.

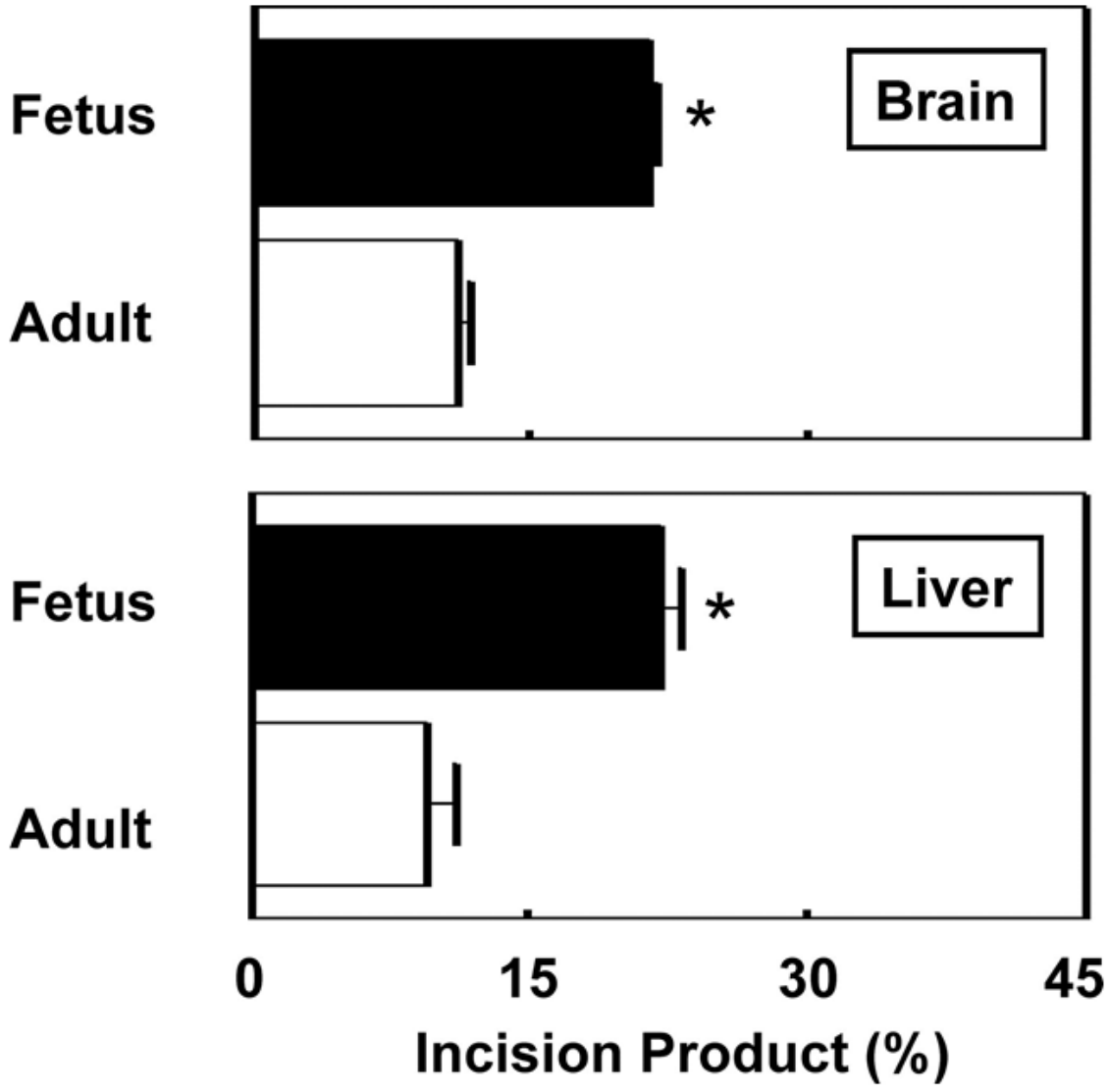

Figure 1.8: OGG1 activity in adult and fetal mouse organs
OGG1 is more active in fetal brains and livers compared to adult brains and livers in mice. Brain extracts were incubated with biotin-labelled oligonucleotides containing 8-oxodG and resolved using 15% PAGE, prior to densitometric analysis. * indicates a difference from the respective adult tissue ($p < 0.05$).

The tumour suppressor protein BRCA1 is also capable of up-regulating a number of DNA repair enzymes, including OGG1 (Saha *et al.*, 2010). Nuclear extracts incubated with BRCA1 were found to have increased OGG1 mRNA and protein levels, as well as increased activity, while BRCA1 knockdown decreased protein levels and activity. This increase appears to be reliant on the transcription factor OCT1, as OCT1-silencing abolished the BRCA1-mediated increase in OGG1 protein levels.

The mitochondrial deacetylase Sirtuin 3 has been shown to maintain OGG1 by deacetylating it, preventing its degradation (Cheng *et al.*, 2013). Silencing of Sirtuin 3 in glioblastoma cell cultures resulted in increased 8-oxodG levels, as well as decreased growth and proliferation.

Cadmium has been show to decrease OGG1 expression and activity in a number of different species and models (Zharkov & Rosenquist, 2002; Potts *et al.*, 2003; Youn *et al.*, 2005; Bravard *et al.*, 2006; Singh *et al.*, 2009; Kim *et al.*, 2012; Smith *et al.*, 2013). This inhibition appears to be mediated by a number of different mechanisms, including decreasing the binding of the transcription factor Sp1 to the OGG1 promoter, and irreversibly inactivating OGG1, potentially via oxidation (Zharkov & Rosenquist, 2002; Youn *et al.*, 2005; Bravard *et al.*, 2006). It is currently unknown whether MeHg is capable of inhibiting OGG1 activity or of decreasing OGG1 expression, but in light of what is known about cadmium, this would be worth investigating, although a study of Saudi Arabian men found that while environmental exposure to cadmium was

associated with increased OGG1 mRNA, environmental exposure to Hg had no effect (Al Bakheet *et al.*, 2013). However, since the subjects were exposed to possible metal contamination from a nearby mine, it is difficult to make any conclusions about the relative contributions of MeHg and Hg. Furthermore, MeHg may still be capable of directly inhibiting OGG1 function, or increasing its degradation.

Acetaminophen is also capable of inhibiting DNA repair by OGG1 in C6 glioma cells by decreasing protein levels without affecting mRNA levels (Wan *et al.*, 2004). The exact mechanism by which this occurs is unknown, but the study authors suggest that it may be due to binding of acetaminophen or one of its metabolites to OGG1, resulting in a protein adduct which is subsequently targeted for degradation. This hypothesis is supported by other adducts of acetaminophen or its metabolites to other enzymes, including glutamine synthetase, glutamate dehydrogenase, and aldehyde dehydrogenase (review: Zhou *et al.*, 2005).

1.3.5 Mode of Action

OGG1 appears to slide along the DNA backbone inducing bends to locate 8-oxodG (Chen *et al.*, 2002b). 8-oxodG is pushed out of the DNA helix into the active site of the enzyme, forming a lesion-recognition complex, leaving a space in the helix that is temporarily filled by Asn149 forming a hydrogen bond with the paired cytosine, which also interacts with Arg154, Arg204 and Tyr203 (Bruner *et al.*, 2000; David *et al.*, 2007). Gly42 appears to be especially crucial in distinguishing 8-oxodG from guanine, while

Cys253, Gln315 and Phe319 help to exclude adenine, cytosine and thymine (Bruner *et al.*, 2000). Lys249 is crucial for the removal of 8-oxodG (Nash *et al.*, 1997; Bruner *et al.*, 2000). OGG1 is a bifunctional glycosylase, so called as it both acts as a glycosylase by excising the damaged DNA base, and also as an AP endonuclease by cleaving the 5'-phosphodiester bond of the DNA backbone (**fig. 1.9**) (Pascucci *et al.*, 2002; Klungland & Bjelland, 2007). The resulting gap is filled in with the correct nucleotide by DNA polymerase β, which also removes the remaining deoxyribose phosphate and recruits an XRCC1/DNA ligase III complex (Caldecott *et al.*, 1996). DNA ligase III seals the backbone to complete the repair. While OGG1 can also repair 8-oxodG when it is mispaired with thymine, guanine, and adenine, it is less active at these lesions and has reduced AP endonuclease activity, most likely to guard against further mutations incurred by removing 8-oxodG and replacing it with the appropriate base for the original mispair (Bjorâs *et al.*, 1997; Radicella *et al.*, 1997).

1.3.6 Models of OGG1 deficiency

Ogg1 knockout mice have been generated as models of deficient DNA repair (Klungland *et al.*, 1999; Minowa *et al.*, 2000). One strain was generated through the excision of the hairpin-helix-hairpin coding section and insertion of a neomycin-resistance gene/polyadenylation signal (*Neo*) in embryonic stem cells from 129SV mice which were injected into C57BL/6 blastocysts (Klungland *et al.*, 1999). The second model was generated by excision of the 3' half of exon 2 and the entirety of exon 3 and the insertion of a *pgk-neo* cassette in J1 embryonic stem cells that were similarly injected into C57BL/6 blastocysts (Minowa *et al.*, 2000). The knockout animals exhibit 2-

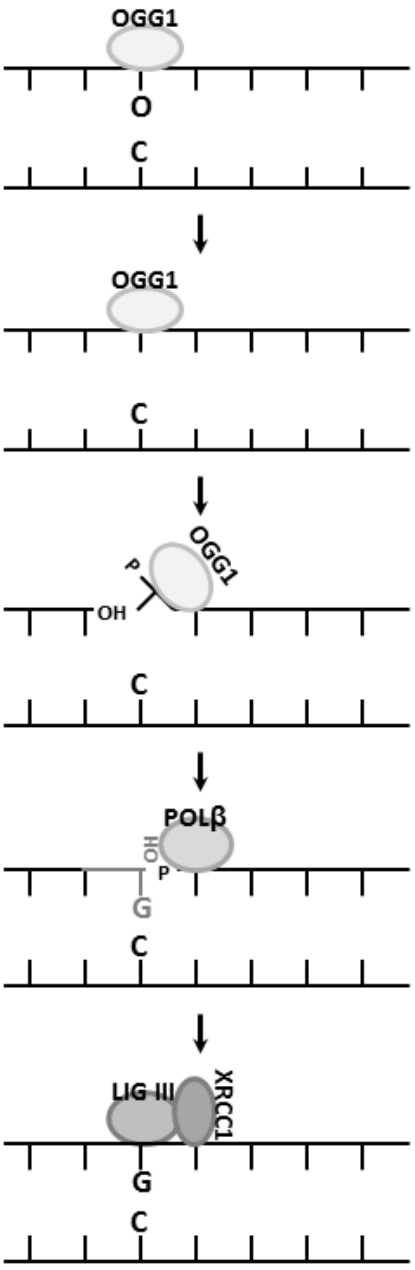

Figure 1. 9: Short-patch BER of 8-oxodG by OGG1
Abbreviations: **C**, deoxycytosine; **LIG III**, DNA ligase III; **O**, 8-oxo-2'-deoxyguanine; **OGG1**, oxoguanine glycosylase 1; **POLβ**, DNA polymerase β; **XRCC1**, x-ray repair cross-complementing protein 1

fold to 7-fold higher levels of 8-oxodGuo in the liver, as well as a modest increase in the spontaneous mutation rate, but no increases in spontaneous tumour incidence or other age-related diseases (Klungland *et al.*, 1999; Minowa *et al.*, 2000). Despite a 9-fold increase in mitochondrial 8-oxodG levels in the liver, there is no change in mitochondrial DNA mutations, nor any loss of function in *myh -/-* x *ogg1 -/-* mice, suggesting that any disease phenotypes are caused by the loss of DNA repair in the nucleus rather than the mitochondria (de Souza-Pinto *et al.*, 2001; Halsne *et al.*, 2012).

As previously discussed, female *ogg1* -/- mice appear to be more susceptible to motor coordination deficits after *in utero* methamphetamine exposure (Wong *et al.*, 2008). Additionally, *ogg1* -/- mouse embryonic fibroblasts exhibit decreased growth, and increased DNA damage and cell death after MeHg exposure (Ondovcik *et al.*, 2012).

1.3.7 The role of OGG1 in disease

1.3.7.1 OGG1 in cancer

As might be expected with a DNA repair enzyme, OGG1 appears to play a role in a number of cancers. The loss of function ser326cys mutation of OGG1 in humans has been associated with an increased risk of colon and lung cancer in humans with 2 mutated gene copies, and decreased sperm health in men who have smoked more than 5 pack-years (Wei *et al.*, 2011; Kershaw & Hodges, 2012; Sameer *et al.*, 2012; Ji *et al.*, 2013). Loss of heterozygosity or deletions from the short arm of chromosome 3, on which *Ogg1* is situated, are often commonly found in renal cancers and small cell lung

cancers (Lu *et al.*, 1997; Audebert *et al.*, 2000). Additionally, it was found in a study of breast cancer patients that a lack of OGG1 expression in the tumour tissue was associated with a particularly aggressive phenotype (Karihtala *et al.*, 2012). Mutations in *Ogg1* have also been observed in lung and gastrointestinal cancers (Ishida *et al.*, 1999b; Przybylowska *et al.*, 2013).

While they do have increased 8-oxodG levels and a slightly higher spontaneous mutation rate, *ogg1* -/- mice do not appear to have increased tumour incidence (Klungland *et al.*, 1999). They are, however, more susceptible to skin cancer after UVB exposure (Kunisada *et al.*, 2005). The cross-bred progeny of *myh* -/- x *ogg1* -/- mice have both a shortened lifespan and increased tumourigenesis, particularly in the lungs and ovaries, and lymphomas (Xie *et al.*, 2004). Increased incidences of lung and small intestine cancers have been reported in these mice (Russo *et al.*, 2004).

1.3.7.2 OGG1 in Development

Our laboratory has found that the progeny of *ogg1* -/- mice are more susceptible to the adverse developmental effects of *in utero* methamphetamine exposure (Wong et al. 2008). A single maternal i.p. dose of methamphetamine on GD 17 resulted in increased 8-oxodGuo levels in the fetal brain, and the same dose caused motor-coordination deficits in the female -/- mice, both compared to saline controls as well as methamphetamine-treated female +/+ mice (Wong et al. 2008). Deficits were not observed in the males, suggesting a sex-selective component to methamphetamine

toxicity, or DNA repair impairment. These deficits continued to 12 weeks of age, suggesting that a single exposure to an oxidizing xenobiotic during gestation can result in lasting or permanent deficits in vulnerable individuals.

1.3.7.3 OGG1 in mild cognitive impairment (MCI) and Alzheimer's Disease (AD)

Decreased nuclear OGG1 levels have been found in the parietal lobes of MCI and late AD patients and in the frontal lobes of late AD patients, in addition to decreased nuclear OGG1 activity in the frontal, parietal, and temporal lobes of both MCI and late AD patients (Shao *et al.*, 2008). In a small sample of AD patients and age-matched controls, four of fourteen AD patients were found to have loss of function mutations in *Ogg1*, while no such mutations were observed in the control population (Mao *et al.*, 2007). There have been conflicting results regarding the levels of fapyG in AD and MCI patients, but increased levels of 8-oxodG have been found in various brain regions of MCI and AD patients (Gabbita *et al.*, 1998; Wang *et al.*, 2005b; Wang *et al.*, 2006). Increased 8-oxodG and 8-oxodGuo have also been measured in the urine and cerebrospinal fluid of Mixed Dementia patients (Gackowski *et al.*, 2008).

1.3.7.4 OGG1 in Parkinson's Disease

Increased 8-oxodGuo levels have been found in the substantia nigra of Parkinson's Disease (**PD**) patients (Alam *et al.*, 1997). The proportion of OGG1-positive neurons in the substantia nigra appears to be higher in PD patients, although it seems unclear if this is due to OGG1 being upregulated, or whether neurons that do not express OGG1

are more susceptible to death (Fukae *et al.*, 2005). Regardless, it appears that OGG1 may be protective or involved in a protective response against the onset of PD.

1.3.7.5 OGG1 in Huntington's Disease

There is conflicting evidence as to whether or not the ser326cys mutation of OGG1 is a risk factor for Huntington's Disease (**HD**). While one previous study has found that the mutation is associated with CAG sequence expansions in the HD gene huntingtin, a recognized determinant of age of HD onset, another study failed to replicate these findings (Coppedè *et al.*, 2010; Taherzadeh-Fard *et al.*, 2010).

1.3.8 Other related enzymes and repair pathways

MutT homolog 1 (**MTH1**) removes 8-oxodGuo from the nucleotide pool and MutY homolog (**MYH**) removes 8-oxodG after it has been mistakenly paired with adenine during replication (David *et al.*, 2007). Tyr165Cys and Gly382Asp mutations in MYH, first identified in a British family (family N), are loss of function mutations which have resulted in a predisposition to colon cancer (Al-Tassan *et al.*, 2002; Chmiel *et al.*, 2003). Both of these enzymes play important roles in protecting the cell against mutagenesis, but in non-mutagenic mechanisms of toxicity, such as during development or in relatively non-replicating cell populations, such as mature neurons, OGG1, as the only enzyme unrelated to replication, is likely to be the most important.

Endonuclease VIII-like 1 (**NEIL1**), like OGG1, is a bifunctional glycosylase that is capable of recognizing many lesions, including fapyG (Ide & Kotera, 2004). Although NEIL1 appears to be relatively inefficient at repairing 8-oxodG, there is evidence that it can stimulate the release of OGG1 from abasic sites, presumably increasing the repair capacity of OGG1 by freeing it to excise other lesions (Mokkapati *et al.*, 2004). However, in cases of 8-oxodG lesions that are resistant to excision by OGG1, such as near the 3' end of a DNA single strand break, NEIL1 appears to be more efficient at excising the lesion (Parsons *et al.*, 2005). Although *neil1* -/- mice exhibited no defects in learning in a Morris water maze task, they had inherently decreased memory retention compared to wild-type mice, although no changes or deficits were observed in the open-field test, the rotarod test, novel object recognition, or fear conditioning (Canugovi *et al.*, 2012). NEIL1 can be inhibited by copper and iron at exposure levels that have no effect on OGG1 (Hegde *et al.*, 2010). There is currently no published research on the effects of Hg or MeHg on NEIL1 function, but it may be an interesting avenue to pursue, in light of what we know of the effects of copper and iron.

FPG is a bacterial functional, though not structural, OGG1 homolog. It is more efficient at excising both 8-oxodG and fapyG compared to OGG1, having a greater substrate affinity and faster rate of action (Frosina, 2006). Previous studies in our laboratory have indicated that Fpg-expressing human embryonic kidney cells are more susceptible to MeHg, exhibiting increased cytotoxicity, and decreased survival and growth (Ondovcik *et al.*, 2013a). Similarly to the increased toxicity also observed in *hOgg1*-expressing cells, this may be due to an increase in the abasic sites generated by Fpg activity.

Other forms of DNA repair include long-patch BER, nucleotide excision repair (**NER**), mismatch repair (**MMR**), and DNA double-strand break (**DSB**) repair. While short-patch BER identifies and removes only the damaged base, in long-patch BER, DNA strands between 2 – 12 nucleotides are replaced. Long-patch BER begins similarly to short-patch BER, but oxidation of the abasic site can render DNA polymerase β incapable of removing the 5'-deoxyribose (Klungland & Lindahl, 1997). Aided by Replication Factor C (**RFC**), binding of the processivity factor PCNA at the damage site results in the recruitment of DNA polymerase δ/ε, which replaces a short strand of DNA, resulting in a 'flap' oligonucleotide (review: Pascucci *et al.*, 1999; Mailand *et al.*, 2013). This flap is excised by flap endonuclease 1 (**FEN1**), leaving a backbone nick that is repaired by DNA ligase I, aided by Replication Protein A (**RPA**) (Pascucci *et al.*, 1999).

NER repairs strands with at least 2 damaged bases, as well as bulky DNA lesions that distort the helical structure, including DNA adducts and cyclic lesions. The two forms of NER are global genome repair (**GGR**) and TCR. In GGR, DNA damage is identified by XPC-RAD23B, while TCR recognizes damaged DNA on the transcribed strand, via the stalling of RNA polymerase (Lagerwerf *et al.*, 2011). Stalling of RNA polymerase recruits the proteins Cockayne Syndrome A (**CSA**) and CSB (Sarker *et al.*, 2005). While the exact role that CSB plays in TCR is still being elucidated, it may assist in recruiting downstream repair proteins, and may assist in chromatin remodelling or strand annealing, while CSA appears to be responsible for ubiquitinating CSB and terminating repair (Groisman *et al.*, 2006). Our laboratory has shown that CSB-deficient progeny

exposed *in utero* to methamphetamine accumulate more 8-oxodGuo in fetal brain than their wild-type littermates, and that the methamphetamine-exposed CSB-deficient female progeny exhibit deficits in motor coordination after birth (McCallum *et al.*, 2011). There is also evidence that CSB may play a role in regulating expression of other DNA repair enzymes, including OGG1. *In vitro* studies have demonstrated that knockdown of CSB is followed by decreased OGG1 mRNA and protein levels (Javeri *et al.*, 2011). CSB also appears to co-localize with NEIL1 and increase its repair activity (Muftuoglu *et al.*, 2009).

Beyond this recognition step, it appears that both GGR and TCR employ the same mechanism to repair DNA lesions (review: Compe & Egly, 2012). The Transcription factor II Human (**TFIIH**) complex is recruited to the damage site, and prompts opening of the helix, creating a bubble to which RPA and XPA bind (Krasikova *et al.*, 2010). TFIIH recruits the endonuclease XPG before it is released and XPA recruits the XPF-ERCC1 complex (Coin *et al.*, 2008). XPF-ERCC1 cuts DNA from the 5’ end of the damage site and XPG cuts from the 3’ end, before DNA polymerases δ, ε, or κ complete the correct nucleotide sequence (Ogi *et al.*, 2010). The backbone nick can be repaired by DNA ligase III and XRCC1 or DNA ligase I and FEN1 (Moser *et al.*, 2007; Mocquet *et al.*, 2008).

MMR primarily resolves base pair mismatches that occur during replication, as well as insertion/deletion loops, where improper annealing of repeated-sequence motifs during

replication can result in an extra-helical bubble (review: Jiricny, 2006). The MutSα complex, consisting of the proteins MSH2 and MSH6, recognizes base pair mismatches and short insertion/deletion loops, while MutSβ, which consists of MSH2 and MSH3, recognizes longer insertion/deletion loops (Acharya *et al.*, 1996; Zhang *et al.*, 2005). Other proteins and protein complexes that are involved include PCNA, exonuclease 1 (**EXO1**), FEN1, DNA polymerase δ/ε, RPA, and RFC, and the MutLα complex, which consists of the proteins MLH1 and PMS1 (Zhang *et al.*, 2005). MutLα, which is recruited by MutSα and MutSβ, appears to assist in regulating excision by EXO1 at the mismatch site (Zhang *et al.*, 2005).

DNA DSBs can be directly produced by γ-irradiation and UVB exposure, but they can also be caused by xenobiotics and ROS (Flowers *et al.*, 1997; Srinivasan *et al.*, 2001). DSBs can also be formed by replication fork collapses, where a SSB or DNA lesion is encountered during replication, resulting in the formation of a DSB (review: Goodarzi & Jeggo, 2013). However, DSBs are also be deliberately formed during meiosis, V(D)J recombination, which introduces diversity into antibody populations, and class switch recombination, which occurs in response to mature B-cell activation to alter antibody classes. DNA DSB repair consists of nonhomologous end joining (**NHEJ**) and homologous recombination (**HR**).

HR relies on a homologous sister chromatid, and thus can occur during S phase or in dividing cells. In HR, repair is initiated by binding of the MRN complex, which consists of

Mre11, Rad50, and Nbs1 (Paull & Lee, 2005). This complex activates ataxia telangiectasia mutated (**ATM**), which in turn activates CtIP, a resectase that generates a 3' overhang (You *et al.*, 2009). EXO1 may also contribute to this resection (Bolderson *et al.*, 2010). The 3' strand is coated by RPA before BRCA2 removes it, allowing Rad51 to bind to the 3' overhang, with one Rad51 monomer for every 3 base pairs (Wong *et al.*, 2003; Jensen *et al.*, 2010). Rad52 facilitates matching of the strand with the homologous region of the undamaged template strand, and Rad54 appears to facilitate branch migration of the template strand and replicating strand (Mortensen *et al.*, 1996; Bugreev *et al.*, 2006). DNA polymerase and DNA ligase complete the repair. The second damaged strand can either be resynthesized from the newly repaired strand after displacement from the template strand, referred to as synthesis-dependent strand annealing, or alternatively, both damaged strands can be resynthesized simultaneously based on the template strands, resulting in a crossover with junctions that are referred to as Holliday junctions (review: Wechsler *et al.*, 2011). These junctions can be resolved by a number of enzymes.

Unlike HR, NHEJ can take place both in dividing cells and in non-dividing cells, as it does not rely on a homologous sister chromatid. In classical NHEJ, the two broken strands are positioned together by the Ku70-Ku80 dimer, but this results in a greater probability of mutagenesis, as genetic information can be lost (Walker *et al.*, 2001; Mari *et al.*, 2006). The Ku70-Ku80 dimer recruits DNA-PKcs, and together these form the holoenzyme DNA-PK (Spagnolo *et al.*, 2006). DNA-PK autophosphorylates, which appears to result in a structural change allowing access to the damage site (Reddy *et*

al., 2004). Polynucleotide kinase/phosphatase processes the ends to ensure a 5' phosphate and 3' OH, which are necessary for ligation (Jilani *et al.*, 1999; Karimi-Busheri *et al.*, 1999). DNA polymerases λ and μ can fill any gaps that may be present, and ligation is completed by a DNA ligase IV complex, consisting of XRCC4, XLF, and DNA ligase IV (Nick McElhinny *et al.*, 2000; Mahajan *et al.*, 2002; Ramadan *et al.*, 2004; Mari *et al.*, 2006).

Alternative end joining is less understood, but appears to occur in the absence of classical NHEJ proteins, or in cases where DNA lesions or damage at the ends of DSBs prevent binding of classical NHEJ proteins, and when the DNA strands are microhomologous (review: Goodarzi & Jeggo, 2013). The DSBs are bound by MRN and PARP-1 (Iliakis, 2009). The homology between the two strands results in annealing, and the resulting flaps are removed, possibly by XPF-ERCC1 (Della-Maria *et al.*, 2011). DNA ligase III complexed with XRCC1 appears to be the primary ligase that completes repair (Wang *et al.*, 2005a).

Phosphorylation of histone 2A variant H2AX at Ser139 near the DSB site and dephosphorylation at Tyr142, produces γH2AX, which is often used as a marker of DSBs (Rogakou *et al.*, 1998). As γH2AX appears to be produced only in response to DSBs, it is considered a more definitive marker compared to molecules which also exist in undamaged cells (review: Mah *et al.*, 2010). H2AX is phosphorylated by ATM, DNA-PKcs, and ATR, and appears to prompt co-localization of other DNA damage response

proteins, including the MRN complex (Fernandez-Capetillo *et al.*, 2002; Stiff *et al.*, 2004; Stiff *et al.*, 2006). After repair is complete, γH2AX is gradually dephosphorylated by wild-type p53-induced phosphatase 1, protein phosphatase 2A, protein phosphatase 6, or protein phosphatase 4C (Chowdhury *et al.*, 2005; Nakada *et al.*, 2008; Douglas *et al.*, 2010; Moon *et al.*, 2010).

Although H2AX -/- mice are viable, they are more sensitive to radiation, the males are infertile and embryonic fibroblasts from these mice exhibit early senescence and decreased growth (Celeste *et al.*, 2002). They are also more susceptible to genomic instability and early tumourigenesis (Celeste *et al.*, 2002; Celeste *et al.*, 2003).

Our laboratory has found increased γH2AX levels in MeHg-treated *ogg1* -/- mouse embryonic fibroblasts compared to vehicle-treated *ogg1* -/- cells and MeHg-treated wild-type cells, implicating DNA damage, and specifically DSBs, in the toxic mechanism (Ondovcik *et al.*, 2012). Maximal γH2AX levels may be observed within an hour of x-ray irradiation (Firsanov *et al.*, 2012). In our laboratory, however, peak γH2AX levels in MeHg-exposed *ogg1* -/- mouse embryonic fibroblasts were not observed until at least 6 hr after exposure (Ondovcik *et al.*, 2012).

Given the importance of DNA DSB repair, many animal models developed to be knockouts of various enzymes involved in the repair process are not viable, but loss of

function mutations in people and conditional knockout animal models often result in microcephaly, immune deficiencies, and developmental deficits (review: Phillips & McKinnon, 2007). Severe combined immunodeficiency (**SCID**) mice lack DNA-PKcs, and the CA1 and CA3 neurons of the hippocampus are particularly susceptible to cell death after excitotoxicity (Culmsee *et al.*, 2001).

1.4 Methylmercury

MeHg is an environmental toxin to which we are primarily exposed through the consumption of seafood (EPA, 2013). Mercury (**Hg**) released into the water through volcanic or industrial processes, including chloralkali plants and gold mining, is methylated by aquatic bacteria, entering the food chain (Bisogni & Lawrence, 1975; Dangwal, 1993; Hamelin *et al.*, 2011). MeHg bioaccumulates in aquatic organisms, and can result in levels higher than 1 ppm (1 mg/kg) in larger, long-lived, predatory species such as tuna, king mackerel, shark and swordfish (**Table 1.2**) (Mahaffey *et al.*, 2011).

MeHg causes neurodevelopmental deficits in learning and memory, measured by T-mazes, radial arm mazes, Morris water mazes, novel object recognition, and passive avoidance, and deficits in motor coordination and exploratory behaviour, in multiple animal models of *in utero* exposure (Doré *et al.*, 2001; Baraldi *et al.*, 2002; Goulet *et al.*, 2003; Falluel-Morel *et al.*, 2007; Ferraro *et al.*, 2009). Animal studies have also shown earlier neurological declines and symptoms of accelerated aging in rodents and primates, including decreased motor and visual function, as well as the development of previously undetected cognitive deficits (Spyker, 1975; Rice & Hayward, 1999; Newland & Rasmussen, 2000; Yoshida *et al.*, 2008).

Rank	Species	EPA + DHA milligrams/100 g of fish	Hg milligrams/ 1,000 g of fish*
1	Shrimp	390	0.03–0.04
2	Tuna (all, average)	630	0.24–0.48
	Canned‡:		
	Light (skipjack)	128§–270¶	0.11–0.12
	White (albacore)	862§	0.35–0.37
	Fresh:		
	Bluefin (7 kg)	1,173**–1,504††	0.13
	Skipjack (3 kg)	256**–328††	0.17
	Yellowfin (5–20 kg)	100**–120††	0.06–0.31
3	Breaded fish products	0.26	0.135†
4	Salmon	1,590	0.04–0.13
5	Crabs	36	0.06–0.26
6	Catfish	280	0.16†
7	Other fish	54	0.223†
8	Scallops	270	0.05
9	Lobster	360	0.10–0.28
10	Clams	240	0.01–0.06
11	Cod	240	0.06–0.11
12	Oysters	350	0.01–0.07
13	Other shellfish	310	0.061†
14	Flatfish	15	0.092†
15	Unknown fish	53	0.223†
16	Pollock	260	0.02–0.06
17	Mussels	350	0.03–0.08
18	Trout	580	0.14–0.15†
19	Haddock	180	0.03–0.06
20	Crayfish	380	0.03
21	Perch	300	0.09–0.11†
22	Sardines	980	0.02–0.03
23	Swordfish	580	0.98–1.03
24	Bass (freshwater)	640	0.38†
25	Sea bass	490	0.14–0.22
26	Pike	140	0.31†
27	Mackerel (except King)	1,790	0.09–0.220
	King mackerel	401	0.73–1.06
28	Shark	220	0.75–0.99
29	Walleye	530	0.52†
30	Porgy	210	0.08

* Ranges in Hg concentrations represent variability in sample means across different harvesting regions.
† Data from Mahaffey et al., 2004[3] and references therein.
‡ Data from USDA.[167]
§ Tuna canned in water, drained solids.
¶ Tuna canned in oil, drained solids.
** Raw.
†† Cooked, dry heat.

Table 1.2: Levels of Hg and ω3 fatty acids in commonly consumed fish in the United States

Reproduced from Mahaffey et al, Nutr Rev, 2011, with permission from John Wiley and Sons, Inc.

1.4.1 Human Toxicity

1.4.1.1 Minamata

The most famous cases of widespread MeHg poisoning were due to industrial waste spilled in Minamata Bay, Japan by a Chisso Corporation factory between 1953 and 1960. This was the first documented case of MeHg poisoning due to environmental contamination of fish, and MeHg poisoning is still referred to today as Minamata Disease (Harada, 1995). Common symptoms of adult poisoning included paresthesia (numbing and tingling in the extremities), ataxia (uncoordinated gait), narrowing of the visual field, slurred speech, and impaired hearing (Harada, 1995). It became apparent, however, that infants exposed to MeHg *in utero* were more susceptible to MeHg toxicity (Harada, 1978). Symptoms in infants, which were described as a form of cerebral palsy, became apparent by six months of age and included symptoms similar to those observed in adults, such as motor coordination deficits and slurred speech, but in more severe cases, resulted in complete loss of motor control or paralysis, microcephaly, and severe developmental deficits (Harada, 1978). Adult toxicity was associated with hair levels above 50 ppm, and levels in the brain after autopsy ranged from 2.6 to 24.8 ppm (Harada, 1995).

Upon autopsy, it was found that MeHg caused cell death focally in the adult brain, particularly in the visual cortex, motor cortex, auditory cortex, and the granule cells of the cerebellum, while in infants, the damage was more widespread, encompassing the entire brain (Choi *et al.*, 1978; Takeuchi & Eto, 1999). Decreased cerebral and cerebellar volume, as well as decreased neuronal number and neuronal disorganization

have been observed both clinically and in animal models of *in utero* MeHg exposure (Choi *et al.*, 1978; Kakita *et al.*, 2002, 2003; Falluel-Morel *et al.*, 2007).

1.4.1.2 Iraq

Bread made from grain contaminated with MeHg-containing fungicides caused widespread MeHg poisoning in Iraq between 1971 and 1972 (Bakir *et al.*, 1973; Amin-Zaki *et al.*, 1974; Clarkson *et al.*, 1976). The contaminated grain was intended for use as seed grain, and not for human consumption. Similarly to the findings in Minamata, in adults, the most common symptoms were paresthesia, ataxia, narrowing of the visual field, slurred speech, and impaired hearing (Bakir *et al.*, 1973). Levels of MeHg in infant and cord blood samples approached or exceeded levels 2-fold greater than maternal levels, a finding that has been duplicated in multiple clinical studies (**fig. 1.10**) (Amin-Zaki *et al.*, 1974; Stern & Smith, 2003; Björnberg *et al.*, 2005). Common symptoms in infants included blindness, loss of hearing, hyperactive reflexes, generalized paralysis, and severe neurodevelopmental deficits (Amin-Zaki *et al.*, 1974). Blood levels of at least 0.3 ppm were associated with symptoms of MeHg poisoning in the mothers, and it was estimated that maternal hair levels above 10 ppm would be likely to result in MeHg toxicity after birth (Amin-Zaki *et al.*, 1974; Cox *et al.*, 1989).

While these large scale human poisonings were instrumental in demonstrating the dangers of MeHg poisoning, on a daily basis, we are exposed to far less MeHg. Other

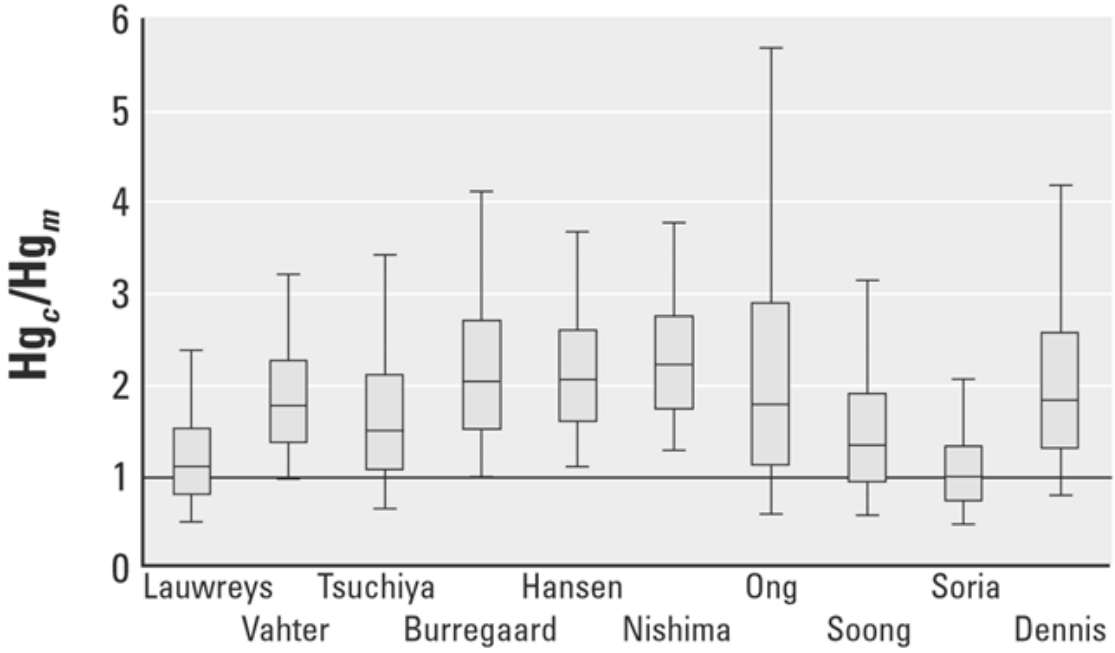

Figure 1.10: Ratios of cord blood Hg to maternal blood Hg from multiple clinical studies
Each bar represents the ratio of cord blood Hg: maternal blood Hg from a separate clinical study. First authors from each study are indicated under each bar. Boxes represent the 25^{th} – 75^{th} percentile, internal lines represent the median, and T-bars represent the 5^{th} to 95^{th} percentile. The aggregate cord blood Hg: maternal blood Hg is 1.86.

Reproduced from Stern and Smith, 2003, with permission from Environmental Health Perspectives.

populations have thus become more common models for chronic, low exposure to MeHg, including those of the Faroe and Seychelles Islands.

1.4.1.3 Faroe Islands

The Faroe Islands are a self-governing archipelago of the Kingdom of Denmark. Having a relatively homogenous population with a heavy reliance on the sea for food, the Faroe Islands have been used as a model for the effects of chronic, low-level MeHg exposure in children (Grandjean *et al.*, 1997; Stern *et al.*, 2004). Fish consumption, particularly cod (0.07 ppm Hg), is the primary source of exposure to MeHg in the Faroe Islands, although pilot whale (3.3 ppm Hg) is occasionally eaten as well (Grandjean *et al.*, 1992).

The most comprehensive studies have followed a cohort of 1022 singleton births from 1986 – 1987 (Grandjean *et al.*, 1992; Grandjean *et al.*, 1997; Debes *et al.*, 2006). Following Hg measurements in cord blood (mean 22.9 µg/L) and maternal hair (mean 4.27 ppm), the children were assessed at 7 and 14 years of age for a variety of learning, memory, motor coordination, and language parameters. At 7 years of age, higher maternal hair concentrations were associated with subtle deficits in memory, language, and motor coordination, measured by the California Verbal Learning Test, the Bender Visual Motor Gestalt Test, the Boston Naming Test and Finger Tapping (Grandjean *et al.*, 1997). These associations were still present when data from children with mothers with hair levels above 10 ppm were excluded, suggesting that subtle MeHg effects may occur at lower exposures. At 14 years of age, these associations continued to be

present, suggesting that *in utero* MeHg exposure may result in long-term effects on cognition (Debes *et al.*, 2006). Maternal fish consumption during pregnancy was associated with a non-significant improvement with the tested outcomes, a finding which has been echoed in multiple studies on MeHg-induced developmental abnormalities (Oken *et al.*, 2005; Strain *et al.*, 2008; Davidson *et al.*, 2011). In these studies, postnatal Hg exposure, measured in hair, had little to no association with test performance, suggesting that prenatal exposure to MeHg causes subtle but persistent neurological deficits. The suggestion that increased maternal fish consumption may be protective is an important potential complication in studies of MeHg neurodevelopmental toxicity, discussed further in section 1.4.1.4. Recently it has also been reported in the Faroe Islands population that MeHg exposure in adults is associated with greater cardiovascular health risks, including increased blood pressure and thickness of the interior arterial walls (Choi *et al.*, 2009). Interestingly, increased cardiovascular risk has also been observed in the Faroese children exposed *in utero*, indicated by decreased heart rate variability and increased blood pressure (Sørensen *et al.*, 1999; Grandjean *et al.*, 2004).

1.4.1.4 Seychelles Islands

The Republic of Seychelles consists of an archipelago east of Africa in the Indian Ocean. Like the Faroe Islands, seafood constitutes a large part of the residents' diet, although in the Seychelles this tends to consist of smaller fish species instead of whale (van Wijngaarden *et al.*, 2012).

Unlike the studies of the Faroe Islands population, multiple studies in the Seychelles Islands, where the mean MeHg levels in maternal hair are 6.8 ppm, have failed to find consistent cognitive deficits associated with MeHg (Davidson *et al.*, 1995; Myers *et al.*, 1997; Davidson *et al.*, 2000; Davidson *et al.*, 2011; Strain *et al.*, 2012). In the past, the study authors have argued that these results suggest that MeHg is not a public health concern (Myers & Davidson, 1998).

It has been proposed that higher concentrations of nutrients such as ω3 fatty acids in the fish eaten by residents of the Seychelles Islands may be masking the detrimental effects of MeHg, resulting in the discrepancy between conclusions drawn from the Faroe Islands research and the Seychelles Islands research. Certainly the Faroe Islands study and studies in the US have suggested that increased maternal fish consumption during pregnancy may have a beneficial effect on cognitive outcomes in infants, even as increased maternal MeHg levels have a detrimental effect (Oken *et al.*, 2005; Strain *et al.*, 2008; Davidson *et al.*, 2011). Consequently, the Seychelles researchers have stratified the data to measure the effect of long chain polyunsaturated fatty acids (**LCPUFA**) and maternal MeHg levels separately (Strain *et al.*, 2008; Lynch *et al.*, 2011; Stokes-Riner *et al.*, 2011). These studies suggest that prenatal exposure to LCPUFA, particularly the ω3 fatty acid docosahexaenoic acid (**DHA**), improves performance according to the Psychomotor Development Index of the Bayley Scales of Infant Development at 9 and 30 months of age, while increased exposure to MeHg causes subtle deficits. However, at 5 years of age, language function measured by the Preschool Language Scale-Revised Edition showed only a protective effect of LCPUFA

without a decrease in score associated with increased MeHg exposure (Strain *et al.*, 2012).

However, another confounding factor that must be noted is that the Seychelles Islands and Iraq studies consistently rely on maternal hair MeHg levels to determine exposure, whereas the Faroe Islands researchers noted that cord blood MeHg levels were a more consistent predictor of neurobehavioural outcome than maternal hair levels (Grandjean & Budtz-Jørgensen, 2007, 2010). It has also been observed that the Faroe Islands population is relatively homogenous, which would suggest a relative lack of genetic diversity, potentially resulting in a particularly susceptible population. By learning more about the mechanism of toxicity of MeHg, we may be able to determine whether there are genetic components of risk that render the Faroe Islands population more susceptible to MeHg than people in the Seychelles Islands.

1.4.1.5 Other populations

1.4.1.5.1 United States of America

Project VIVA is a longitudinal study in the US following various health parameters of mothers and children. Although MeHg is not a primary focus of their work, they have published two studies examining the correlation between maternal hair Hg levels, fish consumption, and visual recognition memory in infants (Oken *et al.*, 2005). In a test designed to measure a 6-month old infant's ability to distinguish a novel picture from a familiar picture, the researchers determined that while a greater proportion of fish in the

maternal diet was beneficial, higher maternal hair Hg levels were associated with lower scores in the test. Average maternal hair Hg levels were 0.55 ppm, below the level associated with the Reference Dose (**RfD**) established by the U.S. Environmental Protection Agency (**EPA**), of 1.2 ppm (Rice *et al.*, 2000). The researchers concluded that the most benefit was incurred through diets high in fish, and suggested that the best choices would be fish species high in ω3 fatty acid levels, but also low in MeHg, similar to the conclusions drawn by the researchers in the Seychelles and Faroe Islands. As might be expected, small, oily fish species generally maximize the ratio of beneficial fatty acids to MeHg levels (**fig. 1.11**) (Mahaffey *et al.*, 2011). Further studies in this cohort at three years of age still found that higher Hg levels were associated with poorer scores on the Peabody Picture Vocabulary Test and the Wide Range Assessment of Motor Visual Ability (Oken *et al.*, 2008). Similarly to the results in the Faroese population, a workshop organized by the EPA to assess the cardiovascular risk associated with MeHg concluded that the current evidence supports further study into the incidence of myocardial infarction after MeHg exposure (Roman *et al.*, 2011).

These results are particularly important since there is consistent evidence that subpopulations in North America achieve MeHg body burdens that exceed the RfD of 0.1 µg/kg/day (equivalent to approximately 1.2 ppm in hair and 3.5 – 5.8 µg/L in cord blood) (Rice *et al.*, 2000; Mahaffey, 2005). The RfD is an estimate of the upper boundary of daily exposure that is unlikely to lead to significant health effects (EPA, 1993). A study of Japanese American and Korean American women of childbearing age in Washington state found that the mean total mercury (**THg**) levels in the hair of the

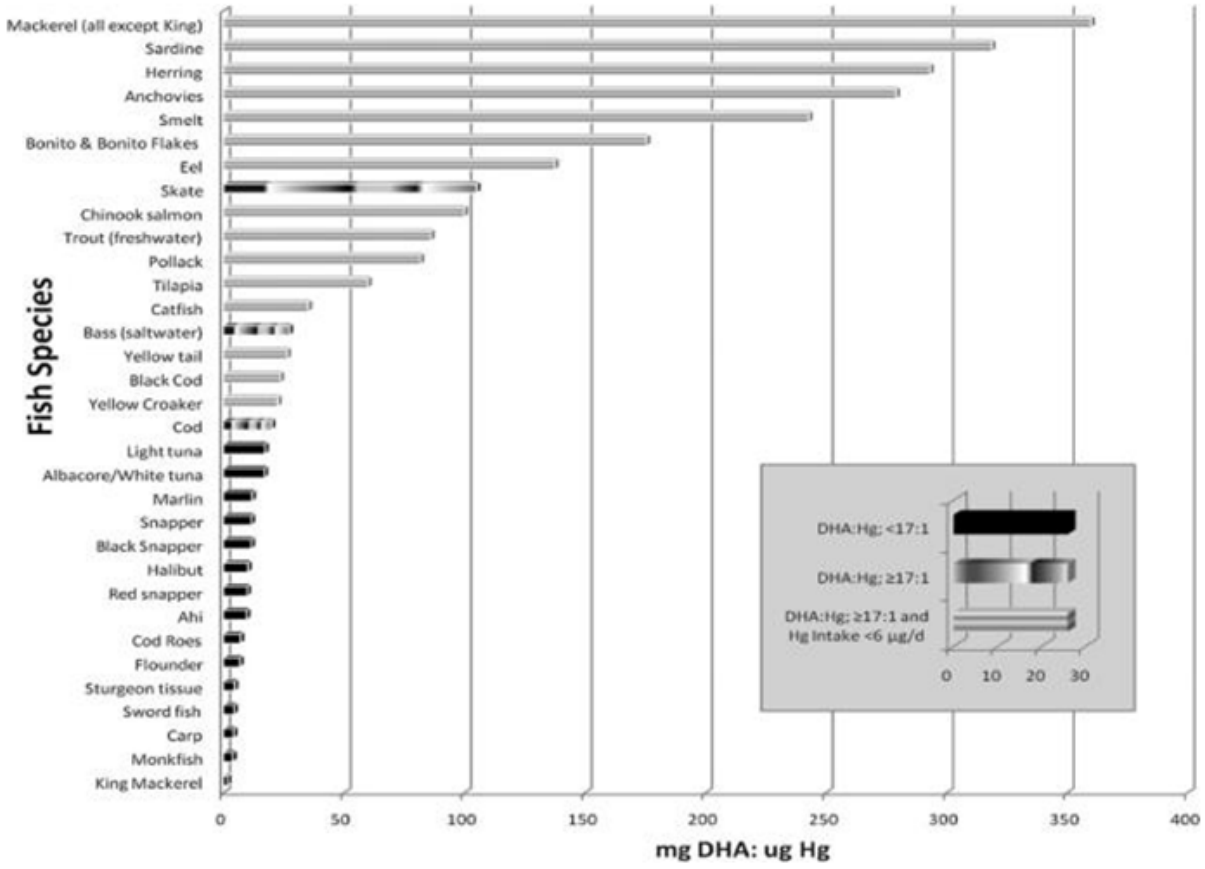

Figure 1.11: Ratios of Hg:DHA in commonly consumed fish in the United States

Reproduced from Mahaffey et al, Nutr Rev, 2011, with permission from John Wiley and Sons, Inc.

Japanese population (1.23 ppm) slightly exceeded the levels associated with the RfD, while 10 – 20% of the Korean population exceeded these THg levels (mean: 0.61 ppm) (Tsuchiya *et al.*, 2008). Despite the similar frequency of fish consumption between the populations, the Japanese population demonstrated a greater risk, emphasizing that, even disregarding socioeconomic considerations, diverse cultural backgrounds can influence diets, resulting in subpopulations that are at significant risk. Additionally genetic differences in the level or activity of transporters and detoxifying enzymes could alter absorption or excretion rates. It is of paramount importance, then, to carefully design study populations to derive an accurate representation of risk.

1.4.1.5.2 Grassy Narrows

The most famous MeHg poisoning in Canada is attributed to waste runoff from the Dryden Paper Company Ltd. in northern Ontario spilling into the Wabigoon-English River system from 1962 to 1970 (Takeuchi *et al.*, 1977). This river system is historically important to the Ojibway tribe in the surrounding communities both for their diet as well as their livelihood, including commercial fishing and acting as guides for sports fishermen (Harada *et al.*, 2011). Researchers from Minamata were asked to confirm that Minamata disease was present in the community. Mean blood Hg levels were 70 µg/L, more than 10-fold higher than the level associated with the EPA RfD, 5.8 µg/L, and many of the classical symptoms of Minamata Disease were found, including paresthesia, hearing impairment, visual disturbances, and ataxia (Takeuchi *et al.*, 1977). MeHg contamination was also found in the surrounding wildlife that depend on the river system for their diet, with MeHg levels exceeding 16 ppm in some fish, and 96

ppm in the livers of turkey vultures (Fimreite & Reynolds, 1973; Harada *et al.*, 2011). Levels exceeding 1 ppm are considered extremely high, and are generally only observed in large, long-lived predatory fish (**Table 1.2**).

Although the contamination occurred over 40 years ago, MeHg levels in the fish, including walleye with mean levels exceeding 0.5 ppm, remain elevated (Kinghorn *et al.*, 2007). Furthermore, follow-up studies by Dr. Harada in 2002 and 2010 found that Minamata disease symptoms were still present in significant portions of the population (Harada *et al.*, 2005; Harada *et al.*, 2011). By 2002, 43% of the previous study population from 1975 had passed away, although the causes of death were not known (Harada *et al.*, 2005). Hypertension was also found in 37.5% of the study population in 2010, although the researchers noted that it was difficult to draw definitive conclusions about likely causes, given the unfortunately poor access to medical care in the area (Harada *et al.*, 2011).

1.4.1.5.3 Spain

Studies in Spain of the total Hg in cord blood have indicated that approximately 60% of developing infants may be currently exposed to levels of MeHg greater than the EPA RfD of 0.1 µg/kg/day (Ortega-García *et al.*, 2009; Ramon *et al.*, 2011). Higher Hg levels in infant hair (mean 0.96 ppm) were associated with lower memory, verbal, and general cognition scores according to the McCarthy Scales of Children's Abilities (Freire *et al.*, 2010). In this study, contrary to the Seychelles Islands studies, higher fish consumption

was also associated with lower general cognition scores, particularly for whitefish and fried fish. These results, therefore, further reinforce that it may be of paramount importance to consider the nutritional content of fish, particularly the LCPUFA, when measuring the toxic effects of MeHg.

1.4.1.5.4 Gold Mining

A particularly alarming source of MeHg exposure is through small-scale gold mining. Hg, when combined with a slurry of ore, forms an amalgam with gold that can then be heated to isolate the gold. This results both in the release of Hg into the water and Hg vapour into the air. It is estimated that worldwide, 1000 metric tonnes of Hg are released each year, of which 650 metric tonnes are deposited in soil and waterways (Telmer & Veiga, 2009). While there are emerging studies on the effects of this Hg exposure in the surrounding populations and environment, it is often difficult to recruit participants, particularly as these mining practices are often illicit (Bose-O'Reilly *et al.*, 2010). Furthermore, many of these studies report THg exposure only, and it can be difficult to determine the relative exposures to different forms of Hg, since there is an increased chance of Hg vapour exposure. This form of exposure is likely to continue, given the incentive of the high price of gold, and the lack of access to safer refining methods (Schmidt, 2012). In discussing the likelihood of MeHg-initiated neurodevelopmental effects, therefore, it is necessary to remember that while populations in wealthier, developed nations may be aware enough to begin limiting MeHg exposure, some of the most vulnerable populations in the world remain at serious risk.

1.4.2 Treatment

Historically, treatments for MeHg poisoning have included thiol resins and chelators, which bind and inactivate MeHg, and hemodialysis, which removes it from the blood (Clarkson *et al.*, 1981; Lund *et al.*, 1984). Chelation is less invasive and relatively well-tolerated, so MeHg poisoning now is primarily treated through the use of chelators, particularly dimercaptosuccinic acid (**DMSA**), dimercaptopropanesulfonate (**DMPS**), and NAC (Koh *et al.*, 2002; Madejczyk *et al.*, 2007; Aremu *et al.*, 2008; Bridges *et al.*, 2009; Zalups & Bridges, 2009; Falluel-Morel *et al.*, 2012). These chelators have thiol groups that readily bind to MeHg. Studies suggest that the MeHg-chelator complexes involving NAC and DMPS are transported from the blood into the proximal tubule cells of the kidney via the organic anion transporter-1 (**OAT1**), and then into the tubular fluid using multidrug-resistance protein 2 (**MRP2**), hastening excretion (Koh *et al.*, 2002; Madejczyk *et al.*, 2007; Zalups & Bridges, 2009). A similar role for MRP2 has been found for excretion of MeHg-DMSA complexes (Zalups & Bridges, 2009). It also appears that treatment with NAC may reduce fetal exposure to MeHg in rats and some of its toxic effects in the developing hippocampus, including reduced DNA synthesis and increased apoptosis (Aremu *et al.*, 2008; Falluel-Morel *et al.*, 2012).

1.4.2.1 MeHg Detoxification

The detoxification of MeHg by selenium, MT, and GSH is discussed in section 1.2.4.1.

1.4.3 Pharmacokinetics

1.4.3.1 Absorption, distribution and excretion

MeHg is well-absorbed (>90%) both by oral and intraperitoneal routes (Clarkson, 1972; Walsh, 1982), and is measurable in the liver, kidneys, red blood cells, hair, and brain (Young *et al.*, 2001). In trout, rodents, dolphins, and humans, upwards of 90% of the MeHg in blood appears to be bound in red blood cells, primarily to hemoglobin and GSH (Giblin & Massaro, 1975; Rabenstein *et al.*, 1982; Yasutake *et al.*, 1989; Berglund *et al.*, 2005; Pedrero Zayas *et al.*, 2013). It is estimated that in humans the brain:blood Hg ratio is 5:1, and that the blood:hair Hg ratio is 1:250, although there is a great deal of interindividual variation (Castoldi *et al.*, 2003; Chain, 2012). The half-life of MeHg in humans has been estimated at approximately 45 – 70 days, versus 7-14 in mice and 20 in rats (Aberg *et al.*, 1969; Clarkson *et al.*, 1981; Ballatori *et al.*, 1995; Smith & Farris, 1996; Sundberg *et al.*, 1998).

MeHg is primarily excreted from the body in feces (Aberg *et al.*, 1969; Ballatori *et al.*, 1995). The excretion of MeHg in the feces appears to be largely dependent on its conjugation to GSH by GSTs (Schläwicke Engström *et al.*, 2008; Lee *et al.*, 2010). This MeHg-GSH complex is transported into bile by MRP2, where it undergoes enterohepatic circulaton until it is demethylated by body microflora and excreted in feces as mercuric Hg (Ballatori *et al.*, 1995). The slow demethylation of MeHg results in inorganic mercury (**IHg**), which can stay in the body, particularly bound to selenide compounds in the central nervous system, for years (Sasakura & Suzuki, 1998; Suzuki *et al.*, 1998).

1.4.3.2 Transport

Studies in mice suggest that MeHg is far more efficient than IHg at crossing the blood-brain barrier (**BBB**) and entering the brain (Sundberg *et al.*, 1999). This may be due to the fact that MeHg when complexed to L-cysteine appears to be an excellent substrate for LAT1, one of 2 L-type large neutral amino acid transporters, presumably because the complex mimics methionine (Hirayama, 1980; Kerper *et al.*, 1992; Simmons-Willis *et al.*, 2002; Yin *et al.*, 2008). A similar study in *C. elegans* suggests that functionally homologous transporters to LAT1 are similarly responsible for MeHg transport in this species (Caito *et al.*, 2013). LAT1 does not appear to be expressed across all tissues, as rat heart, lung, kidney, and liver have all been found to lack LAT1 mRNA (Boado *et al.*, 1999). As LAT1 is not present in the kidney and liver, which are the primary organs for MeHg accumulation, other transporters are required. In the kidneys, this transport appears to be mediated by the System $B^{0,+}$ amino acid transporters and OAT1, while the exact mechanism of transport into the liver remains unknown, although it likely involves an amino acid or GSH-conjugate transporter, since uptake of MeHg into the liver is increased with coadministration of cysteine or GSH (**Table 1.3**) (Bridges & Zalups, 2010). Once in the brain, MeHg accumulates in the astrocytes and other glial cells, likely via transportation by LAT1, and is demethylated to IHg over a period of months in the adult monkey (Aschner *et al.*, 1990; Charleston *et al.*, 1995).

LAT1 also appears to transport MeHg across the placenta from the mother to the developing fetus, and LAT-1 mRNA can be detected early in development, before

Organ	Direction of transport	Known mechanism(s)	Postulated mechanism(s)	Postulated species of CH_3Hg^+
Brain	Uptake at blood–brain barrier	System L	Other amino acid transporters	S-conjugates of thiol-containing amino acids
Erythrocytes	Uptake	None at present	OATs, D-glucose diffusive transporter, Cys facilitated transporter, Cl^- transporter	S-conjugates of GSH and/or Cys
Intestine	Absorption from lumen	None at present	OATs, amino acid and peptide transporters; MRP3 on basolateral membrane	S-conjugates of GSH, Cys, and/or CysGly
	Secretion into lumen	None at present	Amino acid transporters (e.g. System L), GSH transporters	S-conjugates of Cys and/or GSH
Kidney	Uptake at apical membrane	System $B^{0,+}$	Other amino acid transporters	S-conjugates of Cys and Hcy
	Uptake at basolateral membrane	OAT1	OAT3	S-conjugates of Cys, Hcy, and NAC
	Secretion at apical membrane	MRP2	MRP4	S-conjugates of thiol-containing amino acids, peptides and/or DMPS and DMSA

Table 1.3: Mechanisms of MeHg transport in various organs and cells
Continued on next page

Adapted from Bridges and Zalups, 2010, with permission from Taylor & Francis.

Organ	Direction of transport	Known mechanism(s)	Postulated mechanism(s)	Postulated species of CH_3Hg^+
Liver	Uptake at sinusoidal membrane	None at present	Amino acid transporters, GSH transporters	S-conjugates of thiol-containing amino acids, and/or GSH
	Export at canalicular membrane	MRP2	—	S-conjugates of thiol-containing amino acids, peptides and/or DMPS and DMSA
Placenta	Uptake at apical (maternal) membrane	None at present	Amino acid transporters	S-conjugates of thiol-containing amino acids
	Export at basolateral (fetal) membrane	None at present	Amino acid transporters	S-conjugates of thiol-containing amino acids
	Uptake at basolateral (fetal) membrane	None at present	OAT4	S-conjugates of thiol-containing amino acids and GSH
	Export at apical (maternal) membrane	None at present	MRP2	S-conjugates of thiol-containing amino acids, peptides, and/or DMPS and DMSA

Table 1.3: Mechanisms of MeHg transport in various organs and cells
Continued

Adapted from Bridges and Zalups, 2010, with permission from Taylor & Francis.

implantation (Kajiwara *et al.*, 1996; Chrostowski *et al.*, 2009). Furthermore, MeHg appears to be preferentially transported across the placenta to the fetus, with umbilical cord and infant blood levels at birth sometimes reaching more than twice the levels in maternal blood (**fig. 1.10**) (Stern & Smith, 2003; Björnberg *et al.*, 2005). The increased levels of MeHg in the fetus relative to the mother may partially account for the increased sensitivity of the developing fetus. The levels of MeHg appear to drop in breast-fed infants over the first 13 weeks of age, suggesting that exposure through breast milk is less than that *in utero* (Björnberg *et al.*, 2005). This decrease in MeHg levels in the offspring after birth and before weaning is also observed in rats (Newland & Reile, 1999). Co-exposure to PCBs, however, may increase transfer of MeHg to the breastfeeding infant, possibly through the elevation of maternal serum albumin levels, to which MeHg appears to be bound when it travels into the breast milk (Lee *et al.*, 2009).

1.4.4 Possible Mechanisms of Toxicity

1.4.4.1 Excitotoxicity

MeHg is known to disrupt glutamate homeostasis in the brain (**fig. 1.12**). MeHg accumulates in astrocytes in the brain, likely as a substrate for LAT-1 when it is complexed to cysteine, and can inhibit the reuptake of glutamate, as well as the metabolism of glutamate into glutamine by decreasing the activity of glutamine synthetase (Garman *et al.*, 1975; Aschner *et al.*, 1990; Farina *et al.*, 2003; Kwon & Park, 2003; Mutkus *et al.*, 2005). MeHg appears to inhibit astrocytic glutamate uptake by the glutamate/aspartate transporter (**GLAST**) (Allen *et al.*, 2001; Mutkus *et al.*, 2005). While GLAST protein expression is increased after MeHg exposure, activity is decreased,

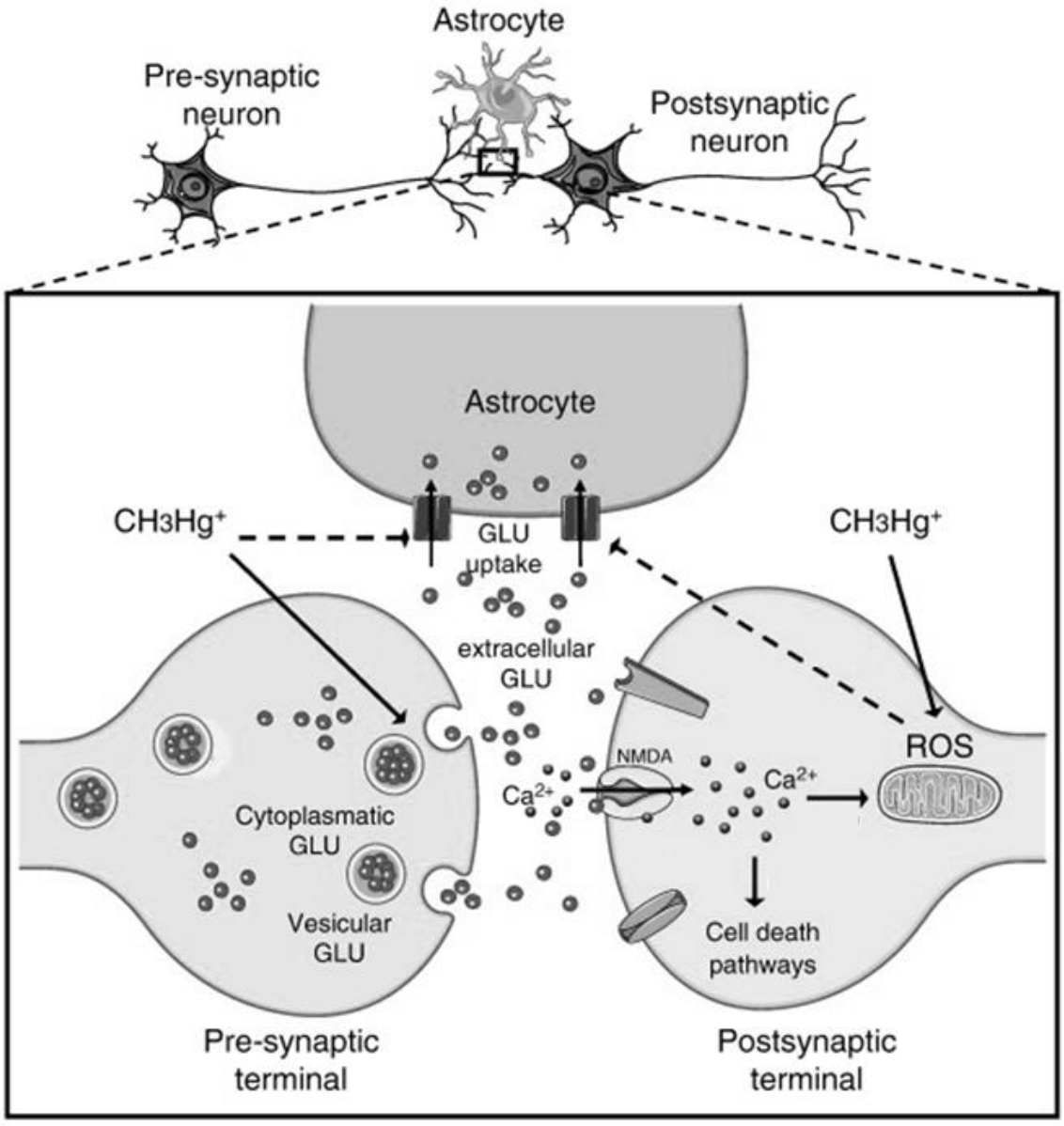

Figure 1.12: Disruption of glutamate homeostasis by MeHg
Abbreviations: **CH_3Hg+**, methylmercury; **GLU**, glutamate

Reprinted and modified from Life Sciences, Vol. 89, Farina et al. Mechanisms of methylmercury-induced neurotoxicity: Evidence from experimental studies, pp. 555 – 563, © 2011, with permission from Elsevier.

suggesting that MeHg can directly inactivate the protein, or cause posttranslational modifications that inactivate the protein or internalize it, rendering it incapable of transporting glutamate (Mutkus *et al.*, 2005). Primary cortical neuron cultures from 1-day old rats exposed to 4 mg/kg MeHg *in utero* were susceptible to increased toxicity after incubation with glutamate, further suggesting that glutamate can modulate MeHg toxicity (Ferraro *et al.*, 2009).

Increased NMDA receptor activation by glutamate results in intracellular influx of Ca^{2+}, leading to excitotoxicity (Ramanathan & Atchison, 2011). Oxidative stress is an important component of excitotoxicity, discussed in section 1.4.4.4, although it is not the only excitotoxic mechanism (review: Lau & Tymianski, 2010). The influx of Ca^{2+} into the neuron may also trigger apoptosis by mitochondrial activation of caspases and by the release of the apoptosis-inducing factor (**AIF**) by the Ca^{2+}-dependent activation of calpains (Polster *et al.*, 2005). NOS, particularly neuronal NOS (**nNOS**) are also induced by Ca^{2+} influx (Sattler *et al.*, 1999). The production of NO may overactivate the DNA repair enzyme PARP1, which can also cause AIF to be released (Zhang *et al.*, 1994; Yu *et al.*, 2002). Furthermore, NO may cause cell death by the S-nitrosylation of GAPDH, which causes it to bind to the ubiquitin ligase Siah1, resulting in nuclear translocation and activation of nuclear proteins including the proapoptotic p53 (Hara *et al.*, 2005). It has also been suggested that the nitrosylation of GAPDH, which is crucial for glycolysis, may result in a toxic loss of cellular respiration, a particularly dangerous outcome for such a sensitive system as the brain (Molina y Vedia *et al.*, 1992). In addition to these mechanisms, NO can form peroxynitrite with superoxide, which can

bind to proteins, lipid, and DNA (Radi *et al.*, 1991a, b). Zinc has also been implicated in excitotoxicity (Nutini *et al.*, 2011). Zinc influx into the neuron during excitotoxicity appears to be ROS-dependent, but it can itself inhibit both glycolysis and the citric acid cycle, as well as further initiate ROS production by disrupting the mitochondrial electron transport chain and stimulating NADPH oxidase activity, resulting in increased $O_2^{\cdot-}$ and H_2O_2 production (Sensi *et al.*, 1999a; Sensi *et al.*, 1999b; Brown *et al.*, 2000; Noh & Koh, 2000; Sheline *et al.*, 2000; Dineley *et al.*, 2008; Nutini *et al.*, 2011).

It also appears that excitotoxicity may be a potential mechanism of IHg toxicity, as *in vitro* studies have shown that co-exposure to subtoxic levels of glutamate and mercuric chloride can be toxic in cerebellar cultures (Matyja & Albrecht, 1993).

1.4.4.2 Thiol binding

Thiol groups, whether in proteins or other molecules, readily bind to MeHg (Hughes, 1957). This particular mechanism may be closely related with disruptions in signal transduction. The redox states of many proteins dictate their activity, as described in section 1.2.3. One such protein, thioredoxin (**TRX**), may be of particular interest, as it can be bound and inactivated by MeHg. When its thiol groups are oxidized, TRX releases the enzyme apoptosis signal-regulating kinase 1 (**ASK1**), which can then initiate apoptosis (Saitoh *et al.*, 1998). The binding of MeHg to TRX thiol groups may also cause it to dissociate from ASK1, eventually resulting in apoptosis (Usuki *et al.*, 2011). Similarly, MeHg appears to induce expression of genes generally associated

with Notch signaling by binding to thiols in related proteins (Rand *et al.*, 2008). In addition to potentially causing protein dysfunction, MeHg binding to thiols can result in the depletion of thiols and important antioxidant molecules, including GSH, resulting in increased susceptibility to oxidative stress.

1.4.4.3 Signal Transduction

Neuronal disorganization and increased astrocyte number have been observed in fetal autopsies after MeHg poisoning (Choi *et al.*, 1978; Peckham & Choi, 1988), as well as in rodent models (Zimmer *et al.*, 2012; Guo *et al.*, 2013). Disruption of signal transduction cascades at critical developmental junctures may contribute to this disorganization (review: Usuki & Fujimura, 2012). At doses lower than the one I employed, neuronal migration appears to be impaired without any effect on proliferation or apoptosis (Guo *et al.*, 2013). A decrease in expression of the proteins Rac1 and Cdc42, which stimulate dendrite and axon extension, and RhoA, which stimulates dendrite and axon retraction, may be implicated (Fujimura *et al.*, 2009). Studies in cortical progenitor cell cultures have also shown that MeHg may be capable of inducing astrocytic differentiation, possibly via the JAK/STAT signaling pathway (Kakita *et al.*, 2003; Jebbett *et al.*, 2013). MeHg has also been shown to increase Notch signalling and activation of downstream Notch-targeted genes in *Drosophila*, resulting in decreased axonal growth (Bland & Rand, 2006; Engel *et al.*, 2012). In rat neural stem cells, this activation appears to inhibit neuronal differentiation (Tamm *et al.*, 2008). Inhibition of the selenium-dependent antioxidative enzyme TRX reductase 1 by MeHg can lead to the

dissociation of oxidized TRX from ASK1, stimulating the JNK and p38 pathways, which can result in apoptosis (Usuki *et al.*, 2011).

1.4.4.4 Oxidative Stress

One important consideration is that each of the previous mechanisms can contribute to oxidative stress through the generation of ROS, or alternatively can be precipitated by oxidative stress. Increased mitochondrial activity during excitotoxicity can result in the generation of ROS through leakage in the electron transport chain, particularly from Complex I and Complex III (**fig. 1.3**) (Balaban *et al.*, 2005). Increased Ca^{2+} influx can also result in increased phospholipase A activity, which can indirectly generate superoxide through the formation of arachidonic acid; increased production of NO through the activation of NOS; and increased production of $O_2^{\cdot}$ by the activation of NADPH oxidase (**fig. 1.13**) (Sattler *et al.*, 1999; Shanker *et al.*, 2002; Demaurex & Scorrano, 2009; Sun *et al.*, 2010). As discussed in sections 1.2.3 and 1.4.4.3, oxidation of proteins in signal transduction pathways can alter their activity, potentially initiating apoptosis in the case of TRX. Similarly, the binding of MeHg to thiols in antioxidant molecules can disrupt protein function, potentially resulting in increased susceptibility to ROS.

In addition to causing increased ROS levels in neurons by disrupting astrocytic glutamate homeostasis, there is also evidence that MeHg causes oxidative stress in astrocyte cultures, and results obtained with the mitochondria-specific fluorescent probe

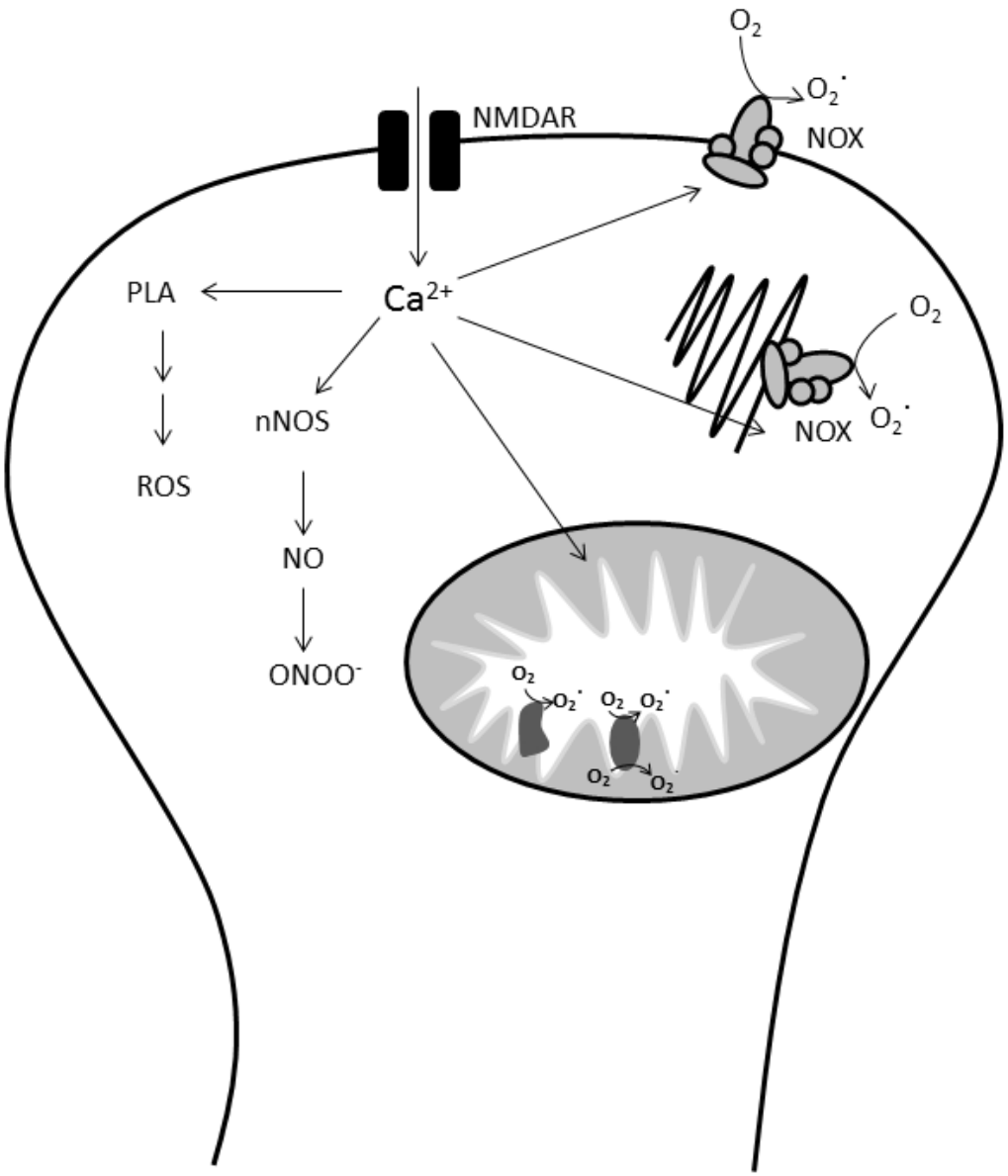

Figure 1.13: Formation of ROS induced by Ca^{2+} influx from NMDA receptor activation
Abbreviations: **NMDAR**, NMDA receptor; **nNOS**, neuronal nitric oxide synthase; **NO**, nitric oxide, **NOX**, NADPH oxidase; **PLA**, phospholipase A; **ROS**, reactive oxygen species

CM-H2-XRos suggest that mitochondria are a major source of ROS after MeHg exposure (Shanker *et al.*, 2004a). ROS in astrocytes can reduce the uptake of glutamate, possibly by oxidizing glutamate transporters and decreasing their activity, which can in turn result in excitotoxicity due to the increase in extracellular glutamate levels (Volterra *et al.*, 1994a; Volterra *et al.*, 1994b). However, MeHg is also capable of increasing arachidonic acid levels in primary hippocampal neuron cultures, apparently through the increase in cytosolic phospholipase A2 protein expression (Shanker *et al.*, 2004b). This can increase ROS levels in the neuron due to the metabolism of arachidonic acid by prostaglandin H synthase (Wells *et al.*, 2005).

MeHg can also reduce the activity of a number of antioxidative enzymes in the fetal brain, including GSH-Px and TRX reductase (Watanabe *et al.*, 1999; Farina *et al.*, 2003; Grotto *et al.*, 2009; Usuki *et al.*, 2011). The reduction of activity in these enzymes may be in large part due to the sequestration of selenium by MeHg (Grotto *et al.*, 2009). This irreversible inactivation of these protective enzymes due to the binding of MeHg to selenium in the active sites can render the cell more susceptible to ROS and oxidative stress.

In vivo exposure to antioxidants, previously discussed in section 1.2.4.1 has also been shown to decrease MeHg-initiated neurotoxicity, further suggesting that oxidative stress is an important component of the mechanism of toxicity.

Previously in our laboratory, it has been demonstrated that *ogg1* -/- cells are more susceptible to MeHg, implicating DNA oxidation in the *in vitro* mechanism of toxicity for the first time (Ondovcik *et al.*, 2012). Furthermore, overexpression of OGG1 *in vitro* can also result in increased susceptibility to MeHg (Ondovcik *et al.*, 2013a). As the number of abasic sites is increased in these cells, it appears that the increased activity of OGG1 after oxidative DNA damage due to MeHg exposure cannot adequately be compensated for by other downstream enzymes in the DNA repair pathway, resulting in increased cytotoxicity. This possibility is supported by previous studies demonstrating that overexpression of OGG1 results in decreased cell survival in response to H_2O_2, but also decreased 8-oxoG levels (Furtado *et al.*, 2012).

1.5 The Hippocampus

1.5.1 Structure and Development

The hippocampus (**fig. 1.14**) is a region of the medial temporal lobe that is crucial for learning and memory. The hippocampal formation consists of the CA1 and CA3 regions in the cornu ammonis, the dentate gyrus, and the subiculum, while the parahippocampal region encompasses the presubiculum, parasubiculum, entorhinal cortex, perirhinal cortex, and postrhinal cortex (**fig. 1.15**) (van Strien *et al.*, 2009). Communication in the hippocampus appears to be relatively unidirectional, beginning in the entorhinal cortex, which projects to all the regions of the hippocampal formation (Amaral & Lavenex, 2007). The axons, referred to as mossy fibres, of the granule cells in the dentate gyrus project to the pyramidal neurons in the CA3, which project to the pyramidal neurons in the CA1, which in turn project to the subiculum and entorhinal cortex (Amaral & Lavenex, 2007).

In the mouse, the pyramidal cells of the CA1 and CA3 begin forming on gestational day (**GD**) 10.5, with the peak of proliferation of hippocampal cells occurring around GD 15 (Angevine, 1965). Cells continue forming and migrating to the CA1 and CA3 regions until birth, and until approximately 20 days postpartum for the dentate gyrus (Angevine, 1965). In the rat, pyramidal cells begin forming on GD 16, with the peak of CA1 and CA3 cell formation between GD 17 – 19, ending at birth (Bayer, 1980). The granule cells of the dentate gyrus begin forming on GD 17, and continue forming into adulthood, although up to 90% of the granule cell population is formed by postnatal day 18 (Bayer, 1980). In humans, the CA1 and CA3 regions can be distinguished by the 16th embryonic

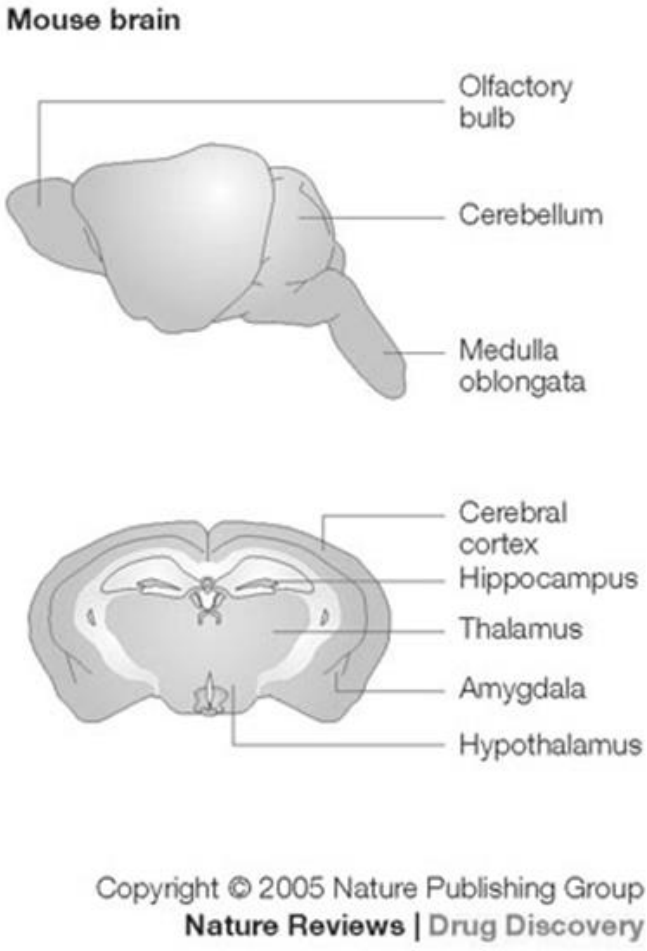

Fig. 1.14: Anatomy of the mouse hippocampus and amygdala

Adapted by permission from MacMillan Publishers Ltd: Nature Reviews Drug Discovery, Cryan and Andrews, © 2005.

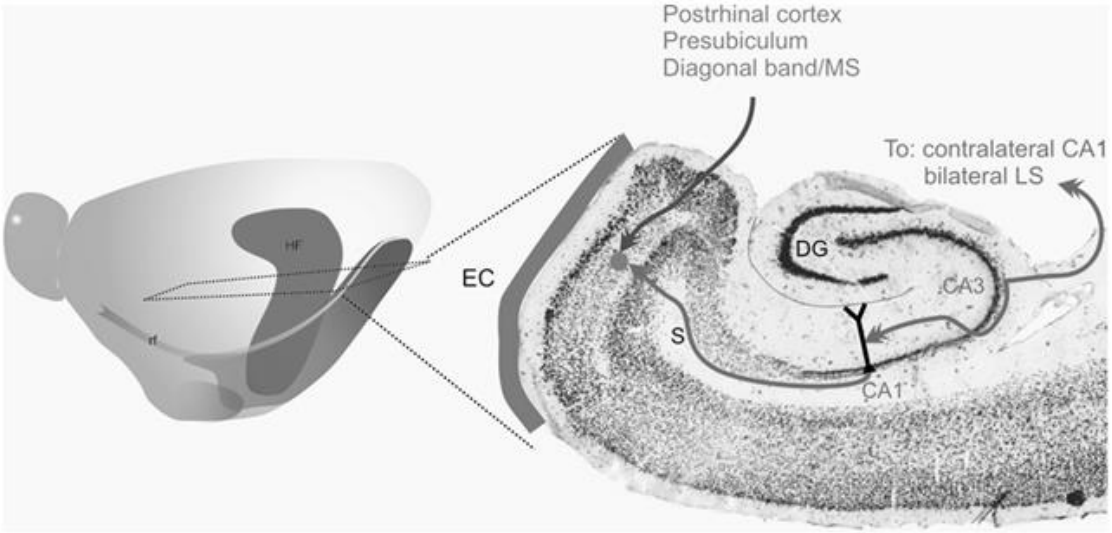

Fig. 1.15: Anatomy of the rodent hippocampal formation and parahippocampal region
Abbreviations: **CA1**, Cornu Ammonis 1; **CA3**, Cornu Ammonis 3; **DG**, dentate gyrus; **EC**, entorhinal cortex; **HF**, hippocampal formation; **LS**, lateral septum; **MS**, medial septum; **S**, subiculum

Adapted from Ohara et al. 2009, with permission from Frontiers Research Foundation.

week, while the dentate gyrus appears to begin forming at the 13th week, with major formation continuing to the 33rd – 34th weeks, and into adulthood (Arnold & Trojanowski, 1996).

1.5.2 Glutamate and Glutamatergic Neurons

The amino acid glutamate is considered the main excitatory neurotransmitter, although it can also be metabolized to the inhibitory neurotransmitter γ-aminobutyric acid (**GABA**) by glutamate decarboxylases (**GAD**) (**fig. 1.16**) (Shetty & Turner, 1998). As it is largely unable to cross the BBB, glutamate must be synthesized in the brain (Smith *et al.*, 1987; review: Smith, 2000). The lack of glutamate transport into the brain is believed to be a protective measure to prevent excess amounts of glutamate in the extracellular fluid, which could result in excitotoxic insult (Hawkins, 2009). Glutamate can be synthesized via a number of different pathways. Other amino acids are transported into the brain, and can, along with α-ketoglutarate, derived from glucose metabolism, be used to form glutamate through the actions of branched-chain amino acid transferase in the mitochondria of astrocytes and cytoplasm of neurons, and aspartate aminotransferase in neurons (Kugler, 1987; Mason *et al.*, 1995; Daikhin & Yudkoff, 2000; García-Espinosa *et al.*, 2007). Glutamate dehydrogenase converts glutamate to α-ketoglutarate and ammonia, and also catalyzes the opposite reaction in both astrocytes and neurons (**fig. 1.17**) (Kugler & Schleyer, 2004; Zaganas *et al.*, 2012). Glutamate can also be recycled in the brain; it is transported into astrocytes and other glial cells by transporters including GLAST and GLT-1 and metabolized to glutamine by glutamine synthetase (**fig. 1.16**) (Rothstein *et al.*, 1994; Daikhin & Yudkoff, 2000).

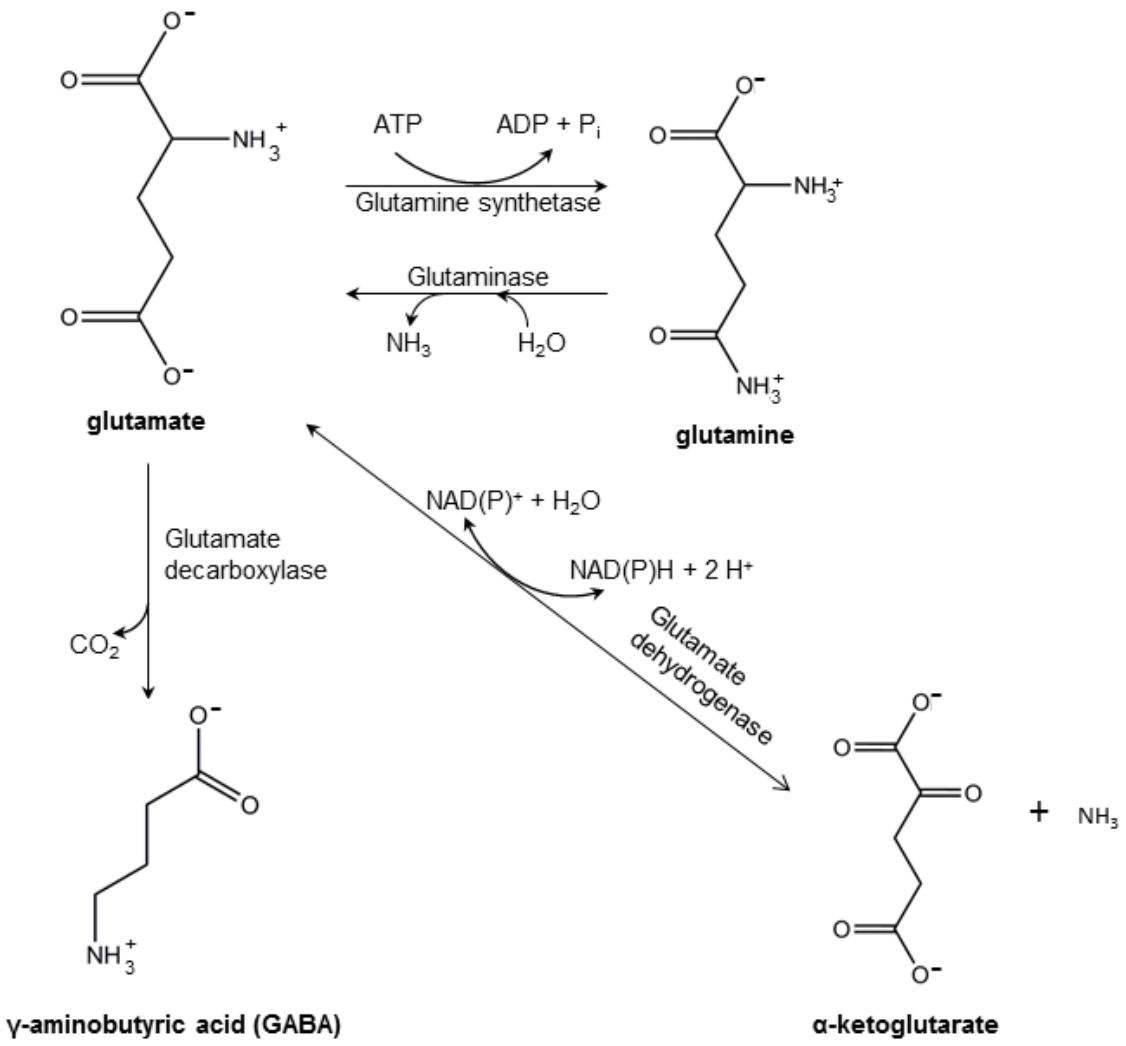

Fig. 1.16: Metabolism of glutamate to glutamine, GABA, and α-ketoglutarate

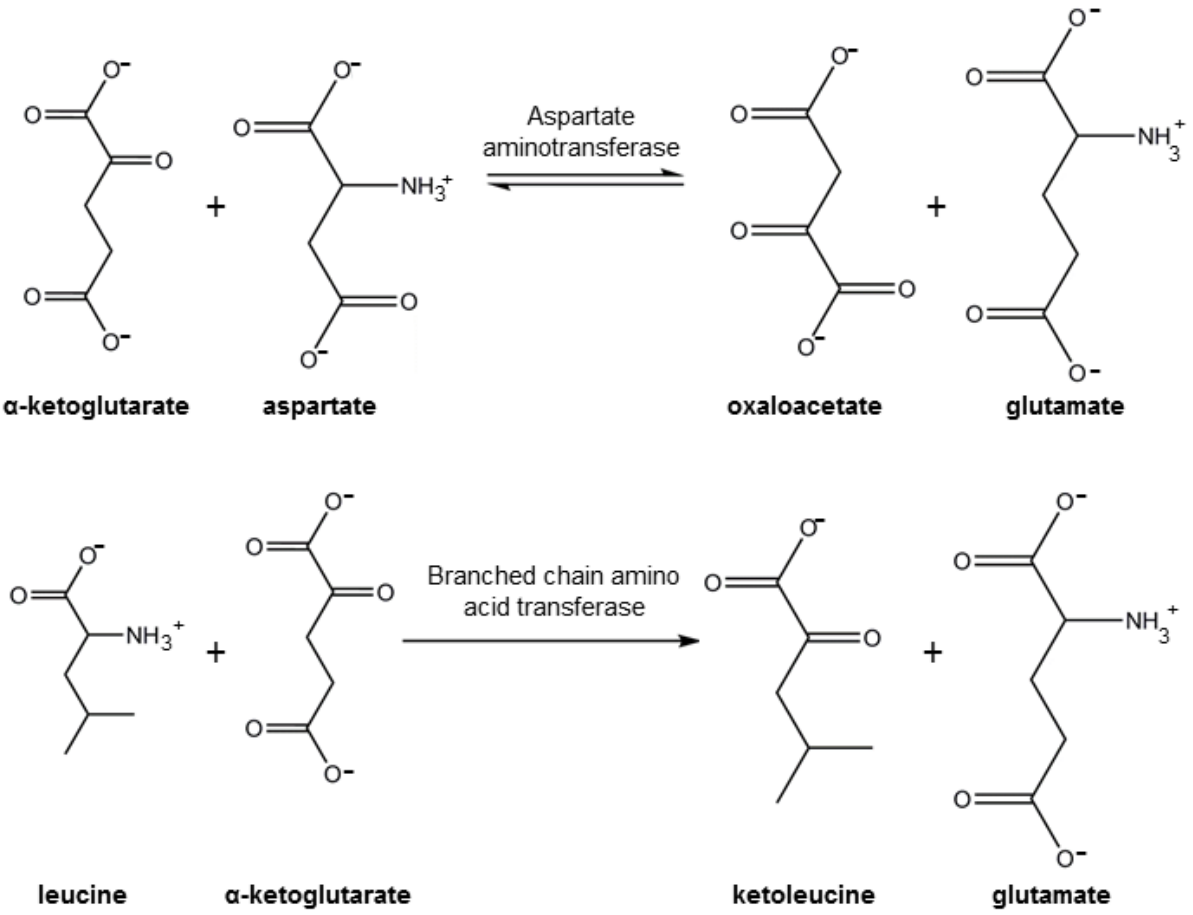

Fig. 1.17: Glutamate synthesis by aspartate aminotransferase and branched chain amino acid transferase

When glutamine is released by glial cells and uptaken by glutamatergic neurons, it can be used in the resynthesis of glutamate by glutaminases (**fig. 1.16**) (Daikhin & Yudkoff, 2000). This cycle allows glutamate to be preserved in the brain without the risk of elevated glutamate in the extracellular space, which could cause excitotoxicity.

While glutamate can be released into the synapse by excitation of a presynaptic glutamatergic neuron, it can also be released from astrocytes. The release of glutamate from astrocytes can enhance the magnitude of the post-synaptic response, referred to as synaptic strength (Jourdain *et al.*, 2007). Increases in intra-astrocytic Ca^{2+} levels can also stimulate the release of glutamate (Parpura & Haydon, 2000). As discussed in sections 1.4.4.1 and 1.4.4.4, this astrocytic release of glutamate is believed to contribute to the mechanism of toxicity of MeHg.

Glutamate has a number of different receptors, both fast-acting ionotropic (N-methyl-D-aspartate (**NMDA**), kainic acid, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (**AMPA**)) and slow-acting metabotropic (review: Lau & Tymianski, 2010). NMDA receptors are transmembrane heterotetramers, consisting of NR1, NR2, and NR3 subunits. When glutamate is bound to NR2 and glycine is bound to NR1, and the neuronal membrane is depolarized to -50 mV, possibly by the activation of AMPA receptor by glutamate, the Mg^{2+} blocking the receptor pore is released, allowing the influx of Ca^{2+} and Na^{+} and the efflux of K^{+} . This influx of Ca^{2+} allows for the activation of Ca^{2+}-dependent enzymes. While these enzymes do not contribute to fast excitatory

postsynaptic potentials, they are essential for long-term potentiation (**LTP**), whereby presynaptic and postsynaptic modifications result in long-lasting enhancement of the postsynaptic response for a given input (review: Bliss & Collingridge, 2013). LTP contributes to learning and memory when the enhancement of the postsynaptic response occurs in a neural circuit that is associated with a specific learning paradigm, or context. However, the induction of Ca^{2+}-dependent enzymes, including phospholipase A2, NADPH oxidase, and NOS, can contribute to excitotoxicity by increasing the formation of ROS and reactive nitrogen species in the neuron (Sattler *et al.*, 1999; Demaurex & Scorrano, 2009; Sun *et al.*, 2010). Ca^{2+} also increases mitochondrial respiration, which results in greater ATP production and also ROS formation (Balaban *et al.*, 2005).

Glutamatergic neurons in the hippocampus are essential for learning and memory. Intraperitoneal injections of NMDA receptor antagonists prior to passive avoidance training results in impaired learning (Venable & Kelly, 1990). Administration of the NMDA receptor antagonist MK-801 in both mice and rats before passive avoidance training, but not between training and retention trials, induced deficits, suggesting that glutamate and NMDA receptors are primarily required for passive avoidance learning (Venable & Kelly, 1990). A Cre/*loxP* conditional NMDA receptor subunit 1 knockout mouse model, where CA1 pyramidal neurons were selectively targeted, require more time to find a submerged platform in the Morris water maze (Tsien *et al.*, 1996).

Additional neurotransmitters that contribute to learning and memory include acetylcholine, dopamine, serotonin, and noradrenaline. Cholinergic neurons project to the hippocampus primarily from the medial septum and diagonal band of Broca, although there are some cholinergic neurons within the hippocampus, as well (Osten *et al.*, 2007). Dopaminergic neurons project to the hippocampus from the substantia nigra, and ventral tegmental area (Herlenius & Lagercrantz, 2004); serotonergic neurons project to the hippocampus primarily from the median raphe nucleus (Azmitia, 1981; Ohmura *et al.*, 2010); and noradrenergic inputs arise primarily from the locus coeruleus (Osten *et al.*, 2007).

1.5.3 Learning and Memory

Whether the hippocampus is primarily involved in learning and short-term memory, as opposed to long-term consolidation of memory, has been often debated. According to the consolidation theory, the hippocampus is primarily involved in forming memories, communicating with the neocortex to store long-term memories there, eventually playing no role in long-term memory (Squire *et al.*, 2004). New memories are initially associated with neural circuitry contained within the hippocampus. Over time, the hippocampus connects neural circuits in the neocortex, which are associated with a specific part of the memory, at which point the circuitry within the hippocampus is extinguished (McClelland *et al.*, 1995). This theory is supported by subjects with hippocampal lesions, including patient HM, who developed anterograde amnesia and temporally graded retrograde amnesia, suggesting that the hippocampus stores recent memories, in addition to forming new ones (Scoville and Milner, 1957). However, the hippocampus

also appears to be necessary for the retrieval of some older memories, resulting in the multiple trace theory, which suggests that the hippocampus and neocortex are both involved in long-term memory, and that communication between the two regions is essential for proper recall (Nadel *et al.*, 2000; Moscovitch *et al.*, 2005). Furthermore, according to this theory, previously formed knowledge that is encoded in the cortex can inform learning and memory driven by the hippocampus.

However, the novel object recognition and passive avoidance tests I performed in my studies involved learning and memory over only 1 – 24 hr, so the differences between these two theories of long-term memory formation and recall are not likely to be relevant to my work. Novel object recognition measures an animal's capacity to distinguish a novel object from a familiar object. Hippocampal lesions result in maximal novel object recognition impairment (Clark *et al.*, 2000). Multiple studies have suggested that this form of recognition memory is relatively robust compared to spatial memory, and resistant to mild forms of traumatic brain injury and hippocampal lesioning (Broadbent *et al.*, 2004; Zhao *et al.*, 2012). Rats exposed to a single dose of 8 mg/kg MeHg *in utero* have impaired novel object recognition, as well as increased NR-2B subunit expression in the hippocampus, though not the cortex (Baraldi *et al.*, 2002).

Passive avoidance testing determines whether animals can learn to associate certain environments with a negative stimulus, such as a mild foot shock, and therefore learn to avoid the stimulus. In the initial acquisition trial, animals are placed in a testing arena

consisting of two chambers. Upon entering a designated chamber, the animals are given a mild foot shock. In subsequent testing trials, the time that elapses before the animal re-enters the chamber where the shock was administered is designated the step-through latency, and is used as a measure of the animal's capacity to learn to avoid the foot shock. This learning is dependent on not only the hippocampus, but also the amygdala and cerebral cortex (Fukuchi *et al.*, 1987; Dunn & Everitt, 1988; Huang *et al.*, 2013). Hippocampal lesions impair passive avoidance learning (Best & Orr, 1973). Passive avoidance learning appears to be more sensitive to traumatic brain injury than novel object recognition, and therefore may be a more sensitive test to determine subtle genotype and xenobiotic effects (Zhao *et al.*, 2012). Although CA1 and CA3 injury both compromise novel object recognition and passive avoidance performance, injury to the dentate gyrus appears to compromise passive avoidance impairment more than novel object recognition (Zhao *et al.*, 2012).

One often overlooked aspect of the passive avoidance test is that, in addition to performance deficits reflecting impaired cognition, a loss of somatosensory function may result in the appearance of a cognitive deficit when no actual deficit is present. This may occur if, as can happen with MeHg poisoning, there is a loss of sensation in the foot. Two possible methods of ascertaining that somatosensory function is normal, beyond observing a physical reaction to the foot shock, are the hot plate test and the use of von Frey filaments. In the hot plate test, animals are placed on a plate that either maintains a constant temperature, or one that is gradually heated until a nociceptive response is elicited, such as licking or shaking of the animal's hind paws (Eddy et al.

1950). von Frey filaments are calibrated to bend after they have exerted a specific amount of force. These filaments are pressed against the rear surface of the animal's foot, and the smallest filament that elicits a lifting or licking response is considered the touch threshold, allowing researchers to quantify somatosensory response. As neither of these tests uses an electric current to elicit a response, it is possible that there are minor differences between the responses to these tests and the response in the passive avoidance test. However, they do provide a more quantitative measure of somatosensory function, and in combination with the reaction of the animals in the passive avoidance test, can help determine whether there are any somatosensory alterations that can confound passive avoidance results.

1.5.4 Related Brain Regions

Communication between the hippocampus and cerebral cortex is essential for learning and memory. Three month-old offspring from rat dams exposed to 8 mg/kg MeHg by gavage, but not 4 mg/kg MeHg, were found to have impaired passive avoidance learning, without any changes in sensitivity measured by the hot plate test (Ferraro *et al.*, 2009). Furthermore, cells from primary cortical neuron cultures from 1-day old rats exposed to the high dose of MeHg were also less viable and had a greater amount of nuclear condensation, suggesting apoptosis, than either controls or cultures from rats exposed to low-dose MeHg. While these results implicate the cortex in the MeHg effect on passive avoidance learning, cultures from the hippocampus and amygdala were not studied and may have been more important than the cortex for this MeHg effect.

The septal nuclei act as relays between multiple brain regions, including the hippocampus. The hippocampus projects to the ventral tegmental area via the lateral septal nucleus (Luo *et al.*, 2011). The medial septal nucleus receives inputs from the hypothalamus, the raphe nuclei, and the locus coeruleus (Bland *et al.*, 1994; McKenna & Vertes, 2001) and projects cholinergic and GABAergic neurons to the CA1 and the dentate gyrus (Amaral & Lavenex, 2007). Lesions of the medial septal nucleus can result in impaired learning and memory in a number of maze tasks, as well as a slight impairment in passive avoidance performance (KAADA *et al.*, 1962; Miyamoto *et al.*, 1987). Injection of the neuropeptide Substance P, which is commonly associated with nociception but also increases learning, into the medial septal area immediately after the acquisition trial in the passive avoidance test improves learning (Stäubli & Huston, 1980; Hasenöhrl *et al.*, 2000). Cholinergic neuron loss in the medial septal area of rats does not appear to disrupt novel object recognition, although object location memory is impaired (Cai *et al.*, 2012).

The amygdala (**fig. 1.14**) in the medial temporal lobe is crucial for fear-based learning. Neural activity is increased in the amygdala during the acquisition phase of passive avoidance (Huang *et al.*, 2013). Similarly to the medial septal area, injection of Substance P into the amygdala can improve passive avoidance learning, and can also improve long-term retention (Kertes *et al.*, 2009). Lesions of the amygdala, either induced surgically or by injections of the excitotoxin ibotenic acid, prior to passive avoidance testing results in impaired learning or retention (Russo *et al.*, 1976; Dunn & Everitt, 1988).

Section 2: Methods

2.1 Chemicals

Methylmercury (II) chloride and PBN were purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario). For the DNA oxidation study, 8-hydroxy-2'-deoxyguanosine was obtained from Cayman Chemical Co. (Ann Arbor, Michigan), 2'-deoxyguanosine, 2'-deoxythymidine and Nuclease P1 were purchased from Sigma-Aldrich Canada Co. Calf intestinal alkaline phosphatase was purchased from Invitrogen (Burlington, Ontario), Proteinase K from BioShop (Burlington, Ontario), and HPLC-grade methanol from MedStore (University of Toronto).

2.2 Mice

For the MeHg and PBN studies, virgin 6 week-old CD-1 animals (Charles River Canada, Sherbrooke, Quebec) were acclimatized for 1 week before breeding. *Ogg1* -/- mice were generously donated by Dr. Thomas Lindahl (Cancer Research UK, London, UK) via Dr. Christi A. Walter (University of Texas Health Science Center at San Antonio, San Antonio, TX) and maintained in a colony by +/- x +/- breeding pairs. Mice were housed in plastic cages on HEPA filter racks with corncob bedding and *ad libitum* access to water and food (2018 Teklad Global 18% Protein Rodent Diet, Harlan, Mississauga, Ontario) on a 12 hour light/dark cycle. Up to three CD-1 females were time-mated with one male overnight, or two *ogg1* +/- females with one +/- male overnight, with the presence of a vaginal plug the next morning designating GD 1. The use of +/- x +/- matings for the *ogg1* behavioural studies resulted in a much higher number of +/- offspring, compared to the number of +/+ and -/- offspring, approximating the expected Mendelian ratio of 1:2:1. For the immunohistochemical studies, wild-type *ogg1* females

were time-mated overnight with wild-type *ogg1* males and knockout *ogg1* females with knockout *ogg1* males. Pregnant females were housed separately and on GD 17 were injected intraperitoneally (**i.p.**) with 4 mg/kg MeHg or an equivalent volume of phosphate-buffered saline (**PBS**) vehicle. Selected CD-1 dams were pretreated with PBN (45 mg/kg i.p.) or its saline vehicle 2 hours prior to MeHg. Litter and offspring deaths for each treatment are summarized in **Table 2.1**. All animal studies were approved by the University of Toronto Animal Care Committee in accordance with the standards of the Canadian Council on Animal Care.

2.3 Genotyping of *ogg1* mice

DNA was isolated from adult ear punches or fetal tail clips using a crude NaOH/EDTA extraction. Primers used to amplify the *ogg1* gene band (500 bp) were *ogg1*-sense (5′-ACTGCATCTGCTTAATGGCC-3′) and *ogg1*-antisense (5′-CGAAGGTCAGCACTGAACAG-3′). Primers used to amplify the knockout gene (300 bp) were neo-sense (5′-CTGAATGAACTGCAGGACGA-3′) and neo-antisense (5′-CTCTTCGTCCAGATCATCCT-3′). The samples were run in a thermal coupler (Eppendorf Mastercycler Gradient; Eppendorf Scientific, Mississauga, ON) using the following conditions: 94° C for 1 minute; followed by 94° C for 1 minute, 58° C for 1.5 minutes, and 72° C for 2 minutes for 30 cycles; followed by 94°C for 10 minutes. PCR samples were run on a 1.5% agarose gel made with 1x Tris-HCl-acetic acid-EDTA and ethidium bromide. The gel was run at 120 V for 45 minutes and viewed and photographed under UV light.

Treatment	Litters cannibalized	Offspring deaths after weaning				Number of offspring tested	
		Before object recognition (6 wk)		Before passive avoidance (4 mth)			
		Number (offspring)	Cause of death	Number (offspring)	Cause of death	Object recognition test	Passive avoidance test
CD-1 litters							
Vehicle	2	0	-	7	Drowning	32	18
MeHg	3	1	Euthanized due to severe dermatitis	0	-	31	35
PBN + vehicle	0	0	-	0	-	26	48
PBN + MeHg	1	0	-	1	Euthanized due to severe dermatitis	24	46
***ogg1* litters:**							
Vehicle	0	1	Drowning	1	Found dead	47	46
MeHg	2	0	-	1	Euthanized due to severe dermatitis	67	56

Table 2.1: Litter characteristics and offspring death

2.4 DNA oxidation

The dams from the studies with MeHg and PBN, as well as those with MeHg and *ogg1* mice, were sacrificed 6 hours after MeHg or PBS exposure and fetal brains were collected, flash frozen in liquid nitrogen and stored at -80° C until analysis. The 6 hour time point was based on a previous study measuring 8-oxodGuo formation in fetal brains 2, 6, and 12 hours after MeHg exposure, which suggested that 6 hr after MeHg exposure would represent the peak of 8-oxodGuo formation in the fetal brain (**Appendix 1, fig. A1.1**). All fetal brains from the CD-1 litters were collected and 2 – 3 brains from each litter were randomly selected for analysis. All fetal brains were collected from the *ogg1* litters, and after genotyping, all +/+ and -/- brains from each litter were analyzed. Production of the oxidative DNA lesion 8-oxodGuo was quantified using HPLC with tandem mass spectrometry (**MS**) standardized to 2'-deoxyguanosine (**dGuo**) measured by HPLC-UV. DNA extraction was based on a method by (Ravanat *et al.*, 2002) designed to minimize artefactual 8-oxodGuo production. Briefly, fetal brains were homogenized in 1 mL of lysis buffer (320 mM sucrose, 5 mM $MgCl_2$, 10 mM Tris-HCl, 0.1 mM desferoxamine, 1% Triton X-100, pH 7.5) and centrifuged at 1,000 x g for 10 minutes. After this step was repeated, the pellets were homogenized in 200 uL of enzyme reaction solution (1% w/v SDS, 5 mM EDTA-Na_2, 0.15 mM desferoxamine, 10 mM Tris-HCl, pH 8.0) and incubated at 50° C for 1 hour with RNAse A and T, and subsequently with Proteinase K. DNA was precipitated using a sodium iodide solution (7.6 M NaI, 40 mM Tris-HCl, 20 mM EDTA-Na_2, 0.3 mM desferoxamine, pH 8.0) and isopropyl alcohol. The DNA pellets were resolubilized in 20 mM sodium acetate, pH 5.2. A 100 µg aliquot of DNA was digested to deoxynucleotides by incubation with nuclease P1 for 1 hour at 37° C, and subsequently to deoxynucleosides with calf intestinal

alkaline phosphatase. To quantify dGuo content, the samples were quantified using an binary Series 200 HPLC system (PerkinElmer Instruments) with UV detection (280 µm) equipped with a 5 µm Supelcosil LC-18-T column (25 cm x 4.6 mm, Sigma Aldrich). Samples were filtered (0.22 µm) and eluted using a mobile phase of 92.5% $NaPO_4$, pH 5.5 buffer and 7.5% methanol at a flow rate of 0.8 mL/min. The chromatographs were analyzed using the TotalChrom chromatography software version 6.2.0 (PerkinElmer Instruments). To measure 8-oxodGuo, samples were run using an Agilent 1100 series HPLC (Mississauga, ON) with an AB Sciex API4000 QTRAP triple quadrupole mass spectrometer under the control of ANALYST® software (AB Sciex, Concord, ON). Samples were passed through a Gemini® NX-C18 column (100 x 4.6 mm, 3 µm pore, Phenomenex, Torrance, CA, USA) on a water:methanol gradient with a flow rate of 0.6 mL/min for 9.5 min. The multiple reaction monitoring (**MRM**) transition used to quantify 8-oxodGuo was m/z 284.0→168.0 in the positive ion selection mode to capture the parent compound (**Q1**), 8-oxodGuo, and the product ion (**Q3**), 8-oxo-2'-deoxyguanine. Data from both runs were combined and expressed as fmol 8-oxodGuo per µg dG. In previous time course studies of DNA oxidation in CD-1 mice, dams were injected with 0, 4, or 8 mg/kg MeHg, and fetal brains were collected for analysis at 2, 6, and 12 hours after MeHg exposure (**Appendix 1, fig. A1.1**). In this study, 8-oxodGuo was measured by HPLC-EC using a Series 200 HPLC system (PerkinElmer Instruments) equipped with a 5 µm Supelcosil LC-18-T column (25 cm x 4.6 mm, Sigma Aldrich), an electrochemical detector (Coulochem II) with a guard cell (100 mV) and an analytical cell (400 mV) (Coulochem, ESA Inc., Chelmsford, MA). The 8 mg/kg dose was later discarded because of maternal toxicity. Reported values are given for the nucleosides (8-oxodGuo and dGuo).

2.5 Object recognition test

The dams were allowed to deliver spontaneously and the pups were weaned at 3 weeks of age. The offspring were tested in the object recognition test at 6 weeks of age. All animal cages were randomized prior to testing. Cage identifiers were covered and cages were assigned random letter and number identifiers. The custom made testing arena consisted of an opaque 40 x 40 x 40 cm chamber with an open top and a camera positioned above the chamber (**fig. 2.1**). The animals were habituated in the testing room for 1 hour prior to each trial, and were habituated to the testing chamber with 10 min exploration sessions at 24 hour intervals for 3 days prior to testing. On the testing day, the animals underwent two sessions in the arena. During the first familiarization session, two identical objects were placed along the far side of the chamber, 8 cm away from the walls. The CD-1 mice from the MeHg and PBN studies were allowed to explore the arena and the objects for 3 minutes. The *ogg1* mice were allowed to explore the arena and the objects for 5 minutes. The time for the familiarization trial for the *ogg1* animals was increased to optimize baseline performance in the wild-type control mice (**Appendix 2, fig. A2.1**). The mice were removed to their home cage and after an interval of 1 hour were replaced in the testing arena for the recall trial, now containing 1 familiar and 1 novel object, in the same positions as the objects in the learning session. The mice were again allowed to explore the arena and objects for 3 minutes, and this session was recorded by the camera. After the recordings were independently randomized, the amount of time the animals spent exploring each object was measured. Animals that explored the objects for less than 10 seconds in either session were rejected. The scores for each animal were calculated by determining the proportion of

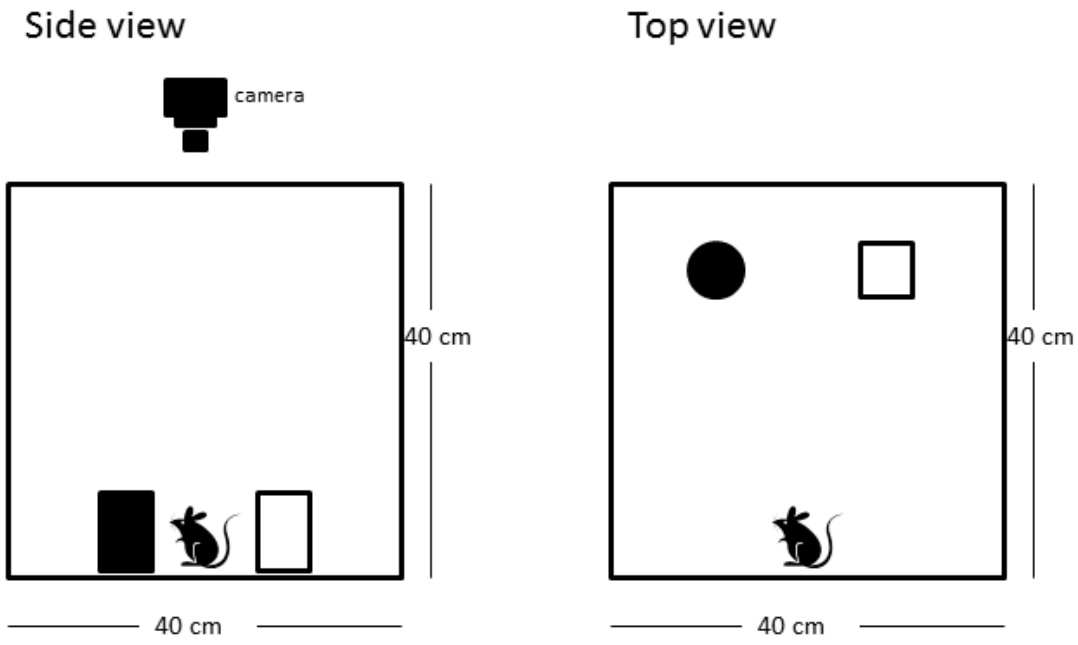

Figure 2.1: Object recognition apparatus
The testing chamber for the novel object recognition test consisted of a 40 x 40 x 40 cm chamber with opaque walls. The top of the chamber was open and a camera was suspended above it. Two objects (identical objects in the familiarization trial, and one familiar and one novel object in the recall trial) were placed 8 cm from the far wall of the chamber. CD-1 mice were allowed to explore the chamber for 3 minutes in the familiarization trial, and *ogg1* mice were allowed to explore the chamber for 5 minutes in the familiarization trial.

time the animals spent exploring the novel object compared to the amount of time spent exploring both objects in the second session. In a previous pilot study, CD-1 animals exposed to 0 or 4 mg/kg MeHg were tested at 4 weeks of age (**Appendix 1, fig. A1.2**).

2.6 Passive avoidance test

Dams were allowed to deliver spontaneously and the pups were weaned at three weeks of age. The offspring were tested in the passive avoidance test at 4 months of age. All animal cages were randomized prior to testing. The custom made testing arena consisted of 2 chambers, each 20 x 20 x 20 cm, one dark and one light (**fig. 2.2**). The chambers were connected by a manually operated door, and the floor of each chamber consisted of suspended steel rods that were attached to a power supply. The mice habituated to the testing room for 1 hour before each trial. After habituation, the mice were placed in the light chamber, facing away from the connecting door, and allowed to explore the light chamber for 20 seconds before the door was opened. Once the dark chamber was entered, the door was closed, and a small current of 1 mA was applied for across the rods in the dark chamber, resulting in a mild foot shock. The CD-1 animals from the MeHg and PBN study were shocked for 4 seconds, and 3 trials were conducted at 24 hour intervals. The *ogg1* animals were shocked for 6 seconds, and 5 trials were conducted at 24 hour intervals. The duration of the foot shock and number of trials was increased in the *ogg1* animals to optimize baseline performance in the wild-type control animals (**Appendix 2, fig. A2.2**). During the last trial, the animal's latency to enter the dark chamber was recorded as its testing score, to a maximum of 300

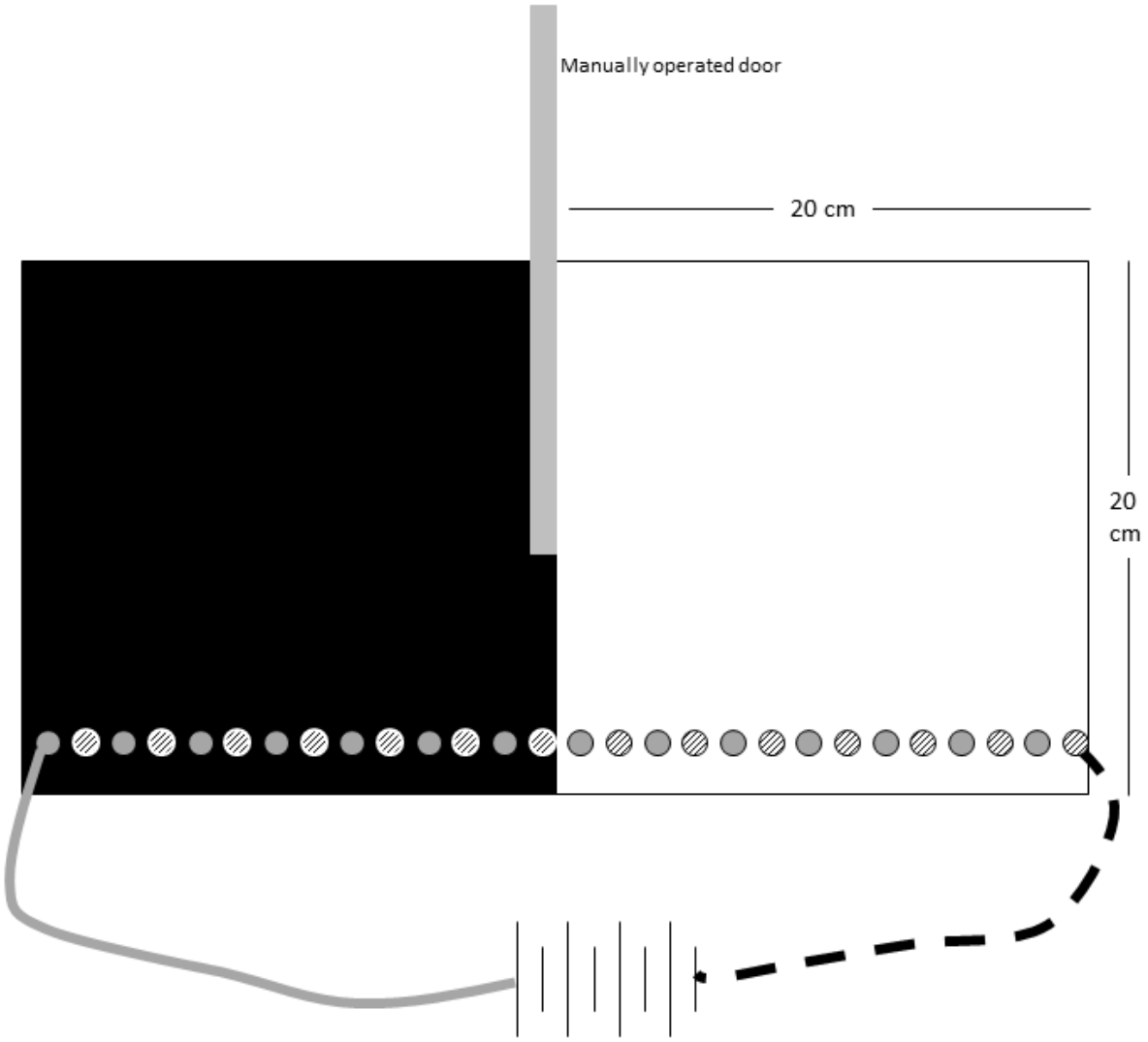

Figure 2.2: Passive avoidance apparatus
The testing chamber for the novel object recognition test consisted of 2 20 x 20 x 20 cm chambers, one light and one dark, connected by a manually operated door. The floor in both chambers consisted of steel rods. Alternating rods were connected to the positive and negative electrodes of a power supply set at 1 mA.

seconds. In a previous pilot study, CD-1 animals exposed to 0 or 4 mg/kg MeHg were tested at 7months of age (**Appendix 1, fig. A1.3**).

2.7 von Frey test

One of the hallmarks of MeHg poisoning in adults is numbness and tingling in the extremities (Ninomiya *et al.*, 2005; Takaoka *et al.*, 2008). We therefore assessed tactile sensitivity to confirm that any deficit observed in passive avoidance was attributable to a deficit of learning and memory, and not an inability to feel the foot shock. The animals from the MeHg and PBN studies, as well as those from the MeHg and *ogg1* studies were tested 24 hour after the last passive avoidance trial. All animal cages were randomized prior to testing. The testing arena consisted of a small chamber with a plastic mesh floor, sufficiently large to allow the von Frey filaments through. The mice were habituated in the testing room for 1 hr prior to testing, and for 1 minute in the testing arena. Calibrated von Frey filaments (North Coast Medical, Morgan Hill, California), which bend after a specific amount of pressure are applied on them, were pressed against the plantar surface of each rear foot twice, by increasing size and force until the mice reacted by lifting the affected foot at least twice. The force applied by the successful filament was recorded as the score for each animal.

2.8 Immunohistochemistry

Ogg1 dams were injected i.p. with 100 mg/kg bromodeoxyuridine (**BrdU**) 1 hour prior to sacrifice, which occurred 6 hours after the MeHg or vehicle dose. The 6 hour timepoint

was based on a previous DNA oxidation in MeHg-exposed CD-1 mice which suggested that a 6 hour exposure to MeHg would yield peak 8-oxodGuo levels in the fetal brain (**Appendix 1, fig. A1.1**), and is the same as the time point chosen for the DNA oxidation studies (see Section 2.4). Fetuses from +/- x +/- matings, +/+ x +/+ matings and -/- x -/- matings were analyzed. After genotyping, all +/+ and -/- fetuses were selected for analysis from the +/- x +/- litters, while fetuses from the +/+ x +/+ matings and -/- x -/- matings were randomly selected for analysis. Fetal brains were fixed with 4% paraformaldehyde before being paraffin-embedded. Matched serial 5 µm coronal sections of the hippocampus were used for analysis. Antigen retrieval using 10 mM citrate buffer (pH 6.0) was used for γH2AX staining and using pepsin digestion, followed by incubation in 2N HCl and 0.1 M Na-Borate for BrdU staining. Samples were blocked in 3% bovine serum albumin and 3% Tween 20 in 20 mM MgCl2 with 5% goat serum prior to incubation overnight with 1:200 mouse anti-γH2AX as a marker of DNA DSBs (Millipore, Billerica, MA), 1:200 rat anti-BrdU as a marker of cellular migration into the developing hippocampal region (Clontech, Mountain View, CA) or 1:500 mouse anti-TUJ1 as a marker of differentiated neurons (Covance, Emeryville, CA) at 4° C. The samples were subsequently incubated for 1 hr at room temperature with 1:500 goat anti-mouse Alexa 555 (Invitrogen, Burlington, ON), 1:500 goat anti-rat Alexa 568 (Invitrogen) or 1:200 goat anti-mouse Alexa 488 (Invitrogen), before being mounted with Vector Mounting Medium with DAPI (Vector Laboratories, Burlington, ON). DNA fragmentation as a marker for apoptosis was measured using terminal deoxynucleotidyl transferase dUTP nick end labeling (**TUNEL**) (*In Situ* Cell Death Detection Kit, Fluorescein, Roche, Mississauga, ON). Slides were viewed on an Imager.Z1 Epifluorescence microscope (Carl Zeiss Canada Ltd., Toronto, ON) and photos were

taken at 10x and 20x magnification. After being randomized by an independent observer, the developing hippocampal area in the γH2AX-, BrdU- and TUNEL photos was measured using AxioVision SE64 Rel. 4.8 (Carl Zeiss Canada Ltd.), and the number of positive cells was manually counted.

2.9 Survival

One litter each from the treatment groups for the MeHg and PBN studies (saline, MeHg, PBN + saline and PBN + MeHg) were housed in groups of up to four to a cage and monitored for survival to 16 months of age.

2.10 Statistics

In the MeHg and PBN studies, comparisons between treatment groups and the vehicle control animals were analyzed using 1-way analysis of variance (**ANOVA**) with a post-hoc Dunnett's test. Comparisons between different treatment groups were analyzed using 1-way ANOVA with a post-hoc Fisher's LSD test. When appropriate, statistical differences between data were analyzed using an unpaired Student's t-test. Survival curves were analyzed using the log-rank (Mantel-Cox) test (GraphPad Prism 5.0; GraphPad Software Inc., La Jolla, CA). Analysis by 2-way ANOVA with a post-hoc Bonferonni test was used to determine the effects of*ogg1* genotype and MeHg treatment, and the interaction of genotype and treatment. The effect of *ogg1* genotype on passive avoidance learning in the female mice was analyzed using 1-way ANOVAwith a post-hoc Dunnett's test. When appropriate, statistical differences

between data were analyzed using an unpaired two-tailed Student's t-test (GraphPad Prism 5.0, GraphPad Software Inc. La Jolla, CA). The level of significance was determined to be at $p \leq 0.05$, except for the survival study, where significance was determined to be at $p < 0.0125$.

Section 3: Results

3.1 Reduction in methylmercury-initiated neurodevelopmental deficits by maternal PBN pretreatment in CD-1 mice may not involve oxidatively damaged DNA

Aaron Shapiro assisted with the HPLC with tandem MS for the 8-oxodGuo study.

3.1.1 MeHg does not increase 8-oxodGuo levels in GD 17 fetal brain

Changes in 8-oxodGuo levels in whole fetal brain after exposure to MeHg or vehicle, with or without PBN pretreatment, were measured to determine whether MeHg or PBN modulate DNA oxidation in the fetus. *In utero* exposure to MeHg on GD 17 (70 ± 47 fmol 8-oxodGuo/µg dGuo) did not result in higher 8-oxodGuo levels in whole fetal brain samples compared to vehicle-treated controls (51 ± 27 fmol 8-oxodGuo/ug dGuo) (1-way ANOVA with post-hoc Dunnett's test, $p > 0.05$) (**fig. 3.1**). Furthermore, there were no significant effects of PBN treatment on 8-oxodGuo levels, either in vehicle- or MeHg-treated fetuses. We can be confident that the lack of difference between the treatment groups was not due to a lack of sensitivity in our assay, as the samples from the *ogg1* -/- animals, which were analyzed concurrently with the samples from the MeHg- and PBN-treated animals, exhibited significantly higher levels of 8-oxodGuo than the samples from the *ogg1* +/+ animals (see Section 3.2.1, fig. 3.6).

3.1.2 MeHg causes cognitive deficits in the object recognition test that are prevented by PBN pretreatment

To determine whether MeHg or PBN would affect cognition in the offspring after exposure during gestation, the ability of the mice to distinguish novel objects from familiar objects was measured using the object recognition test. Cognitive deficits in the

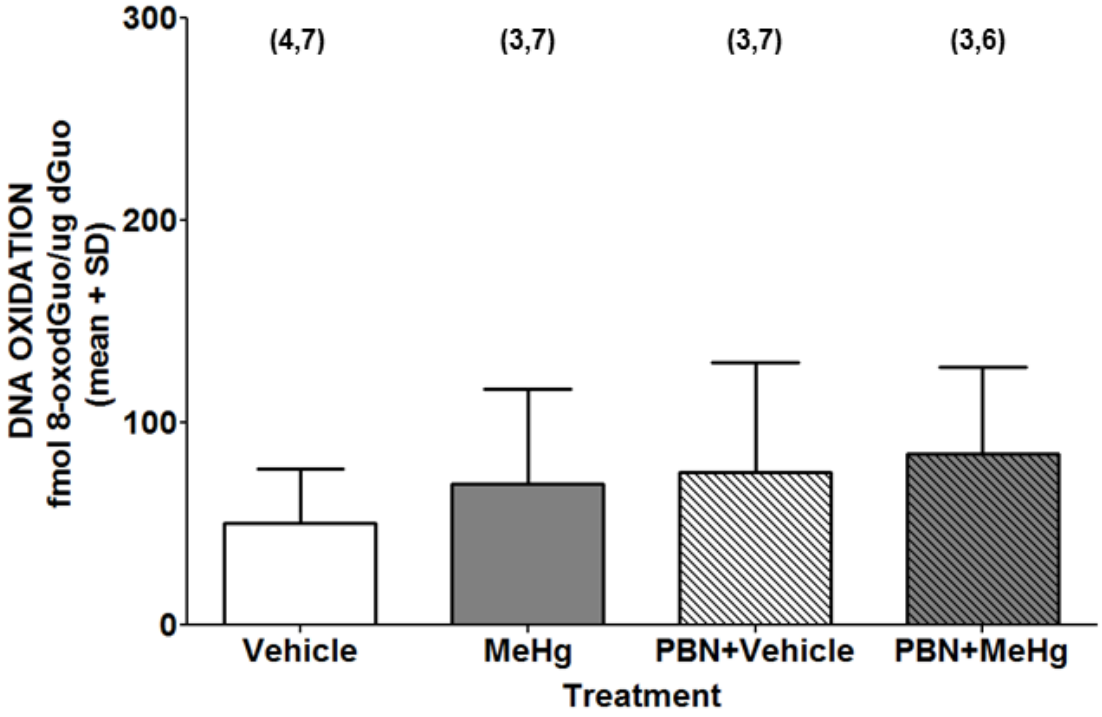

Figure 3.1: No increase in DNA oxidation in GD 17 fetal brains after *in utero* exposure to MeHg treatment, with or without PBN pretreatment.

Pregnant dams were injected i.p. on GD 17 with 4 mg/kg MeHg or PBS, with or without i.p. pretreatment with 45 mg/kg PBN 2 hr prior to MeHg or PBS. Fetal brains were collected after 6 hr and analyzed for DNA oxidation, reflected by 8-oxodGuo levels. No significant differences were observed between saline controls and MeHg-treated animals. Similarly, no effect of PBN pretreatment was observed. (x,y) = number of litters, number of fetuses

object recognition test were seen in the MeHg-treated 6-week old offspring (51 ± 12%) compared to the vehicle controls (59 ± 13%) (**fig. 3.2**) (1-way ANOVA with post-hoc Dunnett's test, $p = 0.046$). The score expected for a maximal deficit in this test would be 50%, which would represent an equal time spent exploring the novel and familiar objects, or in other words, a complete inability to distinguish the two types of object. These results suggest that this single dose of MeHg was sufficient to cause a maximal deficit. Treatment with PBN prior to MeHg completely protected against this deficit, with a mean score of 58 ± 14%, virtually identical to the mean score of the vehicle-treated animals (1-way ANOVA with post-hoc Fisher's LSD, $p < 0.05$). The post-hoc Fisher's LSD was used to compare the MeHg- and PBN-pretreated MeHg-treated animals as the post-hoc Dunnett's test only compared treatment groups to the vehicle-treated control animals. Animals treated with PBN prior to vehicle also performed similarly to vehicle-treated animals, suggesting that PBN alone does not affect performance in this test. No sex-dependent differences were observed between the treatment groups (1-way ANOVA, $p > 0.05$).

3.1.3 MeHg causes cognitive deficits in the passive avoidance test that are unaffected by PBN pretreatment

To further elucidate the effects of MeHg and PBN on cognition, 4-month old mice were tested using the passive avoidance test. MeHg (145 ± 125 s) caused a cognitive deficit compared to vehicle controls (241 ± 98 s), with a 40% decrease in the latency to enter the dark chamber (1-way ANOVA with post-hoc Dunnett's test, $p<0.05$) (**fig. 3.3**). Pretreatment with PBN (mean score: 174 ± 122 s) did not significantly reduce the MeHg-initiated deficit, although a post-hoc Dunnett's test showed no difference between

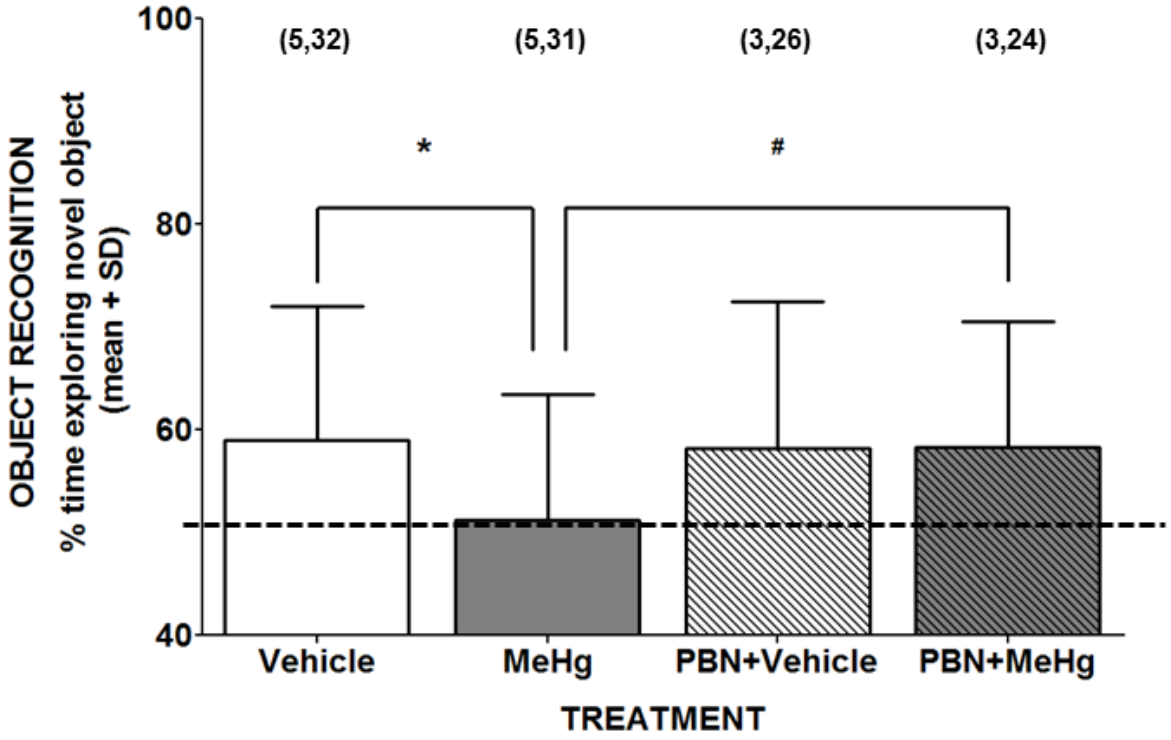

Figure 3.2: Cognitive deficits in postnatal novel object recognition after *in utero* exposure to MeHg, and protection by pretreatment with PBN.

Pregnant dams were injected i.p. on GD 17 as described in fig. 1. The dams were allowed to deliver spontaneously, and the offspring were weaned at 3 weeks of age. The offspring were evaluated in the object recognition test at 6 weeks of age. Scores indicate the proportion of time the animals explored the novel object, compared to the cumulative exploration time for both objects. The dashed line indicates a score of 50, the expected score for a maximally impaired animal.

* indicates a difference from vehicle controls ($p < 0.05$, 1-way ANOVA with post-hoc Dunnett's Multiple Comparison test). # indicates a difference from the group exposed to MeHg alone ($p = 0.046$, 1-way ANOVA with post-hoc Fisher's LSD).

(x,y) = number of litters, number of offspring

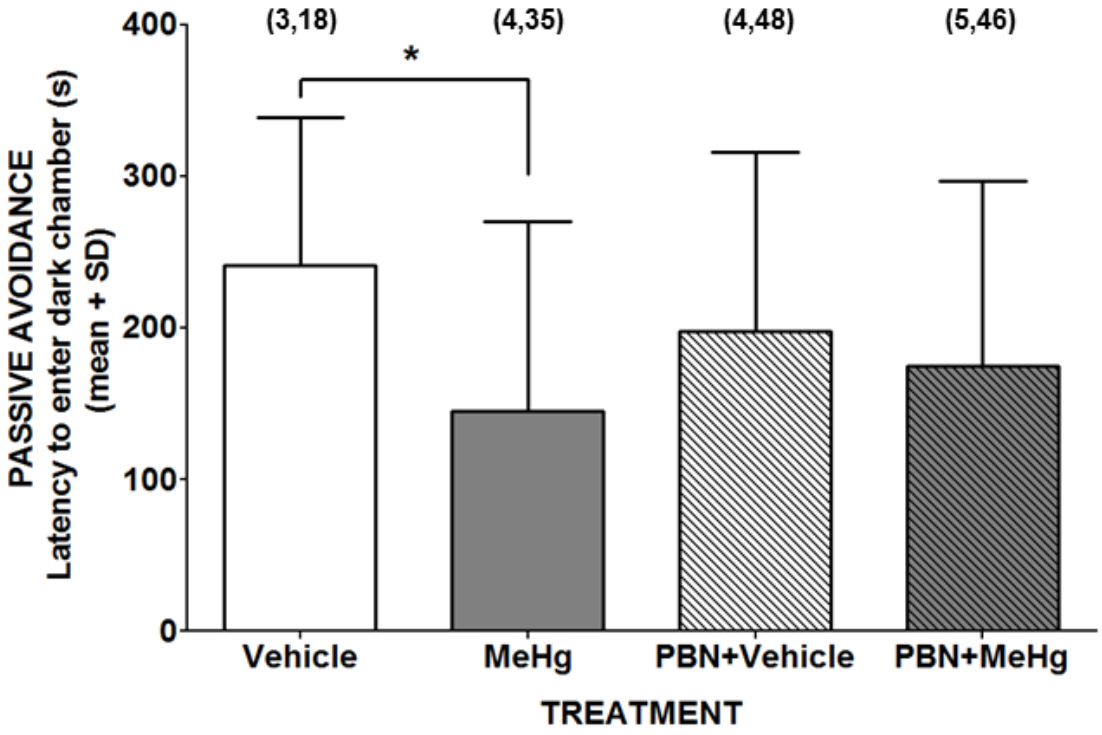

Figure 3.3: Cognitive deficits in postnatal passive avoidance after *in utero* exposure to MeHg, and no protection by pretreatment with PBN.

Pregnant dams were injected i.p. on GD 17 as described in fig. 1. The dams were allowed to deliver spontaneously, and the offspring were weaned at 3 weeks of age. Animals were evaluated in the passive avoidance test at 4 months of age. Scores indicate the latency to enter the dark chamber on the third trial.

* indicates a difference from vehicle controls ($p < 0.05$, 1-way ANOVA with post-hoc Dunnett's Multiple Comparison test). (x,y) = number of litters, number of offspring

the vehicle group and the PBN-pretreated MeHg group. No sex-dependent differences were observed between the treatment groups (1-way ANOVA, $p > 0.05$).

3.1.4 MeHg does not cause deficits in tactile sensitivity

To determine whether the deficits observed in the passive avoidance test were due to a cognitive deficit or an inability of the mice to detect the sensation of the foot shock, tactile sensitivity was measured using von Frey filaments. *In utero* exposure to MeHg did not cause any deficits in tactile sensitivity in the von Frey test, which would be represented by an increase in the force applied to the foot to elicit a reaction (1-way ANOVA with post-hoc Dunnett's test, $p > 0.05$) (**fig. 3.4**). To the contrary, the MeHg-treated animals were slightly more sensitive than the vehicle-treated animals when analyzed by t-test (mean scores: 1.9 ± 0.8 g and 3.2 ± 1.6 g, respectively) ($p=0.02$). No significant difference was observed between the PBN plus vehicle and the PBN plus MeHg groups, or among any of the other treatment groups. These results suggest that the deficit observed in the passive avoidance test is a cognitive deficit, and not due to a difference in sensitivity. No sex-dependent differences were observed between the treatment groups (1-way ANOVA, $p > 0.05$).

3.1.5 MeHg shortens lifespan, an effect that is blocked by PBN pretreatment

One litter from each treatment group was followed to 16 months of age to determine whether MeHg or PBN would shorten lifespan. *In utero* exposure to a single maternal dose of MeHg resulted in a substantially shorter lifespan than that for offspring exposed

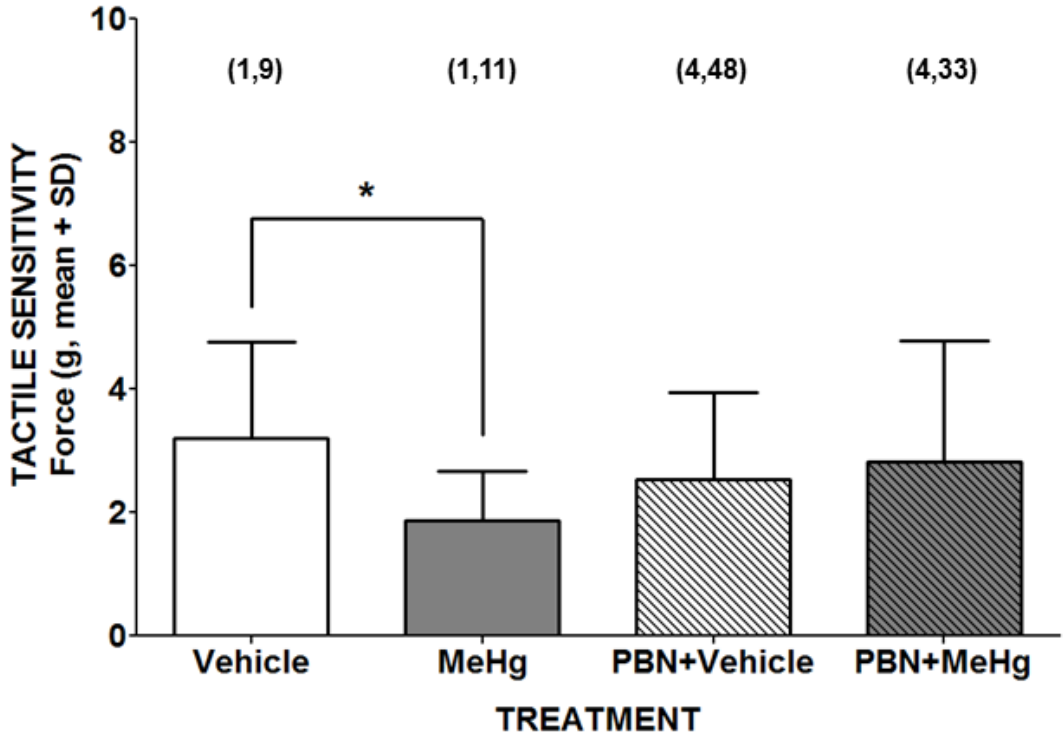

Figure 3.4: No decrease in tactile sensitivity after *in utero* exposure to MeHg.
Pregnant dams were injected i.p. on GD 17 as described in fig. 1. The dams were allowed to deliver spontaneously, and the offspring were weaned at 3 weeks of age. At 24 hr after the last trial of the passive avoidance test, animals were tested with von Frey filaments. Scores indicate the amount of force applied to the rear foot that was necessary to elicit a reaction from the animal. A higher score indicates less tactile sensitivity.
* indicates a difference from vehicle controls ($p < 0.05$, Student's t-test). (x,y) = number of litters, number of offspring

to vehicle (Mantel-Cox log-rank test, $p<0.001$) (**fig. 3.5**). This MeHg-initiated decrease in lifespan was completely blocked by maternal pretreatment with a single dose of PBN (Mantel-Cox log-rank test, $p<0.0125$). By 16 months of age, % survival for the vehicle-treated animals was 86%, versus 10% in the MeHg-treated group, 73% in the PBN plus vehicle group and 77% in the PBN plus MeHg group.

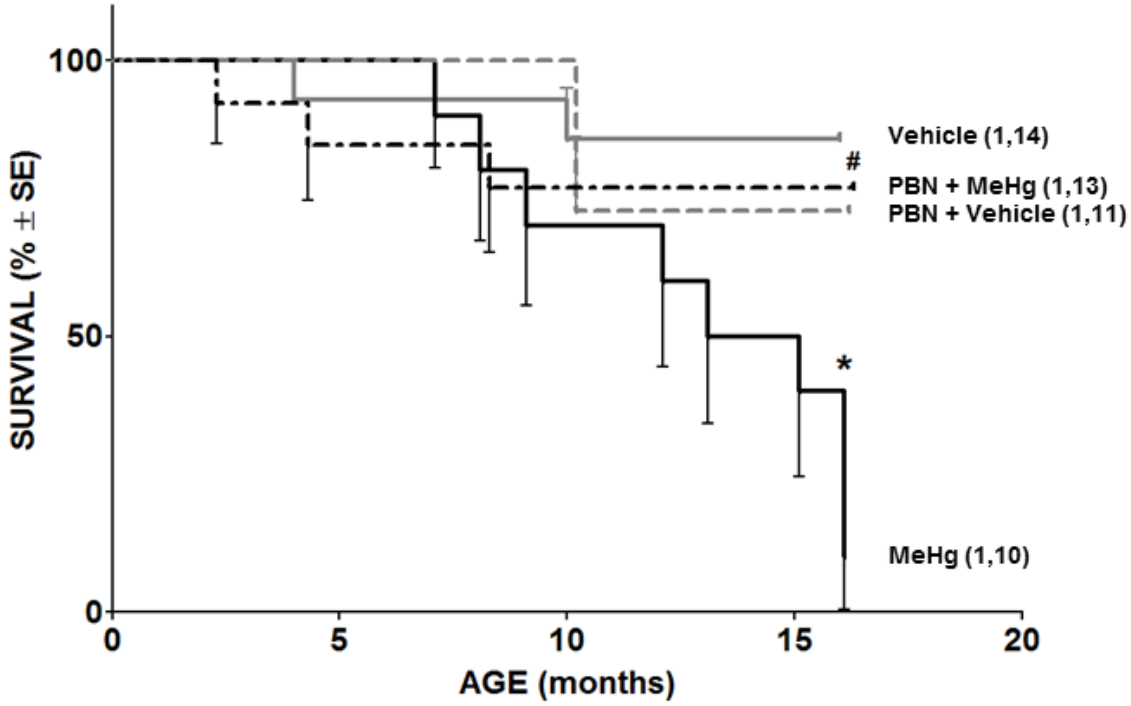

Figure 3.5: PBN pretreatment blocks the decrease in lifespan caused by *in utero* exposure to MeHg. Pregnant dams were injected i.p. on GD 17 as described in fig. 1. The dams were allowed to deliver spontaneously, and the offspring were weaned at 3 weeks of age. Animals were monitored for survival to 16 months of age.

* indicates a difference from vehicle controls ($p < 0.0125$, Mantel-Cox test). # indicates a difference from the group exposed to MeHg alone ($p < 0.0125$, Mantel-Cox test). (x,y) = number of litters, number of offspring

3.2 Mice deficient in OGG1 are more susceptible to cognitive deficits after *in utero* MeHg exposure

Aaron Shapiro assisted with the HPLC with tandem MS for the 8-oxodGuo study. Hannah Choi, Hudson Liao, and Nestor Sabat assisted with staining the immunohistochemical slides.

3.2.1 *Ogg1* genotype affects DNA oxidation levels, but MeHg does not

8-oxodGuo levels in whole fetal brain samples were measured to determine whether they would be changed by MeHg treatment or *ogg1* genotype. *Ogg1* genotype was a significant factor in increasing 8-oxodGuo levels in the fetal brain (2-way ANOVA, $p=0.0033$), but neither MeHg treatment nor the interaction of genotype and treatment were significant (2-way ANOVA, $p > 0.05$) (**fig. 3.6**). DNA oxidation levels in the *ogg1* -/- mice (140 ± 65 fmol 8-oxodGuo/μg dGuo) were 2.4-fold higher than in the wild-type mice (59 ± 32 fmol 8-oxodGuo/μg dGuo).

3.2.2 Neither *ogg1* genotype nor MeHg treatment affect novel object recognition

Cognitive deficits due to *ogg1* genotype, whether alone or in combination with MeHg treatment, were measured using the object recognition test. The factors treatment, genotype, and interaction were not significant (2-way ANOVA, $p > 0.05$), indicating that neither MeHg treatment nor *ogg1* genotype caused a deficit in novel object recognition (**fig. 3.7**). The scores for the vehicle-treated (+/+: 59 ± 7%, +/-: 62 ± 11%, -/-: 63 ± 10%) and the MeHg-treated (+/+: 62 ± 12%, +/-: 59 ± 15%, -/-: 56 ± 15%) groups suggest that

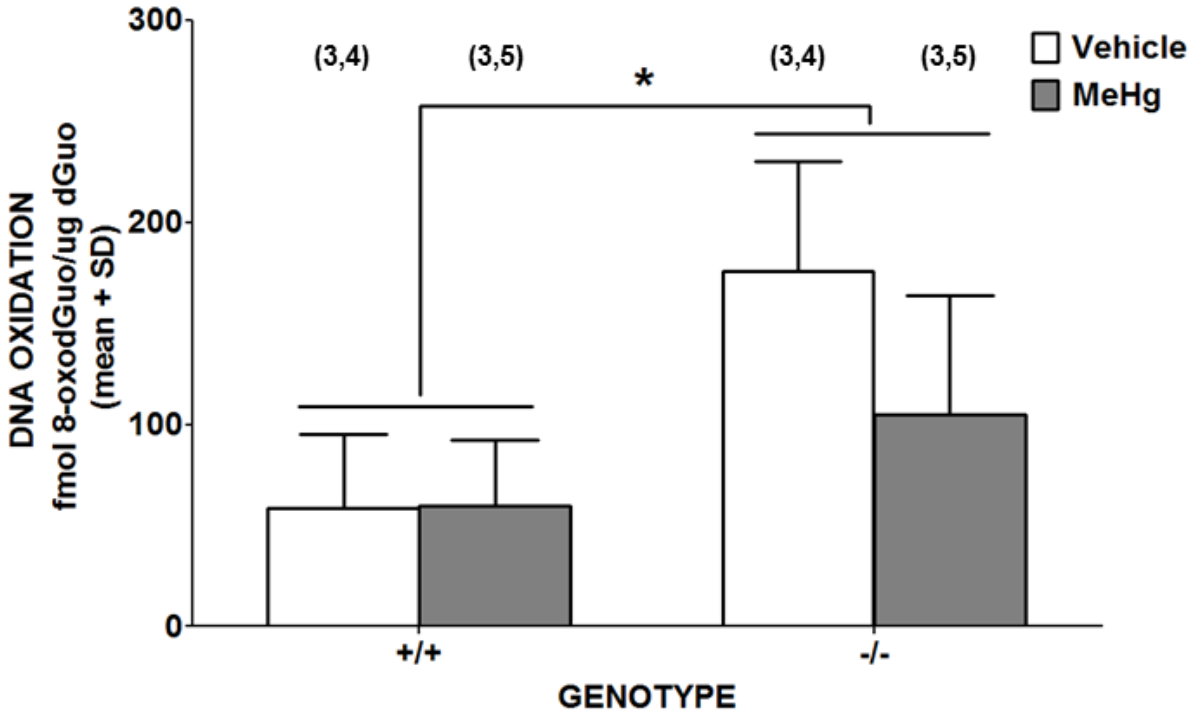

Figure 3.6: *Ogg1* -/- fetuses have higher endogenous brain levels of 8-oxodGuo, but there is no increase after *in utero* MeHg exposure.
Pregnant dams were injected i.p. on GD 17 with 4 mg/kg MeHg or vehicle and sacrificed 6 hr later. Fetal brains were collected and analyzed for DNA oxidation, represented by 8-oxodGuo levels.
* indicates a difference from +/+ samples (p = 0.033, 2-way ANOVA). (x,y) = number of litters, number of fetuses

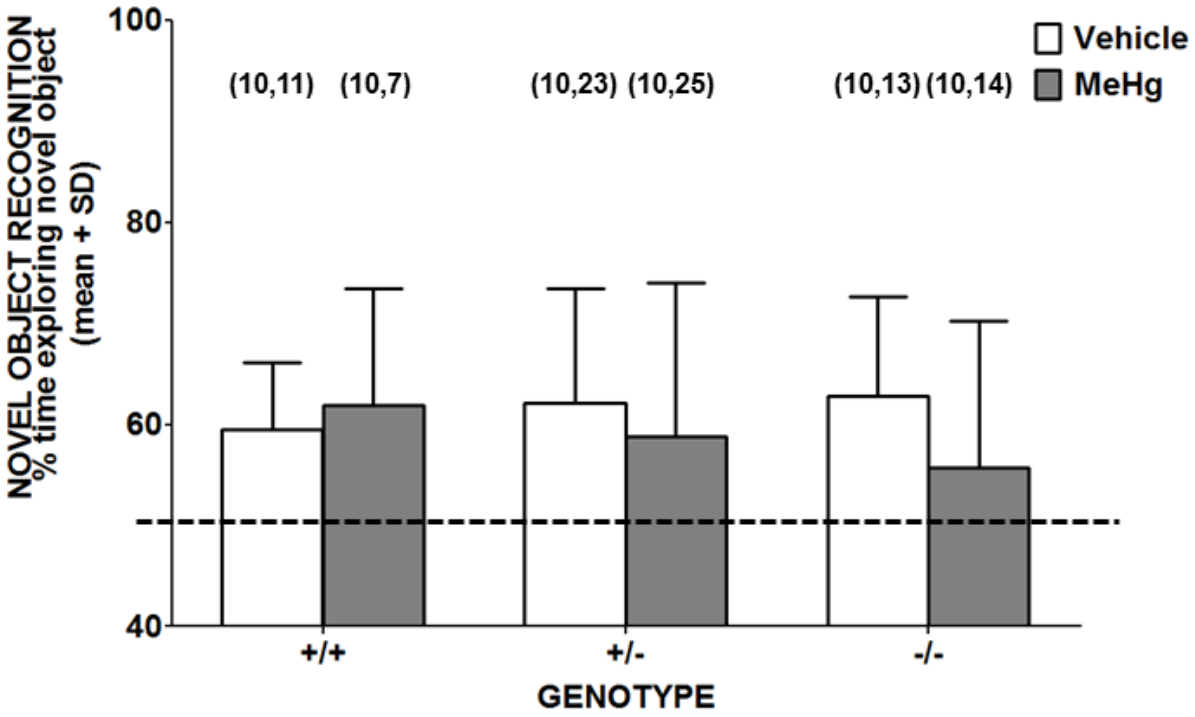

Figure 3.7: Neither *ogg1* genotype nor *in utero* MeHg treatment result in cognitive deficits in novel object recognition.

Pregnant dams were injected i.p. on GD 17 with 4 mg/kg MeHg or vehicle and allowed to spontaneously deliver. Offspring were weaned at 3 weeks of age and evaluated in the object recognition test at 6 weeks of age. Scores indicate the proportion of time the animals spent exploring the novel object, compared to the cumulative exploration time of the novel and familiar objects. The dashed line indicates a score of 50, the expected score for a maximally impaired animal. No differences in performance were seen either due to MeHg treatment or *ogg1* genotype.
(x,y) = number of litters, number of offspring

the animals were capable of distinguishing the novel object. The expected score for a cognitively impaired animal would be 50%, indicating equal time spent exploring the novel and familiar objects, or in other words, a complete inability to distinguish the novel object from the familiar. Furthermore, no differences in score were observed in a comparison of the OGG1-deficient (+/- and -/-) vehicle-treated and OGG1-deficient MeHg-treated animals (data not shown; 2-way ANOVA, $p > 0.05$), suggesting that under these conditions, there is no cumulative or synergistic deficit caused by deficient DNA repair and MeHg treatment. When the data were stratified by sex, neither males nor females were uniquely susceptible to modulation by either *ogg1* genotype or MeHg treatment (data not shown; 2-way ANOVA, $p > 0.05$).

3.2.3 Passive avoidance performance is affected in MeHg-treated *ogg1* deficient animals

To further elucidate what effect, if any, *ogg1* genotype, alone or in combination with MeHg, would have on cognition, the mice were also tested using the passive avoidance test. No effect of *ogg1* genotype, MeHg treatment, or the interaction of treatment and genotype on passive avoidance was seen using a 2-way ANOVA. (**fig. 3.8**). However, the MeHg-treated *ogg1* +/- animals (219 ± 109 s) performed significantly more poorly than the vehicle-treated *ogg1* +/- animals (269 ± 71 s) ($p = 0.034$, Student's t-test).

To further elucidate the effects of *ogg1* genotype and MeHg treatment, the data were stratified to compare the MeHg- (267 ± 58 s) and vehicle-treated (253 ± 85 s) wild-type mice with the OGG1-deficient (+/- and -/-) MeHg-treated mice (209 ± 106 s) and OGG1-

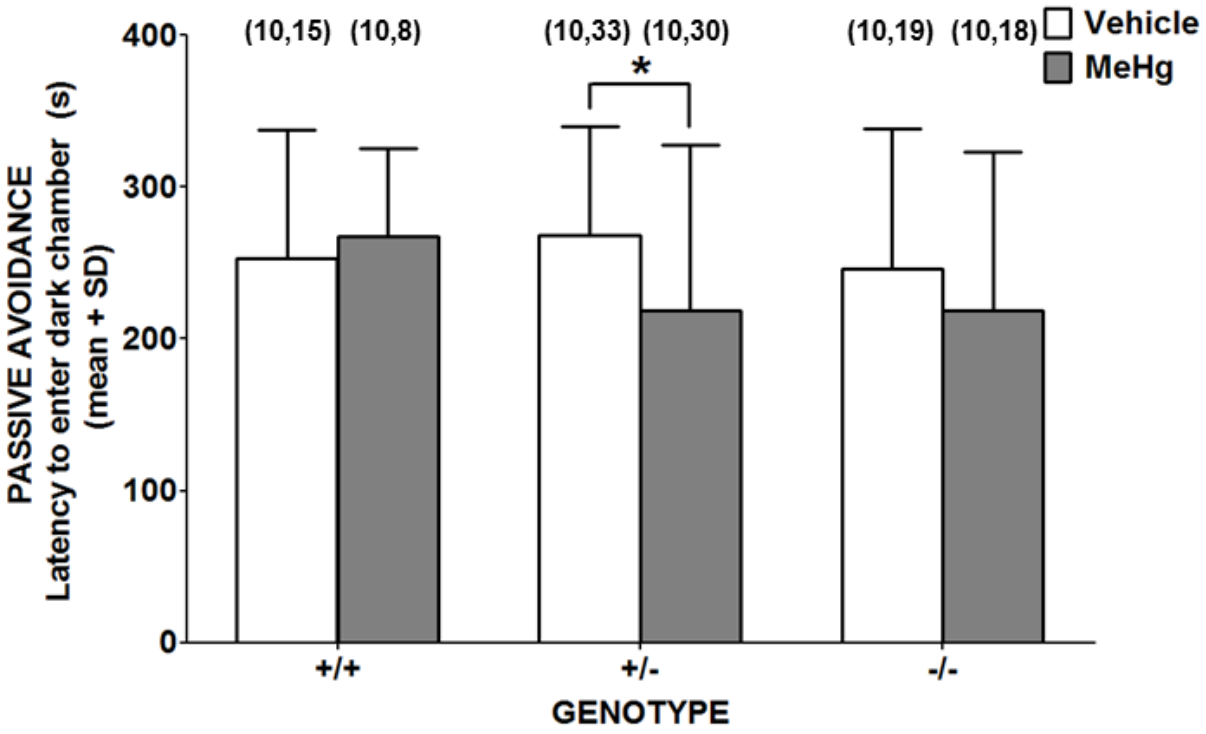

Figure 3.8: *Ogg1* +/- animals treated with MeHg *in utero* have cognitive deficits in passive avoidance. Pregnant dams were injected i.p. with 4 mg/kg MeHg or vehicle on GD 17 and allowed to spontaneously deliver. Offspring were weaned at 3 weeks of age and evaluated in the passive avoidance test at 4 months of age. Scores indicate latency to enter the dark chamber on the fifth trial. No differences in score were observed due to *ogg1* genotype or MeHg treatment by 2-way ANOVA. However, in the *ogg1* +/- mice only, the MeHg-treated animals exhibited a cognitive deficit compared to the vehicle-treated animals.
* indicates a difference from vehicle controls of the same genotype ($p = 0.0341$, Student's t-test).
(x,y) = number of litters, number of offspring

deficient vehicle-treated mice (260 ± 79 s) (**fig. 3.9**). Although the factors genotype, treatment, and interaction were not significant according to analysis by 2-way ANOVA, a post-hoc Bonferonni test revealed that the MeHg-treated OGG1 deficient animals performed significantly more poorly than the vehicle-treated OGG1 deficient animals ($p > 0.05$), providing the first *in vivo* evidence that a deficiency in the repair of oxidative DNA damage may result in increased susceptibility to MeHg toxicity.

When the data were stratified by sex, the males demonstrated no modulation by *ogg1* genotype or MeHg treatment (data not shown; 2-way ANOVA, $p > 0.05$). As there was no difference between the scores of the female MeHg-treated and vehicle-treated *ogg1* wild-type progeny (277 ± 52 s and 269 ± 65 s, respectively), nor between the female MeHg-treated and vehicle-treated *ogg1* -/- progeny (185 ± 119 s and 183 ± 133 s, respectively), the data for each pair were combined and stratified by genotype, regardless of treatment. The *ogg1* -/- females (184 ± 120 s) performed significantly more poorly than the *ogg1* +/+ females (271 ± 59 s), with a 32% decrease in the latency to enter the dark chamber (1-way ANOVA with post-hoc Dunnett's test, $p = 0.05$) (**fig. 3.10**), providing the first evidence that, regardless of treatment, *ogg1* -/- female mice are uniquely susceptible to cognitive deficits. There was also a trend towards the MeHg-treated *ogg1* +/- females performing more poorly than the vehicle-treated *ogg1* +/- females (student's t-test, $p = 0.075$), again suggesting that DNA oxidation may be involved in the mechanism of toxicity of MeHg.

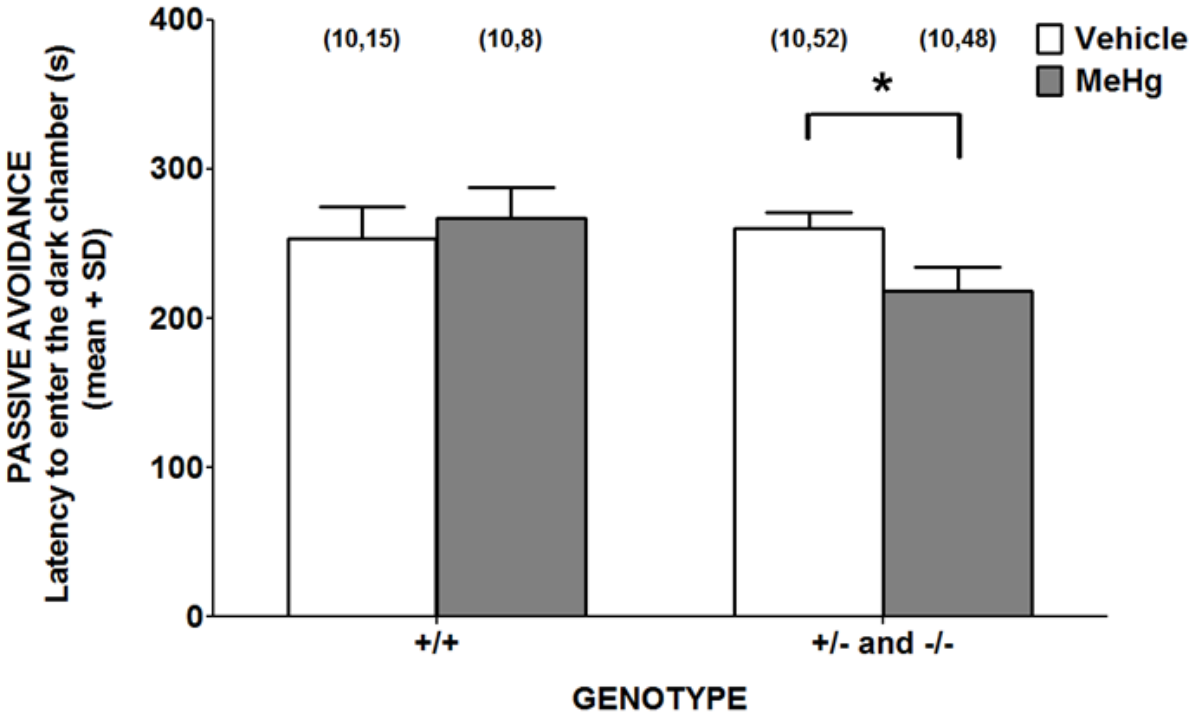

Figure 3.9: OGG1-deficient animals treated with MeHg *in utero* have cognitive deficits in passive avoidance.

Scores indicate latency to enter the dark chamber on the fifth trial. No differences in score were observed due to *ogg1* genotype or MeHg treatment by 2-way ANOVA. MeHg-treated *ogg1* wild-type mice did not exhibit cognitive deficits in passive avoidance compared to vehicle-treated *ogg1* wild-type mice. * indicates a difference from vehicle treated-animals of the same genotype ($p < 0.05$, 2-way ANOVA with post-hoc Bonferonni test). (x,y) = number of litters, number of offspring

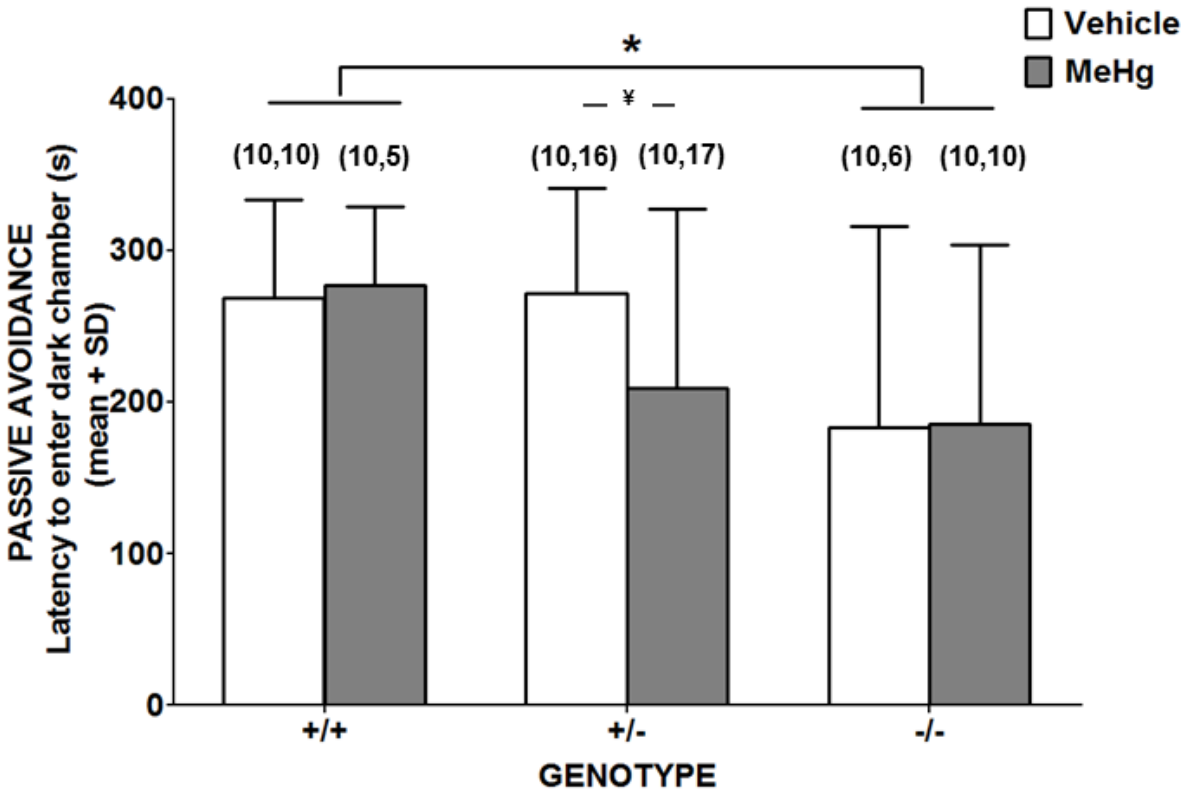

Figure 3.10: Female *ogg1* -/- mice have cognitive deficits in passive avoidance compared to female *ogg1* wild-type mice.

The scores for the female animals were stratified by genotype and treatment to determine if sex-selective cognitive deficits were occurring.

* indicates a difference from +/+ animals, regardless of treatment ($p = 0.05$, 1-way ANOVA). ¥ indicates a trend towards a difference compared to vehicle-treated animals of the same genotype ($p = 0.075$, student's t-test).

(x,y) = number of litters, number of offspring

3.2.4 Neither *ogg1* genotype nor MeHg treatment result in somatosensory changes

von Frey filaments were used to assess somatosensory changes to confirm the cognitive deficit observed in passive avoidance. The factors genotype, treatment, and interaction were not significant (2-way ANOVA, $p > 0.05$) (**fig. 3.11**). The force necessary to elicit a reaction from the animals for the vehicle-treated (+/+: 1.8 ± 0.3 g, +/-: 2.2 ± 1.0 g, -/-: 1.9 ± 0.4 g) and MeHg-treated (+/+: 1.9 ± 0.3 g, +/-: 2.0 ± 0.9 g, -/-: 2.0 ± 1.0 g) animals were virtually identical. Furthermore, no difference was observed in the scores of the OGG1-deficient vehicle-treated (2.1 ± 0.9 g) and OGG1-deficient MeHg-treated animals (2.0 ± 1.0 g) (2-way ANOVA, $p > 0.05$). This suggests that the deficit in passive avoidance observed in the MeHg-treated *ogg1* deficient animals was in fact a deficit in learning and memory, and not due to a difference in tactile sensitivity. Additionally, as no sex-dependent differences were observed, it is likely that the deficit in passive avoidance observed in the females is also due to cognitive deficits, and not differences in tactile sensitivity (data not shown; 2-way ANOVA, $p > 0.05$).

3.2.5 MeHg treatment, but not *ogg1* genotype results in increased apoptosis in the fetal developing hippocampal region

To determine whether *ogg1* genotype, alone or in combination with MeHg treatment, would affect levels of apoptosis in the developing hippocampus, GD 17 coronal brain slices were TUNEL-stained (**fig. 3.12a**). MeHg treatment resulted in a 26% increase in the number of TUNEL-positive cells (+/+: $2.5 \times 10^{-5} \pm 1.0 \times 10^{-5}$ positive cells/μm^2, -/-: $2.1 \times 10^{-5} \pm 8.5 \times 10^{-6}$ positive cells/μm^2) compared to the vehicle-treated samples (+/+: $1.8 \times 10^{-5} \pm 4.1 \times 10^{-6}$ positive cells/μm^2, -/-: $1.7 \times 10^{-5} \pm 6.1 \times 10^{-6}$ positive cells/μm^2),

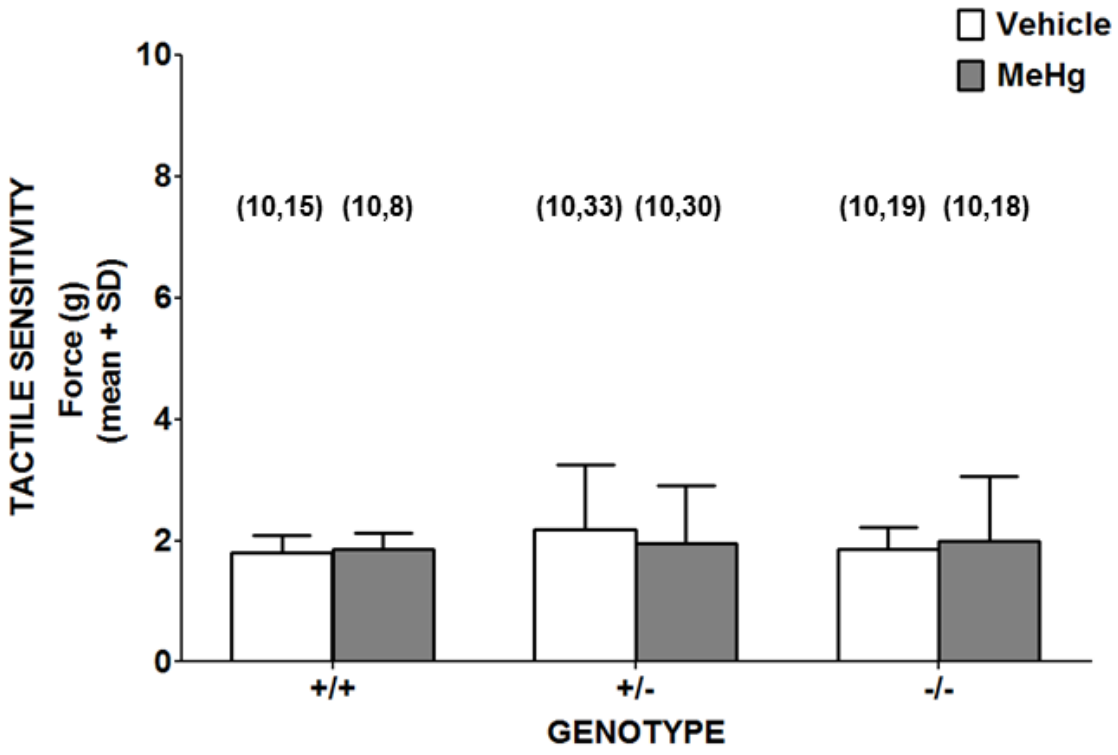

Figure 3.11: Neither *in utero* MeHg treatment nor *ogg1* genotype affect tactile sensitivity.
At 24 hr after passive avoidance testing, animals were tested with von Frey filaments. Scores indicate the force applied to the rear foot that was necessary to elicit a response. No changes in tactile sensitivity were observed due to MeHg treatment or *ogg1* genotype. (x,y) = number of litters, number of offspring

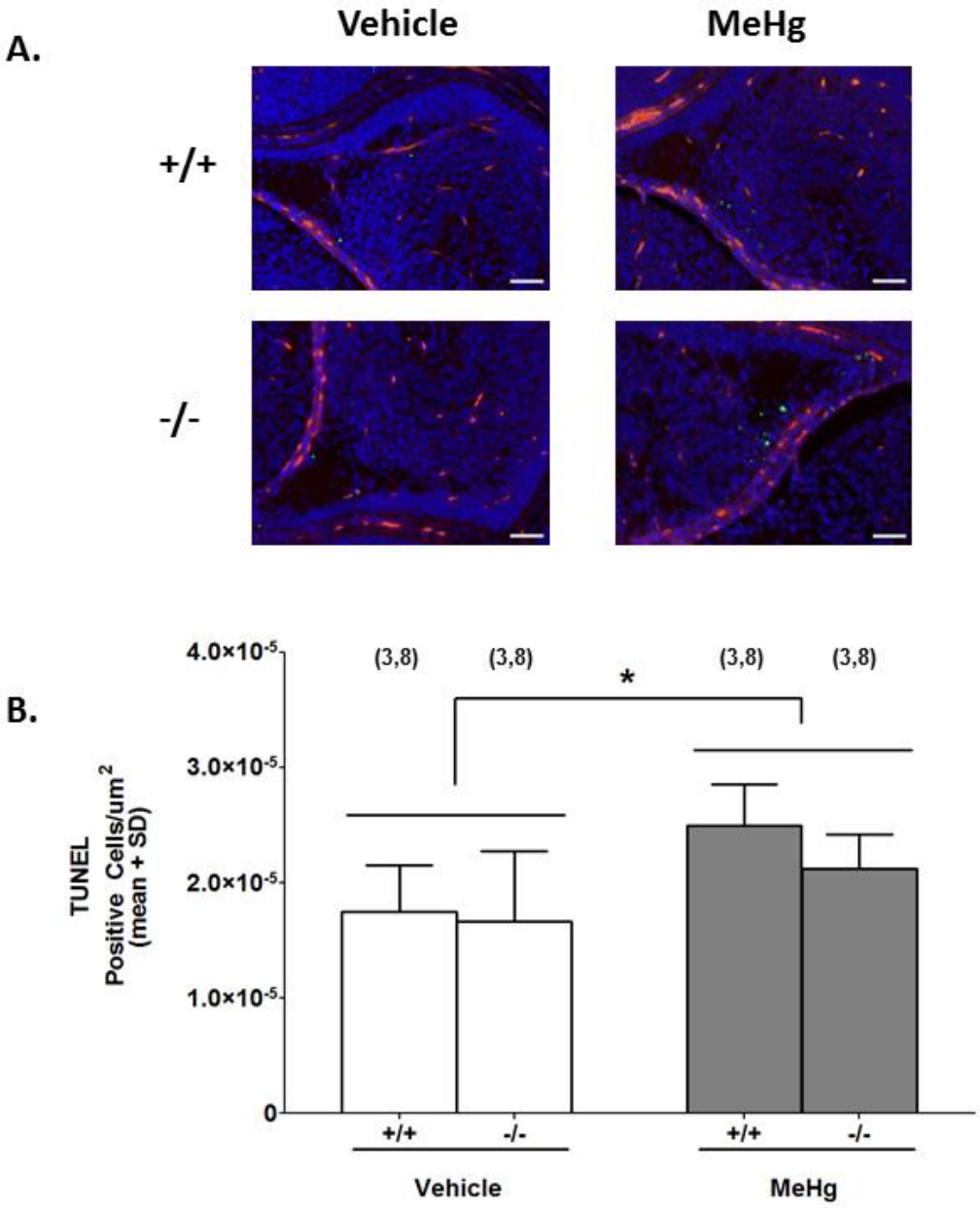

Figure 3.12: *In utero* MeHg exposure, but not *ogg1* genotype, causes increased apoptosis in the developing hippocampal region.

Pregnant dams were injected i.p. with 4 mg/kg MeHg or vehicle on GD 17, and sacrificed 6 hr later. The fetal brains were collected and fixed in 4% paraformaldehyde prior to being embedded in paraffin. **A.** Apoptosis, represented by TUNEL staining, was measured in the developing hippocampal region. Green staining indicates TUNEL; blue staining indicates DAPI; pink staining is non-specific. Scale bar = 50 µm. **B.** * indicates a difference from vehicle-treated samples (p = 0.033, 2-way ANOVA). (x,y) = number of litters, number of fetuses

regardless of *ogg1* genotype in the developing hippocampal area (**fig. 3.12b**) (2-way ANOVA, p = 0.033). No difference was observed due to *ogg1* genotype, and no significant interaction between MeHg treatment and *ogg1* genotype was observed by 2-way ANOVA. These results indicate, not only that MeHg causes increased apoptosis in the developing hippocampal area, but also that, at this developmental stage, the *ogg1* genotype does not appear to result in increased apoptosis in the developing hippocampal area.

3.2.6 MeHg treatment, but not *ogg1* genotype, may result in increased DNA double-strand breaks in the fetal developing hippocampal region

To determine if levels of DNA DSBs are affected by *ogg1* genotype, with or without MeHg treatment, GD 17 coronal brain slices were analyzed for γH2AX levels in the developing hippocampal region (**fig. 3.13a**). After DNA DSBs occur, histone H2AX is phosphorylated at the serine139 position at the damage site, resulting in γH2AX. The *ogg1* -/- (vehicle: $1.1 \times 10^{-5} \pm 4.9 \times 10^{-6}$ positive cells/μm^2, MeHg: $1.6 \times 10^{-5} \pm 9.1 \times 10^{-6}$ positive cells/μm^2) animals did not have an increased number of γH2AX-positive cells compared to the *ogg1* +/+ animals (vehicle: $9.0 \times 10^{-6} \pm 4.8 \times 10^{-6}$ positive cells/μm^2, MeHg: $1.6 \times 10^{-5} \pm 1.3 \times 10^{-5}$ positive cells/μm^2). However, similarly to our TUNEL results, we found that there was a trend towards MeHg treatment resulting in a 60% increase in γH2AX staining, suggesting that MeHg may cause DNA DSBs in the developing hippocampal area (**fig. 3.13b**) (2-way ANOVA, p = 0.086). No difference or trend towards a difference was observed due to *ogg1* genotype, suggesting that, at this

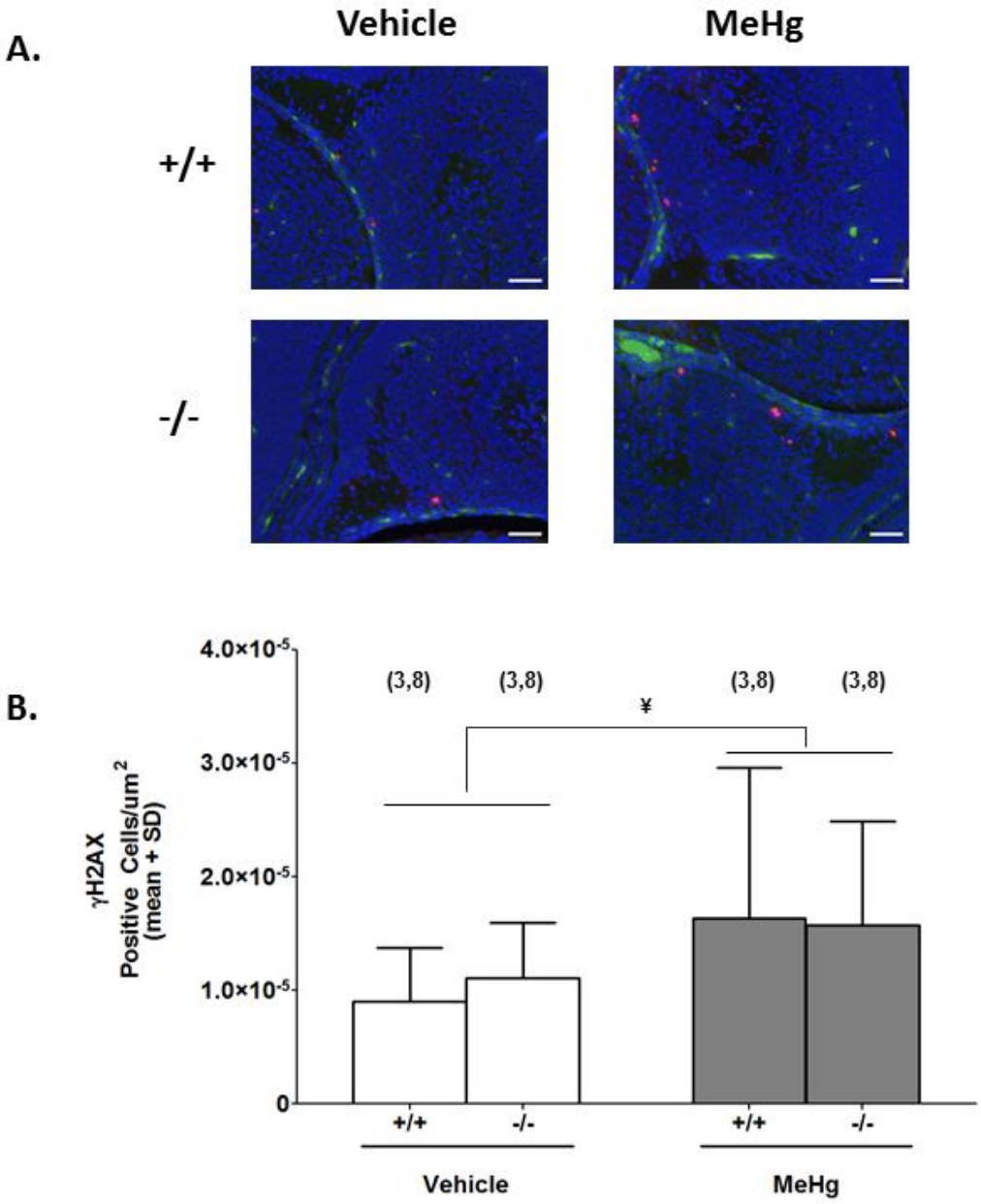

Figure 3.13: *In utero* MeHg exposure may cause increased DNA DSBs in the developing hippocampal region.

Pregnant dams were injected i.p. with 4 mg/kg MeHg or vehicle on GD 17, and sacrificed 6 hr later. The fetal brains were collected and fixed in 4% paraformaldehyde prior to being embedded in paraffin. **A.** DNA DSBs, represented by γH2AX, were measured in the developing hippocampal region. Pink staining indicates γH2AX; blue staining indicates DAPI; green staining is non-specific. Scale bar = 50 µm. **B.** No significant changes in DNA damage were observed due to *ogg1* genotype or MeHg treatment. However, similarly to the TUNEL results, there was a trend towards MeHg treatment resulting in increased DNA damage, regardless of treatment (¥, p = 0.086, 2-way ANOVA). (x,y) = number of litters, number of fetuses

developmental stage, a lack of OGG1 does not result in increased DNA DSBs in the developing hippocampal area.

3.2.7 *Ogg1* -/- mice exhibit higher numbers of newly replicated cells in the fetal developing hippocampal region, but this is reduced by MeHg

To determine whether *ogg1* genotype, with or without MeHg treatment, affects cellular proliferation or migration in the developing hippocampus, pregnant dams were injected with BrdU and the fetal brains were collected and analyzed for BrdU expression in coronal slices around the developing hippocampal region (**fig. 3.14a**). While neither the factor genotype nor treatment were significant (2-way ANOVA, $p > 0.05$), the interaction of *ogg1* genotype and MeHg treatment had a significant effect on the concentration of BrdU-positive cells in the area of the developing hippocampus (2-way ANOVA, $p = 0.034$). The vehicle-treated *ogg1* -/- samples exhibited a 41% increase in the concentration of positive cells ($2.4 \times 10^{-4} \pm 9.2 \times 10^{-5}$ positive cells/μm^2) compared to vehicle-treated *ogg1* wild-type samples ($1.7 \times 10^{-4} \pm 3.0 \times 10^{-5}$ positive cells/μm^2) (student's t-test, $p = 0.0094$). MeHg-treatment resulted in a 29% decrease in the concentration of positive cells in the *ogg1* -/- samples ($1.7 \times 10^{-4} \pm 2.6 \times 10_{-5}$ positive cells/μm^2) (student's t-test, $p = 0.004$) (**fig. 3.14b**).

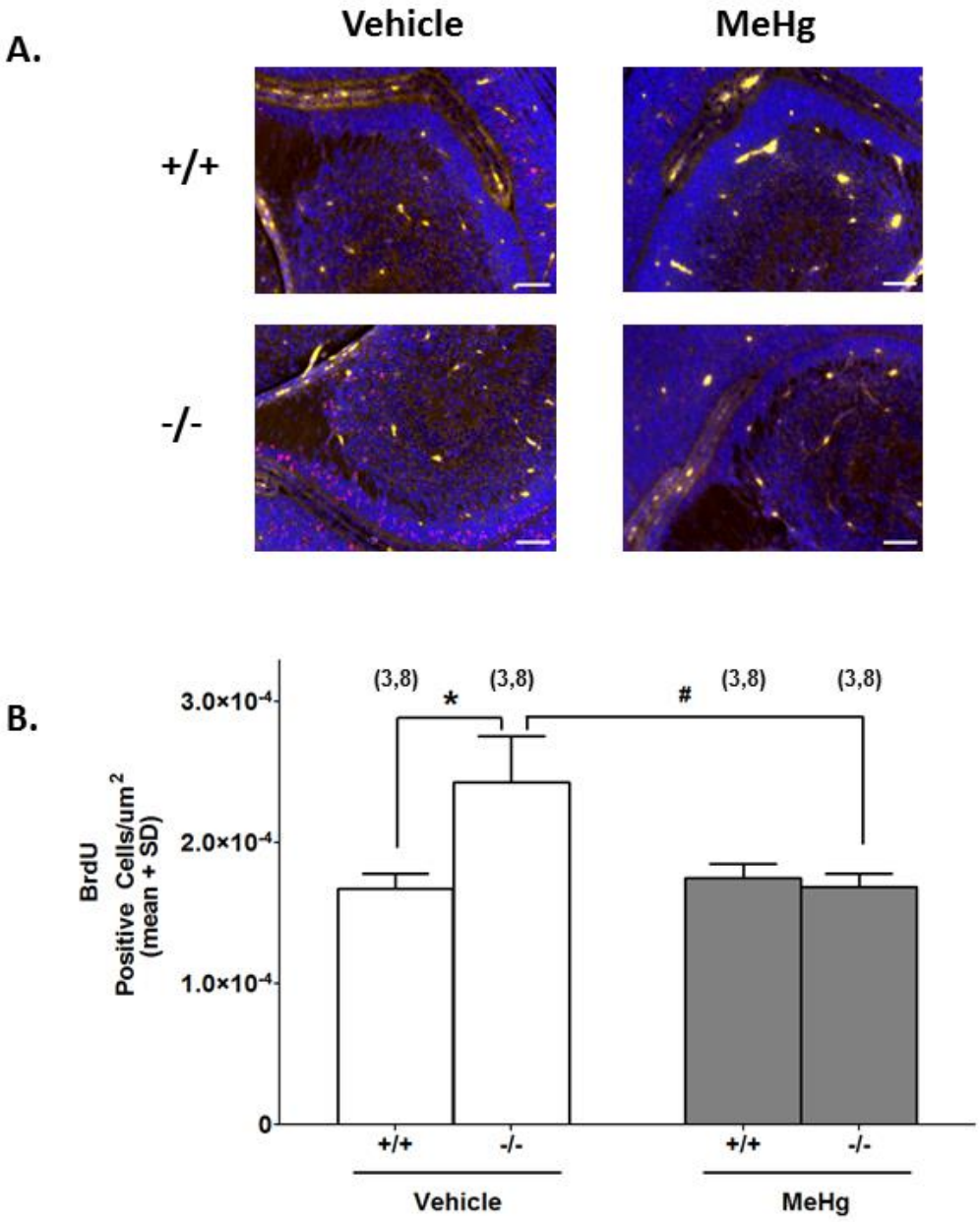

Figure 3.14: *Ogg1* -/- fetuses have increased cellular proliferation or migration to the developing hippocampal region, an effect which is blocked by *in utero* MeHg exposure.
Pregnant dams were injected i.p. with 4 mg/kg MeHg or vehicle on GD 17, and sacrificed 6 hr later. The fetal brains were collected and fixed in 4% paraformaldehyde prior to being embedded in paraffin. **A.** Cellular proliferation, represented by BrdU staining, was measured in the developing hippocampal region. Pink staining indicates BrdU; blue staining indicates DAPI; yellow staining is non-specific. Scale bar = 50 µm. **B.** The interaction of *ogg1* genotype and MeHg treatment was significant ($p = 0.034$, 2-way ANOVA). # indicates a difference from vehicle control of the same genotype ($p = 0.004$, Student's t-test). * indicates a difference from wild-type samples with the same treatment ($p = 0.0093$, Student's t-test). (x,y) = number of litters, fetuses

Section 4: Discussion

The results from my MeHg and PBN studies following *in utero* exposure provide the first *in vivo* evidence that the free radical spin trapping agent PBN can protect against the neurodevelopmental toxicity of MeHg, suggesting a role for ROS in the pathogenic mechanism. Moreover, I provided evidence that MeHg exposure *in utero* may reduce lifespan, an effect that also appears to involve ROS, as the effect on lifespan was ameliorated by maternal PBN treatment. This is the first evidence in an animal model reflecting the reduced lifespan informally reported for humans exposed to higher levels of MeHg (Tamashiro *et al.*, 1984; Harada, 1995; Harada *et al.*, 2005). However, it was unclear whether DNA oxidation was involved, particularly since the levels of the specific 8-oxodGuo lesion in my whole fetal brain samples remained unchanged after MeHg exposure.

My studies of the effects of *in utero* MeHg exposure on mice deficient in OGG1, a major DNA repair enzyme, provided the most direct *in vivo* evidence to date that DNA oxidation may be involved in the mechanism of toxicity of MeHg exposure *in utero*. I also provided the first evidence that female *ogg1* -/- mice are innately more prone to cognitive deficits, demonstrating that DNA repair in the fetus is crucial for normal neurodevelopment.

4.1 MeHg and PBN

4.1.1 MeHg, PBN, and Cognition

In these studies, I have shown that a single *in utero* exposure to a low dose of MeHg during gestation is sufficient to cause long-term postnatal cognitive deficits. Specifically, MeHg causes cognitive deficits as measured by the object recognition test and the passive avoidance test, although only the deficit in the object recognition test was blocked by maternal PBN pretreatment. A single, low dose was chosen as a "threshold" model, instead of a subchronic or chronic dosing regimen, which is more akin to common human exposures. This threshold approach was used to facilitate a focus on mechanisms of teratological initiation, avoiding the potential confounding effects due to the activation of secondary and tertiary pathways. Using a relatively low dose of MeHg also allowed me to focus on sensitive pathogenic events, avoiding the confounding effects of additional mechanisms recruited at higher exposures to MeHg that are less relevant to our lower dietary exposure levels. The dose used is equal to, or lower than that used in many other neurodevelopmental studies in rodent models (Kakita *et al.*, 2000; Doré *et al.*, 2001; Goulet *et al.*, 2003). Although psychomotor deficits are among the first effects of *in utero* MeHg exposure found in humans, we chose not to employ the rotarod to test motor coordination in mice as other investigators have found that exposure *in utero* to cumulative doses of MeHg 3 – 10 fold greater than the dose we employed in mice resulted in cognitive deficits, but not motor coordination deficits, in the adult offspring (Doré *et al.*, 2001; Goulet *et al.*, 2003; Stringari *et al.*, 2006)

The ability of maternal administration of the free radical spin trap PBN to prevent the MeHg-initiated cognitive deficit in object recognition, but not in passive avoidance, suggests several possibilities for the mechanism of toxicity. The two most obvious are that PBN does not act within a MeHg-affected brain region or pathway that is necessary for learning and memory in passive avoidance, unlike that for object recognition; or, that PBN acts within a region or pathway that is necessary for object recognition, but not passive avoidance. Further to this second possibility is that PBN may act within a MeHg-affected region or pathway that is necessary for both tests, but not to a sufficient extent to restore the deficit seen in passive avoidance. Another possibility is that the MeHg-initiated deficit in passive avoidance involves a ROS-independent mechanism.

The amygdala has previously been shown to be crucial for passive avoidance learning, as both chemical and surgical lesions of the amygdala result in acquisition deficits (Russo *et al.*, 1976; Dunn & Everitt, 1988; Roozendaal *et al.*, 1993). Furthermore, prenatal MeHg exposure in rats can result in decreased neuron number in the amygdala (Kakita *et al.*, 2000). Although PBN has been shown to protect against neuronal lesions in the amygdala in an epilepsy model in adult rats, the dose of PBN was 3-fold higher than that used in my studies (Peterson *et al.*, 2005). It is therefore possible that we would have seen protection against MeHg-initiated passive avoidance deficits if a higher dose of PBN had been used. Another potential target in passive avoidance is the dentate gyrus, as neuronal number in the dentate gyrus appears to be more highly correlated with passive avoidance performance than novel object recognition in a study of traumatic brain injury in mice (Zhao *et al.*, 2012). It is unclear how effective PBN is in

protecting the dentate gyrus, as opposing results have been reported in various rat models (Leib *et al.*, 1996; Hossmann *et al.*, 2001; Loeffler *et al.*, 2001). Neonatal models of meningitis have shown that cumulative doses between 200 and 600 mg/kg protect against apoptosis in the dentate gyrus and possibly protected against increases in superoxide around the dentate gyrus (Leib *et al.*, 1996). Subchronic administration of relatively high doses of PBN, resulting in a total dose of 1500 mg/kg over a period of 5 days, actually resulted in increased apoptosis in the dentate gyrus of neonatal rats infected with meningitis (Loeffler *et al.*, 2001). This cumulative dose, however, was more than 20-fold higher than the dose I employed, and may have little to no relevance to my studies. However, the protective effects of PBN may decrease in adult rats, as injections of 300 mg/kg PBN had only equivocal protective effects against ischemia-initiated changes in the dentate gyrus (Hossmann *et al.*, 2001). Another study of ischemia in rats using a cumulative dose of 30 mg/kg PBN, closer to the dose I employed, found protection against neuronal death in the CA1 and CA3 regions of the hippocampus, but only a non-significant protection in the dentate gyrus (Li *et al.*, 2001). Generally, however, the doses of PBN used in these studies far exceeded my dose of 45 mg/kg. I chose to use a low dose of PBN to avoid any confounding results due to other effects aside from trapping ROS, discussed in section 1.2.4.1.1. The dose used in these studies was based on previous studies from our lab which showed that PBN pretreatment decreased protein and lipid oxidation levels caused by ROS-initiating teratogens at a dose that alone caused no apparent maternal or fetal toxicity (Liu & Wells, 1994).

A 200 mg/kg dose of PBN was reported to reduce a passive avoidance deficit and hippocampal levels of lipid peroxidation in a mouse sleep deprivation model in adult mice (Silva *et al.*, 2004), supporting the possibility that my dose of PBN was insufficient to prevent the passive avoidance deficit. Furthermore, in other rodent models, doses of 100 mg/kg PBN have been able to prevent deficits in passive avoidance performance in neonatal rat models of neuroinflammation (Fan *et al.*, 2008; Fan *et al.*, 2010). Previous studies in mice have shown that passive avoidance performance is more sensitive than object recognition performance to mild traumatic brain injury, suggesting that a dose of PBN that is sufficient to protect against deficits in object recognition may be insufficient to rescue passive avoidance behaviour (Zhao *et al.*, 2012). Both prenatal and perinatal exposure to MeHg is known to decrease hippocampal size and cell number, which may be the root cause of the resulting cognitive deficits (Kakita *et al.*, 2000; Falluel-Morel *et al.*, 2007). PBN has been shown to protect against apoptosis and toxicity in the hippocampus, albeit often at higher doses than that used in my study (Leib *et al.*, 1996; Fan *et al.*, 2008). It is therefore possible that the dose of PBN I used prevented hippocampal damage sufficiently to rescue novel object recognition, but not passive avoidance, even though both may have resulted from a ROS-initiated mechanism.

It is likely that the deficits we observed in the passive avoidance test are due to a deficit in learning and memory, and are not confounded by somatosensory deficits, as no deficit in tactile sensitivity was detected by the von Frey test. This confirmation is generally lacking in MeHg studies, and is important given that digital numbness is one of the first signs of MeHg poisoning in adults (Ninomiya *et al.*, 2005; Takaoka *et al.*,

2008). Thus, my results provide the first evidence that, at least for *in utero* MeHg exposure, cognitive deficits exhibit a measurably lower threshold than that for somatosensory deficits.

4.1.2 MeHg, PBN, and DNA Oxidation

Previous *in vitro* studies have suggested that PBN is capable of partially protecting against MeHg-initiated increases in ROS formation in cultured astrocytes (Shanker & Aschner, 2003). However, it is important to note that decreasing ROS levels may not be the only mechanism by which PBN acts. In fact, there is rarely any direct evidence that PBN is affecting ROS, and it appears to have many other possible effects. A 100 mg/kg dose of PBN has been shown to reduce lesion size in the dentate gyrus of adult rats after intrahippocampal injections with β-amyloid peptide fragments, although it had no effect on increased lipid peroxidation after injection of both β-amyloid and iodoacetate (Montiel *et al.*, 2006). An identical dose in neonatal rats in a model of neuroinflammation prevented impaired neurological reflexes and improved performance in a modified passive avoidance test, as well as protecting against neuronal loss in the CA1 (Fan *et al.*, 2010). However, as the authors did not analyze levels of ROS or oxidative damage, it is difficult to determine whether these protective effects were due to ROS trapping, or whether PBN protects against neuroinflammation by other mechanisms. A study of glutamate efflux in the ventral tegmental area following a low dose of the ROS-initiating drug amphetamine found no increase in hydroxyl radical levels, although 60 mg/kg PBN still eradicated the increase in glutamate efflux and prevented behavioural sensitization (Wolf *et al.*, 2000).

Other studies have found that MeHg exposure increases 8-oxodGuo levels *in vitro* and in *in vivo* adult rat cerebral cortex (Belletti *et al.*, 2002; Xu *et al.*, 2012). Another study found that urinary and serum Hg levels were positively correlated with urinary 8-oxodGuo levels (Chen *et al.*, 2005). However, as the subjects of this study were exposed to Hg in their workplaces, the extent to which MeHg was responsible for this correlation is unclear. Previous *in vitro* studies in our laboratory provided evidence for a role for OGG1 in protecting against MeHg-initiated apoptosis and cell cycle disruption (Ondovcik *et al.*, 2012; Ondovcik *et al.*, 2013a; Ondovcik *et al.*, 2013b). Contrary to my expectations and my previous *in vivo* pilot study (**Appendix 1, fig. A1.1**) suggesting that I had chosen an appropriate time point for peak 8-oxodGuo levels initiated by MeHg, I did not find an increase in 8-oxodGuo levels in fetal brain after MeHg exposure. As I was able to detect measurable increases in 8-oxodGuo formation in my study with DNA repair-deficient *ogg1* -/- mice, which contained samples run concurrently with these samples, it is unlikely that my method of quantification did not have the sensitivity to detect a change in 8-oxodGuo levels in these fetal brains (**Appendix 1, fig. A1.4**). Furthermore, as my baseline measurements of 8-oxodGuo in control animals were 2 fold lower with our new protocol using HPLC with tandem MS compared to HPLC with electrochemical (**EC**) detection, it appears that our new protocol may be more sensitive and accurate. However, further study, perhaps using the immunohistochemical approach discussed in section 4.4, is necessary to confirm that MeHg did not cause increases in 8-oxodGuo in fetal brain.

My current results do not preclude a pathogenic role for MeHg-initiated DNA oxidation, particularly as I did find that, after MeHg exposure, OGG1-deficient animals displayed cognitive deficits that were not exhibited by similarly exposed *ogg1* wild-type animals. A potential limitation to my study was that I was only able to study 8-oxodGuo levels in the whole fetal brain. It is possible that MeHg caused tissue-, cell- or gene-specific increases in oxidative DNA damage in brain target tissues, which were masked by a global lack of change in the brain. Previous studies in our laboratory have shown that in adult mice, different brain regions have substantially different baseline levels of 8-oxodGuo, and also have variable increases in 8-oxodGuo level after exposure to a ROS-initiating xenobiotic (Jeng *et al.*, 2006). It is also possible that oxidative DNA damage and OGG1 may play a role in modulating MeHg toxicity, but 8-oxodGuo is not the appropriate lesion to study despite its apparent role in neurodevelopmental deficits caused by *in utero* exposure to ROS-initiating drugs like amphetamine analogs. OGG1 is also crucial in repairing 2,6-diamino-4-hydroxy-5-formamidopyrimidine (**fapyG**), a ring-opened lesion resulting from oxidative damage (Hu *et al.*, 2005; Krishnamurthy *et al.*, 2008). While fapy lesions are less studied than 8-oxoG, they can be mutagenic and are associated with mild cognitive impairment and Alzheimer's Disease (Kalam *et al.*, 2006; Wang *et al.*, 2006).

4.1.3 MeHg, PBN, and Lifespan

I have shown that *in utero* exposure to a single dose of MeHg that causes postnatal cognitive defects may also reduce lifespan. Although only one litter was observed for each treatment group, leaving open the possibly confounding "litter effect" of an

anomalous pregnancy, the results are biologically plausible, and are congruent with the outcomes for neurodevelopmental deficits among the different treatment groups. Moreover, the difference in survival was striking, and this is the first evidence in an animal model that a single MeHg exposure *in utero* may have lasting consequences, not only for function or cognition, but also for quality and length of life. To date there have been no human studies that have been able to exhaustively examine whether long-term MeHg exposure decreases lifespan, beyond the initial observations of increased mortality in the first few years after high exposure (Tamashiro *et al.*, 1984; Harada, 1995). However, informal reports suggest that MeHg exposure may result in a shorter lifespan (Harada *et al.*, 2005). Unfortunately, the documentation to confirm that MeHg exposure contributes to decreasing lifespan, and is not simply incidental, does not appear to exist. The majority of the MeHg-treated animals showed no overt signs of distress or morbidity prior to being found dead during routine daily checks, suggesting that the cause of death was not cancer or another form of progressive debilitating disease. It is possible that cardiovascular toxicity contributed to the shorter lifespan observed in the MeHg-treated mice, compared to vehicle controls and MeHg-exposed mice pretreated with PBN. This speculation is supported by a post-mortem examination of one of the mice, which was found with a distended abdomen, apparently caused by a large volume of blood in the abdominal cavity (**Appendix 1, Table A1.1**). This finding suggests that a ruptured vascular aneurysm may have been the cause of death.

There is growing evidence from diverse populations that adult exposure to MeHg is associated with adverse cardiovascular effects, including increased blood pressure,

decreased heart rate variability, increased resting heart rate and myocardial infarction (Choi *et al.*, 2009; Roman *et al.*, 2011; Valera *et al.*, 2013). Moreover, increased blood pressure and decreased heart rate variability have been observed in 7-year old Faroese children after prenatal MeHg exposure, with some effects lasting to 14 years of age (Sørensen *et al.*, 1999; Grandjean *et al.*, 2004).

The apparent ability of PBN pretreatment to prevent a MeHg-initiated decrease in lifespan may be attributed either to a general ability of a single dose of PBN to prolong life, or to a specific effect of PBN in countering MeHg toxicity. Currently, there is no evidence suggesting that a single exposure to PBN *in utero* is capable of prolonging lifespan, and there is conflicting evidence on whether repetitive treatment with PBN or other nitrones are effective at prolonging lifespan (Dubey *et al.*, 1995; Saito *et al.*, 1998; Miller *et al.*, 2007). While one study found that dilute concentrations of PBN in the drinking water of aged mice extended their lifespan by a month over control levels (Saito *et al.*, 1998), another study in fruit flies found that low concentrations of PBN in drinking water did not extend lifespan, while high concentrations significantly shortened lifespan (Dubey *et al.*, 1995). A multi-centre study by the Intervention Testing Program of the National Institute of Aging found that a PBN analog, 4-OH-α-phenyl-N-tert-butylnitrone, had no effect on lifespan in mice (Miller *et al.*, 2007). If the effects of MeHg on lifespan are in fact due to enhanced cardiovascular toxicity, it is possible that maternal pretreatment with a single dose of PBN may protect against the shortened lifespan by blocking MeHg-initiated molecular lesions leading to postnatal alterations in cardiovascular innervation and/or contractility. Most of the current research on the use

of PBN to protect against adverse cardiovascular events is directed at acute effects of PBN in adult models administered after ischemia, reperfusion injury and stroke (review: Floyd *et al.*, 2008). More research, therefore, is required to elucidate the mechanism by which PBN may counter this developmental aspect of MeHg toxicity.

4.1.4 Conclusions

In vitro studies examining the potential protective effects of PBN have provided equivocal results. Concentrations of PBN that partially protect against increases in ROS in astrocytes had no appreciable effect on MeHg-initiated inhibition of glutamate uptake into these cells (Allen *et al.*, 2001; Shanker & Aschner, 2003). I have shown, however, that PBN does appear to protect against some of the cognitive deficits initiated by *in utero* exposure to MeHg.

I have shown in these studies that a single, low dose of MeHg late in gestation can have lasting consequences for both cognition and lifespan. The ability of the free radical spin trap PBN to rescue the resulting cognitive deficits and potentially the shortened lifespan suggest that ROS are involved in the mechanism of toxicity. It remains unclear, however, whether oxidative damage to fetal DNA contributes to the mechanism of these postnatal consequences of *in utero* MeHg exposure.

4.2 MeHg and OGG1

4.2.1 MeHg, OGG1, and DNA Oxidation

As might be expected, the *ogg1* -/- mice had higher levels of 8-oxodGuo in fetal brain compared to wild-type mice. This suggests that cognitive and functional deficits observed after birth may be caused in part by 8-oxodG. These results are contrary to our previous study of these OGG1-deficient animals, in which the vehicle-treated *ogg1* -/- fetuses did not have higher levels of 8-oxodGuo than the vehicle-treated *ogg1* wild-type fetuses (Wong *et al.*, 2008). Since that time, however, we have refined our protocol using HPLC-MS/MS for quantifying 8-oxodGuo, and the 8-oxodGuo levels measured in the vehicle-treated *ogg1* wild-type fetuses for these studies were more than 3-fold lower than those we previously measured. It is possible that our previous assay using HPLC-EC may have lacked the sensitivity required to measure the differences in 8-oxodGuo between the *ogg1* wild-type and *ogg1* -/- fetuses. In any event, MeHg treatment did not increase 8-oxodGuo formation in the whole fetal brains that we analyzed using HPLC-MS/MS.

Given the cognitive deficit observed in the MeHg-treated OGG1-deficient animals, however, it seems likely that DNA oxidation is involved in the mechanism of toxicity of MeHg, despite the apparent lack of increased 8-oxodGuo levels. Furthermore, we have previously shown that *ogg1* -/- mouse embryonic fibroblasts (**MEFs**) are more susceptible to the toxic effects of MeHg than wild-type cells (Ondovcik *et al.*, 2012, 2013b). This suggests that DNA oxidation is involved, even though we did not see increases in 8-oxodGuo levels in our whole fetal brain samples.

As previously discussed, the two possibilities that might account for this apparent contradiction are that 8-oxodGuo increases occur in specific regions of interest, such as the hippocampus, amygdala or septal nuclei, or even particular sites within specific genes, and that I was unable to detect these differences in my whole brain samples; or that a different DNA lesion repaired by OGG1 is responsible for the MeHg-initiated deficits. Previously we have shown that baselines levels of 8-oxodGuo, as well as increases in 8-oxodGuo levels, vary across brain regions in the adult mouse after exposure to an oxidizing agent (Jeng *et al.*, 2006). It is possible that this occurs in the fetal mouse, as well. OGG1 is also vital for repairing fapyG, a ring-opened lesion (Hu *et al.*, 2005; Krishnamurthy *et al.*, 2008; Liu *et al.*, 2011). FapyG is an oxidative lesion that is formed from the same intermediate as 8-oxodG (Munk *et al.*, 2007). While it has not been as extensively researched as 8-oxodG, there is increasing evidence that fapy lesions may be increased in patients with Mild Cognitive Impairment and Alzheimer's Disease (Wang *et al.*, 2005b; Wang *et al.*, 2006).

4.2.2 MeHg, OGG1, and Cognition

The effect of MeHg on cognition in these mice was analyzed using the object recognition test and the passive avoidance test. Using our MeHg treatment regimen, no cognitive deficit in novel object recognition was observed in the *ogg1* -/- mice, nor did there appear to be any cognitive deficit due to deficient DNA repair alone. This is in contrast to my previous studies with CD-1 mice, as well as a study in rats exposed *in utero* to an 8 mg/kg dose, twice the dose that I used (Baraldi *et al.*, 2002). Deficits in

visual recognition have also been correlated with MeHg exposure in infants in the United States (Oken *et al.*, 2005).

However, we were able to detect a significant cognitive deficit in passive avoidance in MeHg-exposed DNA repair-deficient *ogg1* mice lacking either one or both copies of the gene. Although the cognitive deficit initiated by MeHg was small, it is consistent with the subtle nature of the deficits observed in exposed humans (Grandjean *et al.*, 1997; Debes *et al.*, 2006). These results indicate that, despite the lack of global increase in 8-oxodGuo levels in our fetal brain samples, DNA oxidation may play a role in MeHg-initiated neurodevelopmental deficits. This further supports our speculation that the fapyG lesion and/or regional differences in 8-oxodGuo levels may be involved.

These results were in contrast to my previous experiments using this same MeHg dosing regimen in CD-1 mice, which showed that both novel object recognition and passive avoidance performance were adversely affected after MeHg exposure. However, I did find in those studies that pretreatment with PBN restored novel object recognition without restoring passive avoidance performance, as with the OGG1-deficient mice. I speculated that this might be due to PBN blocking ROS effects in a particular region or pathway involved in both tests to a degree that would allow proper novel object recognition, but insufficient to prevent deficits in passive avoidance performance. This possibility is supported by previous research showing that passive avoidance performance appears to be more sensitive than novel object recognition to

mild traumatic brain injury in mice (Zhao *et al.*, 2012). If true, it suggests that in this particular strain of mouse, the injury caused by the combination of MeHg treatment and deficient OGG1 is relatively small. However, it may also be that our results were confounded by inherent characteristics of this mouse strain, which arises from a mixed C57BL/6 and 129SV background. It has been previously shown that 129SV mice exhibit relatively high anxiogenic behaviours, and poorer performance on a number of cognitive tests compared to other inbred strains (Brooks *et al.*, 2005). These inherent characteristics may have contributed to poorer performances by the vehicle-treated animals, and it should be noted that testing parameters were adjusted for the control animals to achieve acceptable threshold performance. This may have resulted in the masking of cognitive deficits in the MeHg-treated animals, and in the OGG1-deficient animals. Thus is it possible that deficient OGG1 in another mouse strain would have resulted in a more significant MeHg-initiated deficit.

We were also able to determine that regardless of MeHg treatment, the female but not the male *ogg1* -/- mice were innately prone to poor performance in passive avoidance compared to the wild-type mice. This is the first evidence that there is an innate sex-selective tendency towards a cognitive deficit in this strain of mice. These results are supported by previous studies in our laboratory showing that *ogg1* -/- female mice are more susceptible to the effects of *in utero* exposure to methamphetamine on motor coordination, manifested by poor performance on the rotarod compared to wild-type mice (Wong *et al.*, 2008). Furthermore, there was a trend towards the MeHg-treated female *ogg1* +/- performing more poorly than the vehicle-treated female *ogg1* +/-,

further suggesting that DNA oxidation may be involved in the mechanism of toxicity, even though no differences were observed between the MeHg-treated and vehicle-treated mice from the *ogg1* wild-type and -/- genotypes.

One often overlooked aspect of passive avoidance is that there are two possible causes of what is perceived as a cognitive deficit in this test. The first is that an actual cognitive deficit results in a decreased latency to enter the dark chamber compared to controls; however, if a somatosensory deficit is present, it is possible that this decreased latency is in fact due to the mice not receiving the same aversive stimulus as the control animals. This is a particularly important distinction to make with MeHg, as numbness and tingling in the extremities are the hallmarks of adult poisoning (Ninomiya *et al.*, 2005; Takaoka *et al.*, 2008)

As we found no differences in tactile sensitivity with the von Frey filaments due to MeHg treatment, *ogg1* genotype or sex, we can conclude that the deficits we observed in the passive avoidance test were truly deficits of learning and memory, and that these deficits are caused by MeHg doses that are insufficient to cause numbness.

4.2.3 MeHg, OGG1, and the Developing Hippocampal Area

It has been previously shown that MeHg increases apoptosis in the developing hippocampus of neonatal rats (Falluel-Morel *et al.*, 2007; Sokolowski *et al.*, 2011).

Studies from our laboratory have also found that these effects were exacerbated in *ogg1* -/- MEFs compared to MeHg-treated *ogg1* wild-type cells (Ondovcik *et al.*, 2012, 2013b). Similarly, using TUNEL staining, I found that *in utero* exposure to MeHg appears to increase apoptosis in the developing hippocampal area, although I did not see any interaction between the *ogg1* genotype and MeHg treatment at this timepoint. I also did not find that the *ogg1* genotype alone affected apoptosis at this developmental stage. The apoptosis visually appears to be in the vicinity of the developing dentate gyrus, both in the cells that have already migrated to the dentate gyrus, and in those in the process of migrating there. The latter group may also consist of cells in the developing alveus. Unfortunately, without the use of markers specific to these regions, it is difficult to definitively determine which subregion is most affected. Multiple studies have demonstrated that damage to the dentate gyrus affects passive avoidance performance, however, and since the OGG1-deficient mice demonstrated a deficit in this test after MeHg exposure, it seems plausible that the apoptotic cells were part of, or migrating to, the developing dentate gyrus (Ma *et al.*, 1998; Zhao *et al.*, 2012).

I also found that there was a trend towards increased DNA DSBs in the MeHg-treated samples, represented by cells positive for γH2AX. The *ogg1* genotype did not appear to have an effect, or to interact with MeHg treatment. These results are similar to the apoptosis results. It may be that I failed to see a difference as cells with DNA DSBs were already apoptotic and no longer contained γH2AX. Previously we have found that *ogg1* -/- MEFs are more susceptible to DNA DSBs after MeHg exposure than their wild-type counterparts (Ondovcik *et al.*, 2012, 2013b). While no other *in vivo* studies have

measured γH2AX formation after MeHg exposure, another organic Hg compound, thimerosal, has been found to increase DSBs *in vitro* (Wu *et al.*, 2008).

The lack of difference in TUNEL and γH2AX staining between the wild-type and -/- animals is suggestive. Given the cognitive deficit observed in the MeHg-treated OGG1-deficient animals, and the innate deficit observed in the vehicle-treated *ogg1* -/- female animals, it would not be unreasonable to expect to see a genotype effect on apoptosis or DNA DSBs. However, without analysis over multiple timepoints, it is not possible to definitively dismiss either of these endpoints from being involved. It is possible that measurements at different timepoints would reveal that *ogg1* -/- animals are more susceptible than their wild-type counterparts after MeHg exposure, particularly as *ogg1* -/- MEFs in culture do exhibit increased DNA DSBs and apoptosis after MeHg exposure compared to wild-type cells (Ondovcik *et al.*, 2012). However, the lack of interaction between MeHg treatment and *ogg1* genotype in these measures, despite their combined effect in the passive avoidance test, suggest that more subtle measures of damage than apoptosis should be examined. Other possible effects of MeHg exposure in OGG1 deficient animals might include abnormal cellular proliferation or migration into the developing hippocampal region, abnormal neuronal connections or impairment of LTP in the hippocampus.

This possibility is supported by our observations of the effect of both MeHg and *ogg1* genotype on cellular migration or proliferation. At this timepoint, the vehicle-treated *ogg1*

-/- samples had increased BrdU-positive cells in the developing hippocampal region compared to the vehicle-treated *ogg1* wild-type samples. However, MeHg treatment completely abolished this increase. This suggests that after MeHg exposure, either less cellular proliferation is occurring, or there is less migration of new cells into the developing hippocampal region. These results are consistent with previous studies in our laboratory using *ogg1* -/- mouse embryonic fibroblasts plated at confluent concentrations (Ondovcik *et al.*, 2013b). However these results are in contrast to other studies that have shown that mice deficient in NEIL3, another enzyme that repairs oxidative DNA damage, have impaired hippocampal neurogenesis as adults (Regnell *et al.*, 2012). Results from other studies also suggest that increases in DNA oxidation are associated with impaired neuronal proliferation or migration (Narasimhaiah *et al.*, 2005; Xie *et al.*, 2008). It therefore seems possible that the increased proliferation or migration observed in the vehicle-treated *ogg1* -/- samples may have been a compensatory mechanism. MeHg has been previously shown to decrease or interfere with both cellular proliferation and migration in the developing rodent brain, including in the developing hippocampal region (Kakita *et al.*, 2002; Burke *et al.*, 2006; Falluel-Morel *et al.*, 2007). Furthermore, exposure to similar doses of MeHg in neonatal rats have decreased levels of cyclin D1, D3 and E, all of which are involved in G1-S phase transition (Burke *et al.*, 2006; Falluel-Morel *et al.*, 2007). It therefore is possible that in the vehicle-treated *ogg1* -/- samples, the increased cellular proliferation or migration occurs in response to some undetermined measure of damage caused by impaired DNA repair, and that MeHg prevents this increase, perhaps by increased apoptosis in migrating cells. However, since the MeHg-treated wild-type samples were not different

from the vehicle-treated wild-type samples, a more complex mechanism may be involved.

4.2.4 Conclusions

I have shown in these studies that animals deficient in OGG1 are more susceptible to the effects of *in utero* exposure to MeHg. This provides the first direct *in vivo* evidence that DNA oxidation may be involved in the mechanism of toxicity of MeHg. Furthermore, I have provided the first evidence of a sex-selective cognitive phenotype in this strain of mouse, where female *ogg1* -/- mice have impaired cognition compared to the female *ogg1* wild-type mice. These results confirm that protection against oxidative DNA damage is essential for normal neurodevelopment. This suggests that differences in DNA repair status in general populations may affect cognitive development, resulting in a varied spectrum of neurodevelopmental effects. Currently, there have been no studies examining genetic differences that may explain why children from the Faroe Islands exposed *in utero* to MeHg are susceptible to deficits in learning and language, while children from the Seychelles Islands are not. Similarly, there have been no studies examining the effect of OGG1 polymorphisms on childhood neurodevelopmental parameters. It is possible that these differences, in addition to exposure to different levels of other nutrients *in utero*, such as ω3 fatty acids, may contribute to the conflicting results observed in the Faroe Islands and Seychelles Islands populations.

4.3 MeHg, PBN, and OGG1

The conclusions I have drawn from my studies with MeHg and PBN, and with MeHg exposure in OGG1-deficient animals are consistent. Taken on their own, the DNA oxidation quantification and cognitive tests in the MeHg and PBN studies suggest that DNA oxidation is not involved in the mechanism of toxicity, though not conclusively. Although my cognitive tests in the OGG1-deficient animals suggest that oxidative damage to DNA is in fact involved in the mechanism of toxicity, the results from the DNA oxidation study in these mice confirm what I showed in the CD-1 animals treated with MeHg; namely, that there is no increase of 8-oxodGuo in whole brain samples. As I discussed earlier, the two likely explanations for this are that regional or local increases in 8-oxodG are responsible for the cognitive deficits, or that another lesion that is repaired by OGG1, such as fapyG, is responsible.

In the MeHg and PBN studies, I found that MeHg exposure resulted in cognitive deficits in both novel object recognition and passive avoidance, but that pretreatment with PBN improved only novel object recognition. In the *ogg1* knockout strain, however, I did not find any cognitive deficit in novel object recognition, regardless of MeHg treatment, *ogg1* genotype or sex. I did find that OGG1-deficient animals were sensitive to MeHg exposure in passive avoidance, and it appeared that the females may be particularly susceptible to cognitive deficits. The results from the MeHg-exposed OGG1 deficient animals are similar to those from the PBN-pretreated MeHg-exposed CD-1 animals, suggesting that this MeHg exposure in the OGG1-deficient animals may result in similar neurological injuries as the PBN-pretreated MeHg-exposed CD-1 animals. Furthermore,

these results suggest that although I did not observe any changes in DNA oxidation after MeHg and PBN exposure, PBN may still have protected against MeHg toxicity by trapping ROS. Previously, I discussed the possibility that the pretreatment with PBN might sufficiently protect the developing brain to block the MeHg-initiated deficit in novel object recognition, but not sufficiently to maintain normal passive avoidance. It therefore is possible that in the OGG1-deficient animals, MeHg exposure causes sufficient damage to affect passive avoidance, but not enough to affect novel object recognition. However, this would suggest that the background strain of the *ogg1* mice is less susceptible to MeHg than the CD-1 mice. The *ogg1* knockout animals are from a mixed C57BL/6 and 129/SV background (Klungland *et al.*, 1999). C57BL/6 mice have repeatedly been shown to be susceptible to MeHg, generally at higher doses than the one I employed (Doré *et al.*, 2001; Goulet *et al.*, 2003). One study in C57BL/6 mice showed severe motor coordination deficits, decreased exploratory behaviour and memory deficits in the Morris water maze with a cumulative maternal dose of 0.11 mg/kg (Montgomery *et al.*, 2008). This dose is more than 30-fold lower than the dose I used; however, these results contradict previous studies showing that higher doses of MeHg had no effect on the rotarod test and little to no effect in the open field test (Doré *et al.*, 2001; Goulet *et al.*, 2003). While 129/SV mice are less commonly used in MeHg studies, they are susceptible to structural deficits and embryolethality after higher doses of MeHg (Spyker & Smithberg, 1972). This would suggest that the lack of measurable toxicity in the *ogg1* wild-type animals was not due to a lack of susceptibility in the mouse strain.

Another possibility is that the relatively poor baseline performance of the *ogg1* wild-type animals decreased the sensitivity of the cognitive tests I used. For both the object recognition test and the passive avoidance test, the testing parameters for the *ogg1* mice were changed to allow the wild-type animals to achieve acceptable baseline scores (**Appendix 2, figs. A2.1 and A2.2**). It is possible that this poorer cognitive performance may have made it more difficult to discern subtle cognitive deficits, and that in a different mouse strain deficient in OGG1, a greater apparent susceptibility to MeHg would have been seen. This possibility is supported by a previous study showing that 129SV mice both have higher anxiogenic behaviours and require more training to learn a number of cognitive tests (Brooks *et al.*, 2005). Furthermore, previous studies have also shown that C57BL/6J mice perform poorly at passive avoidance (Mathis *et al.*, 1994). Though there are studies that show that the F2 offspring of 129SvxC57BL/6 mice have improved baseline performance over both parent strains in the Morris water maze, these colony conditions are different from ours and may not accurately reflect the behaviour of our mice (Wolfer *et al.*, 1997; de Bruin *et al.*, 2006). Furthermore, because those models were bred to select for mutations in genes other than *ogg1*, it is possible that the genetic predisposition to poor cognitive behaviour was lost in those models, but not ours (Gerlai, 1996).

It does not seem surprising that the MeHg-initiated deficits in these mice were not especially severe, given that the deficits observed in humans with low, chronic exposure are also quite subtle. In the Faroe Islands, there appears to be a consistent negative effect of MeHg exposure on a small number of parameters, including language memory

and fine motor coordination, that persists at least until adolescence (Grandjean *et al.*, 1997; Debes *et al.*, 2006). This effect, however, is not large. Until recently, findings in the Seychelles Child Development Study suggested that MeHg had no adverse effect on neurodevelopment, and have sometimes been used as evidence that MeHg is of little concern (Stern *et al.*, 2004; Davidson *et al.*, 2011). It now appears that high consumption of LCPUFA may mask subtle developmental toxicity (Strain *et al.*, 2008; Stokes-Riner *et al.*, 2011). A number of studies from other populations, including Spain, the United States, and Hong Kong, have all shown subtle but significant deficits (Oken *et al.*, 2005; Freire *et al.*, 2010; Lam *et al.*, 2013). Furthermore, I employed a single, low dose of MeHg in order to be able to study the initiation of toxicity instead of downstream secondary and tertiary responses to MeHg exposure. Had I conducted subchronic or chronic studies, it is possible that the deficits I observed would have been more significant. This would have allowed me to potentially target a different gestational day that might have resulted in a stronger deficit. I chose GD 17 because previous studies in our lab showed that in these mice, OGG1 activity was significant at this developmental age (Wong *et al.*, 2008). This gestational age is slightly after the peak of cellular proliferation for the developing hippocampus in mice, so it is possible that an earlier exposure would cause a more substantial deficit (Angevine, 1965). This might be of particular importance as the dose I used was among the lowest used in MeHg studies in mice (Kim *et al.*, 2000a; Doré *et al.*, 2001; Goulet *et al.*, 2003; Onishchenko *et al.*, 2007; Gao *et al.*, 2008; Yoshida *et al.*, 2008; Liang *et al.*, 2009). Although I was unable to measure MeHg levels in the fetal brains after exposure, previous studies in mice where MeHg levels have been measured suggest that the dose I used would produce achievable, but high MeHg brain levels in humans (Burbacher *et al.*, 1990; Doré *et al.*,

2001; Goulet *et al.*, 2003). However, a direct comparison of MeHg concentrations across species is likely not appropriate, due to species differences in metabolism, half-life, and susceptibility, and an estimate based on dose-translation calculations for differences in body surface area suggest that human exposure would be approximately 10% of mouse exposure (Reagan-Shaw *et al.*, 2008). Based on this estimate, the dose I used translated to humans would represent a reasonably achievable dose. Regardless of the size of the deficit, however, my results showing that PBN was capable of rescuing some of these deficits, and that the OGG1-deficient animals were more susceptible, introduce interesting possibilities for subpopulations of people who may be at increased risk of exhibiting MeHg-initiated neurodevelopmental toxicity. Although it may be difficult to analyze study subjects for impaired DNA repair or antioxidant enzymes, it is important to note that either of these may contribute to increased susceptibility.

4.3.1 Conclusions

I hypothesized that ROS, and oxidative damage to DNA in particular, are essential components of the mechanism of toxicity of MeHg. I also hypothesized that, as a consequence, pretreatment with the free radical spin trap PBN would protect against neurodevelopmental deficits caused by MeHg, and that impaired DNA repair in the fetus, represented by mice lacking OGG1, would result in animals that were more sensitive to the toxic effects of MeHg. In my studies, I have shown that pretreatment with PBN protects against some of the cognitive deficits caused by *in utero* exposure to MeHg, which suggests that ROS may be involved. However, by showing that OGG1-deficient animals are more sensitive to the toxic effects of MeHg, I have provided the

first direct evidence that ROS, and more specifically, oxidative damage to DNA, may be involved in the mechanism of toxicity. Furthermore, I have shown in female *ogg1* -/- mice that impaired DNA repair can result in cognitive deficits regardless of treatment with MeHg. Taken together, the results from my studies suggest that when considering populations that may be at risk of neurodevelopmental deficits, we must consider that individuals with deficient DNA repair may be peculiarly at risk, both to neurodevelopmental deficits caused by *in utero* exposure to ROS-initiating xenobiotics, but also to those caused by endogenous sources of oxidative DNA damage.

4.4 Future Directions

The purpose of my thesis was to determine if ROS and DNA oxidation are involved in the mechanism of toxicity caused by *in utero* exposure to MeHg. I have shown that pretreatment with a free radical spin trap protects animals from cognitive deficits initiated by MeHg treatment, and that there may also be protective effects on long-term survival. Similarly, animals deficient in the repair of oxidatively damaged DNA were shown to be more sensitive to the long-term cognitive effects of MeHg, and under certain conditions, animals deficient in DNA repair are more sensitive to cognitive deficits regardless of treatment. However, more work is necessary to illustrate the details of how DNA oxidation contributes to MeHg-initiated toxicity.

I was unable to show that MeHg treatment resulted in increased global levels of 8-oxodGuo in my fetal brain samples. However, since the *ogg1* +/- and -/- DNA repair-

deficient animals demonstrated a cognitive deficit after treatment with MeHg, I was able to conclude that DNA oxidation may be involved. Since *ogg1* is essential for the repair of 8-oxodG and fapyG, it seems likely that one or both of these lesions is involved in the pathogenic mechanism. As 8-oxodGuo levels remained unchanged in the whole brain samples we analyzed, the next logical step would be to analyze 8-oxodG levels within regions of the brain. In the adult mouse brain this can be achieved by microdissecting the brain and analyzing each region separately using the methods I used for the whole fetal brains. However, microdissecting the developing fetal brain and obtaining enough tissue for analysis with HPLC-UV and HPLC with tandem MS is impractical. While we have had little success previously with immunohistochemistry using antibodies for 8-oxodG, immunohistochemical localization is a potentially fruitful approach to consider, particularly in light of my analysis with TUNEL and antibodies for γH2AX and BrdU. Costaining with these antibodies will help to establish whether 8-oxodG is a pathogenic lesion, or whether any increases in 8-oxodG levels are incidental to the mechanism of toxicity.

Another possibility is that fapyG is the primary lesion involved in the mechanism of toxicity. Although less is known about fapyG, it can be quantified by gas chromatography/ mass spectrometry (GC/MS) (Jaruga *et al.*, 2000). However, if the crucial changes in DNA oxidation are in fact formed in specific brain regions or genes, as I have suggested for 8-oxodGuo, GC/MS of whole fetal brain samples may not be adequately sensitive and may require too much tissue (Liu *et al.*, 2010). There does not

appear to be a commercial anti-fapyG antibody at this time, but in the future it would be a valuable addition to these studies.

Ideally, these studies would be done in concert with expanded studies into the cellular consequences of MeHg exposure. Though I was able to demonstrate that MeHg causes increased apoptosis in the developing hippocampal region, and that DNA DSBs are also likely increased, a time course after MeHg exposure might be more informative, particularly in regards to the effects of genotype in the *ogg1* -/- samples. Furthermore, as I observed previously, it would be helpful to identify which subregions of the hippocampus are primarily affected; markers for the dentate gyrus would be particularly useful, as this seemed to be the region most affected by MeHg exposure. Costaining to determine which, if any, cell types are peculiarly sensitive, such as neuronal targets of dopaminergic or cholinergic neurotransmission or astrocytes, would help us to further understand the mechanism of toxicity. Additionally, there are other brain regions that seem likely to be affected by MeHg, given that I was able to show cognitive deficits in passive avoidance, including the amygdala, and potentially the septal nuclei (Roozendaal *et al.*, 1993; Elvander-Tottie *et al.*, 2006). Examinations of histological changes at later timepoints, perhaps in the first two weeks after birth, would help us to understand the consequences of increased apoptosis and changes in cellular proliferation or migration. It has been previously established that *in utero* and neonatal exposure to MeHg cause decreased hippocampal volume and neuronal number in the developing hippocampus (Kakita *et al.*, 2000; Falluel-Morel *et al.*, 2007; Falluel-Morel *et al.*, 2012). Fetal Minamata Disease, although caused by much higher levels of MeHg, is

also characterized by smaller brain size and atrophy (Harada, 1978). If it appears that more subtle effects may be occurring, we have previously discussed the possibility of collaborating with Dr. Zhengping Jia at the Hospital for Sick Children to study effects on LTP and pre-pulse facilitation in the CA1 region of these animals at 4 months of age, when I have been able to show deficits in cognition.

One important factor in my project is the single dose of MeHg that I used in order to determine whether DNA oxidation was a direct consequence of exposure, or a secondary effect. Our primary route of exposure, however, is low, chronic, and not necessarily regular. Using a chronic route of exposure, therefore, would give a more accurate picture of the true risks associated with decreased DNA repair in the *ogg1* -/- mice. While I was able to show a small, significant cognitive deficit in the OGG1-deficient animals after a single dose, we may find that with chronic exposure, these deficits are exacerbated.

If possible, these studies with chronic administration should be continued to determine the effects of MeHg on lifespan, and to see whether the *ogg1* -/- mice are more severely affected. Though it was initially unexpected, the observation that MeHg might have decreased lifespan in the MeHg-treated CD-1 litter that was followed to 16 months was one of the most interesting and novel outcomes of the MeHg and PBN studies. However, due to time and financial constraints, I was unable to follow up on this study with additional litters. This initial observation should be expanded on, particularly as the

PBN-pretreated group had a lifespan closer to the vehicle-treated animals, in contrast to the shorter lifespan observed with the litter treated with MeHg alone. There is growing evidence for long-term cardiovascular risks after MeHg exposure both during development and in adulthood (Sørensen *et al.*, 1999; Grandjean *et al.*, 2004; Choi *et al.*, 2009; Roman *et al.*, 2011). As I discussed earlier, it seems plausible that a vascular event may have been the cause of death for some of the MeHg-treated animals, as there were no signs of illness prior to death. Furthermore, it is suggestive that one of the animals was found with a large quantity of blood in the abdomen, perhaps due to a ruptured vascular aneurysm. It would therefore be instructive to follow these animals to determine if they have any signs of increased cardiovascular risk, including increased blood pressure and heart rate, and decreased heart rate variability (Thireau *et al.*, 2008; Feng & DiPetrillo, 2009; Ho *et al.*, 2011).

Section 5: References

Aberg B, Ekman L, Falk R, Greitz U, Persson G & Snihs JO. (1969). Metabolism of methyl mercury (203Hg) compounds in man. *Arch Environ Health* **19,** 478-484.

Abramov JP & Wells PG. (2011). Embryonic catalase protects against endogenous and phenytoin-enhanced DNA oxidation and embryopathies in acatalasemic and human catalase-expressing mice. *FASEB J* **25,** 2188-2200.

Acharya S, Wilson T, Gradia S, Kane MF, Guerrette S, Marsischky GT, Kolodner R & Fishel R. (1996). hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. *Proc Natl Acad Sci U S A* **93,** 13629-13634.

Al Bakheet SA, Attafi IM, Maayah ZH, Abd-Allah AR, Asiri YA & Korashy HM. (2013). Effect of long-term human exposure to environmental heavy metals on the expression of detoxification and DNA repair genes. *Environ Pollut* **181,** 226-232.

Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, Hodges AK, Davies DR, David SS, Sampson JR & Cheadle JP. (2002). Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors. *Nat Genet* **30,** 227-232.

Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P & Halliwell B. (1997). Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J Neurochem* **69,** 1196-1203.

Allen JW, Mutkus LA & Aschner M. (2001). Methylmercury-mediated inhibition of 3H-D-aspartate transport in cultured astrocytes is reversed by the antioxidant catalase. *Brain Res* **902,** 92-100.

Amaral D & Lavenex P. (2007). Hippocampal Neuroanatomy. In *The Hippocampus Book*, ed. Andersen P, Morris R, Amaral D, Bliss T & O'Keefe J. Oxford University Press, New York.

Amin-Zaki L, Elhassani S, Majeed MA, Clarkson TW, Doherty RA & Greenwood M. (1974). Intra-uterine methylmercury poisoning in Iraq. *Pediatrics* **54,** 587-595.

Anderson DE, Yuan XJ, Tseng CM, Rubin LJ, Rosen GM & Tod ML. (1993). Nitrone spin-traps block calcium channels and induce pulmonary artery relaxation independent of free radicals. *Biochem Biophys Res Commun* **193,** 878-885.

Angevine JB. (1965). Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Exp Neurol Suppl***,** Suppl 2:1-70.

Arai K, Morishita K, Shinmura K, Kohno T, Kim SR, Nohmi T, Taniwaki M, Ohwada S & Yokota J. (1997). Cloning of a human homolog of the yeast OGG1 gene that is involved in the repair of oxidative DNA damage. *Oncogene* **14,** 2857-2861.

Aremu DA, Madejczyk MS & Ballatori N. (2008). N-acetylcysteine as a potential antidote and biomonitoring agent of methylmercury exposure. *Environ Health Perspect* **116,** 26-31.

Arnold SE & Trojanowski JQ. (1996). Human fetal hippocampal development: I. Cytoarchitecture, myeloarchitecture, and neuronal morphologic features. *J Comp Neurol* **367,** 274-292.

Aschner M, Eberle NB, Goderie S & Kimelberg HK. (1990). Methylmercury uptake in rat primary astrocyte cultures: the role of the neutral amino acid transport system. *Brain Res* **521,** 221-228.

Audebert M, Chevillard S, Levalois C, Gyapay G, Vieillefond A, Klijanienko J, Vielh P, El Naggar AK, Oudard S, Boiteux S & Radicella JP. (2000). Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. *Cancer Res* **60,** 4740-4744.

Azmitia EC. (1981). Bilateral serotonergic projections to the dorsal hippocampus of the rat: simultaneous localization of 3H-5HT and HRP after retrograde transport. *J Comp Neurol* **203,** 737-743.

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, Tikriti S, Dahahir HI, Clarkson TW, Smith JC & Doherty RA. (1973). Methylmercury poisoning in Iraq. *Science* **181,** 230-241.

Balaban RS, Nemoto S & Finkel T. (2005). Mitochondria, oxidants, and aging. *Cell* **120,** 483-495.

Ballatori N, Gatmaitan Z & Truong AT. (1995). Impaired biliary excretion and whole body elimination of methylmercury in rats with congenital defect in biliary glutathione excretion. *Hepatology* **22,** 1469-1473.

Baraldi M, Zanoli P, Tascedda F, Blom JM & Brunello N. (2002). Cognitive deficits and changes in gene expression of NMDA receptors after prenatal methylmercury exposure. *Environ Health Perspect* **110 Suppl 5,** 855-858.

Bayer SA. (1980). Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* **190,** 87-114.

Belletti S, Orlandini G, Vettori MV, Mutti A, Uggeri J, Scandroglio R, Alinovi R & Gatti R. (2002). Time course assessment of methylmercury effects on C6 glioma cells: submicromolar concentrations induce oxidative DNA damage and apoptosis. *J Neurosci Res* **70,** 703-711.

Berglund M, Lind B, Björnberg KA, Palm B, Einarsson O & Vahter M. (2005). Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. *Environ Health* **4,** 20.

Best PJ & Orr J. (1973). Effects of hippocampal lesions on passive avoidance and taste aversion conditioning. *Physiol Behav* **10,** 193-196.

Beyrouty P & Chan HM. (2006). Co-consumption of selenium and vitamin E altered the reproductive and developmental toxicity of methylmercury in rats. *Neurotoxicol Teratol* **28,** 49-58.

Bisogni JJ & Lawrence AW. (1975). Kinetics of mercury methylation in aerobic and anaerobic aquatic environments. *J Water Pollut Control Fed* **47,** 135-152.

Bjelland S & Seeberg E. (2003). Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutat Res* **531,** 37-80.

Bjorâs M, Luna L, Johnsen B, Hoff E, Haug T, Rognes T & Seeberg E. (1997). Opposite base-dependent reactions of a human base excision repair enzyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. *EMBO J* **16,** 6314-6322.

Björnberg KA, Vahter M, Berglund B, Niklasson B, Blennow M & Sandborgh-Englund G. (2005). Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. *Environ Health Perspect* **113,** 1381-1385.

Bland BH, Oddie SD, Colom LV & Vertes RP. (1994). Extrinsic modulation of medial septal cell discharges by the ascending brainstem hippocampal synchronizing pathway. *Hippocampus* **4,** 649-660.

Bland C & Rand MD. (2006). Methylmercury induces activation of Notch signaling. *Neurotoxicology* **27,** 982-991.

Bliss TV & Collingridge GL. (2013). Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. *Mol Brain* **6,** 5.

Boado RJ, Li JY, Nagaya M, Zhang C & Pardridge WM. (1999). Selective expression of the large neutral amino acid transporter at the blood-brain barrier. *Proc Natl Acad Sci U S A* **96,** 12079-12084.

Board PG & Menon D. (2013). Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim Biophys Acta* **1830,** 3267-3288.

Bokoch GM & Knaus UG. (2003). NADPH oxidases: not just for leukocytes anymore! *Trends Biochem Sci* **28,** 502-508.

Bolderson E, Tomimatsu N, Richard DJ, Boucher D, Kumar R, Pandita TK, Burma S & Khanna KK. (2010). Phosphorylation of Exo1 modulates homologous recombination repair of DNA double-strand breaks. *Nucleic Acids Res* **38,** 1821-1831.

Bose-O'Reilly S, Drasch G, Beinhoff C, Rodrigues-Filho S, Roider G, Lettmeier B, Maydl A, Maydl S & Siebert U. (2010). Health assessment of artisanal gold miners in Indonesia. *Sci Total Environ* **408,** 713-725.

Bravard A, Vacher M, Gouget B, Coutant A, de Boisferon FH, Marsin S, Chevillard S & Radicella JP. (2006). Redox regulation of human OGG1 activity in response to cellular oxidative stress. *Mol Cell Biol* **26,** 7430-7436.

Bridges CC, Joshee L & Zalups RK. (2009). Effect of DMPS and DMSA on the placental and fetal disposition of methylmercury. *Placenta* **30,** 800-805.

Bridges CC & Zalups RK. (2010). Transport of inorganic mercury and methylmercury in target tissues and organs. *J Toxicol Environ Health B Crit Rev* **13,** 385-410.

Brigelius-Flohé R & Maiorino M. (2013). Glutathione peroxidases. *Biochim Biophys Acta* **1830,** 3289-3303.

Broadbent NJ, Squire LR & Clark RE. (2004). Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A* **101,** 14515-14520.

Brooks SP, Pask T, Jones L & Dunnett SB. (2005). Behavioural profiles of inbred mouse strains used as transgenic backgrounds. II: cognitive tests. *Genes Brain Behav* **4,** 307-317.

Brown AM, Kristal BS, Effron MS, Shestopalov AI, Ullucci PA, Sheu KF, Blass JP & Cooper AJ. (2000). Zn2+ inhibits alpha-ketoglutarate-stimulated mitochondrial respiration and the isolated alpha-ketoglutarate dehydrogenase complex. *J Biol Chem* **275,** 13441-13447.

Bruner SD, Norman DP & Verdine GL. (2000). Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* **403,** 859-866.

Bugreev DV, Mazina OM & Mazin AV. (2006). Rad54 protein promotes branch migration of Holliday junctions. *Nature* **442,** 590-593.

Burbacher TM, Rodier PM & Weiss B. (1990). Methylmercury developmental neurotoxicity: a comparison of effects in humans and animals. *Neurotoxicol Teratol* **12,** 191-202.

Burke K, Cheng Y, Li B, Petrov A, Joshi P, Berman RF, Reuhl KR & DiCicco-Bloom E. (2006). Methylmercury elicits rapid inhibition of cell proliferation in the developing brain and decreases cell cycle regulator, cyclin E. *Neurotoxicology* **27,** 970-981.

Cai L, Gibbs RB & Johnson DA. (2012). Recognition of novel objects and their location in rats with selective cholinergic lesion of the medial septum. *Neurosci Lett* **506,** 261-265.

Caito SW, Zhang Y & Aschner M. (2013). Involvement of AAT transporters in methylmercury toxicity in Caenorhabditis elegans. *Biochem Biophys Res Commun* **435,** 546-550.

Caldecott KW, Aoufouchi S, Johnson P & Shall S. (1996). XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. *Nucleic Acids Res* **24,** 4387-4394.

Canugovi C, Yoon JS, Feldman NH, Croteau DL, Mattson MP & Bohr VA. (2012). Endonuclease VIII-like 1 (NEIL1) promotes short-term spatial memory retention and protects from ischemic stroke-induced brain dysfunction and death in mice. *Proc Natl Acad Sci U S A* **109,** 14948-14953.

Castoldi AF, Coccini T & Manzo L. (2003). Neurotoxic and molecular effects of methylmercury in humans. *Rev Environ Health* **18,** 19-31.

Celeste A, Difilippantonio S, Difilippantonio MJ, Fernandez-Capetillo O, Pilch DR, Sedelnikova OA, Eckhaus M, Ried T, Bonner WM & Nussenzweig A. (2003). H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell* **114,** 371-383.

Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC & Nussenzweig A. (2002). Genomic instability in mice lacking histone H2AX. *Science* **296,** 922-927.

Chain EPoCitF. (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA JOURNAL* **10**.

Chan AC. (1993). Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol* **71,** 725-731.

Charleston JS, Body RL, Mottet NK, Vahter ME & Burbacher TM. (1995). Autometallographic determination of inorganic mercury distribution in the cortex of the calcarine sulcus of the monkey Macaca fascicularis following long-term subclinical exposure to methylmercury and mercuric chloride. *Toxicol Appl Pharmacol* **132,** 325-333.

Chen C, Qu L, Li B, Xing L, Jia G, Wang T, Gao Y, Zhang P, Li M, Chen W & Chai Z. (2005). Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2'-deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. *Clin Chem* **51,** 759-767.

Chen D, Cao G, Hastings T, Feng Y, Pei W, O'Horo C & Chen J. (2002a). Age-dependent decline of DNA repair activity for oxidative lesions in rat brain mitochondria. *J Neurochem* **81,** 1273-1284.

Chen D, Lan J, Pei W & Chen J. (2000). Detection of DNA base-excision repair activity for oxidative lesions in adult rat brain mitochondria. *J Neurosci Res* **61,** 225-236.

Chen GM, Bray TM, Janzen EG & McCay PB. (1990). Excretion, metabolism and tissue distribution of a spin trapping agent, alpha-phenyl-N-tert-butyl-nitrone (PBN) in rats. *Free Radic Res Commun* **9,** 317-323.

Chen L, Haushalter KA, Lieber CM & Verdine GL. (2002b). Direct visualization of a DNA glycosylase searching for damage. *Chem Biol* **9,** 345-350.

Cheng HY, Liu T, Feuerstein G & Barone FC. (1993). Distribution of spin-trapping compounds in rat blood and brain: in vivo microdialysis determination. *Free Radic Biol Med* **14,** 243-250.

Cheng Y, Ren X, Gowda AS, Shan Y, Zhang L, Yuan YS, Patel R, Wu H, Huber-Keener K, Yang JW, Liu D, Spratt TE & Yang JM. (2013). Interaction of Sirt3 with OGG1 contributes to repair of mitochondrial DNA and protects from apoptotic cell death under oxidative stress. *Cell Death Dis* **4,** e731.

Chmiel NH, Livingston AL & David SS. (2003). Insight into the functional consequences of inherited variants of the hMYH adenine glycosylase associated with colorectal cancer: complementation assays with hMYH variants and pre-steady-state kinetics of the corresponding mutated E.coli enzymes. *J Mol Biol* **327,** 431-443.

Choi AL, Weihe P, Budtz-Jørgensen E, Jørgensen PJ, Salonen JT, Tuomainen TP, Murata K, Nielsen HP, Petersen MS, Askham J & Grandjean P. (2009). Methylmercury exposure and adverse cardiovascular effects in Faroese whaling men. *Environ Health Perspect* **117,** 367-372.

Choi BH, Lapham LW, Amin-Zaki L & Saleem T. (1978). Abnormal neuronal migration, deranged cerebral cortical organization, and diffuse white matter astrocytosis of human

fetal brain: a major effect of methylmercury poisoning in utero. *J Neuropathol Exp Neurol* **37,** 719-733.

Chowdhury D, Keogh MC, Ishii H, Peterson CL, Buratowski S & Lieberman J. (2005). gamma-H2AX dephosphorylation by protein phosphatase 2A facilitates DNA double-strand break repair. *Mol Cell* **20,** 801-809.

Chrostowski MK, McGonnigal BG, Stabila JP & Padbury JF. (2009). LAT-1 expression in pre- and post-implantation embryos and placenta. *Placenta* **30,** 270-276.

Clark RE, Zola SM & Squire LR. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* **20,** 8853-8860.

Clarkson TW. (1972). The pharmacology of mercury compounds. *Annu Rev Pharmacol* **12,** 375-406.

Clarkson TW, Amin-Zaki L & Al-Tikriti SK. (1976). An outbreak of methylmercury poisoning due to consumption of contaminated grain. *Fed Proc* **35,** 2395-2399.

Clarkson TW, Magos L, Cox C, Greenwood MR, Amin-Zaki L, Majeed MA & Al-Damluji SF. (1981). Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak. *J Pharmacol Exp Ther* **218,** 74-83.

Coin F, Oksenych V, Mocquet V, Groh S, Blattner C & Egly JM. (2008). Nucleotide excision repair driven by the dissociation of CAK from TFIIH. *Mol Cell* **31,** 9-20.

Compe E & Egly JM. (2012). TFIIH: when transcription met DNA repair. *Nat Rev Mol Cell Biol* **13,** 343-354.

Coppedè F, Migheli F, Ceravolo R, Bregant E, Rocchi A, Petrozzi L, Unti E, Lonigro R, Siciliano G & Migliore L. (2010). The hOGG1 Ser326Cys polymorphism and Huntington's disease. *Toxicology* **278,** 199-203.

Cox C, Clarkson TW, Marsh DO, Amin-Zaki L, Tikriti S & Myers GG. (1989). Dose-response analysis of infants prenatally exposed to methyl mercury: an application of a single compartment model to single-strand hair analysis. *Environ Res* **49,** 318-332.

Culmsee C, Bondada S & Mattson MP. (2001). Hippocampal neurons of mice deficient in DNA-dependent protein kinase exhibit increased vulnerability to DNA damage, oxidative stress and excitotoxicity. *Brain Res Mol Brain Res* **87,** 257-262.

Daikhin Y & Yudkoff M. (2000). Compartmentation of brain glutamate metabolism in neurons and glia. *J Nutr* **130,** 1026S-1031S.

Dangwal SK. (1993). Evaluation and control of mercury vapor exposure in the cell house of chlor alkali plants. *Environ Res* **60,** 254-258.

David SS, O'Shea VL & Kundu S. (2007). Base-excision repair of oxidative DNA damage. *Nature* **447,** 941-950.

Davidson PW, Cory-Slechta DA, Thurston SW, Huang LS, Shamlaye CF, Gunzler D, Watson G, van Wijngaarden E, Zareba G, Klein JD, Clarkson TW, Strain JJ & Myers GJ. (2011). Fish consumption and prenatal methylmercury exposure: cognitive and behavioral

outcomes in the main cohort at 17 years from the Seychelles child development study. *Neurotoxicology* **32,** 711-717.

Davidson PW, Myers GJ, Cox C, Shamlaye CF, Marsh DO, Tanner MA, Berlin M, Sloane-Reeves J, Cernichiari E & Choisy O. (1995). Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. *Neurotoxicology* **16,** 677-688.

Davidson PW, Palumbo D, Myers GJ, Cox C, Shamlaye CF, Sloane-Reeves J, Cernichiari E, Wilding GE & Clarkson TW. (2000). Neurodevelopmental outcomes of Seychellois children from the pilot cohort at 108 months following prenatal exposure to methylmercury from a maternal fish diet. *Environ Res* **84,** 1-11.

de Bruin N, Mahieu M, Patel T, Willems R, Lesage A & Megens A. (2006). Performance of F2 B6x129 hybrid mice in the Morris water maze, latent inhibition and prepulse inhibition paradigms: Comparison with C57Bl/6J and 129sv inbred mice. *Behav Brain Res* **172,** 122-134.

de Freitas AS, Funck VR, Rotta MoS, Bohrer D, Mörschbächer V, Puntel RL, Nogueira CW, Farina M, Aschner M & Rocha JB. (2009). Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Res Bull* **79,** 77-84.

de Souza-Pinto NC, Eide L, Hogue BA, Thybo T, Stevnsner T, Seeberg E, Klungland A & Bohr VA. (2001). Repair of 8-oxodeoxyguanosine lesions in mitochondrial dna depends on the oxoguanine dna glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial dna of OGG1-defective mice. *Cancer Res* **61,** 5378-5381.

Debes F, Budtz-Jørgensen E, Weihe P, White RF & Grandjean P. (2006). Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicol Teratol* **28,** 536-547.

Della-Maria J, Zhou Y, Tsai MS, Kuhnlein J, Carney JP, Paull TT & Tomkinson AE. (2011). Human Mre11/human Rad50/Nbs1 and DNA ligase IIIalpha/XRCC1 protein complexes act together in an alternative nonhomologous end joining pathway. *J Biol Chem* **286,** 33845-33853.

Demaurex N & Scorrano L. (2009). Reactive oxygen species are NOXious for neurons. *Nat Neurosci* **12,** 819-820.

Dhénaut A, Boiteux S & Radicella JP. (2000). Characterization of the hOGG1 promoter and its expression during the cell cycle. *Mutat Res* **461,** 109-118.

Dineley KE, Devinney MJ, Zeak JA, Rintoul GL & Reynolds IJ. (2008). Glutamate mobilizes [Zn2+] through Ca2+ -dependent reactive oxygen species accumulation. *J Neurochem* **106,** 2184-2193.

Dizdaroglu M. (2005). Base-excision repair of oxidative DNA damage by DNA glycosylases. *Mutat Res* **591,** 45-59.

Doré FY, Goulet S, Gallagher A, Harvey PO, Cantin JF, D'Aigle T & Mirault ME. (2001). Neurobehavioral changes in mice treated with methylmercury at two different stages of fetal development. *Neurotoxicol Teratol* **23,** 463-472.

Douglas P, Zhong J, Ye R, Moorhead GB, Xu X & Lees-Miller SP. (2010). Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates gamma-H2AX. *Mol Cell Biol* **30,** 1368-1381.

Dubey A, Forster MJ & Sohal RS. (1995). Effect of the spin-trapping compound N-tert-butyl-alpha-phenylnitrone on protein oxidation and life span. *Arch Biochem Biophys* **324,** 249-254.

Dunn LT & Everitt BJ. (1988). Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behav Neurosci* **102,** 3-23.

EDDY NB, TOUCHBERRY CF & LIEBERMAN JE. (1950). Synthetic analgesics; methadone isomers and derivatives. *J Pharmacol Exp Ther* **98,** 121-137.

Elvander-Tottie E, Eriksson TM, Sandin J & Ogren SO. (2006). N-methyl-D-aspartate receptors in the medial septal area have a role in spatial and emotional learning in the rat. *Neuroscience* **142,** 963-978.

Endoh H, Kato N, Fujii S, Suzuki Y, Sato S, Kayama T, Kotake Y & Yoshimura T. (2001). Spin trapping agent, phenyl N-tert-butylnitrone, reduces nitric oxide production in the rat brain during experimental meningitis. *Free Radic Res* **35,** 583-591.

Engel GL, Delwig A & Rand MD. (2012). The effects of methylmercury on Notch signaling during embryonic neural development in Drosophila melanogaster. *Toxicol In Vitro* **26,** 485-492.

EPA US. (1993). Reference Dose (RfD): Description and Use in Health Risk Assessments.

EPA US. (2013). Mercury: Human Exposure.

Falluel-Morel A, Lin L, Sokolowski K, McCandlish E, Buckley B & DiCicco-Bloom E. (2012). N-acetyl cysteine treatment reduces mercury-induced neurotoxicity in the developing rat hippocampus. *J Neurosci Res* **90,** 743-750.

Falluel-Morel A, Sokolowski K, Sisti HM, Zhou X, Shors TJ & Dicicco-Bloom E. (2007). Developmental mercury exposure elicits acute hippocampal cell death, reductions in neurogenesis, and severe learning deficits during puberty. *J Neurochem* **103,** 1968-1981.

Fan LW, Tien LT, Mitchell HJ, Rhodes PG & Cai Z. (2008). Alpha-phenyl-n-tert-butyl-nitrone ameliorates hippocampal injury and improves learning and memory in juvenile rats following neonatal exposure to lipopolysaccharide. *Eur J Neurosci* **27,** 1475-1484.

Fan LW, Tien LT, Zheng B, Pang Y, Rhodes PG & Cai Z. (2010). Interleukin-1beta-induced brain injury and neurobehavioral dysfunctions in juvenile rats can be attenuated by alpha-phenyl-n-tert-butyl-nitrone. *Neuroscience* **168,** 240-252.

Farina M, Campos F, Vendrell I, Berenguer J, Barzi M, Pons S & Suñol C. (2009). Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. *Toxicol Sci* **112,** 416-426.

Farina M, Frizzo ME, Soares FA, Schwalm FD, Dietrich MO, Zeni G, Rocha JB & Souza DO. (2003). Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. *Toxicol Lett* **144,** 351-357.

Feng M & DiPetrillo K. (2009). Non-invasive blood pressure measurement in mice. *Methods Mol Biol* **573,** 45-55.

Ferger B, Teismann P, Earl CD, Kuschinsky K & Oertel WH. (2000). The protective effects of PBN against MPTP toxicity are independent of hydroxyl radical trapping. *Pharmacol Biochem Behav* **65,** 425-431.

Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, Naka K, Xia Z, Camerini-Otero RD, Motoyama N, Carpenter PB, Bonner WM, Chen J & Nussenzweig A. (2002). DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol* **4,** 993-997.

Ferraro L, Tomasini MC, Tanganelli S, Mazza R, Coluccia A, Carratù MR, Gaetani S, Cuomo V & Antonelli T. (2009). Developmental exposure to methylmercury elicits early cell death in the cerebral cortex and long-term memory deficits in the rat. *Int J Dev Neurosci* **27,** 165-174.

Fimreite N & Reynolds LM. (1973). Mercury contamination of fish in northwestern Ontario. *The Journal of Wildlife Management* **37,** 62-68.

Firsanov D, Vasilishina A, Kropotov A & Mikhailov V. (2012). Dynamics of γH2AX formation and elimination in mammalian cells after X-irradiation. *Biochimie* **94,** 2416-2422.

Flowers L, Ohnishi ST & Penning TM. (1997). DNA strand scission by polycyclic aromatic hydrocarbon o-quinones: role of reactive oxygen species, Cu(II)/Cu(I) redox cycling, and o-semiquinone anion radicals,. *Biochemistry* **36,** 8640-8648.

Floyd RA, Chandru HK, He T & Towner R. (2011). Anti-cancer activity of nitrones and observations on mechanism of action. *Anticancer Agents Med Chem* **11,** 373-379.

Floyd RA, Kopke RD, Choi CH, Foster SB, Doblas S & Towner RA. (2008). Nitrones as therapeutics. *Free Radic Biol Med* **45,** 1361-1374.

Freire C, Ramos R, Lopez-Espinosa MJ, Díez S, Vioque J, Ballester F & Fernández MF. (2010). Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. *Environ Res* **110,** 96-104.

Frosina G. (2006). Prophylaxis of oxidative DNA damage by formamidopyrimidine-DNA glycosylase. *Int J Cancer* **119,** 1-7.

Fujimoto Y, Sakamoto N, Kitaura H, Ikeda E & Fujita T. (1988). Stimulatory action of ascorbic acid on methyl mercury-induced lipid peroxidation in rabbit kidney cortex slices. *Res Commun Chem Pathol Pharmacol* **60,** 121-124.

Fujimura M, Usuki F, Sawada M, Rostene W, Godefroy D & Takashima A. (2009). Methylmercury exposure downregulates the expression of Racl and leads to neuritic degeneration and ultimately apoptosis in cerebrocortical neurons. *Neurotoxicology* **30,** 16-22.

Fukae J, Takanashi M, Kubo S, Nishioka K, Nakabeppu Y, Mori H, Mizuno Y & Hattori N. (2005). Expression of 8-oxoguanine DNA glycosylase (OGG1) in Parkinson's disease and related neurodegenerative disorders. *Acta Neuropathol* **109,** 256-262.

Fukuchi I, Kato S, Nakahiro M, Uchida S, Ishida R & Yoshida H. (1987). Blockade of cholinergic receptors by an irreversible antagonist, propylbenzilylcholine mustard (PrBCM), in the rat cerebral cortex causes deficits in passive avoidance learning. *Brain Res* **400,** 53-61.

Funato Y, Michiue T, Asashima M & Miki H. (2006). The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. *Nat Cell Biol* **8,** 501-508.

Funato Y & Miki H. (2007). Nucleoredoxin, a novel thioredoxin family member involved in cell growth and differentiation. *Antioxid Redox Signal* **9,** 1035-1057.

Furtado C, Kunrath-Lima M, Rajão MA, Mendes IC, de Moura MB, Campos PC, Macedo AM, Franco GR, Pena SD, Teixeira SM, Van Houten B & Machado CR. (2012). Functional characterization of 8-oxoguanine DNA glycosylase of Trypanosoma cruzi. *PLoS One* **7,** e42484.

Gabbita SP, Lovell MA & Markesbery WR. (1998). Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J Neurochem* **71,** 2034-2040.

Gackowski D, Rozalski R, Siomek A, Dziaman T, Nicpon K, Klimarczyk M, Araszkiewicz A & Olinski R. (2008). Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. *J Neurol Sci* **266,** 57-62.

Gao Y, Ding Y, Shi R & Tian Y. (2008). Effects of methylmercury on postnatal neurobehavioral development in mice. *Neurotoxicol Teratol* **30,** 462-467.

García-Espinosa MA, Wallin R, Hutson SM & Sweatt AJ. (2007). Widespread neuronal expression of branched-chain aminotransferase in the CNS: implications for leucine/glutamate metabolism and for signaling by amino acids. *J Neurochem* **100,** 1458-1468.

Garman RH, Weiss B & Evans HL. (1975). Alkylmercurial encephalopathy in the monkey (Saimiri sciureus and Macaca arctoides): a histopathologic and autoradiographic study. *Acta Neuropathol* **32,** 61-74.

Gerlai R. (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci* **19,** 177-181.

Giblin FJ & Massaro EJ. (1975). The erythrocyte transport and transfer of methylmercury to the tissues of the rainbow trout (Salmo gairdneri). *Toxicology* **5,** 243-254.

Girard PM, D'Ham C, Cadet J & Boiteux S. (1998). Opposite base-dependent excision of 7,8-dihydro-8-oxoadenine by the Ogg1 protein of Saccharomyces cerevisiae. *Carcinogenesis* **19,** 1299-1305.

Goodarzi AA & Jeggo PA. (2013). The repair and signaling responses to DNA double-strand breaks. *Adv Genet* **82,** 1-45.

Goode EL, Ulrich CM & Potter JD. (2002). Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* **11,** 1513-1530.

Goulet S, Doré FY & Mirault ME. (2003). Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. *Neurotoxicol Teratol* **25,** 335-347.

Grandjean P & Budtz-Jørgensen E. (2007). Total imprecision of exposure biomarkers: implications for calculating exposure limits. *Am J Ind Med* **50,** 712-719.

Grandjean P & Budtz-Jørgensen E. (2010). An ignored risk factor in toxicology: The total imprecision of exposure assessment. *Pure Appl Chem* **82,** 383-391.

Grandjean P, Murata K, Budtz-Jørgensen E & Weihe P. (2004). Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of a Faroese birth cohort. *J Pediatr* **144,** 169-176.

Grandjean P, Weihe P, Jørgensen PJ, Clarkson T, Cernichiari E & Viderø T. (1992). Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. *Arch Environ Health* **47,** 185-195.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sørensen N, Dahl R & Jørgensen PJ. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* **19,** 417-428.

Greenberg MM. (2012). The formamidopyrimidines: purine lesions formed in competition with 8-oxopurines from oxidative stress. *Acc Chem Res* **45,** 588-597.

Groisman R, Kuraoka I, Chevallier O, Gaye N, Magnaldo T, Tanaka K, Kisselev AF, Harel-Bellan A & Nakatani Y. (2006). CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. *Genes Dev* **20,** 1429-1434.

Grotto D, Barcelos GR, Valentini J, Antunes LM, Angeli JP, Garcia SC & Barbosa F. (2009). Low levels of methylmercury induce DNA damage in rats: protective effects of selenium. *Arch Toxicol* **83,** 249-254.

Guo BQ, Yan CH, Cai SZ, Yuan XB & Shen XM. (2013). Low level prenatal exposure to methylmercury disrupts neuronal migration in the developing rat cerebral cortex. *Toxicology* **304,** 57-68.

Halliwell B & Gutteridge JMC. (2007). *Free Radicals in Biology and Medicine*. Oxford University Press, New York.

Halsne R, Esbensen Y, Wang W, Scheffler K, Suganthan R, Bjørås M & Eide L. (2012). Lack of the DNA glycosylases MYH and OGG1 in the cancer prone double mutant mouse does not increase mitochondrial DNA mutagenesis. *DNA Repair (Amst)* **11,** 278-285.

Hamelin S, Amyot M, Barkay T, Wang Y & Planas D. (2011). Methanogens: principal methylators of mercury in lake periphyton. *Environ Sci Technol* **45,** 7693-7700.

Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y, Takahashi M, Cheah JH, Tankou SK, Hester LD, Ferris CD, Hayward SD, Snyder SH & Sawa A. (2005). S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol* **7,** 665-674.

Harada M. (1978). Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology* **18,** 285-288.

Harada M. (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* **25,** 1-24.

Harada M, Fujino T, Oorui T, Nakachi S, Nou T, Kizaki T, Hitomi Y, Nakano N & Ohno H. (2005). Followup study of mercury pollution in indigenous tribe reservations in the Province of Ontario, Canada, 1975-2002. *Bull Environ Contam Toxicol* **74,** 689-697.

Harada M, Hanada M, Tajiri M, Inoue Y, Hotta N, Fujino T, Takaoka S & Ueda K. (2011). Mercury poisoning in First Nations groups in Ontario, Canada: 35 years of Canadian Minamata Disease. *Journal of Minamata Studies* **3,** 3 - 30.

Hasenöhrl RU, Souza-Silva MA, Nikolaus S, Tomaz C, Brandao ML, Schwarting RK & Huston JP. (2000). Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides* **34,** 272-280.

Hawkins RA. (2009). The blood-brain barrier and glutamate. *Am J Clin Nutr* **90,** 867S-874S.

Hayes JD, Chanas SA, Henderson CJ, McMahon M, Sun C, Moffat GJ, Wolf CR & Yamamoto M. (2000). The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. *Biochem Soc Trans* **28,** 33-41.

Hegde ML, Hegde PM, Holthauzen LM, Hazra TK, Rao KS & Mitra S. (2010). Specific Inhibition of NEIL-initiated repair of oxidized base damage in human genome by copper and iron: potential etiological linkage to neurodegenerative diseases. *J Biol Chem* **285,** 28812-28825.

Herlenius E & Lagercrantz H. (2004). Development of neurotransmitter systems during critical periods. *Exp Neurol* **190 Suppl 1,** S8-21.

Hirayama K. (1980). Effect of amino acids on brain uptake of methyl mercury. *Toxicol Appl Pharmacol* **55,** 318-323.

Ho D, Zhao X, Gao S, Hong C, Vatner DE & Vatner SF. (2011). Heart Rate and Electrocardiography Monitoring in Mice. *Curr Protoc Mouse Biol* **1,** 123-139.

Hossmann KA, Oschlies U, Schwindt W & Krep H. (2001). Electron microscopic investigation of rat brain after brief cardiac arrest. *Acta Neuropathol* **101,** 101-113.

Hu J, de Souza-Pinto NC, Haraguchi K, Hogue BA, Jaruga P, Greenberg MM, Dizdaroglu M & Bohr VA. (2005). Repair of formamidopyrimidines in DNA involves different

glycosylases: role of the OGG1, NTH1, and NEIL1 enzymes. *J Biol Chem* **280,** 40544-40551.

Huang AC, Shyu BC, Hsiao S, Chen TC & He AB. (2013). Neural substrates of fear conditioning, extinction, and spontaneous recovery in passive avoidance learning: a c-fos study in rats. *Behav Brain Res* **237,** 23-31.

Hughes WL. (1957). A physicochemical rationale for the biological activity of mercury and its compounds. *Ann N Y Acad Sci* **65,** 454-460.

Ide H & Kotera M. (2004). Human DNA glycosylases involved in the repair of oxidatively damaged DNA. *Biol Pharm Bull* **27,** 480-485.

Iliakis G. (2009). Backup pathways of NHEJ in cells of higher eukaryotes: cell cycle dependence. *Radiother Oncol* **92,** 310-315.

Ishida T, Hippo Y, Nakahori Y, Matsushita I, Kodama T, Nishimura S & Aburatani H. (1999a). Structure and chromosome location of human OGG1. *Cytogenet Cell Genet* **85,** 232-236.

Ishida T, Takashima R, Fukayama M, Hamada C, Hippo Y, Fujii T, Moriyama S, Matsuba C, Nakahori Y, Morita H, Yazaki Y, Kodama T, Nishimura S & Aburatani H. (1999b). New DNA polymorphisms of human MMH/OGG1 gene: prevalence of one polymorphism among lung-adenocarcinoma patients in Japanese. *Int J Cancer* **80,** 18-21.

Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD & Yamamoto M. (1999). Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* **13,** 76-86.

Jaruga P, Speina E, Gackowski D, Tudek B & Olinski R. (2000). Endogenous oxidative DNA base modifications analysed with repair enzymes and GC/MS technique. *Nucleic Acids Res* **28,** E16.

Javeri A, Lyons JG, Huang XX & Halliday GM. (2011). Downregulation of Cockayne syndrome B protein reduces human 8-oxoguanine DNA glycosylase-1 expression and repair of UV radiation-induced 8-oxo-7,8-dihydro-2'-deoxyguanine. *Cancer Sci* **102,** 1651-1658.

Jebbett NJ, Hamilton JW, Rand MD & Eckenstein F. (2013). Low level methylmercury enhances CNTF-evoked STAT3 signaling and glial differentiation in cultured cortical progenitor cells. *Neurotoxicology* **38C,** 91-100.

Jeng W, Ramkissoon A, Parman T & Wells PG. (2006). Prostaglandin H synthase-catalyzed bioactivation of amphetamines to free radical intermediates that cause CNS regional DNA oxidation and nerve terminal degeneration. *FASEB J* **20,** 638-650.

Jensen RB, Carreira A & Kowalczykowski SC. (2010). Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature* **467,** 678-683.

Jeong HG, Youn CK, Cho HJ, Kim SH, Kim MH, Kim HB, Chang IY, Lee YS, Chung MH & You HJ. (2004). Metallothionein-III prevents gamma-ray-induced 8-oxoguanine accumulation in normal and hOGG1-depleted cells. *J Biol Chem* **279,** 34138-34149.

Ji G, Yan L, Liu W, Qu J & Gu A. (2013). OGG1 Ser326Cys polymorphism interacts with cigarette smoking to increase oxidative DNA damage in human sperm and the risk of male infertility. *Toxicol Lett* **218,** 144-149.

Jilani A, Ramotar D, Slack C, Ong C, Yang XM, Scherer SW & Lasko DD. (1999). Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3'-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. *J Biol Chem* **274,** 24176-24186.

Jin X, Chan HM, Lok E, Kapal K, Taylor M, Kubow S & Mehta R. (2008). Dietary fats modulate methylmercury-mediated systemic oxidative stress and oxidative DNA damage in rats. *Food Chem Toxicol* **46,** 1706-1720.

Jiricny J. (2006). The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* **7,** 335-346.

Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V & Volterra A. (2007). Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci* **10,** 331-339.

Juárez BI, Portillo-Salazar H, González-Amaro R, Mandeville P, Aguirre JR & Jiménez ME. (2005). Participation of N-methyl-D-aspartate receptors on methylmercury-induced DNA damage in rat frontal cortex. *Toxicology* **207,** 223-229.

KAADA BR, RASMUSSEN EW & KVEIM O. (1962). Impaired acquisition of passive avoidance behavior by subcallosal, septal, hypothalamic, and insular lesions in rats. *J Comp Physiol Psychol* **55,** 661-670.

Kajiwara Y, Yasutake A, Adachi T & Hirayama K. (1996). Methylmercury transport across the placenta via neutral amino acid carrier. *Arch Toxicol* **70,** 310-314.

Kakita A, Inenaga C, Sakamoto M & Takahashi H. (2002). Neuronal migration disturbance and consequent cytoarchitecture in the cerebral cortex following transplacental administration of methylmercury. *Acta Neuropathol* **104,** 409-417.

Kakita A, Inenaga C, Sakamoto M & Takahashi H. (2003). Disruption of postnatal progenitor migration and consequent abnormal pattern of glial distribution in the cerebrum following administration of methylmercury. *J Neuropathol Exp Neurol* **62,** 835-847.

Kakita A, Wakabayashi K, Su M, Yoneoka Y, Sakamoto M, Ikuta F & Takahashi H. (2000). Intrauterine methylmercury intoxication. Consequence of the inherent brain lesions and cognitive dysfunction in maturity. *Brain Res* **877,** 322-330.

Kalam MA, Haraguchi K, Chandani S, Loechler EL, Moriya M, Greenberg MM & Basu AK. (2006). Genetic effects of oxidative DNA damages: comparative mutagenesis of the imidazole ring-opened formamidopyrimidines (Fapy lesions) and 8-oxo-purines in simian kidney cells. *Nucleic Acids Res* **34,** 2305-2315.

Karihtala P, Kauppila S, Puistola U & Jukkola-Vuorinen A. (2012). Absence of the DNA repair enzyme human 8-oxoguanine glycosylase is associated with an aggressive breast cancer phenotype. *Br J Cancer* **106,** 344-347.

Karimi-Busheri F, Daly G, Robins P, Canas B, Pappin DJ, Sgouros J, Miller GG, Fakhrai H, Davis EM, Le Beau MM & Weinfeld M. (1999). Molecular characterization of a human DNA kinase. *J Biol Chem* **274,** 24187-24194.

Kerper LE, Ballatori N & Clarkson TW. (1992). Methylmercury transport across the blood-brain barrier by an amino acid carrier. *Am J Physiol* **262,** R761-765.

Kershaw RM & Hodges NJ. (2012). Repair of oxidative DNA damage is delayed in the Ser326Cys polymorphic variant of the base excision repair protein OGG1. *Mutagenesis* **27,** 501-510.

Kertes E, László K, Berta B & Lénárd L. (2009). Effects of substance P microinjections into the globus pallidus and central nucleus of amygdala on passive avoidance learning in rats. *Behav Brain Res* **198,** 397-403.

Khan MA & Wang F. (2010). Chemical demethylation of methylmercury by selenoamino acids. *Chem Res Toxicol* **23,** 1202-1206.

Kim BM, Rhee JS, Seo JS, Kim IC, Lee YM & Lee JS. (2012). 8-Oxoguanine DNA glycosylase 1 (OGG1) from the copepod Tigriopus japonicus: molecular characterization and its expression in response to UV-B and heavy metals. *Comp Biochem Physiol C Toxicol Pharmacol* **155,** 290-299.

Kim CY, Nakai K, Kasanuma Y & Satoh H. (2000a). Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury. *Neurotoxicol Teratol* **22,** 397-403.

Kim JR, Yoon HW, Kwon KS, Lee SR & Rhee SG. (2000b). Identification of proteins containing cysteine residues that are sensitive to oxidation by hydrogen peroxide at neutral pH. *Anal Biochem* **283,** 214-221.

Kinghorn A, Solomon P & Chan HM. (2007). Temporal and spatial trends of mercury in fish collected in the English-Wabigoon river system in Ontario, Canada. *Sci Total Environ* **372,** 615-623.

Kitsera N, Stathis D, Lühnsdorf B, Müller H, Carell T, Epe B & Khobta A. (2011). 8-Oxo-7,8-dihydroguanine in DNA does not constitute a barrier to transcription, but is converted into transcription-blocking damage by OGG1. *Nucleic Acids Res* **39,** 5926-5934.

Klungland A & Bjelland S. (2007). Oxidative damage to purines in DNA: role of mammalian Ogg1. *DNA Repair (Amst)* **6,** 481-488.

Klungland A & Lindahl T. (1997). Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J* **16,** 3341-3348.

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T & Barnes DE. (1999). Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci U S A* **96,** 13300-13305.

Koh AS, Simmons-Willis TA, Pritchard JB, Grassl SM & Ballatori N. (2002). Identification of a mechanism by which the methylmercury antidotes N-acetylcysteine and

dimercaptopropanesulfonate enhance urinary metal excretion: transport by the renal organic anion transporter-1. *Mol Pharmacol* **62,** 921-926.

Korbas M, O'Donoghue JL, Watson GE, Pickering IJ, Singh SP, Myers GJ, Clarkson TW & George GN. (2010). The chemical nature of mercury in human brain following poisoning or environmental exposure. *ACS Chem Neurosci* **1,** 810-818.

Krasikova YS, Rechkunova NI, Maltseva EA, Petruseva IO & Lavrik OI. (2010). Localization of xeroderma pigmentosum group A protein and replication protein A on damaged DNA in nucleotide excision repair. *Nucleic Acids Res* **38,** 8083-8094.

Krishnamurthy N, Haraguchi K, Greenberg MM & David SS. (2008). Efficient removal of formamidopyrimidines by 8-oxoguanine glycosylases. *Biochemistry* **47,** 1043-1050.

Kugler P. (1987). Cytochemical demonstration of aspartate aminotransferase in the mossy-fibre system of the rat hippocampus. *Histochemistry* **87,** 623-625.

Kugler P & Schleyer V. (2004). Developmental expression of glutamate transporters and glutamate dehydrogenase in astrocytes of the postnatal rat hippocampus. *Hippocampus* **14,** 975-985.

Kunisada M, Sakumi K, Tominaga Y, Budiyanto A, Ueda M, Ichihashi M, Nakabeppu Y & Nishigori C. (2005). 8-Oxoguanine formation induced by chronic UVB exposure makes Ogg1 knockout mice susceptible to skin carcinogenesis. *Cancer Res* **65,** 6006-6010.

Kuraoka I, Suzuki K, Ito S, Hayashida M, Kwei JS, Ikegami T, Handa H, Nakabeppu Y & Tanaka K. (2007). RNA polymerase II bypasses 8-oxoguanine in the presence of transcription elongation factor TFIIS. *DNA Repair (Amst)* **6,** 841-851.

Kwon OS & Park YJ. (2003). In vitro and in vivo dose-dependent inhibition of methylmercury on glutamine synthetase in the brain of different species. *Environ Toxicol Pharmacol* **14,** 17-24.

Lagerwerf S, Vrouwe MG, Overmeer RM, Fousteri MI & Mullenders LH. (2011). DNA damage response and transcription. *DNA Repair (Amst)* **10,** 743-750.

Lai EK, Crossley C, Sridhar R, Misra HP, Janzen EG & McCay PB. (1986). In vivo spin trapping of free radicals generated in brain, spleen, and liver during gamma radiation of mice. *Arch Biochem Biophys* **244,** 156-160.

Lam HS, Kwok KM, Chan PH, So HK, Li AM, Ng PC & Fok TF. (2013). Long term neurocognitive impact of low dose prenatal methylmercury exposure in Hong Kong. *Environ Int* **54,** 59-64.

Lame ME, Kalgutkar AS & LaFontaine M. (2004). Intravenous pharmacokinetics and metabolism of the reactive oxygen scavenger alpha-phenyl-N-tert-butyl nitrone (PBN) in the cynomolgus monkey. *Drug Metabol Drug Interact* **20,** 11-24.

Larsen E, Reite K, Nesse G, Gran C, Seeberg E & Klungland A. (2006). Repair and mutagenesis at oxidized DNA lesions in the developing brain of wild-type and Ogg1-/- mice. *Oncogene* **25,** 2425-2432.

Lau A & Tymianski M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch* **460,** 525-542.

Le Page F, Guy A, Cadet J, Sarasin A & Gentil A. (1998). Repair and mutagenic potency of 8-oxoG:A and 8-oxoG:C base pairs in mammalian cells. *Nucleic Acids Res* **26,** 1276-1281.

Lee BE, Hong YC, Park H, Ha M, Koo BS, Chang N, Roh YM, Kim BN, Kim YJ, Kim BM, Jo SJ & Ha EH. (2010). Interaction between GSTM1/GSTT1 polymorphism and blood mercury on birth weight. *Environ Health Perspect* **118,** 437-443.

Lee SK, Hamer D, Bedwell CL, Lohitnavy M & Yang RS. (2009). Effect of PCBs on the lactational transfer of methyl mercury in mice: PBPK modeling. *Environ Toxicol Pharmacol* **27,** 75-83.

Leib SL, Kim YS, Chow LL, Sheldon RA & Täuber MG. (1996). Reactive oxygen intermediates contribute to necrotic and apoptotic neuronal injury in an infant rat model of bacterial meningitis due to group B streptococci. *J Clin Invest* **98,** 2632-2639.

Li PA, He QP, Nakamura L & Csiszar K. (2001). Free radical spin trap alpha-phenyl-N-tert-butyl-nitron inhibits caspase-3 activation and reduces brain damage following a severe forebrain ischemic injury. *Free Radic Biol Med* **31,** 1191-1197.

Liang J, Inskip M, Newhook D & Messier C. (2009). Neurobehavioral effect of chronic and bolus doses of methylmercury following prenatal exposure in C57BL/6 weanling mice. *Neurotoxicol Teratol* **31,** 372-381.

Lin S, Cox HJ, Rhodes PG & Cai Z. (2006). Neuroprotection of alpha-phenyl-n-tert-butyl-nitrone on the neonatal white matter is associated with anti-inflammation. *Neurosci Lett* **405,** 52-56.

Liu D, Croteau DL, Souza-Pinto N, Pitta M, Tian J, Wu C, Jiang H, Mustafa K, Keijzers G, Bohr VA & Mattson MP. (2011). Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions. *J Cereb Blood Flow Metab* **31,** 680-692.

Liu KJ, Kotake Y, Lee M, Miyake M, Sugden K, Yu Z & Swartz HM. (1999). High-performance liquid chromatography study of the pharmacokinetics of various spin traps for application to in vivo spin trapping. *Free Radic Biol Med* **27,** 82-89.

Liu L & Wells PG. (1994). In vivo phenytoin-initiated oxidative damage to proteins and lipids in murine maternal hepatic and embryonic tissue organelles: potential molecular targets of chemical teratogenesis. *Toxicol Appl Pharmacol* **125,** 247-255.

Liu M, Bandaru V, Bond JP, Jaruga P, Zhao X, Christov PP, Burrows CJ, Rizzo CJ, Dizdaroglu M & Wallace SS. (2010). The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. *Proc Natl Acad Sci U S A* **107,** 4925-4930.

Liu W, Xu Z, Deng Y, Xu B, Wei Y & Yang T. (2013). Protective effects of memantine against methylmercury-induced glutamate dyshomeostasis and oxidative stress in rat cerebral cortex. *Neurotox Res* **24,** 320-337.

Loeffler JM, Ringer R, Hablützel M, Täuber MG & Leib SL. (2001). The free radical scavenger alpha-phenyl-tert-butyl nitrone aggravates hippocampal apoptosis and learning deficits in experimental pneumococcal meningitis. *J Infect Dis* **183,** 247-252.

Lu R, Nash HM & Verdine GL. (1997). A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. *Curr Biol* **7,** 397-407.

Lund ME, Banner W, Clarkson TW & Berlin M. (1984). Treatment of acute methylmercury ingestion by hemodialysis with N-acetylcysteine (Mucomyst) infusion and 2,3-dimercaptopropane sulfonate. *J Toxicol Clin Toxicol* **22,** 31-49.

Luo AH, Tahsili-Fahadan P, Wise RA, Lupica CR & Aston-Jones G. (2011). Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. *Science* **333,** 353-357.

Lynch ML, Huang LS, Cox C, Strain JJ, Myers GJ, Bonham MP, Shamlaye CF, Stokes-Riner A, Wallace JM, Duffy EM, Clarkson TW & Davidson PW. (2011). Varying coefficient function models to explore interactions between maternal nutritional status and prenatal methylmercury toxicity in the Seychelles Child Development Nutrition Study. *Environ Res* **111,** 75-80.

Ma YL, Wang HL, Wu HC, Wei CL & Lee EH. (1998). Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. *Neuroscience* **82,** 957-967.

Madejczyk MS, Aremu DA, Simmons-Willis TA, Clarkson TW & Ballatori N. (2007). Accelerated urinary excretion of methylmercury following administration of its antidote N-acetylcysteine requires Mrp2/Abcc2, the apical multidrug resistance-associated protein. *J Pharmacol Exp Ther* **322,** 378-384.

Mah LJ, El-Osta A & Karagiannis TC. (2010). gammaH2AX: a sensitive molecular marker of DNA damage and repair. *Leukemia* **24,** 679-686.

Mahaffey KR. (2005). Mercury exposure: medical and public health issues. *Trans Am Clin Climatol Assoc* **116,** 127-153; discussion 153-124.

Mahaffey KR, Sunderland EM, Chan HM, Choi AL, Grandjean P, Mariën K, Oken E, Sakamoto M, Schoeny R, Weihe P, Yan CH & Yasutake A. (2011). Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption. *Nutr Rev* **69,** 493-508.

Mahajan KN, Nick McElhinny SA, Mitchell BS & Ramsden DA. (2002). Association of DNA polymerase mu (pol mu) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. *Mol Cell Biol* **22,** 5194-5202.

Mailand N, Gibbs-Seymour I & Bekker-Jensen S. (2013). Regulation of PCNA-protein interactions for genome stability. *Nat Rev Mol Cell Biol* **14,** 269-282.

Maiorino M, Roveri A, Coassin M & Ursini F. (1988). Kinetic mechanism and substrate specificity of glutathione peroxidase activity of ebselen (PZ51). *Biochem Pharmacol* **37,** 2267-2271.

Mao G, Pan X, Zhu BB, Zhang Y, Yuan F, Huang J, Lovell MA, Lee MP, Markesbery WR, Li GM & Gu L. (2007). Identification and characterization of OGG1 mutations in patients with Alzheimer's disease. *Nucleic Acids Res* **35,** 2759-2766.

Mari PO, Florea BI, Persengiev SP, Verkaik NS, Brüggenwirth HT, Modesti M, Giglia-Mari G, Bezstarosti K, Demmers JA, Luider TM, Houtsmuller AB & van Gent DC. (2006). Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proc Natl Acad Sci U S A* **103,** 18597-18602.

Marklund N, Sihver S, Långström B, Bergström M & Hillered L. (2002). Effect of traumatic brain injury and nitrone radical scavengers on relative changes in regional cerebral blood flow and glucose uptake in rats. *J Neurotrauma* **19,** 1139-1153.

Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG & Novotny EJ. (1995). Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J Cereb Blood Flow Metab* **15,** 12-25.

Mathis C, Paul SM & Crawley JN. (1994). Characterization of benzodiazepine-sensitive behaviors in the A/J and C57BL/6J inbred strains of mice. *Behav Genet* **24,** 171-180.

Matyja E & Albrecht J. (1993). Ultrastructural evidence that mercuric chloride lowers the threshold for glutamate neurotoxicity in an organotypic culture of rat cerebellum. *Neurosci Lett* **158,** 155-158.

McCallum GP, Wong AW & Wells PG. (2011). Cockayne syndrome B protects against methamphetamine-enhanced oxidative DNA damage in murine fetal brain and postnatal neurodevelopmental deficits. *Antioxid Redox Signal* **14,** 747-756.

McClelland JL, McNaughton BL & O'Reilly RC. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev* **102,** 419-457.

McKenna JT & Vertes RP. (2001). Collateral projections from the median raphe nucleus to the medial septum and hippocampus. *Brain Res Bull* **54,** 619-630.

Miki H & Funato Y. (2012). Regulation of intracellular signalling through cysteine oxidation by reactive oxygen species. *J Biochem* **151,** 255-261.

Milatovic D, Radic Z, Zivin M & Dettbarn WD. (2000a). Atypical effect of some spin trapping agents: reversible inhibition of acetylcholinesterase. *Free Radic Biol Med* **28,** 597-603.

Milatovic D, Zivin M, Hustedt E & Dettbarn WD. (2000b). Spin trapping agent phenyl-N-tert-butylnitrone prevents diisopropylphosphorofluoridate-induced excitotoxicity in skeletal muscle of the rat. *Neurosci Lett* **278,** 25-28.

Miller RA, Harrison DE, Astle CM, Floyd RA, Flurkey K, Hensley KL, Javors MA, Leeuwenburgh C, Nelson JF, Ongini E, Nadon NL, Warner HR & Strong R. (2007). An Aging Interventions Testing Program: study design and interim report. *Aging Cell* **6,** 565-575.

Minowa O, Arai T, Hirano M, Monden Y, Nakai S, Fukuda M, Itoh M, Takano H, Hippou Y, Aburatani H, Masumura K, Nohmi T, Nishimura S & Noda T. (2000). Mmh/Ogg1 gene

inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc Natl Acad Sci U S A* **97,** 4156-4161.

Miyamoto M, Kato J, Narumi S & Nagaoka A. (1987). Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. *Brain Res* **419,** 19-31.

Mocquet V, Lainé JP, Riedl T, Yajin Z, Lee MY & Egly JM. (2008). Sequential recruitment of the repair factors during NER: the role of XPG in initiating the resynthesis step. *EMBO J* **27,** 155-167.

Mokkapati SK, Wiederhold L, Hazra TK & Mitra S. (2004). Stimulation of DNA glycosylase activity of OGG1 by NEIL1: functional collaboration between two human DNA glycosylases. *Biochemistry* **43,** 11596-11604.

Molina y Vedia L, McDonald B, Reep B, Brüne B, Di Silvio M, Billiar TR & Lapetina EG. (1992). Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. *J Biol Chem* **267,** 24929-24932.

Montgomery KS, Mackey J, Thuett K, Ginestra S, Bizon JL & Abbott LC. (2008). Chronic, low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice. *Behav Brain Res* **191,** 55-61.

Montiel T, Quiroz-Baez R, Massieu L & Arias C. (2006). Role of oxidative stress on beta-amyloid neurotoxicity elicited during impairment of energy metabolism in the hippocampus: protection by antioxidants. *Exp Neurol* **200,** 496-508.

Moon SH, Lin L, Zhang X, Nguyen TA, Darlington Y, Waldman AS, Lu X & Donehower LA. (2010). Wild-type p53-induced phosphatase 1 dephosphorylates histone variant gamma-H2AX and suppresses DNA double strand break repair. *J Biol Chem* **285,** 12935-12947.

Moretto MB, Funchal C, Santos AQ, Gottfried C, Boff B, Zeni G, Pureur RP, Souza DO, Wofchuk S & Rocha JB. (2005). Ebselen protects glutamate uptake inhibition caused by methyl mercury but does not by Hg2+. *Toxicology* **214,** 57-66.

Mortensen UH, Bendixen C, Sunjevaric I & Rothstein R. (1996). DNA strand annealing is promoted by the yeast Rad52 protein. *Proc Natl Acad Sci U S A* **93,** 10729-10734.

Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G & Nadel L. (2005). Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. *J Anat* **207,** 35-66.

Moser J, Kool H, Giakzidis I, Caldecott K, Mullenders LH & Fousteri MI. (2007). Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. *Mol Cell* **27,** 311-323.

Muftuoglu M, de Souza-Pinto NC, Dogan A, Aamann M, Stevnsner T, Rybanska I, Kirkali G, Dizdaroglu M & Bohr VA. (2009). Cockayne syndrome group B protein stimulates repair of formamidopyrimidines by NEIL1 DNA glycosylase. *J Biol Chem* **284,** 9270-9279.

Munk BH, Burrows CJ & Schlegel HB. (2007). Exploration of mechanisms for the transformation of 8-hydroxy guanine radical to FAPyG by density functional theory. *Chem Res Toxicol* **20,** 432-444.

Mutkus L, Aschner JL, Syversen T & Aschner M. (2005). Methylmercury alters the in vitro uptake of glutamate in GLAST- and GLT-1-transfected mutant CHO-K1 cells. *Biol Trace Elem Res* **107,** 231-245.

Myers GJ & Davidson PW. (1998). Prenatal methylmercury exposure and children: neurologic, developmental, and behavioral research. *Environ Health Perspect* **106 Suppl 3,** 841-847.

Myers GJ, Davidson PW, Shamlaye CF, Axtell CD, Cernichiari E, Choisy O, Choi A, Cox C & Clarkson TW. (1997). Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. *Neurotoxicology* **18,** 819-829.

Nadel L, Samsonovich A, Ryan L & Moscovitch M. (2000). Multiple trace theory of human memory: computational, neuroimaging, and neuropsychological results. *Hippocampus* **10,** 352-368.

Nakada S, Chen GI, Gingras AC & Durocher D. (2008). PP4 is a gamma H2AX phosphatase required for recovery from the DNA damage checkpoint. *EMBO Rep* **9,** 1019-1026.

Nakamura J, Purvis ER & Swenberg JA. (2003). Micromolar concentrations of hydrogen peroxide induce oxidative DNA lesions more efficiently than millimolar concentrations in mammalian cells. *Nucleic Acids Res* **31,** 1790-1795.

Narasimhaiah R, Tuchman A, Lin SL & Naegele JR. (2005). Oxidative damage and defective DNA repair is linked to apoptosis of migrating neurons and progenitors during cerebral cortex development in Ku70-deficient mice. *Cereb Cortex* **15,** 696-707.

Nash HM, Lu R, Lane WS & Verdine GL. (1997). The critical active-site amine of the human 8-oxoguanine DNA glycosylase, hOgg1: direct identification, ablation and chemical reconstitution. *Chem Biol* **4,** 693-702.

Nauseef WM. (2013). Detection of superoxide anion and hydrogen peroxide production by cellular NADPH oxidases. *Biochim Biophys Acta*.

Newland MC & Rasmussen EB. (2000). Aging unmasks adverse effects of gestational exposure to methylmercury in rats. *Neurotoxicol Teratol* **22,** 819-828.

Newland MC & Reile PA. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicol Sci* **50,** 106-116.

Nick McElhinny SA, Snowden CM, McCarville J & Ramsden DA. (2000). Ku recruits the XRCC4-ligase IV complex to DNA ends. *Mol Cell Biol* **20,** 2996-3003.

Nicol CJ, Zielenski J, Tsui LC & Wells PG. (2000). An embryoprotective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. *FASEB J* **14,** 111-127.

Niki E. (1987). Interaction of ascorbate and alpha-tocopherol. *Ann N Y Acad Sci* **498,** 186-199.

Niki E & Traber MG. (2012). A history of vitamin E. *Ann Nutr Metab* **61,** 207-212.

Ninomiya T, Imamura K, Kuwahata M, Kindaichi M, Susa M & Ekino S. (2005). Reappraisal of somatosensory disorders in methylmercury poisoning. *Neurotoxicol Teratol* **27,** 643-653.

Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K & Nakabeppu Y. (1999). Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol Biol Cell* **10,** 1637-1652.

Noh KM & Koh JY. (2000). Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. *J Neurosci* **20,** RC111.

Nutini M, Frazzini V, Marini C, Spalloni A, Sensi SL & Longone P. (2011). Zinc pre-treatment enhances NMDAR-mediated excitotoxicity in cultured cortical neurons from SOD1(G93A) mouse, a model of amyotrophic lateral sclerosis. *Neuropharmacology* **60,** 1200-1208.

Ogi T, Limsirichaikul S, Overmeer RM, Volker M, Takenaka K, Cloney R, Nakazawa Y, Niimi A, Miki Y, Jaspers NG, Mullenders LH, Yamashita S, Fousteri MI & Lehmann AR. (2010). Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. *Mol Cell* **37,** 714-727.

Ogura H, Takeuchi T & Morimoto K. (1996). A comparison of the 8-hydroxydeoxyguanosine, chromosome aberrations and micronucleus techniques for the assessment of the genotoxicity of mercury compounds in human blood lymphocytes. *Mutat Res* **340,** 175-182.

Ohara S, Inoue K, Yamada M, Yamawaki T, Koganezawa N, Tsutsui K, Witter MP & Iijima T. (2009). Dual transneuronal tracing in the rat entorhinal-hippocampal circuit by intracerebral injection of recombinant rabies virus vectors. *Front Neuroanat* **3,** 1.

Ohmura Y, Izumi T, Yamaguchi T, Tsutsui-Kimura I, Yoshida T & Yoshioka M. (2010). The serotonergic projection from the median raphe nucleus to the ventral hippocampus is involved in the retrieval of fear memory through the corticotropin-releasing factor type 2 receptor. *Neuropsychopharmacology* **35,** 1271-1278.

Oken E, Radesky JS, Wright RO, Bellinger DC, Amarasiriwardena CJ, Kleinman KP, Hu H & Gillman MW. (2008). Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am J Epidemiol* **167,** 1171-1181.

Oken E, Wright RO, Kleinman KP, Bellinger D, Amarasiriwardena CJ, Hu H, Rich-Edwards JW & Gillman MW. (2005). Maternal fish consumption, hair mercury, and infant cognition in a U.S. Cohort. *Environ Health Perspect* **113,** 1376-1380.

Olsvik PA, Amlund H & Torstensen BE. (2011). Dietary lipids modulate methylmercury toxicity in Atlantic salmon. *Food Chem Toxicol* **49,** 3258-3271.

Ondovcik SL, Preston TJ, McCallum GP & Wells PG. (2013a). Expression of human oxoguanine glycosylase 1 or formamidopyrimidine glycosylase in human embryonic kidney 293 cells exacerbates methylmercury toxicity in vitro. *Toxicol Appl Pharmacol*.

Ondovcik SL, Tamblyn L, McPherson JP & Wells PG. (2012). Oxoguanine glycosylase 1 (OGG1) protects cells from DNA double-strand break damage following methylmercury (MeHg) exposure. *Toxicol Sci* **128,** 272-283.

Ondovcik SL, Tamblyn L, McPherson JP & Wells PG. (2013b). Sensitivity to methylmercury toxicity is enhanced in oxoguanine glycosylase 1 knockout murine embryonic fibroblasts and is dependent on cellular proliferation capacity. *Toxicol Appl Pharmacol* **270,** 23-30.

Onishchenko N, Tamm C, Vahter M, Hökfelt T, Johnson JA, Johnson DA & Ceccatelli S. (2007). Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice. *Toxicol Sci* **97,** 428-437.

Ortega-García JA, Rodriguez K, Calatayud M, Martin M, Vélez D, Devesa V, Sánchez-Alarcon MC, Torres Cantero AM, Galindo-Cascales C, Gil-Vázquez JM, Sánchez-Sauco MF, Sánchez-Solís M, Alfonso-Marsilla B & Romero-Braquehais F. (2009). Estimated intake levels of methylmercury in children, childbearing age and pregnant women in a Mediterranean region, Murcia, Spain. *Eur J Pediatr* **168,** 1075-1080.

Osten P, Wisden W & Sprengel R. (2007). Molecular mechanisms of synaptic function in the hippocampus: neurotransmitter exocytosis and glutamatergic, GABAergic, and cholinergic transmission. In *The Hippocampus Book*, ed. Andersen P, Morris R, Amaral D, Bliss T & O'Keefe J. Oxford University Press, New York.

Osterod M, Hollenbach S, Hengstler JG, Barnes DE, Lindahl T & Epe B. (2001). Age-related and tissue-specific accumulation of oxidative DNA base damage in 7,8-dihydro-8-oxoguanine-DNA glycosylase (Ogg1) deficient mice. *Carcinogenesis* **22,** 1459-1463.

Park EM, Shigenaga MK, Degan P, Korn TS, Kitzler JW, Wehr CM, Kolachana P & Ames BN. (1992). Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc Natl Acad Sci U S A* **89,** 3375-3379.

Parman T, Chen G & Wells PG. (1998). Free radical intermediates of phenytoin and related teratogens. Prostaglandin H synthase-catalyzed bioactivation, electron paramagnetic resonance spectrometry, and photochemical product analysis. *J Biol Chem* **273,** 25079-25088.

Parman T, Wiley MJ & Wells PG. (1999). Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. *Nat Med* **5,** 582-585.

Parpura V & Haydon PG. (2000). Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc Natl Acad Sci U S A* **97,** 8629-8634.

Parsons JL, Zharkov DO & Dianov GL. (2005). NEIL1 excises 3' end proximal oxidative DNA lesions resistant to cleavage by NTH1 and OGG1. *Nucleic Acids Res* **33,** 4849-4856.

Pascucci B, Maga G, Hubscher U, Bjoras M, Seeberg E, Hickson ID, Villani G, Giordano C, Cellai L & Dogliotti E. (2002). Reconstitution of the base excision repair pathway for 7,8-dihydro-8-oxoguanine with purified human proteins. *Nucleic Acids Res* **30,** 2124-2130.

Pascucci B, Stucki M, Jónsson ZO, Dogliotti E & Hübscher U. (1999). Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. *J Biol Chem* **274,** 33696-33702.

Pastoriza-Gallego M, Armier J & Sarasin A. (2007). Transcription through 8-oxoguanine in DNA repair-proficient and Csb(-)/Ogg1(-) DNA repair-deficient mouse embryonic fibroblasts is dependent upon promoter strength and sequence context. *Mutagenesis* **22,** 343-351.

Paull TT & Lee JH. (2005). The Mre11/Rad50/Nbs1 complex and its role as a DNA double-strand break sensor for ATM. *Cell Cycle* **4,** 737-740.

Peckham NH & Choi BH. (1988). Abnormal neuronal distribution within the cerebral cortex after prenatal methylmercury intoxication. *Acta Neuropathol* **76,** 222-226.

Pedrero Zayas Z, Ouerdane L, Mounicou S, Lobinski R, Monperrus M & Amouroux D. (2013). Hemoglobin as a major binding protein for methylmercury in white-sided dolphin liver. *Anal Bioanal Chem*.

Peterson SL, Purvis RS & Griffith JW. (2005). Comparison of neuroprotective effects induced by alpha-phenyl-N-tert-butyl nitrone (PBN) and N-tert-butyl-alpha-(2 sulfophenyl) nitrone (S-PBN) in lithium-pilocarpine status epilepticus. *Neurotoxicology* **26,** 969-979.

Phillips ER & McKinnon PJ. (2007). DNA double-strand break repair and development. *Oncogene* **26,** 7799-7808.

Piao MJ, Kim KC, Choi JY, Choi J & Hyun JW. (2011). Silver nanoparticles down-regulate Nrf2-mediated 8-oxoguanine DNA glycosylase 1 through inactivation of extracellular regulated kinase and protein kinase B in human Chang liver cells. *Toxicol Lett* **207,** 143-148.

Polster BM, Basañez G, Etxebarria A, Hardwick JM & Nicholls DG. (2005). Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. *J Biol Chem* **280,** 6447-6454.

Potts RJ, Watkin RD & Hart BA. (2003). Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. *Toxicology* **184,** 189-202.

Proteggente AR, England TG, Rice-Evans CA & Halliwell B. (2001). Iron supplementation and oxidative damage to DNA in healthy individuals with high plasma ascorbate. *Biochem Biophys Res Commun* **288,** 245-251.

Przybylowska K, Kabzinski J, Sygut A, Dziki L, Dziki A & Majsterek I. (2013). An association selected polymorphisms of XRCC1, OGG1 and MUTYH gene and the level of efficiency oxidative DNA damage repair with a risk of colorectal cancer. *Mutat Res* **745-746,** 6-15.

Rabenstein DL, Isab AA & Reid RS. (1982). A proton nuclear magnetic resonance study of the binding of methylmercury in human erythrocytes. *Biochim Biophys Acta* **720,** 53-64.

Radi R, Beckman JS, Bush KM & Freeman BA. (1991a). Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* **266,** 4244-4250.

Radi R, Beckman JS, Bush KM & Freeman BA. (1991b). Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* **288,** 481-487.

Radicella JP, Dherin C, Desmaze C, Fox MS & Boiteux S. (1997). Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **94,** 8010-8015.

Ralston NV, Ralston CR, Blackwell JL & Raymond LJ. (2008). Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology* **29,** 802-811.

Ramadan K, Shevelev I & Hübscher U. (2004). The DNA-polymerase-X family: controllers of DNA quality? *Nat Rev Mol Cell Biol* **5,** 1038-1043.

Ramanathan G & Atchison WD. (2011). Ca2+ entry pathways in mouse spinal motor neurons in culture following in vitro exposure to methylmercury. *Neurotoxicology* **32,** 742-750.

Ramkissoon A & Wells PG. (2013). Developmental role of nuclear factor E2-related factor 2 in mitigating methamphetamine fetal toxicity and postnatal neurodevelopmental deficits. *Free Radic Biol Med* **65C,** 620-631.

Ramon R, Murcia M, Aguinagalde X, Amurrio A, Llop S, Ibarluzea J, Lertxundi A, Alvarez-Pedrerol M, Casas M, Vioque J, Sunyer J, Tardon A, Martinez-Arguelles B & Ballester F. (2011). Prenatal mercury exposure in a multicenter cohort study in Spain. *Environ Int* **37,** 597-604.

Rand MD, Bland CE & Bond J. (2008). Methylmercury activates enhancer-of-split and bearded complex genes independent of the notch receptor. *Toxicol Sci* **104,** 163-176.

Ravanat JL, Douki T, Duez P, Gremaud E, Herbert K, Hofer T, Lasserre L, Saint-Pierre C, Favier A & Cadet J. (2002). Cellular background level of 8-oxo-7,8-dihydro-2'-deoxyguanosine: an isotope based method to evaluate artefactual oxidation of DNA during its extraction and subsequent work-up. *Carcinogenesis* **23,** 1911-1918.

Reagan-Shaw S, Nihal M & Ahmad N. (2008). Dose translation from animal to human studies revisited. *FASEB J* **22,** 659-661.

Reddy YV, Ding Q, Lees-Miller SP, Meek K & Ramsden DA. (2004). Non-homologous end joining requires that the DNA-PK complex undergo an autophosphorylation-dependent rearrangement at DNA ends. *J Biol Chem* **279,** 39408-39413.

Regnell CE, Hildrestrand GA, Sejersted Y, Medin T, Moldestad O, Rolseth V, Krokeide SZ, Suganthan R, Luna L, Bjørås M & Bergersen LH. (2012). Hippocampal adult neurogenesis is maintained by Neil3-dependent repair of oxidative DNA lesions in neural progenitor cells. *Cell Rep* **2,** 503-510.

Rice DC & Hayward S. (1999). Comparison of visual function at adulthood and during aging in monkeys exposed to lead or methylmercury. *Neurotoxicology* **20,** 767-784.

Rice G, Swartout J, Mahaffey K & Schoeny R. (2000). Derivation of U.S. EPA's oral Reference Dose (RfD) for methylmercury. *Drug Chem Toxicol* **23,** 41-54.

Riis B, Risom L, Loft S & Poulsen HE. (2002). OGG1 mRNA expression and incision activity in rats are higher in foetal tissue than in adult liver tissue while 8-oxo-2'-deoxyguanosine levels are unchanged. *DNA Repair (Amst)* **1,** 709-717.

Rogakou EP, Pilch DR, Orr AH, Ivanova VS & Bonner WM. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* **273,** 5858-5868.

Roman HA, Walsh TL, Coull BA, Dewailly É, Guallar E, Hattis D, Mariën K, Schwartz J, Stern AH, Virtanen JK & Rice G. (2011). Evaluation of the cardiovascular effects of methylmercury exposures: current evidence supports development of a dose-response function for regulatory benefits analysis. *Environ Health Perspect* **119,** 607-614.

Roos DH, Puntel RL, Santos MM, Souza DO, Farina M, Nogueira CW, Aschner M, Burger ME, Barbosa NB & Rocha JB. (2009). Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: involvement of oxidative stress and glutamatergic system. *Toxicol In Vitro* **23,** 302-307.

Roozendaal B, Koolhaas JM & Bohus B. (1993). The central amygdala is involved in conditioning but not in retention of active and passive shock avoidance in male rats. *Behav Neural Biol* **59,** 143-149.

Rosenquist TA, Zharkov DO & Grollman AP. (1997). Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci U S A* **94,** 7429-7434.

Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N & Kuncl RW. (1994). Localization of neuronal and glial glutamate transporters. *Neuron* **13,** 713-725.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG & Hoekstra WG. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179,** 588-590.

Russo MT, De Luca G, Degan P, Parlanti E, Dogliotti E, Barnes DE, Lindahl T, Yang H, Miller JH & Bignami M. (2004). Accumulation of the oxidative base lesion 8-hydroxyguanine in DNA of tumor-prone mice defective in both the Myh and Ogg1 DNA glycosylases. *Cancer Res* **64,** 4411-4414.

Russo NJ, Kapp BS, Holmquist BK & Musty RE. (1976). Passive avoidance and amygdala lesions: relationship with pituitary-adrenal system. *Physiol Behav* **16,** 191-199.

Saha T, Rih JK, Roy R, Ballal R & Rosen EM. (2010). Transcriptional regulation of the base excision repair pathway by BRCA1. *J Biol Chem* **285,** 19092-19105.

Saito K, Yoshioka H & Cutler RG. (1998). A spin trap, N-tert-butyl-alpha-phenylnitrone extends the life span of mice. *Biosci Biotechnol Biochem* **62,** 792-794.

Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K & Ichijo H. (1998). Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* **17,** 2596-2606.

Sameer AS, Nissar S, Abdullah S, Chowdri NA & Siddiqi MA. (2012). DNA repair gene 8-oxoguanine DNA glycosylase Ser326Cys polymorphism and colorectal cancer risk in a Kashmiri population. *DNA Cell Biol* **31,** 541-546.

Samuelsson C, Kumlien E, Elfving A, Lindholm D & Ronne-Engström E. (2003). The effects of PBN (phenyl-butyl-nitrone) on GLT-1 levels and on the extracellular levels of amino acids and energy metabolites in a model of iron-induced posttraumatic epilepsy. *Epilepsy Res* **56,** 165-173.

Sarker AH, Tsutakawa SE, Kostek S, Ng C, Shin DS, Peris M, Campeau E, Tainer JA, Nogales E & Cooper PK. (2005). Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: insights for transcription-coupled repair and Cockayne Syndrome. *Mol Cell* **20,** 187-198.

Sasakura C & Suzuki KT. (1998). Biological interaction between transition metals (Ag, Cd and Hg), selenide/sulfide and selenoprotein P. *J Inorg Biochem* **71,** 159-162.

Sattler R, Xiong Z, Lu WY, Hafner M, MacDonald JF & Tymianski M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science* **284,** 1845-1848.

Saxowsky TT, Meadows KL, Klungland A & Doetsch PW. (2008). 8-Oxoguanine-mediated transcriptional mutagenesis causes Ras activation in mammalian cells. *Proc Natl Acad Sci U S A* **105,** 18877-18882.

Schläwicke Engström K, Strömberg U, Lundh T, Johansson I, Vessby B, Hallmans G, Skerfving S & Broberg K. (2008). Genetic variation in glutathione-related genes and body burden of methylmercury. *Environ Health Perspect* **116,** 734-739.

Schmidt CW. (2012). Quicksilver & gold: mercury pollution from artisanal and small-scale gold mining. *Environ Health Perspect* **120,** A424-429.

SCOVILLE WB & MILNER B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* **20,** 11-21.

Sensi SL, Yin HZ, Carriedo SG, Rao SS & Weiss JH. (1999a). Preferential Zn2+ influx through Ca2+-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production. *Proc Natl Acad Sci U S A* **96,** 2414-2419.

Sensi SL, Yin HZ & Weiss JH. (1999b). Glutamate triggers preferential Zn2+ flux through Ca2+ permeable AMPA channels and consequent ROS production. *Neuroreport* **10,** 1723-1727.

Serrander L, Cartier L, Bedard K, Banfi B, Lardy B, Plastre O, Sienkiewicz A, Fórró L, Schlegel W & Krause KH. (2007). NOX4 activity is determined by mRNA levels and reveals a unique pattern of ROS generation. *Biochem J* **406,** 105-114.

Shanker G, Aschner JL, Syversen T & Aschner M. (2004a). Free radical formation in cerebral cortical astrocytes in culture induced by methylmercury. *Brain Res Mol Brain Res* **128,** 48-57.

Shanker G & Aschner M. (2003). Methylmercury-induced reactive oxygen species formation in neonatal cerebral astrocytic cultures is attenuated by antioxidants. *Brain Res Mol Brain Res* **110,** 85-91.

Shanker G, Hampson RE & Aschner M. (2004b). Methylmercury stimulates arachidonic acid release and cytosolic phospholipase A2 expression in primary neuronal cultures. *Neurotoxicology* **25,** 399-406.

Shanker G, Mutkus LA, Walker SJ & Aschner M. (2002). Methylmercury enhances arachidonic acid release and cytosolic phospholipase A2 expression in primary cultures of neonatal astrocytes. *Brain Res Mol Brain Res* **106,** 1-11.

Shao C, Xiong S, Li GM, Gu L, Mao G, Markesbery WR & Lovell MA. (2008). Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. *Free Radic Biol Med* **45,** 813-819.

Sheline CT, Behrens MM & Choi DW. (2000). Zinc-induced cortical neuronal death: contribution of energy failure attributable to loss of NAD(+) and inhibition of glycolysis. *J Neurosci* **20,** 3139-3146.

Shetty AK & Turner DA. (1998). Hippocampal interneurons expressing glutamic acid decarboxylase and calcium-binding proteins decrease with aging in Fischer 344 rats. *J Comp Neurol* **394,** 252-269.

Shibutani S, Takeshita M & Grollman AP. (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349,** 431-434.

Shichiri M, Takanezawa Y, Uchida K, Tamai H & Arai H. (2007). Protection of cerebellar granule cells by tocopherols and tocotrienols against methylmercury toxicity. *Brain Res* **1182,** 106-115.

Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA & Murphy TH. (2003). Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J Neurosci* **23,** 3394-3406.

Silva RH, Abílio VC, Takatsu AL, Kameda SR, Grassl C, Chehin AB, Medrano WA, Calzavara MB, Registro S, Andersen ML, Machado RB, Carvalho RC, Ribeiro ReA, Tufik S & Frussa-Filho R. (2004). Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology* **46,** 895-903.

Simmons-Willis TA, Koh AS, Clarkson TW & Ballatori N. (2002). Transport of a neurotoxicant by molecular mimicry: the methylmercury-L-cysteine complex is a substrate for human L-type large neutral amino acid transporter (LAT) 1 and LAT2. *Biochem J* **367,** 239-246.

Singh B, Chatterjee A, Ronghe AM, Bhat NK & Bhat HK. (2013). Antioxidant-mediated up-regulation of OGG1 via NRF2 induction is associated with inhibition of oxidative DNA damage in estrogen-induced breast cancer. *BMC Cancer* **13,** 253.

Singh KP, Kumari R, Pevey C, Jackson D & DuMond JW. (2009). Long duration exposure to cadmium leads to increased cell survival, decreased DNA repair capacity, and genomic instability in mouse testicular Leydig cells. *Cancer Lett* **279,** 84-92.

Smith JC & Farris FF. (1996). Methyl mercury pharmacokinetics in man: a reevaluation. *Toxicol Appl Pharmacol* **137,** 245-252.

mith QR. (2000). Transport of glutamate and other amino acids at the blood-brain barrier. *J Nutr* **130,** 1016S-1022S.

Smith QR, Momma S, Aoyagi M & Rapoport SI. (1987). Kinetics of neutral amino acid transport across the blood-brain barrier. *J Neurochem* **49,** 1651-1658.

Smith TB, Dun MD, Smith ND, Curry BJ, Connaughton HS & Aitken RJ. (2013). The presence of a truncated base excision repair pathway in human spermatozoa that is mediated by OGG1. *J Cell Sci* **126,** 1488-1497.

Sokol SY. (2011). Maintaining embryonic stem cell pluripotency with Wnt signaling. *Development* **138,** 4341-4350.

Sokolowski K, Falluel-Morel A, Zhou X & DiCicco-Bloom E. (2011). Methylmercury (MeHg) elicits mitochondrial-dependent apoptosis in developing hippocampus and acts at low exposures. *Neurotoxicology* **32,** 535-544.

Spagnolo L, Rivera-Calzada A, Pearl LH & Llorca O. (2006). Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. *Mol Cell* **22,** 511-519.

Spyker JM. (1975). Assessing the impact of low level chemicals on development: behavioral and latent effects. *Fed Proc* **34,** 1835-1844.

Spyker JM & Smithberg M. (1972). Effects of methylmercury on prenatal development in mice. *Teratology* **5,** 181-190.

Squire LR, Stark CE & Clark RE. (2004). The medial temporal lobe. *Annu Rev Neurosci* **27,** 279-306.

Srinivasan A, Lehmler HJ, Robertson LW & Ludewig G. (2001). Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. *Toxicol Sci* **60,** 92-102.

Stern AH, Jacobson JL, Ryan L & Burke TA. (2004). Do recent data from the Seychelles Islands alter the conclusions of the NRC Report on the toxicological effects of methylmercury? *Environ Health* **3,** 2.

Stern AH & Smith AE. (2003). An assessment of the cord blood:maternal blood methylmercury ratio: implications for risk assessment. *Environ Health Perspect* **111,** 1465-1470.

Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Löbrich M & Jeggo PA. (2004). ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res* **64,** 2390-2396.

Stiff T, Walker SA, Cerosaletti K, Goodarzi AA, Petermann E, Concannon P, O'Driscoll M & Jeggo PA. (2006). ATR-dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling. *EMBO J* **25,** 5775-5782.

Stokes-Riner A, Thurston SW, Myers GJ, Duffy EM, Wallace J, Bonham M, Robson P, Shamlaye CF, Strain JJ, Watson G & Davidson PW. (2011). A longitudinal analysis of prenatal exposure to methylmercury and fatty acids in the Seychelles. *Neurotoxicol Teratol* **33,** 325-328.

Strain JJ, Davidson PW, Bonham MP, Duffy EM, Stokes-Riner A, Thurston SW, Wallace JM, Robson PJ, Shamlaye CF, Georger LA, Sloane-Reeves J, Cernichiari E, Canfield RL,

Cox C, Huang LS, Janciuras J, Myers GJ & Clarkson TW. (2008). Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *Neurotoxicology* **29,** 776-782.

Strain JJ, Davidson PW, Thurston SW, Harrington D, Mulhern MS, McAfee AJ, van Wijngaarden E, Shamlaye CF, Henderson J, Watson GE, Zareba G, Cory-Slechta DA, Lynch M, Wallace JM, McSorley EM, Bonham MP, Stokes-Riner A, Sloane-Reeves J, Janciuras J, Wong R, Clarkson TW & Myers GJ. (2012). Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles. *J Nutr* **142,** 1943-1949.

Stringari J, Meotti FC, Souza DO, Santos AR & Farina M. (2006). Postnatal methylmercury exposure induces hyperlocomotor activity and cerebellar oxidative stress in mice: dependence on the neurodevelopmental period. *Neurochem Res* **31,** 563-569.

Stäubli U & Huston JP. (1980). Facilitation of learning by post-trial injection of substance P into the medial septal nucleus. *Behav Brain Res* **1,** 245-255.

Sun GY, Shelat PB, Jensen MB, He Y, Sun AY & Simonyi A. (2010). Phospholipases A2 and inflammatory responses in the central nervous system. *Neuromolecular Med* **12,** 133-148.

Sundberg J, Jönsson S, Karlsson MO, Hallén IP & Oskarsson A. (1998). Kinetics of methylmercury and inorganic mercury in lactating and nonlactating mice. *Toxicol Appl Pharmacol* **151,** 319-329.

Sundberg J, Jönsson S, Karlsson MO & Oskarsson A. (1999). Lactational exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. *Toxicol Appl Pharmacol* **154,** 160-169.

Surh YJ. (2003). Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* **3,** 768-780.

Suzuki KT, Sasakura C & Yoneda S. (1998). Binding sites for the (Hg-Se) complex on selenoprotein P. *Biochim Biophys Acta* **1429,** 102-112.

Sørensen N, Murata K, Budtz-Jørgensen E, Weihe P & Grandjean P. (1999). Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. *Epidemiology* **10,** 370-375.

Taherzadeh-Fard E, Saft C, Wieczorek S, Epplen JT & Arning L. (2010). Age at onset in Huntington's disease: replication study on the associations of ADORA2A, HAP1 and OGG1. *Neurogenetics* **11,** 435-439.

Takaoka S, Kawakami Y, Fujino T, Oh-ishi F, Motokura F, Kumagai Y & Miyaoka T. (2008). Somatosensory disturbance by methylmercury exposure. *Environ Res* **107,** 6-19.

Takeuchi T, D'Itri FM, Fischer PV, Annett CS & Okabe M. (1977). The outbreak of Minamata disease (methyl mercury poisoning) in cats on Northwestern Ontario reserves. *Environ Res* **13,** 215-228.

Takeuchi T & Eto K. (1999). *The Pathology of Minamata Disease: A Tragic Story of Water Pollution*. Kyushu University Press, Fukuoka.

Tamashiro H, Akagi H, Arakaki M, Futatsuka M & Roht LH. (1984). Causes of death in Minamata disease: analysis of death certificates. *Int Arch Occup Environ Health* **54,** 135-146.

Tamm C, Duckworth JK, Hermanson O & Ceccatelli S. (2008). Methylmercury inhibits differentiation of rat neural stem cells via Notch signalling. *Neuroreport* **19,** 339-343.

Tani M, Shinmura K, Kohno T, Shiroishi T, Wakana S, Kim SR, Nohmi T, Kasai H, Takenoshita S, Nagamachi Y & Yokota J. (1998). Genomic structure and chromosomal localization of the mouse Ogg1 gene that is involved in the repair of 8-hydroxyguanine in DNA damage. *Mamm Genome* **9,** 32-37.

Telmer KH & Veiga MM. (2009). World Emissions of Mercury from Artisanal and Small Scale Gold Mining. In *Mercury Fate and Transport in the Global Atmosphere: Emissions, Measurements and Models*, ed. Mason R & Pirrone N, pp. 131-172. Springer US, New York.

Thireau J, Zhang BL, Poisson D & Babuty D. (2008). Heart rate variability in mice: a theoretical and practical guide. *Exp Physiol* **93,** 83-94.

Thompson SA, White CC, Krejsa CM, Diaz D, Woods JS, Eaton DL & Kavanagh TJ. (1999). Induction of glutamate-cysteine ligase (gamma-glutamylcysteine synthetase) in the brains of adult female mice subchronically exposed to methylmercury. *Toxicol Lett* **110,** 1-9.

Tornaletti S, Maeda LS, Kolodner RD & Hanawalt PC. (2004). Effect of 8-oxoguanine on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. *DNA Repair (Amst)* **3,** 483-494.

Trudeau-Lame ME, Kalgutkar AS & LaFontaine M. (2003). Pharmacokinetics and metabolism of the reactive oxygen scavenger alpha-phenyl-N-tert-butylnitrone in the male Sprague-Dawley rat. *Drug Metab Dispos* **31,** 147-152.

Tsien JZ, Huerta PT & Tonegawa S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* **87,** 1327-1338.

Tsuchiya A, Hinners TA, Burbacher TM, Faustman EM & Mariën K. (2008). Mercury exposure from fish consumption within the Japanese and Korean communities. *J Toxicol Environ Health A* **71,** 1019-1031.

Usuki F & Fujimura M. (2012). Effects of methylmercury on cellular signal transduction systems. In *Methylmercury and Neurotoxicity*, ed. Ceccatelli S & Aschner M, pp. 229-240. Springer US, New York, USA.

Usuki F, Yamashita A & Fujimura M. (2011). Post-transcriptional defects of antioxidant selenoenzymes cause oxidative stress under methylmercury exposure. *J Biol Chem* **286,** 6641-6649.

Usuki F, Yasutake A, Umehara F, Tokunaga H, Matsumoto M, Eto K, Ishiura S & Higuchi I. (2001). In vivo protection of a water-soluble derivative of vitamin E, Trolox, against methylmercury-intoxication in the rat. *Neurosci Lett* **304,** 199-203.

Valera B, Dewailly E & Poirier P. (2013). Association between methylmercury and cardiovascular risk factors in a native population of Quebec (Canada): a retrospective evaluation. *Environ Res* **120,** 102-108.

van der Kemp PA, Thomas D, Barbey R, de Oliveira R & Boiteux S. (1996). Cloning and expression in Escherichia coli of the OGG1 gene of Saccharomyces cerevisiae, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. *Proc Natl Acad Sci U S A* **93,** 5197-5202.

van Strien NM, Cappaert NL & Witter MP. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. *Nat Rev Neurosci* **10,** 272-282.

van Wijngaarden E, Myers GJ, Shamlaye CF, Strain JJ & Davidson PW. (2012). The impact of prenatal exposure to methylmercury and maternal nutrition status on child development: findings from the Seychelles Child Development Study. In *Methylmercury and Neurotoxicity*, ed. Ceccatelli S & Aschner M, pp. 37-53. Springer, US, New York, USA.

Venable N & Kelly PH. (1990). Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. *Psychopharmacology (Berl)* **100,** 215-221.

Volterra A, Trotti D & Racagni G. (1994a). Glutamate uptake is inhibited by arachidonic acid and oxygen radicals via two distinct and additive mechanisms. *Mol Pharmacol* **46,** 986-992.

Volterra A, Trotti D, Tromba C, Floridi S & Racagni G. (1994b). Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *J Neurosci* **14,** 2924-2932.

Walker JR, Corpina RA & Goldberg J. (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412,** 607-614.

Walsh CT. (1982). The influence of age on the gastrointestinal absorption of mercuric chloride and methyl mercury chloride in the rat. *Environ Res* **27,** 412-420.

Wan J, Bae MA & Song BJ. (2004). Acetoaminophen-induced accumulation of 8-oxodeoxyguanosine through reduction of Ogg1 DNA repair enzyme in C6 glioma cells. *Exp Mol Med* **36,** 71-77.

Wang H, Rosidi B, Perrault R, Wang M, Zhang L, Windhofer F & Iliakis G. (2005a). DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. *Cancer Res* **65,** 4020-4030.

Wang J, Markesbery WR & Lovell MA. (2006). Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J Neurochem* **96,** 825-832.

Wang J, Xiong S, Xie C, Markesbery WR & Lovell MA. (2005b). Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J Neurochem* **93,** 953-962.

Watanabe C, Yoshida K, Kasanuma Y, Kun Y & Satoh H. (1999). In utero methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. *Environ Res* **80,** 208-214.

Wechsler T, Newman S & West SC. (2011). Aberrant chromosome morphology in human cells defective for Holliday junction resolution. *Nature* **471,** 642-646.

Wei B, Zhou Y, Xu Z, Xi B, Cheng H, Ruan J, Zhu M, Hu Q, Wang Q, Wang Z, Yan Z, Jin K, Zhou D, Xuan F, Huang X, Shao J & Lu P. (2011). The effect of hOGG1 Ser326Cys polymorphism on cancer risk: evidence from a meta-analysis. *PLoS One* **6,** e27545.

Wells PG, Bhuller Y, Chen CS, Jeng W, Kasapinovic S, Kennedy JC, Kim PM, Laposa RR, McCallum GP, Nicol CJ, Parman T, Wiley MJ & Wong AW. (2005). Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. *Toxicol Appl Pharmacol* **207,** 354-366.

Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, Preston TJ, Wiley MJ & Wong AW. (2009). Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. *Toxicol Sci* **108,** 4-18.

Wells PG, McCallum GP, Lam KC, Henderson JT & Ondovcik SL. (2010). Oxidative DNA damage and repair in teratogenesis and neurodevelopmental deficits. *Birth Defects Res C Embryo Today* **90,** 103-109.

Wells PG, Zubovits JT, Wong ST, Molinari LM & Ali S. (1989). Modulation of phenytoin teratogenicity and embryonic covalent binding by acetylsalicylic acid, caffeic acid, and alpha-phenyl-N-t-butylnitrone: implications for bioactivation by prostaglandin synthetase. *Toxicol Appl Pharmacol* **97,** 192-202.

Wolf ME, Xue CJ, Li Y & Wavak D. (2000). Amphetamine increases glutamate efflux in the rat ventral tegmental area by a mechanism involving glutamate transporters and reactive oxygen species. *J Neurochem* **75,** 1634-1644.

Wolfer DP, Müller U, Stagliar M & Lipp HP. (1997). Assessing the effects of the 129/Sv genetic background on swimming navigation learning in transgenic mutants: a study using mice with a modified beta-amyloid precursor protein gene. *Brain Res* **771,** 1-13.

Wong AW, McCallum GP, Jeng W & Wells PG. (2008). Oxoguanine glycosylase 1 protects against methamphetamine-enhanced fetal brain oxidative DNA damage and neurodevelopmental deficits. *J Neurosci* **28,** 9047-9054.

Wong JM, Ionescu D & Ingles CJ. (2003). Interaction between BRCA2 and replication protein A is compromised by a cancer-predisposing mutation in BRCA2. *Oncogene* **22,** 28-33.

Wu X, Liang H, O'Hara KA, Yalowich JC & Hasinoff BB. (2008). Thiol-modulated mechanisms of the cytotoxicity of thimerosal and inhibition of DNA topoisomerase II alpha. *Chem Res Toxicol* **21,** 483-493.

Xie Y, Yang H, Cunanan C, Okamoto K, Shibata D, Pan J, Barnes DE, Lindahl T, McIlhatton M, Fishel R & Miller JH. (2004). Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. *Cancer Res* **64,** 3096-3102.

Xie Y, Yang H, Miller JH, Shih DM, Hicks GG, Xie J & Shiu RP. (2008). Cells deficient in oxidative DNA damage repair genes Myh and Ogg1 are sensitive to oxidants with increased G2/M arrest and multinucleation. *Carcinogenesis* **29,** 722-728.

Xu B, Xu ZF, Deng Y, Liu W, Yang HB & Wei YG. (2012). Protective effects of MK-801 on methylmercury-induced neuronal injury in rat cerebral cortex: involvement of oxidative stress and glutamate metabolism dysfunction. *Toxicology* **300,** 112-120.

Yamashita T, Ando Y, Nakamura M, Obayashi K, Terazaki H, Haraoka K, Guo SX, Ueda M & Uchino M. (2004). Inhibitory effect of α-tocopherol on methylmercury-induced oxidative steress. *Environ Health Prev Med* **9,** 111-117.

Yasutake A, Hirayama K & Inoue M. (1989). Mechanism of urinary excretion of methylmercury in mice. *Arch Toxicol* **63,** 479-483.

Yasutake A & Nakamura M. (2011). Induction by mercury compounds of metallothioneins in mouse tissues: inorganic mercury accumulation is not a dominant factor for metallothionein induction in the liver. *J Toxicol Sci* **36,** 365-372.

Yin Z, Jiang H, Syversen T, Rocha JB, Farina M & Aschner M. (2008). The methylmercury-L-cysteine conjugate is a substrate for the L-type large neutral amino acid transporter. *J Neurochem* **107,** 1083-1090.

Yin Z, Lee E, Ni M, Jiang H, Milatovic D, Rongzhu L, Farina M, Rocha JB & Aschner M. (2011). Methylmercury-induced alterations in astrocyte functions are attenuated by ebselen. *Neurotoxicology* **32,** 291-299.

Yoshida M, Shimizu N, Suzuki M, Watanabe C, Satoh M, Mori K & Yasutake A. (2008). Emergence of delayed methylmercury toxicity after perinatal exposure in metallothionein-null and wild-type C57BL mice. *Environ Health Perspect* **116,** 746-751.

You Z, Shi LZ, Zhu Q, Wu P, Zhang YW, Basilio A, Tonnu N, Verma IM, Berns MW & Hunter T. (2009). CtIP links DNA double-strand break sensing to resection. *Mol Cell* **36,** 954-969.

Youn CK, Kim SH, Lee DY, Song SH, Chang IY, Hyun JW, Chung MH & You HJ. (2005). Cadmium down-regulates human OGG1 through suppression of Sp1 activity. *J Biol Chem* **280,** 25185-25195.

Young JF, Wosilait WD & Luecke RH. (2001). Analysis of methylmercury disposition in humans utilizing a PBPK model and animal pharmacokinetic data. *J Toxicol Environ Health A* **63,** 19-52.

Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM & Dawson VL. (2002). Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science* **297,** 259-263.

Yu WK & Wells PG. (1995). Evidence for lipoxygenase-catalyzed bioactivation of phenytoin to a teratogenic reactive intermediate: in vitro studies using linoleic acid-dependent soybean lipoxygenase, and in vivo studies using pregnant CD-1 mice. *Toxicol Appl Pharmacol* **131,** 1-12.

Zaganas I, Spanaki C & Plaitakis A. (2012). Expression of human GLUD2 glutamate dehydrogenase in human tissues: functional implications. *Neurochem Int* **61,** 455-462.

Zalups RK & Bridges CC. (2009). MRP2 involvement in renal proximal tubular elimination of methylmercury mediated by DMPS or DMSA. *Toxicol Appl Pharmacol* **235,** 10-17.

Zhang J, Dawson VL, Dawson TM & Snyder SH. (1994). Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* **263,** 687-689.

Zhang Y, Yuan F, Presnell SR, Tian K, Gao Y, Tomkinson AE, Gu L & Li GM. (2005). Reconstitution of 5'-directed human mismatch repair in a purified system. *Cell* **122,** 693-705.

Zhao Z, Loane DJ, Murray MG, Stoica BA & Faden AI. (2012). Comparing the predictive value of multiple cognitive, affective, and motor tasks after rodent traumatic brain injury. *J Neurotrauma* **29,** 2475-2489.

Zharkov DO & Rosenquist TA. (2002). Inactivation of mammalian 8-oxoguanine-DNA glycosylase by cadmium(II): implications for cadmium genotoxicity. *DNA Repair (Amst)* **1,** 661-670.

Zhong L, Arnér ES & Holmgren A. (2000). Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. *Proc Natl Acad Sci U S A* **97,** 5854-5859.

Zhou S, Chan E, Duan W, Huang M & Chen YZ. (2005). Drug bioactivation, covalent binding to target proteins and toxicity relevance. *Drug Metab Rev* **37,** 41-213.

Zimmer B, Lee G, Balmer NV, Meganathan K, Sachinidis A, Studer L & Leist M. (2012). Evaluation of developmental toxicants and signaling pathways in a functional test based on the migration of human neural crest cells. *Environ Health Perspect* **120,** 1116-1122.

List of Publications and Abstracts

Note: Lam KC was previously published under Lam K.

Peer Reviewed Papers

1. Lam KC, Shapiro AM, McPherson JP, Wells PG. *Protection by the free radical spin trap PBN against some methylmercury-initiated cognitive deficits may not involve decreasing oxidative damage to DNA*. (2013). (*In preparation*).

2. Lam KC, Shapiro AM, Sabat N, Liao H, Choi H, McPherson JP, Wells PG. *Deficiencies in oxoguanine glycosylase 1 may increase susceptibility to cognitive deficits initiated by in utero exposure to methylmercury.* (2013). (*In preparation*).

3. Wells PG, McCallum GP, **Lam KC**, Henderson JT and Ondovcik SL. *Oxidative DNA damage and repair in teratogenesis and neurodevelopmental deficits*. Birth Defects Res C: Embryo Today. 2010 Jun; 90(2):103-9.

Abstracts

1. Lam KC, Wells PG. The free radical spin trap α-phenyl-N-t-butylnitrone (PBN) reduces postnatal cognitive deficits caused by *in utero* exposure to methylmercury. J Popul Ther Clin Pharmacol 19(2):e286, 2012.

2. Lam K, McCallum GP, Wells PG. Fetal oxidative DNA damage and repair in methylmercury neurodevelopmental deficits. Toxicol. Sci. (Supplement: The Toxicologist) 114(1): 340 (No. 1601), 2010.

3. Lam K, McCallum GP, Wells PG. Fetal Oxidative DNA Damage and Repair in Neurodevelopmental Deficits. Visions in Pharmacology 2009.

4. Lam K, McCallum GP, Wells PG. Temporal pattern of increased oxidative DNA damage in murine fetal brain after *in utero* exposure to methylmercury. Visions in Pharmacology 2008.

5. Lam K, McCallum GP, Wells PG. Temporal pattern of increased oxidative DNA damage in murine fetal brain after *in utero* exposure to methylmercury. Toxicol. Sci. (Supplement: The Toxicologist) 102(1): 381 (No. 1854), 2008.

6. Ondovcik SL, Preston TJ, Ho SK, Henderson JT, **Lam K**, Wells PG. A novel transgenic mouse model expressing a human enzyme for repair of oxidative DNA damage. Toxicol. Sci. (Supplement: The Toxicologist) 102(1): 46 (No. 226), 2008.

7. Lam K, McCallum GP, Wells PG. Methylmercury enhances *in vivo* murine fetal oxidative DNA damage. Birth Defects Res Part A: Clinical and Molecular Teratology 79(5): 417 (P23), 2007

Appendices

Appendix 1: Supplemental Figures

Control		Control + PBN		MeHg		MeHg + PBN	
Age (mth)	Cause of Death	Age (mth)	Cause of Death	Age (mth)	Cause of Death	Age (mth)	Cause of Death
4	Unknown	10	Cage flooded	7	Unknown	3	Dermatitis
10	Unknown	10	Cage flooded	8	Unknown	4	Unknown
		10	Cage flooded	9	Unknown	8	Infected tail
				12	Unknown		
				13	Unknown		
				15	Lung tumour		
				15	Dermatitis		
				16	Unknown - blood-filled abdomen		
				16	Liver tumour		

Table A1.1: Causes of death in CD-1 mice treated with MeHg and PBN
Animals with causes of death designated unknown were found during routine cage checks with no obvious pathologies. One animal found dead was found on postmortem examination to have a large quantity of blood in the abdominal cavity. Animals with dermatitis, infected tail, and tumours were euthanized for quality of life endpoints. See page 138 for commentary.

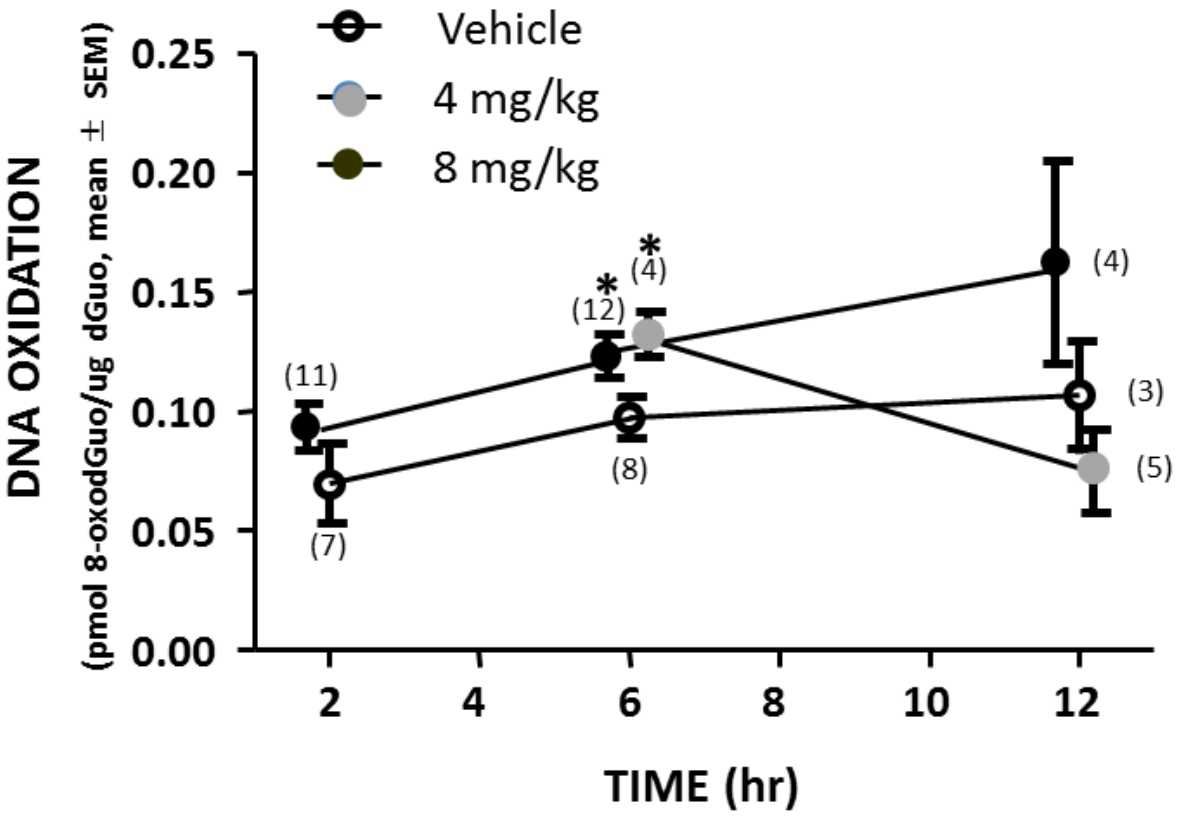

Figure A1.1: DNA Oxidation in CD-1 mice treated with MeHg
Pregnant CD-1 dams were dosed on GD 17 with 0, 4, or 8 mg/kg i.p. MeHg. Fetal brains were collected 2, 6, and 12 hr after MeHg treatment and analyzed using HPLC-UV to quantify deoxyguanosine and HPLC-EC to quantify 8-oxo-dGuo, based on a method by (Ravanat *et al*. 2002). Mice exposed to 4 or 8 mg/kg had increased levels of DNA oxidation at 6 hr after MeHg exposure. * denotes a difference from the control group at the same timepoint. (x) = number of fetuses. See pages 94, 95, 101, and 136 for commentary.

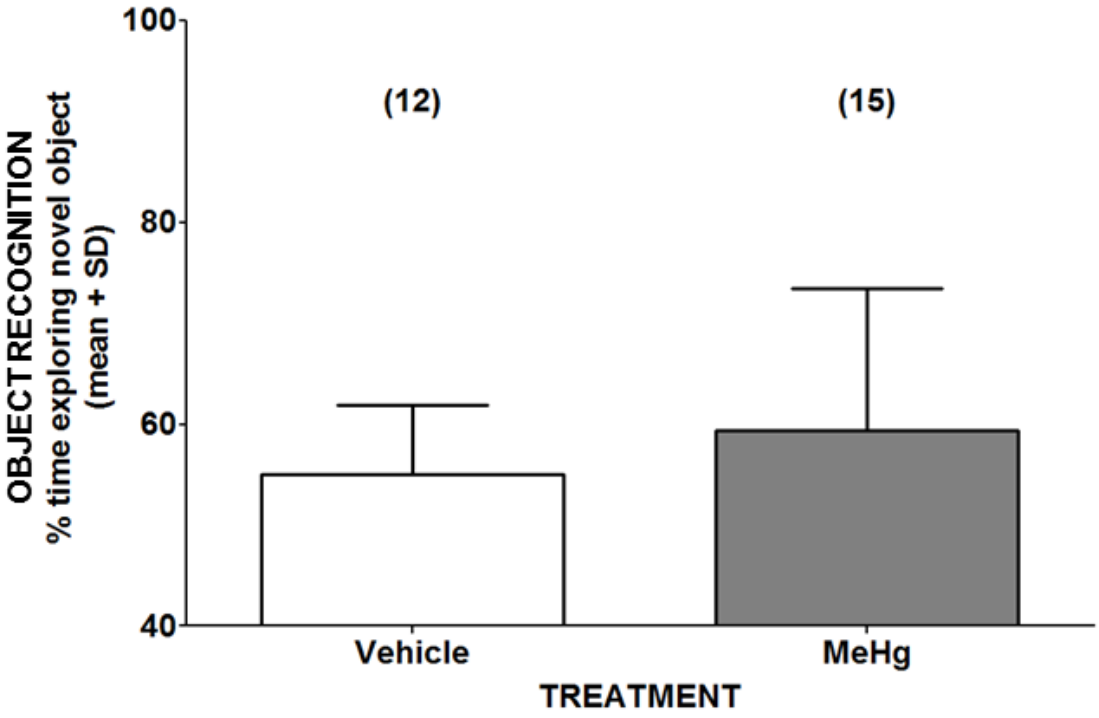

Figure A1.2: Novel object recognition in 4 week-old CD-1 mice treated with MeHg
Pregnant CD-1 dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg. Offspring were tested for novel object recognition using the method described in section 2.4. No differences in novel object recognition were observed between control and MeHg-treated progeny. (x) = number of offspring. See page 98 for commentary.

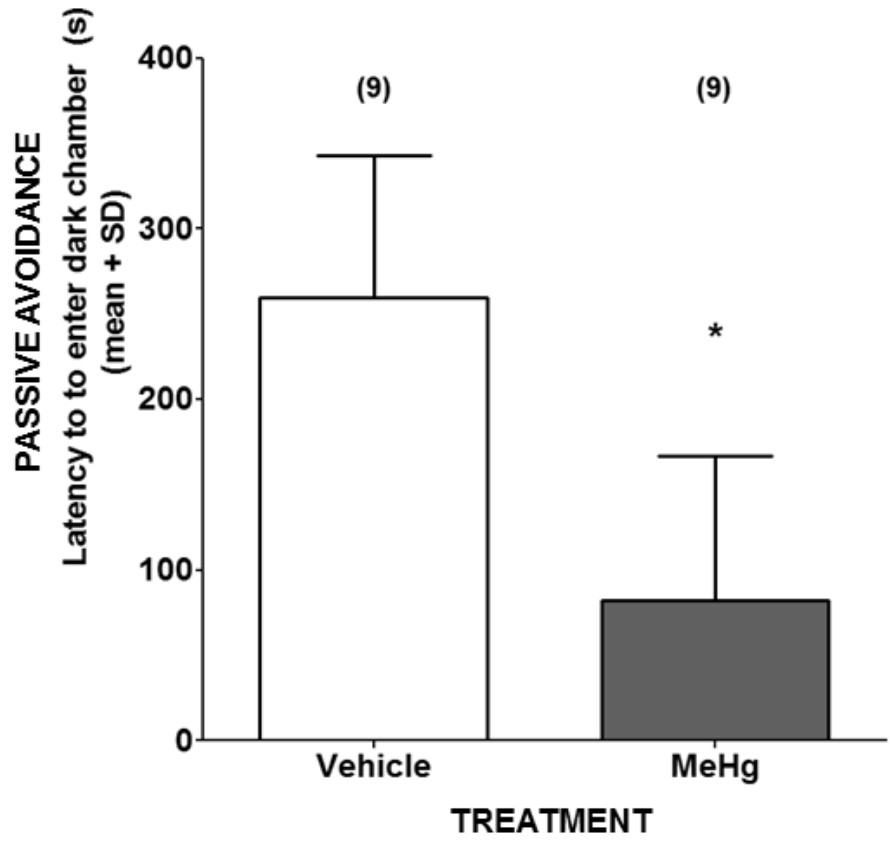

Figure A1.3: Passive avoidance learning in 7 month-old CD-1 mice treated with MeHg
Pregnant CD-1 dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg. Offspring were tested for passive avoidance learning using the method described in section 2.5. Results shown are for the third trial. MeHg treated mice exhibited a significant cognitive deficit. * denotes a difference from the control group $p < 0.05$. (x) = number of offspring. See page 100 for commentary.

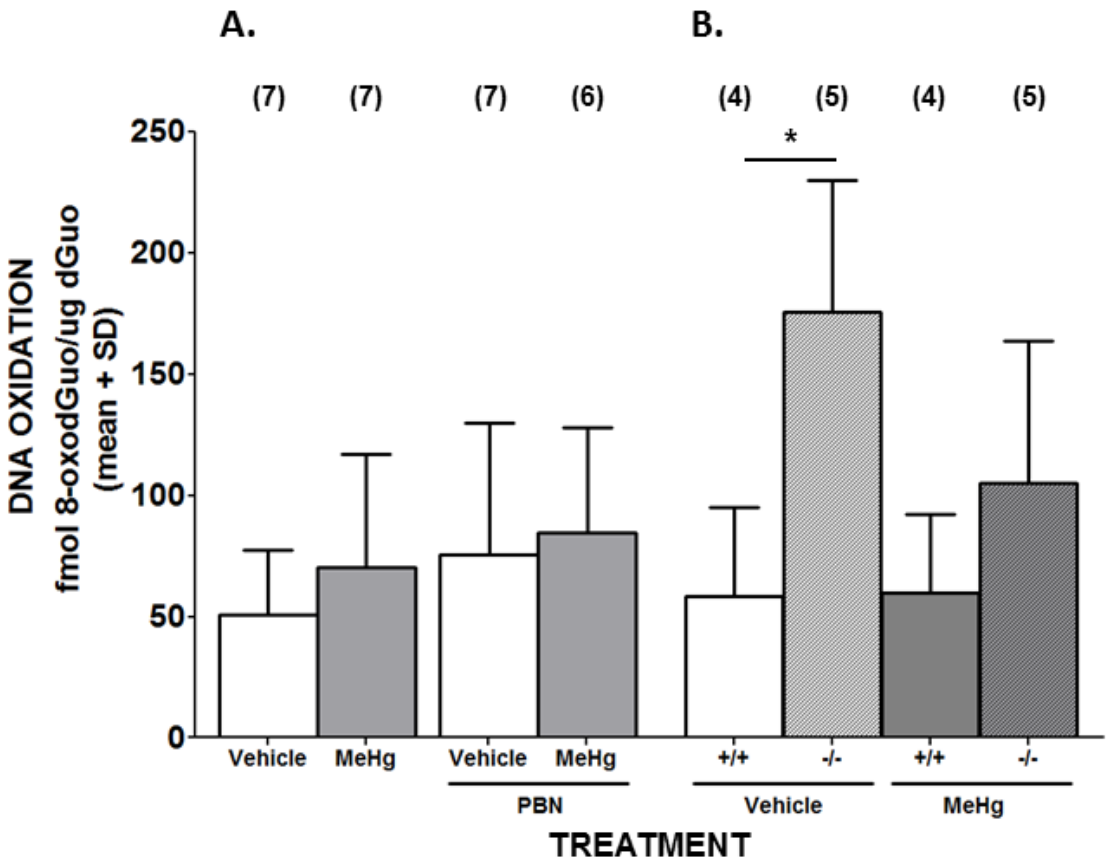

Figure A1.4: Comparison of DNA oxidation in CD-1 mice exposed to MeHg and PBN and *ogg1* mice exposed to MeHg

A. Pregnant CD-1 dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg with or without pretreatment with 45 mg/kg i.p. PBN. **B.** Pregnant heterozygous *ogg1* dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg. Fetal brains were collected 6 hr after MeHg exposure. 8-oxodGuo was measured using HPLC with tandem MS and dGuo was measured using HPLC-UV. *Ogg1* knockout animals had higher levels of DNA oxidation than *ogg1* wild-type animals. No other differences were observed between any other groups. * denotes a difference from the wild-type offspring with the same treatment. (x) = number of offspring. See page 136 for commentary.

Appendix 2: Optimization of Behavioural Tests for *ogg1* Mice

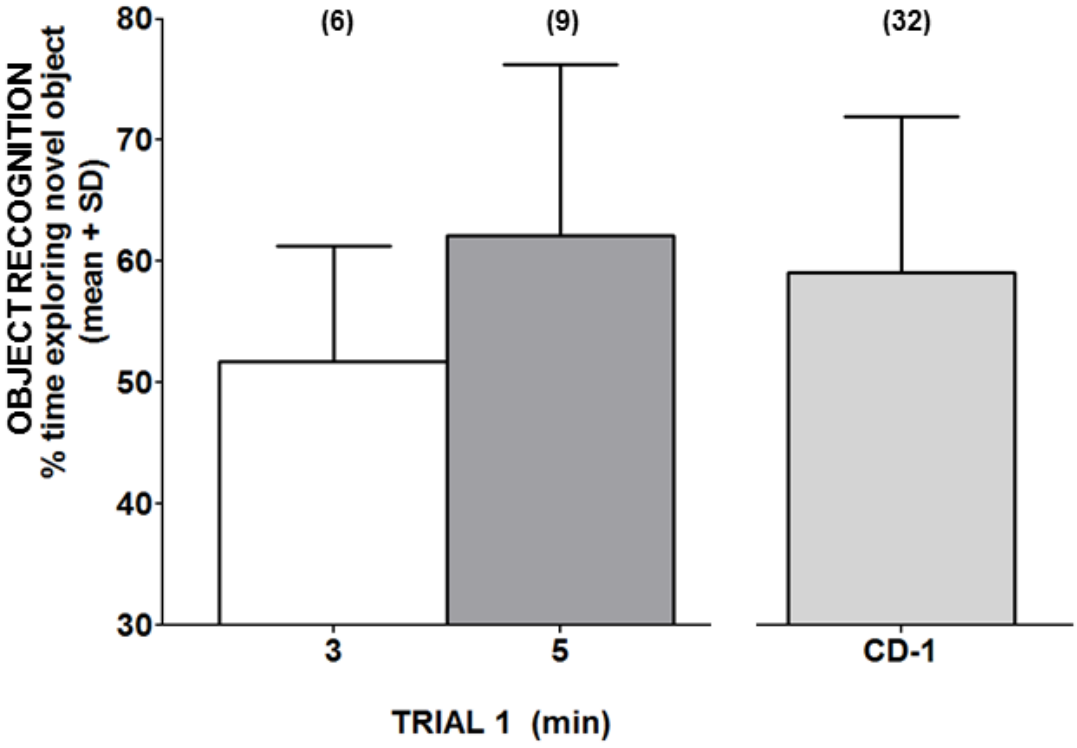

Figure A2.1: Comparison of novel object recognition in vehicle-treated *ogg1* wild-type mice after a 3 minute familiarization trial and a 5 minute familiarization trial

Pregnant heterozygous *ogg1* dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg. Offspring were tested for novel object recognition at 6 weeks of age. After pilot testing, it was determined that a 5 minute familiarization trial was required for optimal baseline performance for the vehicle-treated *ogg1* wild-type mice. For comparison, the performance of the vehicle-treated CD-1 mice after a 3 minute familiarization trial is indicated on the right side of the graph. (x) = number of offspring. See pages 96 and 152 for commentary.

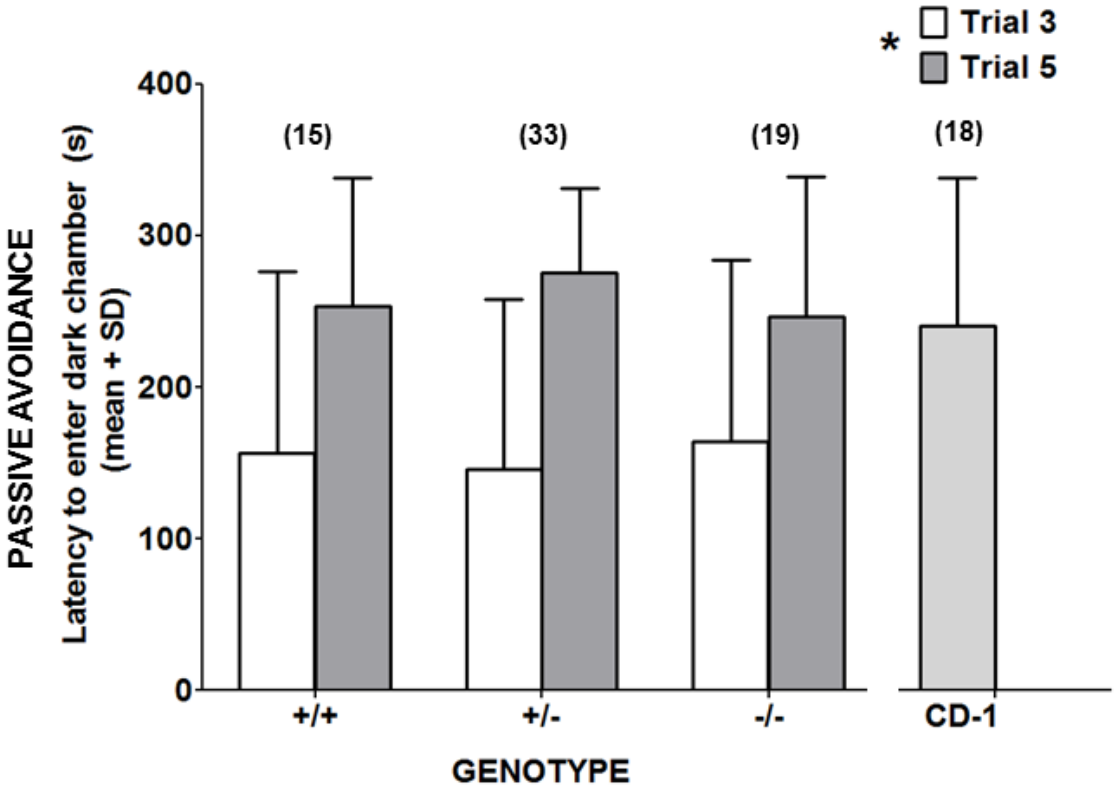

Figure A2.2: Comparison of passive avoidance learning in vehicle-treated *ogg1* wild-type mice after 3 trials and after 5 trials

Pregnant heterozygous *ogg1* dams were dosed on GD 17 with 0 or 4 mg/kg i.p. MeHg. Wild-type offspring were tested for passive avoidance learning at 4 months of age. After pilot testing, it was determined that 5 trials were necessary to optimize baseline performance for the vehicle-treated *ogg1* wild-type mice. For comparison, the performance of the vehicle-treated CD-1 mice after 3 trials is indicated on the right side of the graph. (x) = number of offspring. See pages 98 and 152 for commentary.