Impact of oxidative DNA damage repair on brain function

Monica Dahl Bjørge

Thesis for the degree of Philosophiae Doctor (PhD)

Oslo, October 2016

Department of microbiology
Oslo University Hospital
University of Oslo
Norway

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8333-367-1

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

Dedicated to Sissel

ACKNOWLEDGEMENTS

The work presented in this thesis was conducted from 2010 to 2016 at the Centre for Molecular Biology and Neuroscience, Oslo University Hospital Rikshospitalet, University of Oslo, Norway. The work received financial support from The Medical Student Research Program and Oslo University Hospital.

I would first like to thank my primary supervisor Magnar Bjørås. Thank you for allowing me to do my student research in your laboratory and for all of your support, both in academic and non-academic matters. You have always been available when I have needed advice, and your knowledge seems never-ending.

I would like to thank my co-supervisor Gunn Annette Hildrestrand, primarily for your everlasting patience with me. You and Magnar have taught me everything I know about molecular biology and all of the methods used in this thesis. You have provided me with a very safe learning environment.

Yngve Sejersted, thank you for teaching me about microsurgery and immunohistochemistry. I would also like to thank my other group members for their good advice and support: Raji Suganthan, Veslemøy Rolseth, Katja Scheffler, Luisa Luna, Tine Neurauter, Alex Rowe and Elise Kristiansen. To all the girls at the animal facility: thank you for all your help and technical support. Embjørg Wollen, thank you for your endlessly good mood and your contagious go-ahead spirit when we shared office. Maje Siebke and Lise Sofie Nissen-Meyer, thank you for introducing me to Magnars group.

At last I would like to thank my family and friends. You have been encouraging throughout my work in research, both whilst in medical school and as a PhD student. I could not have done this without your support. Øyvind, you are the love of my life. Thank you for everything.

Monica Dahl Bjørge

CONTENTS

List of papers

This thesis is based on one publication and two manuscripts referred to by Roman numbers throughout the text:

I Katja Schleffer[*#], **Monica D Bjørge**[*], Vuk Palibrk, Yngve Sejersted, Rajikala Suganthan, Anna Kuśnierczyk, Geir Slupphaug and Magnar Bjørås. DNA glycosylases Ogg1 and Mutyh suppress inflammation in brain after hypoxia-ischemia. *Manuscript.*

II **Monica Dahl Bjørge**, Yngve Sejersted, Veslemøy Rolseth, Gunn Annette Hildrestrand, Katja Scheffler, Rajikala Suganthan, Anna Kuśnierczyk, Cathrine Broberg Vågbø, Monica Atneosen-Åsegg, Christine Gran Neurauter, Geir Slupphaug, Lars Eide, Luisa Luna and Magnar Bjørås[#]. NEIL2 is a potential target in management of hypoxic-ischemic encephalopathy. *Manuscript.*

III **Monica D Bjørge**[*], Gunn A Hildrestrand[*], Katja Scheffler[*], Rajikala Suganthan, Veslemøy Rolseth, Anna Kuśnierczyk, Alexander D Rowe, Cathrine B Vågbø, Susanne Vetlesen, Lars Eide, Geir Slupphaug, Yusaku Nakabeppu, Timoty W Bredy, Arne Klungland, Magnar Bjørås[#]. Synergistic Actions of Ogg1 and Mutyh DNA Glycosylases Modulate Anxiety-like Behavior in Mice. Cell reports 13, 2671-2678, December 29, 2015.

[*]Joint first authors. [#]Corresponding authors

Abstract

Base excision repair (BER) is the primary pathway for repair of oxidative DNA damage caused by various agents including reactive oxygen species. BER is initiated by DNA glycosylases that recognize and remove damaged DNA bases, and the function of the glycosylases OGG1, MUTYH, NEIL1, and NEIL2 are the main focus of this thesis.

DNA glycosylases have been suggested to be involved in prevention of neuronal cell death under cerebral hypoxia-ischemia (HI), but the mechanism of neuroprotection is yet to be determined. In the first part of the study, we investigated the role of OGG1, MUTYH, NEIL1, and NEIL2 following HI in newborn mice. We demonstrated that MUTYH- and NEIL1-deficient mice were more sensitive to HI in most regions of the brain, whereas mice lacking OGG1 showed increased sensitivity mostly in the hippocampal area. Removal of NEIL2, on the other hand, led to neuroprotection. We did not discover major discrepancies in quantification of DNA damage in any of the genotypes. RNA sequencing revealed a dysregulation of the inflammatory response in the various knockouts, and increased IL-1β expressed in activated microglia in the hippocampus (HC) of OGG1- and MUTYH-deficient mice. It thus appears that MUTYH has a neuroprotective function, while NEIL2 seems to play a detrimental role in the brain following a HI insult.

Accumulation of oxidative DNA damage is associated with aging and cognitive decline. In the second part of the study, we focused on the function of the OGG1 and MUTYH DNA glycosylases in learning and memory in adult animals. The OGG1/MUTYH double knockout mice were more active and less anxious than wild type mice and both the double knockout and the OGG1 knockout mice displayed impaired learning. We observed no significant differences in DNA damage accumulation between the genotypes. RNA sequencing revealed candidate genes and pathways in anxiety and cognitive functions. Thus, the investigated DNA glycosylases seem to adopt distinct roles in regulation of behavior and cognitive function.

Abbreviations

1/3/7-meA/C/G	1/3/7-methylA/C/G
5ohC/U	5-hydroxyC/U
5caC	5-carboxylcytosine
5fC	5-formylcytosine
5hmC	5-hydroxymethylcytosine
5hmU	5-hydroxymethyluracil
5mC	5-methylcytosine
8-oxoG	7,8-dihydro-8-oxoguanine
A	adenine
AAG	alkyladenine DNA glycosylase
AP	apurinic/apyrimidinic
APE1	AP-endonuclease 1
ATP	adenosine triphosphate
BER	base excision repair
C	cytosine
CA1/3	Cornu Ammonis area 1/3
CpG	cytosine-phosphate-guanine
DDR	DNA damage response
DG	dentate gyrus
DNA	deoxyribonucleic acid
dRP	5'deoxyribose phosphate
DSB	double-strand break
FaPyA	4,6-diamino-5-formamidopyrimidine
FaPyG	2,6-diamino-4-hydroxy-5-formamidopyrimidine
FEN1	flap structure-specific endonuclease 1
G	guanine
H2TH	helix-two-turn-helix
H3K4Me3	histone 3 tri-methylated lysine 4
H3K27Me3	histone 3 tri-methylated lysine 27
HC	hippocampus
HhH	helix-hairpin-helix
HI	hypoxia-ischemia / hypoxic-ischemic
HR	homologous recombination
IHC	immunohistochemistry
IL	interleukin
IPA	Ingenuity pathway Analysis
LIG I/III	DNA ligase I/III
LP-BER	long-patch BER
MBD4	methyl-CpG-binding domain protein 4
MCAO	middle cerebral artery occlusion
MMR	mismatch repair
MTH1	mutT homolog-1
MUTYH	mutY homolog
MWM	morris water maze
NEIL	endonuclease VIII-like
NER	nucleotide excision repair
NHEJ	non-homologous end joining
OGG1	8-oxoguanine DNA glycosylase 1
PARP1	poly (ADP-ribose) polymerase 1
PcG	polycomb group
PCNA	proliferating cell nuclear antigen
POL β/δ/ε	polymerase β/δ/ε
PRCs	polycomb group repressive complexes
RNA	ribonucleic acid
ROS	reactive oxygen species
SMUG1	single-strand selective monofunctional uracil DNA glycosylase
SP-BER	short-patch BER
T	thymine
TDG	thymine DNA glycosylase
TET	ten-eleven translocation
TNF	tumor necrosis factor
TTC	2,3,5-triphenyltetrazolium
UDG	uracil DNA glycosylase
SSB	single strand break

Introduction

Deoxyribonucleic acid (DNA) carries all genetic information crucial for the development and functioning of an organism. DNA damage can cause mutations and cell death, but is also responsible for the genetic diversity in nature. Mutations can be harmful, beneficial, neutral, affect the offspring of the organism, or only the organism itself, all dependent on where in the DNA the mutation is located and in which cells mutations arise. From an evolutionary point of view, mutations are responsible for natural selection and the development of new species. However, at the cellular point of view, unrepaired DNA may cause apoptosis, senescence or genetic instability, which again may lead to aging or disease (Hakem 2008; Hoeijmakers 2001).

DNA damage

DNA is under continuous attack from different intracellular or extracellular DNA damaging agents. Intracellular agents include mainly reactive oxygen species (ROS) originating from metabolism. Exogenous agents include environmental factors such as chemical agents, ionizing radiation, ultraviolet radiation, and thermal disruption (Altieri *et al.,* 2008; Evans *et al.,* 2004; Hakem 2008). In addition, apurinic/ apyrimidinic (AP) sites are generated by spontaneous depurination or as intermediates during repair (Nakamura *et al.,* 1998). The sources of damage and the variety of DNA lesions they produce are shown in Figure 1.

ROS are highly reactive ions and free radicals, and are the major intracellular threat to DNA. ROS include hydroxyl radicals ($^{\bullet}OH$), superoxide radicals ($O_2^{\bullet -}$), and non-radical hydrogen peroxide (H_2O_2) (Evans *et al.,* 2004). ROS can be generated from a variety of endogenous and exogenous sources, most notably as by-products of normal cellular metabolism. They give rise to oxidized bases, AP sites, adducts, crosslinks, and strand breaks (Altieri *et al.*, 2008; Barnes *et al.*, 2004). One way to combat the threat of ROS is through antioxidants. An antioxidant can be defined as "any substrate that delays, prevents or removes oxidative damage to a target molecule" (Gutteridge *et al.*, 2010). Both endogenous and exogenous antioxidants exist. Endogenous are mitochondrial proteins like superoxide dismutases (SODs), which are

considered the first line of defence, and peroxiredoxins. The best sources of exogenous antioxidants are vegetables, fruits and grains (Finkel 2012; Gutteridge *et al.,* 2010). Although excessive ROS are damaging to the cell, they also have important roles in cell signaling and homeostasis (Azzam *et al.,* 2012; Finkel 2012; Hamanaka *et al.,* 2009). A new hypothesis has been proposed suggesting that ROS represent stress signals in response to damage, instead of being the cause of damage (Lagouge *et al.*, 2013). This emphasizes the importance of sustaining the balance between the production of ROS and antioxidants in order for the cell to function properly.

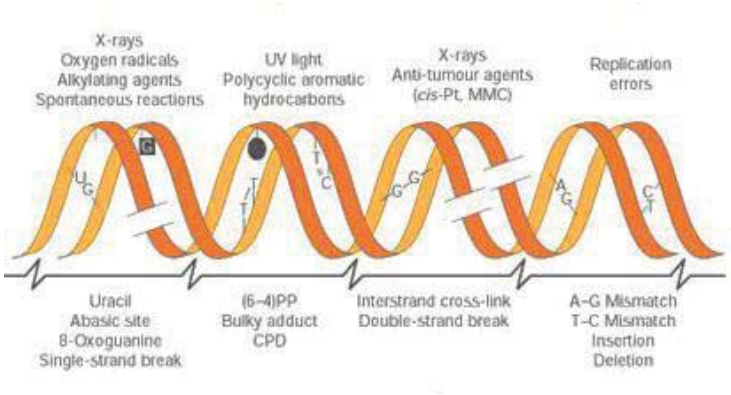

Figure 1. DNA damaging agents and DNA lesions. Different DNA damaging agents presented in relation to the damage they cause. Adapted and modified from Hoeijmakers (2001).

The major extracellular DNA damaging agents are ionizing and ultraviolet (UV) radiation (Altieri *et al.,* 2008). Ionizing radiation disrupt anatomic structures directly or indirectly through radiolysis of water, which generates both ROS and reactive nitrogen species (Azzam *et al.,* 2012). The most abundant direct damage caused by ionizing radiation is double strand breaks (DSBs) (Kumar *et al.,* 2012). UV radiation is divided into three groups depending on wavelength; UVA (320-400 nm), UVB (290-320 nm) and UVC (<290 nm). The UV wavelengths influence the spectrum of DNA damage and mutations, the most common lesion is dipyrimidine dimers (Ikehata *et al.*, 2011; Pfeifer *et al.*, 2005). Elevated temperatures may cause thermal disruption, which increases the rate of depurination and single strand breaks (SSBs). A variety of environmental and industrial chemicals cause a wide spectrum of DNA adducts and

crosslinking of the DNA (Altieri *et al.,* 2008). Some DNA polymerases replicate DNA with variable degree of fidelity, depending on the selection of correct nucleotides or damaged or incorrect nucleotides for incorporation. This is called translesion synthesis. Normally, proofreading and DNA mismatch repair (MMR) ensure high fidelity, but sometimes replication beyond a lesion is preferable to other options such as cell death. Although translesion synthesis may cause precancerous lesions, it may also be useful, as is the case in somatic hypermutation of immunoglobulin genes after exposure to an antigen, allowing mutations in the variable regions at an extraordinary rate, thus ensuring an efficient immune response (Arana *et al.*, 2010; Diaz *et al.*, 2002).

The DNA damage response

To counteract the potential detrimental effects of DNA alterations, the cell harbors a battery of different mechanisms collectively called the DNA damage response (DDR) (Jackson *et al.*, 2009). The DDR includes the detection of DNA lesions, signaling of the presence of a DNA lesion, promotion of repair of the lesion, and resolution of DNA replication problems. These processes are coordinated with ongoing cell physiology and, as shown in Fig. 3, results in cell cycle checkpoint activation, DNA repair, transcriptional program activation, apoptosis or senescence (Harper *et al.*, 2007; Jackson *et al.*, 2009). Defects in the DDR may lead to altered genetic material being passed on to daughter cells. This is seen particularly in cancer, but also in diseases like immunodeficiency, genetic disorders and neurologic disorders (Altieri *et al.*, 2008; Lord *et al.*, 2012). DDR is coordinated by post-translational modifications or direct protein interactions, and dysregulation of any of these processes may contribute to genomic instability (Huen *et al.*, 2008; Nyberg *et al.*, 2002; Warmerdam *et al.*, 2010). Post-translational modifications consist of phosphorylation, ubiquitination and sumoylation of target proteins (Bologna *et al.*, 2013; Kuo *et al.*, 2011).

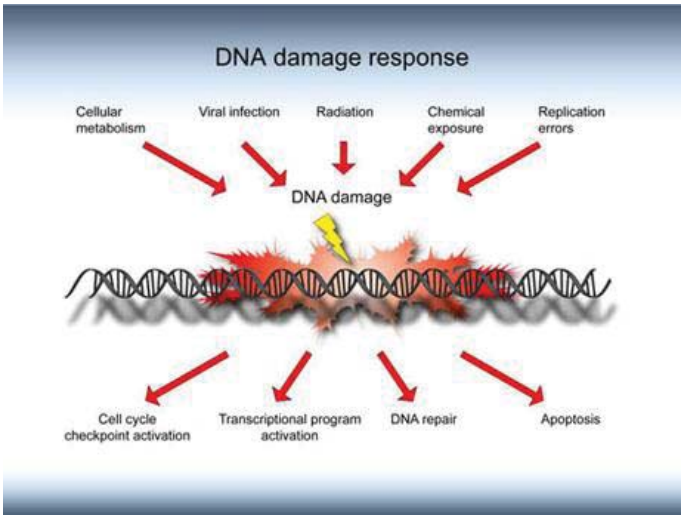

Figure 3. The DNA damage response. The effect of damage from various DNA damaging agents may involve activation of a variety of cellular responses, including cell cycle checkpoint activation, transcriptional program activation, DNA repair and apoptosis. Adapted from: http://www.paterson.man.ac.uk/Research/groups.aspx?id=9.

DNA repair mechanisms

In order to cope with the vast amount of lesions created in the DNA by the different damaging agents, multiple DNA repair pathways exist. These pathways include direct reversal (DR) repair, MMR, nucleotide excision repair (NER), BER, homologous recombination (HR) and non-homologous end joining (NHEJ) (Fig. 4).

DR repair is a single step process that involves only one protein, does not require excision of the damaged bases, and is basically error-free (Eker *et al.,* 2009). MMR removes small deletions and insertions in DNA caused by replication slippage or mismatches caused by DNA polymerase during DNA replication (Hakem 2008; Jiricny 2006). Recent advances in research have revealed that MMR also has an important role in preventing mutations caused by oxidative damage (Brierley *et al.*, 2013).

NER repairs a number of different DNA lesions, including bulky DNA lesions, through a multistep process involving at least 20-30 proteins. There are two subpathways known, global genome NER and transcription-coupled NER (Hoeijmakers 2001; Leibeling *et al.,* 2006). Global

genome NER detects and removes lesions throughout the genome, while transcription-coupled NER repairs genes that are actively transcribed. BER removes small base lesions in the DNA caused mainly by endogenous factors that induce oxidation, alkylation and deamination (Krokan *et al.*, 2013; Seeberg *et al.*, 1995). BER is the main focus of this thesis and will be handled separately in the next chapter. DSBs impose a great threat to the integrity of the genetic information. To deal with this threat, two distinct pathways exist, HR and NHEJ (Kanaar *et al.*, 2008). Naturally occurring DNA ends, telomeres, need to be shielded from being inappropriately processed by these DNA repair pathways (de Lange 2005). HR uses information from an intact sister chromatid as a template for the repair and is the most important pathway during meiotic recombination. NHEJ is a lot faster than HR, but sacrifices some accuracy by joining ends closely positioned to each other without quality control of the information (Kanaar *et al.*, 2008; Stracker *et al.*, 2011). Normal development of immunoglobulin and T-cell receptors are dependent on DSBs and subsequent NHEJ. Defects in NHEJ can therefore lead to immunodeficiency (Blundred *et al.*, 2011).

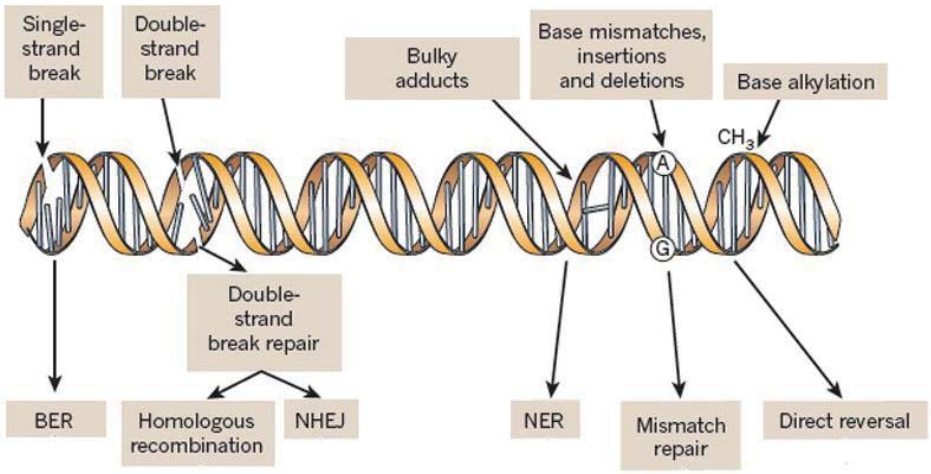

Figure 4. DNA damage repair pathways. Various types of DNA lesions result from different endogenous and exogenous DNA-damaging agents. To repair these lesions, a number of DNA repair pathways exist. These include base excision repair (BER), homologous recombination (HR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), mismatch repair (MMR), and direct reversal (DR) repair. Adapted and modified from (Lord *et al.*, 2012)

Base excision repair

BER is the primary pathway handling endogenous DNA lesions such as small chemical alterations on the DNA bases, AP-sites, and SSBs. In short, BER is initiated by DNA glycosylases, which recognize and excise oxidized and alkylated DNA bases by hydrolyzing the N-glycosidic bond. An AP-endonuclease or an AP-lyase removes the resulting AP-site. The remaining deoxyribose phosphate residue is excised by a phosphodiesterase leaving a gap that is filled by a DNA polymerase and finally the strand is sealed by a DNA ligase (Barnes *et al.*, 2004; Seeberg *et al.*, 1995). There are two main BER pathways, short-patch repair (SP-BER) and long-patch repair (LP-BER) (Fig. 5). In SP-BER, only a single nucleotide is removed. In vitro, the pathway is only dependent on four proteins to function properly; a DNA glycosylase, an AP endonuclease or AP lyase, a DNA polymerase (POL β), and a DNA ligase (Kubota *et al.,* 1996; Seeberg *et al.,* 1995). In vivo, a DNA ligase bound to X-ray repair cross-complementing protein 1 (XRCC1), a non-enzymatic scaffold protein, seals the nick and thereby completes the repair process. DNA ligase (LIG) III is essential in mitochondrial DNA (mtDNA), while in nuclear DNA (nDNA) both LIG I and LIG III can complete the repair process (Gao *et al.,* 2011; Simsek *et al.,* 2011).

In LP-BER, where 2-12 nucleotides are incorporated, a flap is created after nicking of the DNA strand by AP endonuclease 1 (APE1) 5' to the AP site, and DNA polymerases are recruited. POL β is thought to incorporate the first nucleotide, while POL δ or POL ε are the elongating polymerases. Flap structure-specific endonuclease 1 (FEN1) removes the 5' overhanging flap, and the nick is sealed by LIG I. Proliferating cell nuclear antigen (PCNA) is crucial for LP-BER as both POL β and FEN1 are dependent on PCNA to function (Brown *et al.*, 2011; Fortini *et al.*, 2007; Hegde *et al.*, 2008; Robertson *et al.*, 2009). The mechanism of deciding whether to proceed with LP-BER or SP-BER is poorly understood, but it has been shown that LP-BER occurs more frequently at lower adenosine triphosphate (ATP) concentrations near the AP-site (Petermann *et al.,* 2003). It has been proposed that if the intermediate 5'deoxyribose phosphate (dRP) produced by the AP endonuclease activity can be efficiently removed by the dRP lyase activity of POL β, SP-BER will be the preferred pathway (Robertson *et al.,* 2009).

Table 1. Mammalian DNA glycosylases

Protein	Mechanistic class[a]	Structural superfamily[b]	Substrates[c]
NTH1	Bifunctional (β)	HhH	Tg, DHU, FaPyG, 5-oh-U, 5-oh-C, hydantoins in dsDNA
NEIL1	Bifunctional (β/δ)	H2TH	Sp, Gh, Tg, DHU, DHT, 5-oh-U, 5-oh-C, 8-oxoG, FaPyA, FaPyG in ssDNA and dsDNA
NEIL2	Bifunctional (β/δ)	H2TH	Overlap with NEIL1, with minor divergence
NEIL3	Mono-/Bifunctional (β/δ)	H2TH	Sp, Gh, Tg, FaPyA, FaPyG, with preference for ssDNA
OGG1	Mono-/Bifunctional (β)	HhH	8-oxoG opposite C, FaPyG in dsDNA
MUTYH	Monofunctional	HhH	A opposite 8-oxoG, 2-oh-A opposite G in dsDNA
MBD4	Monofunctional	HhH	T or U opposite G at CpG sites, T opposite O^6-meG in dsDNA
AAG/MPG	Monofunctional	AAG	3-meA, 7-meG, 3-meG, hypoxanthine, εA in ssDNA or dsDNA
UNG	Monofunctional	UDG	Uracil, 5-FU in ssDNA or dsDNA
SMUG1	Monofunctional	UDG	Uracil, 5-FU and 5hmU in ssDNA or dsDNA
TDG	Monofunctional	UDG	T, U, and εC opposite G (CpG sites preferably) in dsDNA

[a]Based on Dalhus *et al.* (2009), Krokeide *et al.* (2013), Fromme *et al.* (2004c), and Fromme *et al.* (2004b)

[b]Based on Dalhus *et al.* (2009) and Hegde *et al.* (2008)

[c]Based on Hailer *et al.* (2005), Barnes *et al.* (2004), Jacobs *et al.* (2012), Krokeide *et al.* (2013), Brooks *et al.* (2013), Krishnamurthy *et al.* (2008), Redrejo-Rodriguez *et al.* (2011), Robertson *et al.* (2009), and Zhao *et al*. (2010)

Abbreviations: 5-FU fluorouracil, DHT dihydrothymine, DHU dihydrouracil, dsDNA double stranded DNA, εA/C ethenoA/C, Gh guanidinohydantoin, O^6-meG O^6-methylguanine, Sp spiroiminodihydantoin, ssDNA single stranded DNA, Tg thymine glycol

See text for other abbreviations and details

A total of 11 different mammalian DNA glycosylases are known today (Robertson *et al.,* 2009; Seeberg *et al.,* 1995), and the substrate range of these glycosylases is vast and overlapping, as seen in Table 1. The DNA glycosylases can be divided into groups based on mechanism or structure (Table 1). There are four mammalian structural superfamilies; helix-hairpin-helix (HhH), helix-two-turn-helix (H2TH), alkyladenine DNA glycosylase (AAG), and uracil DNA glycosylase (UDG) (Dalhus *et al.,* 2009; Hegde *et al.,* 2008). The family members of the AAG

and UDG superfamilies are compact single-domain enzymes, while the members of the HhH and H2TH superfamilies contain multiple domains, some of which may serve additional specialized biological roles (Fromme *et al.,* 2004b).

The two mechanistic classes of glycosylases are monofunctional and bifunctional glycosylases. Monofunctional glycosylases catalyze a single step reaction substituting the damaged base with an activated water molecule, creating an abasic site, which is the substrate for an AP endonuclease, like APE1. APE1 nicks the DNA backbone 5' to the AP-site, resulting in a dRP which is removed by POL β. Bifunctional glycosylases substitute the damaged base with an amine nucleophilic active site residue (Fromme *et al.*, 2004b; Fromme *et al.*, 2004c; Hegde *et al.*, 2008). They hold an intrinsic AP lyase activity that catalyze a β-elimination, which consists of a single nick leaving a 3' phosphor α,β unsatured aldehyde (PUA) end, or a β,δ-elimination, which consists of a double nick leaving a phosphate (3'P) end. PUA and 3'P are removed by APE1 and polynucleotide kinase 3'phosphatase, respectively (PKNP) (Fromme *et al.*, 2004c; Hegde *et al.*, 2008). The resulting gap is filled by DNA polymerase activities, by either LP-BER or SP-BER (Hakem 2008; Robertson *et al.,* 2009). The glycosylases removing oxidized DNA base lesions belong to the H2TH and HhH families, and consist of OGG1, NEIL paralogs 1-3, and endonuclease III-like protein (NTH), which are all bifunctional glycosylases with intrinsic AP lyase activity. In addition, SMUG1, a member of the UDG superfamily, has been shown to remove oxidized base lesions (Dalhus *et al.,* 2009).

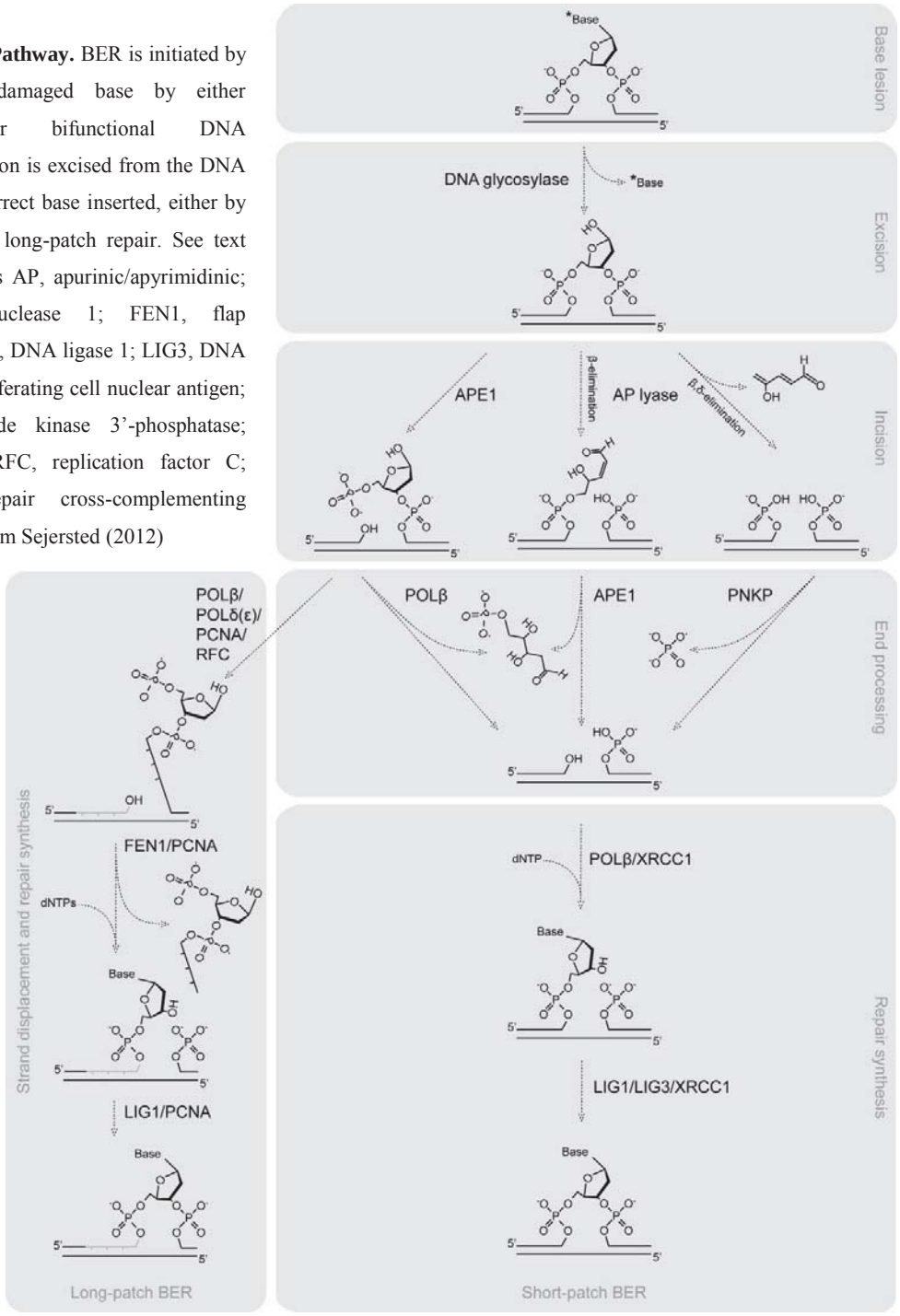

Figure 5. The BER Pathway. BER is initiated by recognition of a damaged base by either monofunctional or bifunctional DNA glycosylases. The lesion is excised from the DNA backbone, and the correct base inserted, either by short-patch repair or long-patch repair. See text body for more details AP, apurinic/apyrimidinic; APE1, AP endonuclease 1; FEN1, flap endonuclease 1; LIG1, DNA ligase 1; LIG3, DNA ligase 3; PCNA, proliferating cell nuclear antigen; PNKP, polynucleotide kinase 3'-phosphatase; POL, polymerase; RFC, replication factor C; XRCC1, X-ray repair cross-complementing protein 1. Adapted from Sejersted (2012)

8-oxoG repair

Guanine (G) has the lowest oxidation potential of the four DNA bases and is therefore more often subjected to oxidation (Devasagayam *et al.,* 1991). 7, 8-dihydro-8-oxoguanine (8-oxoG) is the major mutagenic base damage caused by ROS, and may lead to a G:C to T:A transversion mutation if left unrepaired (Michaels *et al.,* 1992; Moriya *et al.,* 1991; Russo *et al.,* 2007). It has been estimated that about 1000 Gs are oxidized to 8-oxoG in each mammalian genome every day under normal physiological conditions (Lindahl 1993; van Loon *et al.,* 2010).

Three enzymes collaborate to deal with the problem of 8-oxoG; OGG1, MutY homolog (MUTYH) and MutT homolog-1 (MTH1/NUDT1) (Russo *et al.,* 2007). The majority of 8-oxoG:C lesions are repaired by OGG1. OGG1 is localized in both the mitochondria and the nucleus (Boiteux *et al.*, 2000; Evans *et al.*, 2004), and operates differently depending on which base 8-oxoG is coupled with. When coupled with C, the reaction is bifunctional, whilst when paired with adenine (A), the reaction is monofunctional (Bjoras *et al.,* 1997). However, the lyase activity of OGG1 is very weak. Hence, intact AP sites are the major product after cleavage of 8-oxoG by OGG1 (Hegde *et al.,* 2008) (Fig. 4). 8-oxoG is also a substrate for NEIL1, but in comparison to other substrates, the affinity towards 8-oxoG is weak. NEIL1 seems to be more specific towards 8-oxoG:G mispairs (Parsons *et al.,* 2005). MUTYH excises A opposite 8-oxoG, and thereby provides another chance for OGG1 to remove 8-oxoG (Hazra *et al.*, 2001; Klungland *et al.*, 2007; Russo *et al.*, 2007). MUTYH is localized in both the mitochondria and the nucleus (Ohtsubo *et al.,* 2000; Takao *et al.,* 1999). The substrate specificity of MUTYH is of great importance, as excision of A opposite T or excision of C opposite 8-oxoG would lead to increased risk of mutagenesis. MUTYH avoids this by recognizing both 8-oxoG and the misincorporated A, and then initiate the removal of A (Fromme *et al.,* 2004a). MTH1 is the main repair enzyme that hydrolyzes damaged nucleotides in the nucleotide pool, to avoid incorporation into DNA by a polymerase (Nakabeppu *et al.,* 2010). MTH1 is localized in both the nucleus and in the mitochondria, and is expressed in both post-mitotic and proliferative tissue (Kang *et al.,* 1995; Nakabeppu *et al.,* 2006).

In addition to BER, MMR also has an important role in preventing mutations associated with 8-oxoG either by direct removal of 8-oxoG, by removal of A misincorporated opposite 8-oxoG, or a combination of both (Brierley *et al.*, 2013; Russo *et al.*, 2007).

Endonuclease VIII-like DNA Glycosylases

In mammals, there are three structural homologs of the *E.coli* Endonuclease VIII (NEI) and Formamidopyrimidine DNA glycosylase (FPG), NEIL1-3. As NEIL1 and NEIL2 are the enzymes relevant for this thesis, NEIL3 will not be further elaborated upon.

The bifunctional glycosylases NEIL1 and NEIL2 have been well characterized (Bandaru *et al.*, 2002; Das *et al.*, 2004; Dou *et al.*, 2003; Grin *et al.*, 2011; Liu *et al.*, 2010; Morland *et al.*, 2002). They have broad, overlapping substrate specificities, with a preference for oxidized bases in single-stranded DNA, double-stranded DNA and bubble DNA structures (Dalhus *et al.*, 2009; Dou *et al.*, 2003; Hazra *et al.*, 2002a; Hazra *et al.*, 2002b; Hazra *et al.*, 2006; Krishnamurthy *et al.*, 2008; Onizuka *et al.*, 2012; Parsons *et al.*, 2007; Zhao *et al.*, 2010) (Table1). NEIL1 and NEIL2 show a high evolutionary conservation at the N-terminal end. However, at the C-terminal end, there are unstructured domains that enable distinct differences in protein interactions, likely to be of vital importance for their function *in vivo* (Dalhus *et al.,* 2009; Hegde *et al.,* 2008). Human NEIL1 shows high unspecific binding to DNA, suggesting either interaction with cofactors that reduces its non-specific binding or a function at nucleosome-free regions (Odell *et al.,* 2010).

The mRNA expression of NEIL1 and NEIL2 is ubiquitous in mammals, but the expression level varies between different organs (Hazra *et al.,* 2002a; Hazra *et al.,* 2002b; Morland *et al.,* 2002). They are widely expressed in the brain (Rolseth *et al.,* 2008). Both enzymes are localized in the nucleus and are also proposed to participate in maintenance of the mitochondrial DNA (Hazra *et al.,* 2002a; Hazra *et al.,* 2002b; Hu *et al.,* 2005; Mandal *et al.,* 2012; Morland *et al.,* 2002; Sampath *et al.,* 2011; Vartanian *et al.,* 2006). NEIL2 expression remains constant throughout the cell cycle, while NEIL1 expression is quelled upon release from G0 quiescence (Neurauter *et al.,* 2012). NEIL1 is proposed to participate in prereplicative repair due to its interaction with

proteins involved in DNA replication (Hegde *et al.,* 2013). During mitosis, human NEIL1 is co-localized with condensed chromosomes and centrosomes (Hildrestrand *et al.,* 2007). In mammalian cells, NEIL2 has a preferential role in repairing oxidized bases in the transcribed genes (Banerjee *et al.,* 2011). It has been proposed that oxidative stress in the promoter region of NEIL2 regulate its expression by affecting the binding sites for ROS-responsive transcription factors (Kinslow *et al.,* 2010)

Phenotypes of BER-deficient mice

Genetically modified mice deficient of BER enzymes are extensively used to study the implications of DNA damage accumulation, mutations and carcinogenesis, both in individual cells and in tissues (Larsen *et al.,* 2007). There is no obvious phenotype in the different DNA glycosylase single knockout mice, except for the lethal TDG knockout and the UDG-deficient mice, which show increased incidence of B-cell lymphomas at old age (Andersen *et al.,* 2005; Cortazar *et al.,* 2011; Nilsen *et al.,* 2003). However, knocking out the genes in the later stages of BER results in embryonic lethality, with mice surviving only until embryonic days 4.5-18.5 (Fig. 6). This illustrates both the vital role of the enzymes in the downstream component of BER, and the overlapping substrate specificities of the DNA glycosylases (Larsen *et al.,* 2003).

Even though most knockout mouse models of DNA glycosylases do not show an immediate and obvious phenotype, further characterization has revealed damage accumulation in different tissues. Mice deficient of NEIL1 accumulate 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FaPyG) and 4,6-diamino-5-formamidopyrimidine (FaPyA) in liver, kidney and brain (Chan *et al.,* 2009; Jaruga *et al.,* 2010). In addition, they accumulate 8,5'-cyclopurine 2'-deoxynucleosides (cPu) in liver, which is not repairable by BER, suggesting involvement of NEIL1 in NER (Jaruga *et al.,* 2010). *Neil1*$^{-/-}$ mice also have an altered metabolic syndrome phenotype that is confirmed in mice on a high fat diet, thought to be caused by low tolerance to oxidative stress (Sampath *et al.,* 2011; Vartanian *et al.,* 2006). They are further described as equally capable of learning as wild type mice, but are deficient in retaining long-term memory (Canugovi *et al.,* 2012). *Neil2*$^{-/-}$ mice do not show an overt phenotype, but accumulate DNA

damage mostly in transcribed regions and are susceptible to innate inflammation (Chakraborty *et al.,* 2015). *Neil3*$^{-/-}$ mice do not have a profound phenotype (Torisu *et al.,* 2005), but when subjected to perinatal ischemia, *Neil3*$^{-/-}$ mice demonstrated loss of neural stem cells and inability to replace damaged tissue (Sejersted *et al.,* 2011). Further, adult *Neil3*$^{-/-}$ mice displayed decreased proliferation of neural progenitors and impaired learning and memory (Regnell *et al.,* 2012). Naïve NTH1-deficient mice accumulate FaPyA in kidney and liver, and accumulate thymine glycol in liver when exposed to ionizing radiation (Chan *et al.,* 2009; Takao *et al.,* 2002). *Ogg1*$^{-/-}$ mice do not have a tumor predisposition in spite of elevated spontaneous mutation rates and accumulation of 8-oxoG in hepatocytes (Klungland *et al.,* 1999; Minowa *et al.,* 2000). However, they are sensitive to oxidative stress throughout their lifespan and old mice display decreased spontaneous locomotor behavior and brain pathology (Arai *et al.,* 2002; Cardozo-Pelaez *et al.,* 2012; Kunisada *et al.,* 2005). The phenotype of *Mutyh*$^{-/-}$ mice has been subject of some debate, as Xie and colleagues (Xie *et al.,* 2004) observed no increased tumorigenesis in these mice, while Sakamoto *et al.* (2007) reported a significant increase in both spontaneous and stress-induced tumorigenesis of the intestinal tract. In humans, defect in MUTYH leads to accumulation of oxidative damage and a predisposition to colorectal cancer (Al-Tassan *et al.,* 2002; Ruggieri *et al.,* 2013).

Combined deletion of two overlapping DNA glycosylases demonstrates the mutagenic and carcinogenic potential of oxidized base lesions. *Ogg1*$^{-/-}$*Mutyh*$^{-/-}$ mice accumulate 8-oxoG and display a predisposition to tumorigenesis in various tissues (Xie *et al.,* 2004). *Neil1*$^{-/-}$*Nth*$^{-/-}$ mice accumulate FaPy lesions and are prone to hepatocellular and pulmonary tumors (Chan *et al.,* 2009). *Ogg1*$^{-/-}$*Nth*$^{-/-}$ mice accumulate oxidized pyrimidines in mitochondrial DNA, but do not display a predisposition to cancer (Karahalil *et al.,* 2003).

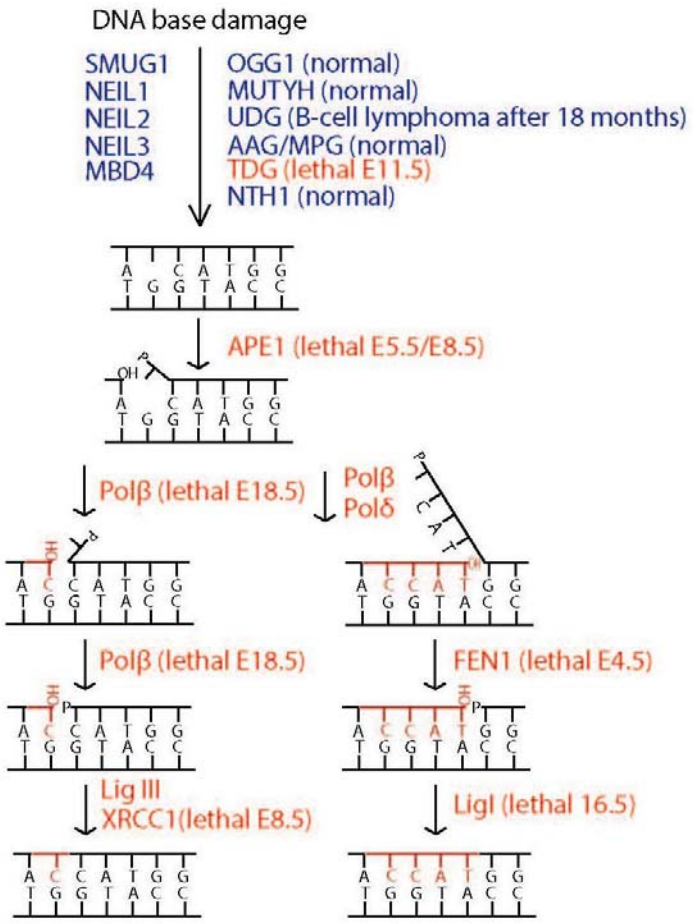

Figure 6. Phenotypes of BER-deficient mice. SP-BER is illustrated to the left, LP-BER to the right. Knocking out DNA glycosylases does not cause an obvious phenotype, except for the lethal TDG knockout and UDG knockout mice, which have an increased incidence of B-cell lymphomas at old age. Gene-targeted knockouts of downstream BER enzymes are embryonically lethal. Abbreviations in text. Adapted and modified from Larsen *et al.* (2007)

Epigenetic DNA modifications

Epigenetic changes to DNA, first defined by Conrad Waddington as "alterations in gene expression without a change in nucleotide sequence", are mediated through processes that are potentially reversible (Henikoff *et al.*, 1997). The processes involved include DNA methylation and histone modifications, chromatin remodeling, and non-coding ribonucleic acid (RNA) (Adwan *et al.*, 2013). DNA methylation is the best characterized epigenetic modification.

Addition of a methyl group at the 5 position of the pyrimidine ring of Cytosines (Cs) creates 5-methylcytosine (5mC) (Razin *et al.*, 1980) and occurs almost exclusively at cytosine-phosphate-guanine (CpG) dinucleotides in eucaryotes. However, methylation of C occurring in a non-CpG context has recently been found to be particularly prevalent in adult mouse and human brain (Varley *et al.,* 2013; Xie *et al.,* 2012). DNA methylation is most abundant in heterochromatin and is normally related to transcriptional repression (Miller *et al.*, 2007). Mattson *et al.* (2003) showed that DNA methylation is important for proliferation and differentiation of neural stem cells. Furthermore, DNA methylation plays an important role in neuronal repair and survival, learning and memory as well as in synaptic plasticity (Fan *et al.,* 2001; Feng *et al.,* 2010; Iskandar *et al.,* 2010).

DNA methylation can be either inherited or created *de novo* by addition of a methyl group to DNA (Bergman *et al.*, 2013; Guerrero-Bosagna *et al.*, 2014; Lardenoije *et al.*, 2015). DNA methyltransferases (DNMT) are responsible for *de novo* DNA methylation by using S-adenosylmethionine (SAM) as the methyl donor, converting SAM to S-adenosylhomocystein (SAH) (Klose *et al.*, 2006; Mastroeni *et al.*, 2010) (Fig. 2). The DNA demethylation pathway is still not completely unraveled and the possibility for multiple overlapping demethylation pathways is not yet excluded (Lardenoije *et al.,* 2015). 5mC is thought to be converted to 5-hydroxymethylcytosine (5hmC) by ten-eleven-translocation (TET) proteins (Booth *et al.*, 2012; Globisch *et al.*, 2010; Kriaucionis *et al.*, 2009; Tahiliani *et al.*, 2009). Contrary to 5mC, 5hmC has generally been associated with transcriptional activation (Chen *et al.,* 2012; Jin *et al.,* 2011; Song *et al.,* 2011). In the adult mammalian brain, 5hmC are low in stem-cell rich areas and high in areas with high density of fully differentiated neurons (Globisch *et al.,* 2010; Orr *et al.,* 2012). Genome-wide distribution of 5hmC also differs from 5mC. In the adult human brain, 5hmC is mostly found at promoter regions while the 5mC levels are highest at intragenic regions (Jin *et al.,* 2011).

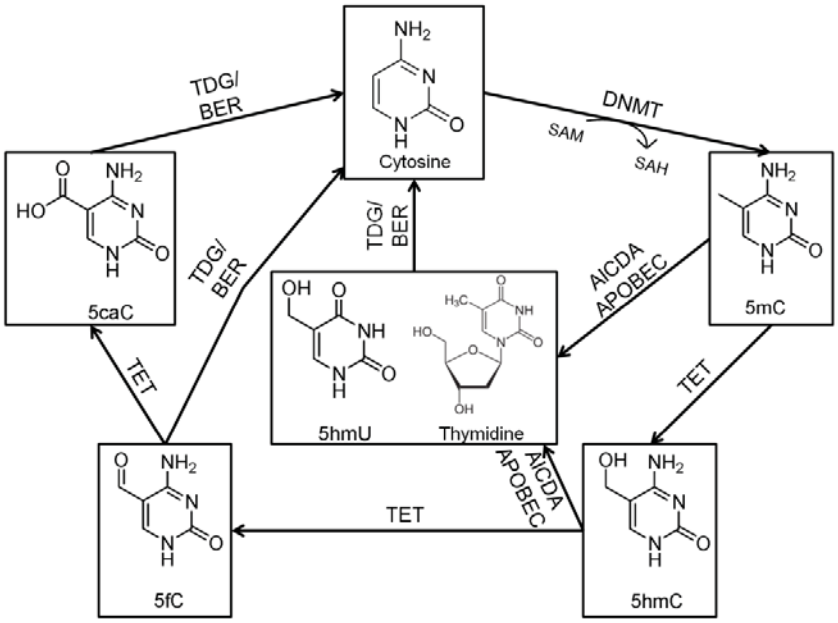

Figure 2. Putative pathways for DNA methylation and DNA demethylation. C is methylated by DNMT to 5mC. 5mC is either oxidized by TET proteins to 5hmC, 5fC, and 5caC or deaminated by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) or activation-induced cytidine deaminase (AICDA) to thymidine or 5hmU. APOBEC and AICDA may also deaminate 5hmC to 5hmU or thymidine. 5caC, 5fC, 5hmU and thymidine are processed by a BER glycosylase, either TDG or SMUG1. Abbreviations in text.

5hmC can be further oxidized by TET proteins to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (He *et al.*, 2011; Ito *et al.*, 2011; Kriaucionis *et al.*, 2009; Tahiliani *et al.*, 2009). Alternatively, 5mC and 5hmC can be deaminated either by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) protein or activation-induced cytidine deaminase (AICDA) creating 5-hydroxymethylUracil (5hmU) (Guo *et al.*, 2011; Popp *et al.*, 2010). The resulting 5hmU:G and thymidine (T):G, as well as 5caC:G and 5fC:G, represent mismatches and can be detected and repaired by single-strand selective monofunctional uracil DNA glycosylase (SMUG1), Methyl-CpG-binding domain protein 4 (MBD4), or thymine DNA glycosylase (TDG) (Cortellino *et al.*, 2011; Guo *et al.*, 2011; Hardeland *et al.*, 2003; Hashimoto *et al.*, 2012; He *et al.*, 2011; Kemmerich *et al.*, 2012; Matsubara *et al.*, 2004). Endonuclease VIII-like (NEIL) glycosylases and 8-oxoguanine DNA glycosylase (OGG1) have been shown to be associated with epigenetic DNA by high throughput screening of epigenetic readers and erasers (Spruijt *et*

al., 2013). Recently, Muller *et al.* (2014) demonstrated that the glycosylases NEIL1, NEIL2 and NEIL3 might provide an alternate BER pathway for DNA demethylation.

Pathophysiology in cerebral hypoxia-ischemia

The brain is particularly vulnerable to oxidative stress. It consumes 20% of the total oxygen used by the body, but has a lower capacity to neutralize ROS (Barzilai 2007). The brain consists of both dividing and non-diving cells. While neurons are terminally differentiated and thereby unable to re-enter the cell cycle, neural stem cells are able to proliferate in response to stress, and, depending on their differentiation status, glial cells may belong to either a dividing or non-dividing cell population (Iyama *et al.*, 2013).

HI causes brain damage by activation of the ischemic cascade. In short, this cascade consists of failure of the mitochondria and subsequent depletion of energy stores, ion pump malfunction, and release of excitatory neurotransmitters. A number of neuroprotective mechanisms are also activated (Deb *et al.,* 2010). Within minutes of blood flow abruption, a cellular edema evolves followed by a vasogenic edema evolving over hours and days (Klatzo 1987). In addition, there is a loss of structural integrity of blood vessels and brain tissue, an activation of the immune system, and activation and migration of microglia (Adibhatla *et al.*, 2010; Deb *et al.*, 2010; Neumann *et al.*, 2009).

A number of different cell death mechanisms exist to protect the organism against genetic instability. These include necrosis, apoptosis, autophagy, and necroptosis (programmed necrosis) (Kroemer *et al.*, 2008; Ouyang *et al.*, 2012; Stracker *et al.*, 2011). Rapid and severe failure to sustain cellular homeostasis and depletion of ATP is thought to be the main cause of necrosis (Edinger *et al.*, 2004; Northington *et al.*, 2011). Apoptosis, autophagy and necroptosis are all mediated by an intracellular program and are collectively called programmed cell death (Ouyang *et al.,* 2012). In addition, senescence, a permanent cell cycle arrest, is another mechanism to protect the organism against disease (Vicencio *et al.,* 2008). Following HI, necrosis is the major cell death phenotype occurring at the core of the insult. However, apoptosis occurs in the tissue surrounding the core, called the penumbra, as well as in vulnerable areas of the brain

(Northington *et al.*, 2011; Ueda *et al.*, 2004). Cell death mechanisms are dependent on the developmental stage in the brain, and apoptosis is more pronounced in the immature brain than in the adult brain (Zhu *et al.,* 2005). In addition, neurons in Cornu Ammonis area 1 (CA1) in the HC of immature brains are more susceptible to apoptosis while neurons in CA3 are prone to autophagic cell death (Ginet *et al.,* 2009). The structural variation of cell death mechanisms and detrimental consequences of HI are termed selective vulnerability, and are evident in the term infant brain where cerebellar Purkinje cells, HC, basal gangliae, and thalami are particularly feeble when subjected to HI (Inder *et al.*, 2000; Okereafor *et al.*, 2008).

The immune system monitors and maintains the homeostasis under normal as well as pathological conditions. Under normal physiological conditions, the blood-brain barrier prevents circulating immune cells from entering the brain. This barrier is dysfunctional under ischemic conditions (Macrez *et al.,* 2011). All steps of the ischemic cascade are strongly affected by an inflammatory reaction involving both locally activated and peripheral immune cells that release cytokines and chemokines. The innate immune system senses so-called damage-associated molecular patterns (DAMPs) released by dying or dead neurons and responds within minutes (Lambertsen *et al.*, 2012; Xu *et al.*, 2014). Various different DAMPs interact with pattern recognition receptors, like Toll-like receptors and nucleotide-binding oligomerization domain receptors causing downstream biological effects by affecting expression of apoptotic and inflammatory genes and regulating release of pro-inflammatory mediators (Abe *et al.*, 2010; Iadecola *et al.*, 2011; Yang *et al.*, 2014a). A crucial step in maintaining the homeostasis of the organism is removal of the dying cells. After damage to the central nervous system, mature microglia are activated, migrate, and concentrate in areas of cell death where the dying cells or their fragments are removed by phagocytosis (Neumann *et al.,* 2009). Microglia are the macrophages of the brain and exhibit ramified morphology in the resting state while the activated microglia alter into an amoeboid appearance once activated (Perry *et al.,* 2010; Thomas 1992; Yang *et al.,* 2014b; Yenari *et al.,* 2010). Activated microglia becomes indistinguishable from circulating macrophages in phenotype (Taylor *et al.*, 2013), eliminate necrotic debris, and secrete pro-inflammatory cytokines like tumor necrosis factor (TNF)α and interleukin (IL)-1β, but also anti-inflammatory cytokines like IL-10 and TNFβ (Chiba *et al.*, 2013; Parada *et al.*, 2013). There

are two types of activated microglia, the classically activated M1 with a pro-inflammatory effect, and the alternatively activated M2 with anti-inflammatory effect (Kigerl *et al.,* 2009). Even though the initial response of microglia to ischemia is release of pro-inflammatory cytokines, suppression of microglia results in larger infarction and a two-fold increase in apoptotic cell death (Lalancette-Hebert *et al.,* 2012).

Following HI, there is a massive production of cytokines and inflammatory mediators in the activated immune cells. These mediators can either exacerbate or counteract the ischemic brain damage. The complexity of the regulatory interplay is compounded by the dual role of many of the mediators. TNFα, IL-1β and IL-6 are dramatically upregulated following ischemic brain injury and they strongly affect the outcome. The major source of TNFα and IL-1β in the ischemic brain is microglia and macrophages, although IL-1β also is released by neurons, astrocytes and endothelial cells (Amantea *et al.,* 2010; Clausen *et al.,* 2008; Lambertsen *et al.,* 2012; Luheshi *et al.,* 2011). TNFα has a neurotoxic effect, as shown in experiments where administration of TNF-binding proteins or neutralizing antibodies to TNF ameliorates cerebral injury (Barone *et al.,* 1997; Lavine *et al.,* 1998; Yang *et al.,* 1998). However, TNF receptor knockouts have been reported to worsen the ischemic brain damage, thus implying TNF to have a neuroprotective role (Bruce *et al.,* 1996; Gary *et al.,* 1998; Taoufik *et al.,* 2007). IL-1β has a more definite neurotoxic effect. Multiple studies have demonstrated neuroprotection by eliminating the effect of IL-1β (Boutin *et al.*, 2001; Hara *et al.*, 1997; Loddick *et al.*, 1996). In spite of the apparent role of IL-1β in ischemic damage to brain tissue, low levels of IL-1β are reported to be important for ischemic preconditioning, an event where tolerance towards subsequent ischemic episodes are developed (Gong *et al.,* 2014; Shin *et al.,* 2009; Wang *et al.,* 2000). IL-6 is a signaling molecule produced in response to inflammation or tissue damage, and is important for induction of various acute phase proteins (Heinrich *et al.,* 1990). However, prolonged synthesis of IL-6 has a pathological effect in autoimmunity and chronic inflammation (Hirano *et al.,* 1987; Nishimoto *et al.,* 2005; Serada *et al.,* 2008). The expression of IL-6 increases following cerebral ischemia (Ali *et al.,* 2000; Berti *et al.,* 2002), and blood levels of IL-6 strongly correlates with stroke severity (Smith *et al.,* 2004; Waje-Andreassen *et al.,* 2005). Several animal studies have suggested a neuroprotective effect of IL-6 following HI, where

administration of IL-6 after middle cerebral artery occlusion (MCAO) ameliorated infarct volumes and neurological deficits (Feng *et al.,* 2015; Loddick *et al.,* 1998). This is in contrast to the findings in a clinical trial, where administration of IL-1 receptor antagonist lowered the plasma levels of IL-6 and improved the clinical outcome (Emsley *et al.,* 2005).

ROS are believed to be important for maintaining inflammatory processes, either by direct signaling or by oxidative modification of lipids, proteins and DNA (Fontes *et al.,* 2015; Poon *et al.,* 2004). Many groups have focused on improving the understanding of the roles of oxidative stress, DNA damage and DNA repair mechanisms during inflammation and immune response. Many DNA repair pathways are involved in regulation of transcription of immune response molecules in addition to the known protection against oxidative stress. It has been shown that enzymes in the BER pathway may influence the inflammatory response. In more detail, Aguila-Aguirre *et al.* (2014) demonstrated a decreased immune response in mice lacking OGG1. Further, the same group has shown that the nonproductive binding of OGG1 to 8-oxoG in promoter sequences might epigenetically modulate the expression of pro-inflammatory genes (Ba *et al.,* 2014). In fact, a decreased production of cytokines and chemokines in *Ogg1*$^{-/-}$ mice has been demonstrated (Mabley *et al.,* 2005). UNG-mutations are associated with impairment in immunoglobulin class-switch recombination important for proper function of the immune system (Imai *et al.,* 2003), as well as production and function of immune cells (Andersen *et al.,* 2005). NEIL1 is suggested to be important for rapid expansion of germinal center B cells and optimal immune response (Mori *et al.,* 2009). A gene variant of MUTYH (AluYb8MUTYH) has been linked to increased oxidative stress and increased IL-1 levels in otherwise healthy individuals (Sun *et al.,* 2010), and increased levels of IL-1β and IL-6 has been found in hemodialysis patients with polymorphisms in OGG1 and MUTYH (Cai *et al.,* 2012).

Neuronal development, learning and memory

Normal brain development and maintenance of functions in the adult brain is dependent on neural plasticity (Sander *et al.,* 2009). Plasticity is the insignia of the immature brain. Even though genesis and migration of neurons are largely completed in the brain of the term infant,

synapse formation and arborization of axons and dendrites still occur at a high level, especially as a response to environmental stimuli, in mature brains (Kolb *et al.*, 2011). Thus, the mature brain has a reduced rate of neurogenesis, but retains a high level of plasticity.

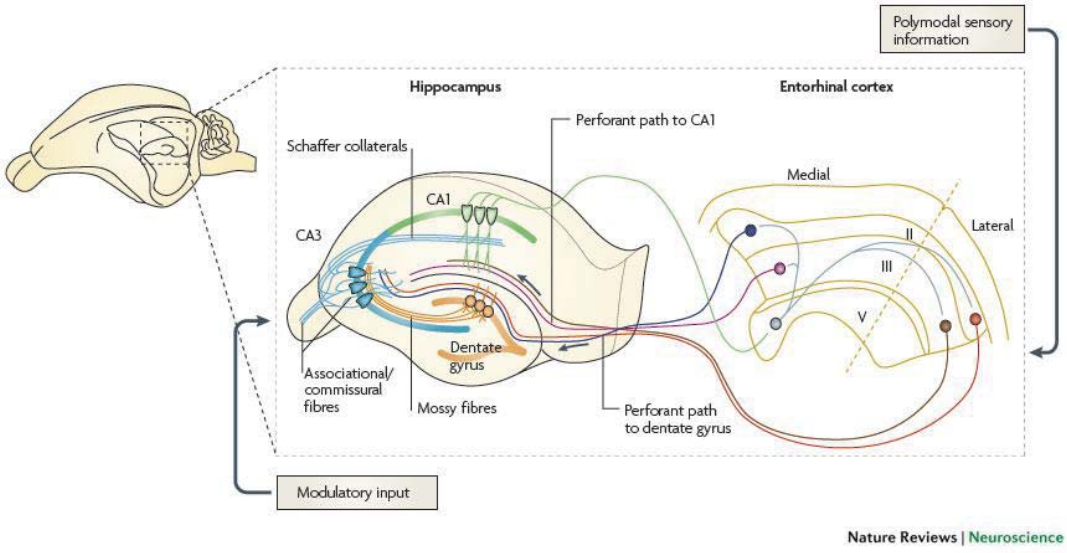

Figure 7. Wiring diagram of the HC: the trisynaptic loop. Sensory information is conveyed from neurons in the entorhinal cortex to the DG by axons of the perforant path. Axons of the granule cells of the DG (Mossy fibers) project to CA3 pyramidal cells. These pyramidal cells project both to CA1 through Schaffer collaterals, as well as to the contralateral HC though commissural fibers. In addition to the trisynaptic loop, there is a perforant path to CA1 from layer III neurons of the entorhinal cortex, which constitutes the monosynaptic loop. Adapted from Neves *et al.* (2008)

The HC and the entorhinal cortex are principally concerned with memory, and information derived from experience is stored in synaptic networks in these regions (Moser *et al.*, 2008; Squire *et al.*, 2004). The HC is normally divided into three major subfields: CA1, CA3, and dentate gyrus (DG) (Fig. 7). There are two defined excitatory pathways: the trisynaptic loop and the monosynaptic loop. The trisynaptic loop is thought to be required for rapid contextual learning while the monosynaptic pathway is sufficient for slow multi-trial spatial learning and other associative memory tasks (Neves *et al.*, 2008; Sander *et al.*, 2009). In order for sensory information to be established as a novel episodic memory, encoding/formation, consolidation and optimization are required. Information storage and consolidation require synaptic plasticity. Synaptic plasticity is the process of experience-dependent changes in synaptic connectivity

believed to underlie learning and memory (Ho *et al.,* 2011). Bliss and Lømo demonstrated synaptic plasticity in 1973 by use of long-term potention (LTP). They showed that high-frequency electrical stimulation of the presynaptic fibers in the perforant pathway resulted in long-lasting responses of postsynaptic neurons (Bliss *et al.*, 1973). However, it is still unclear exactly how memories are retained and stored.

ROS have the potential to oxidize essential molecules, and ROS in the brain have therefore traditionally been thought to have a neurotoxic effect (Butterfield *et al.*, 2002; Mecocci *et al.*, 1993). This is backed by studies demonstrating a decreased cognitive performance when excessive ROS are present, whereas in experiments where superoxide is quenched, behavior deficits tend to normalize (Carney *et al.,* 1991; Dumont *et al.,* 2009; Fukui *et al.,* 2001; Hu *et al.,* 2006; Liu *et al.,* 2003; Massaad *et al.,* 2009). However, ROS have also been suggested to be involved in modulation of memory formation (Carney *et al.,* 1991; Forster *et al.,* 1996; Fukui *et al.,* 2001), and have been demonstrated to modulate LTP (Klann 1998). It is clear that the role of ROS in cerebral function is complex. ROS at balanced levels may contribute to LTP and memory formation depending on the concentration, localization and identification of the specific ROS, whilst excessive amounts may cause damage (Massaad *et al.*, 2011). During aging, the ROS production in the brain increases, while the antioxidant effect decline, leading to a great increase in ROS levels and oxidative stress. (Ames *et al.*, 1993; McGahon *et al.*, 1999; Murray *et al.*, 1998; Poon *et al.*, 2004). The "free radical theory of aging" suggests that accumulation of oxidative damage to the macromolecules is the cause of neural detoriation (Barja 2004; HARMAN 1956). However, this theory has been a subjected of controversy and the link between oxidative DNA damage and cognitive decline is yet to be discovered.

Extensive research has been done to understand the signaling pathways that underlie the experience-dependent alteration of morphology and connectivity of neurons that is thought to be of essence in memory-formation (Goelet *et al.,* 1986; Madabhushi *et al.,* 2015). Neuronal activity causes calcium influx across the plasma membrane triggered by neurotransmitter binding to various receptors (Sabatini *et al.,* 2001). Distinct transcription factors and intracellular signaling pathways are activated depending on the origin of synaptic stimuli and calcium entry

(Bading *et al.*, 1993; Lerea *et al.*, 1993). The activated pathways regulate nuclear gene transcription by modifying expression, localization, or function of transcriptional regulators (West *et al.,* 2002). The activated genes are divided into different subgroups depending on when the change in expression occurs. Early response genes include *Fos*, *Npas4*, *Nr4a1*, and *Egr1*, and late response genes include *Homer1*, *Bdnf*, *Nrn1*, and *Rgs2* (West *et al.*, 2011). It has been suggested that dysregulation of activity-induced transcriptional networks traps the brain in an immature state of synaptic development in some neuropsychiatric and neurological pathological conditions. This implies a role of activity-dependent neuronal plasticity in accurate synaptic development and maturation (West *et al.*, 2011). Recently, Madabhushi *et al*. (2015) suggested a physiological role of DSB formation in learning and memory by demonstrating activity-dependent formation of DSBs in the promoters of early response genes. The generation of these DSB breaks led to expression induction even in the absence of external stimuli.

It is hypothesized that incorporation of new neurons may contribute to learning and memory. The brain is generally an organ with a low proliferation rate, although there are stem-cell rich areas in the HC and SVZ. Even though the mature brain has a lower level of neurogenesis than the newborn brain, environmental stimuli may induce neural stem cells to proliferate and differentiate (Kempermann *et al.,* 2002). The integration of the newborn neurons is regulated by the synaptic activity of the pre-existing neurons (Ge *et al.,* 2006). Multiple reports have proposed that learning and memory in adulthood is dependent on the ability of the neural stem cells to proliferate and of the new neurons to mature, to replace, and to integrate into existing neuronal circuits (Deng *et al.*, 2009; Imayoshi *et al.*, 2008; Jessberger *et al.*, 2008; Kee *et al.*, 2007). However, other groups have shown that there is no effect on learning and memory from adult neurogenesis (Groves *et al.,* 2013; Wojtowicz *et al.,* 2008). Stone *et al.* (2011) showed functional equivalence between granule neurons created during development and during adulthood, indicating a lesser importance of adult neurogenesis on learning and memory. Thus, one cannot yet make a conclusion on the role of adult neurogenesis in learning and memory.

Genetics and epigenetics in anxiety

The HC, amygdala and prefrontal cortex are brain regions commonly associated with anxiety (Oler *et al.,* 2010). Anxiety disorders are a heterogenous group of complex, disabling diseases caused by a combination of environmental and genetic factors. The 12-month prevalence of anxiety disorders are reported to be in the range of 13,6% - 18,1% (Alonso *et al.,* 2004; Kessler *et al.,* 2005). Extensive research has been conducted to reveal the etiology of anxiety, and there is evidence that increased oxidative stress is the molecular foundation of anxiety development (Reviewed in Smaga *et al.,* 2015). There is an upregulation of oxidative stress in the key brain regions following psychological stress (Wilson *et al.,* 2013). Psychological stress in early life is well known as an important risk factor for development of anxiety, but not all who are subjected to psychological stress develops anxiety. This can in part be explained by gene-environment interactions (Nugent *et al.,* 2011). It has been reported an up to three-fold risk increase in first-degree relatives of patients with anxiety disorders in twin- and family-studies (Hettema *et al.,* 2001; Maier *et al.,* 1993). The estimated inherited genetic contribution to the pathogenesis of anxiety disorders ranges up to 30-69 % (Domschke *et al.*, 2012a). The most promising candidate genes for anxiety disorders are amongst others catecol-O-methyltransferase (COMT), serotonin 1A transporter (5-HT_{1A}), glutamic acid decarboxylase 1 (GAD1), monoamine oxidase A (MAO-A), and plexin A2 (PLXNA2) (Domschke *et al.*, 2013a; Hovatta *et al.*, 2008).

Stressful environmental episodes are proposed to influence the methylation pattern and predispose for anxiety disorders like post-traumatic stress disorder (Battaglia 2013; Klengel *et al.,* 2013). Preliminary data have suggested differentially methylation pattern in GAD1 and MAO-A l (Domschke *et al.*, 2012b; Domschke *et al.*, 2013b) although in the case of MAO-A, no difference in methylation was found in antidepressant treatment response (Domschke *et al.,* 2015). Further, gene expression changes has been found in anxiety-associated brain regions revealing top candidate genes like *Fos*, *Ptgds* and *Egr2*, for anxiety disorders (Le-Niculescu *et al.,* 2011).

A problem with the genomic studies is that many of the findings have been difficult to reproduce and the results published by different groups have often been contradictory. Reasons for this might be genetic heterogeneity and that the studies have small sample sizes (Hovatta *et al.*, 2008).

Aims of the thesis

Oxidative damage is associated with aging and degenerative disease and may potentially affect memory. Oxidative lesions accumulate in the DNA during stress and aging and to prevent such lesions from causing disease, they are normally removed by DNA glycosylases.

The overall aim of this study was to characterize the function of the oxidative DNA glycosylases OGG1, MUTYH, NEIL1, and NEIL2 in disease and aging of the brain, both in the acute response to damage and in learning and memory. To address this, we used single- and double-knockout mice of the genes in focus, in combination with a mouse model for hypoxic-ischemic encephalopathy and behavioral tests.

In the first part of the study, we investigated the acute response to hypoxic-ischemic injury in mice deficient of OGG1, MUTYH, NEIL1 and NEIL2. To assess the effects of HI, a model of permanent unilateral carotid artery ligation followed by transient exposure to hypoxia in adult rats was established in 1960 (LEVINE 1960). This method has been modified to be suitable for the more resistant perinatal brains of rats and mice (Rice, III *et al.,* 1981; Sheldon *et al.,* 1998). We compared the total damage after cerebral ischemia in knockout and wild type mice, investigated the cell death processes activated during acute injury, and mapped the DNA damage profiles. DNA damage accumulation was evaluated by immunohistochemistry (IHC), mass spectrometry (MS), and a qPCR-based DNA damage detection assay. Further, we investigated the gene regulatory response in the different knockouts. In OGG1- and MUTYH-deficient mice, we also examined the microglia response to injury.

In the second part of the study, we investigated cognitive function in mice deficient of OGG1, MUTYH or both. We performed behavioral tests focusing on learning and memory in adult knockout and wild type mice. In addition, we examined the morphology of the brain and investigated the DNA damage profiles. We further investigated the transcriptome profile from areas of the brain involved in cognitive function.

Result summary

Paper 1

DNA glycosylases Ogg1 and Mutyh suppress inflammation in brain after hypoxia-ischemia.

Base excision repair initiated by DNA glycosylases have been shown to prevent neuronal cell death under HI. However, the mechanisms of neuroprotection remain to be determined. We compared the damage response in *Ogg1*$^{-/-}$, *Mutyh*$^{-/-}$, and wild type mice after HI. First, we looked at total ischemic damage and found that *Mutyh*$^{-/-}$ mice were more sensitive to HI than wild type mice in most regions of the brain. *Ogg1*$^{-/-}$ mice were also more sensitive to HI, but primarily in the hippocampal area. These findings imply that both OGG1 and MUTYH limit brain damage after HI. To evaluate these findings in more detail, we used IHC to quantify damage mechanisms. We observed no differences between the genotypes when assessing apoptosis. There was a significant decrease in strand breaks, detected as γH2A.x foci, in mice lacking MUTYH, but no significant differences in XRCC1 or Poly(ADP-ribose) polymerase 1 (PARP1) levels between the genotypes. These results suggest that the damage response is not severely altered after hypoxic injury.

We investigated DNA damage accumulation by LC-MS/MS. There was a time-dependent increase of 8-oxoG in the HC of all genotypes three hours post HI as compared to sham-operated animals. However, the only significant altered damage response between the genotypes was increased levels of 8-oxoG in the ipsilateral hemisphere in *Ogg1*$^{-/-}$ mice compared to wild type three hours post HI. No differences were found between the genotypes six hours after HI.

RNA sequencing of ipsilateral HC three hours post HI revealed that most differentially expressed genes (DEGs) were upregulated in *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ HC compared to WT, and that there was a 50% overlap between the mutants. Using ingenuity pathway analysis (IPA) we found that pro-inflammatory mediators were amongst the top upstream regulators. This was confirmed by RT-

qPCR. In untreated OGG1- and MUTYH-deficient HC, we found an enrichment of DEGs that are targets of polycomb repressive complexes (PRCs), which repress transcription. Furthermore, all DEGs being associated with a transcriptional silent state in brain showed a significantly elevated expression three hours post HI as compared to wild type. This suggests that inflammation repression by PRCs may not be functioning properly in the knockouts. Furthermore, we assessed the early microglial response by IHC. We found an increased number of activated microglia expressing IL-1β in the HC of mutant mice. Together, these results demonstrate that OGG1 and MUTYH specifically alter the transcriptional network of the inflammatory response in the HC after HI injury.

Paper 2

NEIL2 is a potential target in management of hypoxic-ischemic encephalopathy

NEIL1, 2 and 3 DNA glycosylases are paralogous enzymes initiating the BER pathway by removing oxidized bases from the DNA. Previously, NEIL1 and NEIL3 have been shown to be involved in protection of the brain during ischemic conditions. We constructed *Neil1*$^{-/-}$, and *Neil2*$^{-/-}$ mice to investigate the role of NEIL1 and NEIL2 in cerebral ischemia in newborn mice. Quantification of infarction by the TTC assay revealed significantly less cell death in perinatal *Neil2*$^{-/-}$ mice compared to wild type mice in the mouse forebrain, including the HC. Perinatal *Neil1*$^{-/-}$ mice, on the other hand, showed increased cell death. We further evaluated the damage mechanisms by investigating brain sections obtained three and six hours post HI. Three hours after HI, we saw an increase in apoptotic cells in the striatum of *Neil1*$^{-/-}$ mice, and a decrease in apoptosis in CA1 of NEIL2-deficient mice. However, at six hours post HI, we did not see any difference in any region between any of the genotypes.

We analyzed the expression of *Neil1* and *Neil2* mRNA in samples from brain subregions harvested one and three days post treatment. The expression of both *Neil1* and *Neil2* was

increased in the ipsilateral hemisphere, with *Neil2* being particularly responsive to cerebral HI and displaying the greatest overall fold change.

We performed IHC to determine the level of strand breaks. We first investigated the amount of DNA strand breaks indicated by γH2AX foci in brain sections. We found decreased number of nuclear foci three hours but not six hours post HI in *Neil1*$^{-/-}$ mice, and no difference in *Neil2*$^{-/-}$ mice. We did not find any differences between the genotypes at either time point in nuclear staining of PARP1, a protein activated by DNA strand breaks. The DNA damage profile was further evaluated by LC-MS/MS. We could not find any difference in global 5ohC as measured by LC-MS/MS. These results taken together imply that neither NEIL1 nor NEIL2 deficiency induce increased genome instability during HI.

In order to examine the transcriptome response, we performed RNA sequencing analysis. There was an overall time-dependent transcriptional response, but the response varied between the investigated genotypes. Three hours post HI, *Neil2*$^{-/-}$ mice demonstrated the most profound changes in gene expression, while after six hours, the greatest number of DEGs was seen in *Neil1*$^{-/-}$ mice. We applied IPA to identify biological processes influenced by NEIL1 and NEIL2 deficiency. Processes involved in inflammation were significantly affected in all genotypes six hours post HI, but the response was seen in both *Neil1*$^{-/-}$and *Neil2*$^{-/-}$ mice already after three hours. In *Neil2*$^{-/-}$ HC we found the most significant enrichment of DEGs in processes involved in apoptosis and cell survival at three hours post HI, while wild type and *Neil1*$^{-/-}$ mice showed the strongest enrichment of DEGs in these functions only at six hours.

Paper 3

Synergistic Actions of Ogg1 and Mutyh DNA Glycosylases Modulate Anxiety-like Behavior in Mice

8-oxoG is a major premutagenic DNA lesion linked to cognitive decline. MUTYH and OGG1 DNA glycosylases both prevent mutations caused by 8-oxoG. To study the effect of compromised DNA repair on behavior, mice deficient of OGG1, MUTYH or both, were

subjected to behavior tests. General activity and movement were monitored in the Open Field test. The *Ogg1*$^{-/-}$*Mutyh*$^{-/-}$ mice were more active than the wild type and single knockout mice. Increased activity was also observed in the Zero Maze. Learning and memory performance was tested in the Morris Water Maze (MWM). The *Ogg1*$^{-/-}$ and *Ogg1*$^{-/-}$*Mutyh*$^{-/-}$ mice needed significantly more time to learn the position of the escape platform as compared to wild type, however; memory did not seem to be affected. In contrast, the *Mutyh*$^{-/-}$ mice had a tendency to learn the position of the platform faster than the wild type mice. Taken together, our observations suggest that DNA glycosylases OGG1 and MUTYH adopt distinct roles in regulating behavior and cognitive functions.

To determine whether accumulation of 8-oxoG lesions could explain behavioral and cognitive differences, we measured 8-oxoG levels in the HC and hypothalamus of wild type and knockout mice by LC-MS/MS analysis. Surprisingly, we found no significant differences in global 8-oxoG levels in any of the brain regions when comparing wild type and knockout mice, suggesting that other functions of DNA glycosylases than genome-wide repair of 8-oxoG are responsible for behavioral changes.

To elucidate genome-wide transcriptional profiles underlying the behavioral phenotype, we applied RNA sequencing of the HC from wild type and mutant mice. Overall, we identified 140 to 190 DEGs in the mutant mice as compared to wild type with the majority being upregulated genes. We filtered all genes that were differentially expressed uniquely in the *Ogg1*$^{-/-}$*Mutyh*$^{-/-}$ double knockout mice to identify genes that potentially underlie the anxiolytic effect observed in these mice and found 43 upregulated genes and 10 downregulated genes. The greatest number of DEGs belonged to signaling pathways that have previously been reported to be top candidate pathways involved in anxiety, specifically in the HC. We also found that a striking percentage of the DEGs that showed pathway hits were enriched in pathways associated with learning and memory in the mutant mice. Among the top hits, we found signaling pathways for which activation is essential for synaptic maintenance and neuronal function.

Discussion

Hypoxia-Ischemia

Cerebral ischemia causes increased levels of oxidative base lesions (Chen *et al.,* 1997; Lan *et al.,* 2003; Liu *et al.,* 1996; Luo *et al.,* 2007). UDG and OGG1 have been shown to protect post-ischemic neurons from the cytotoxic effects of HI damage, while NEIL3 promotes regeneration of neuronal tissue after HI (Endres *et al.,* 2004; Liu *et al.,* 2011; Sejersted *et al.,* 2011). In this study, we demonstrate a neuroprotective effect of OGG1, MUTYH, and NEIL1. For examination of neuronal cell death, 24 hours after injury appears to be the optimal time point to determine the infarcted area in a reproducible manner (Bederson *et al.,* 1986). MUTYH seems to have a greater protective effect than OGG1 and NEIL1 as the HI damage is larger in the *Mutyh*$^{-/-}$ than in the *Ogg1*$^{-/-}$ and *Neil1*$^{-/-}$ mice. NEIL2 on the other hand seems to have a harmful effect, as removal of the gene renders the mice resistant to HI damage. Sheng *et al.* (2012) proposed suppression of MUTYH as an effective strategy for neuroprotection during oxidative stress based on their findings in adult mouse brains subjected to chronic stress. As we have shown, suppression of MUTYH would be detrimental to the perinatal brain following acute HI. Further, it has been proposed that MUTYH is required for induction of cell death under conditions of oxidative stress, based on cell death occurring as a result of MUTYH-induced increase in SSBs following 8-oxoG accumulation in the DNA of both MEF cells and in striatal neurons deficient of OGG1 (Oka *et al.,* 2008). Notably, we found no increased apoptotic cell death in *Ogg1*$^{-/-}$ or *Mutyh*$^{-/-}$ brains. Thus, the general conclusions of the roles of OGG1 and MUTYH during oxidative stress made by Sheng *et al.* (2012) do not apply to acute oxidative stress in newborn mice. We demonstrated a similar damage distribution pattern in the *Ogg1*$^{-/-}$ mice as reported in Liu *et al.* (2011), with the largest increase in damage in the hippocampal area. However, the global increase in damage was not as evident in our experiments, and the total infarcted area was much greater in both wild type and *Ogg1*$^{-/-}$ mice when comparing our results to the results of Liu *et al.* (2011). The explanation probably lies in methodological differences as Liu *et al.* (2011) used five-six months old mice subjected to permanent MCAO without subsequent hypoxia. Further, increased vulnerability to oxidative stress in *Ogg1*$^{-/-}$ mice challenged with a dopaminergic toxin

has been demonstrated (Cardozo-Pelaez *et al.,* 2012). Canugovi *et al.* (2012) showed an increase in cell death in adult *Neil1*$^{-/-}$ mice subjected to MCAO. We demonstrated a similar effect in perinatal *Neil1*$^{-/-}$ mice subjected to unilateral carotid artery occlusion (Paper II). Interestingly, we found an opposite effect in *Neil2*$^{-/-}$ mice, which have not previously been described with regards to HI, where we saw a 27-35% reduction in cell death in various brain regions. A reduction in cell death has also been observed in adult mice deficient of AAG (Ebrahimkhani *et al.,* 2014). It thus seems that especially MUTYH, but also OGG1, UDG, NEIL1 and NEIL3 have a neuroprotective effect during HI, while AAG and NEIL2 have a detrimental effect. The exact mechanisms leading to these outcomes remain unclear, but the protective effect has previously been suggested to be caused by initiation of BER (Chen *et al.,* 1997; Li *et al.,* 2011; Liu *et al.,* 1996; Love *et al.,* 1999). In contrast, Ebrahimkani *et al.* (2014) proposed that AAG-initiated BER acts upstream of PARP1 hyperactivation in HI and sterile inflammation, and that inhibition of AAG might be a good target for limiting damage after HI by reducing toxic BER intermediates, PARP hyperactivation and energy depletion of the cell. We believe that the explanation of the difference in outcome of HI when removing various DNA glycosylases may be due to other functions of the glycosylases than the initiation of canonical BER, as will be discussed in a later section.

Kinslow *et al.* (2010) have suggested that oxidative stress leads to downregulation of *Neil2* based on an in vitro model subjecting normal embryonic human lung fibroblasts to glucose oxidase. Further, *Neil1* has been reported to be downregulated following lethal stroke in the adult human brain (Ghosh *et al.,* 2015). We do not confirm these findings but demonstrate a transient increase in expression of *Neil1*, and a sustained increased expression of *Neil2* in various brain subregions following HI (Paper II). The discrepancy between our results and the previously published results may be due to distinct functions of NEIL1 and NEIL2 under different types of stress, like acute versus chronic stress, which may lead to different consequences for neuronal cell death.

We chose to sacrifice the mice after three and six hours to investigate the initial damage response and cell death mechanisms after HI (Paper I and II). Apoptosis has been shown to appear 5 hours post-infarction in striatum and HC, while not until 24 hours post-infarction in the cortex

(Beilharz *et al.,* 1995). The time point for induction of apoptosis may be influenced by the lack of repair enzymes. The HC is the area most vulnerable to HI stress and this is where we observed most damage after HI. In line with this, we found the highest density of apoptotic cells in CA1, DG, and striatum, but almost no apoptotic cells in cortex, three and six hours post HI. Apoptosis is a protective mechanism that prevents the cytotoxic contents of the cell to leak out and cause increased stress to the surrounding tissue (Taylor *et al.,* 2008). However, it is the sum of neuroprotective and neurotoxic events that ultimately decides the cell fate. We did not find any differences between wild type, *Ogg1*$^{-/-}$, and *Mutyh*$^{-/-}$ in the investigated areas after HI (Paper I). As follows, the increased damage observed in MUTYH-deficient mice is not related to apoptotic cell death. We found increased apoptosis in striatum of *Neil1*$^{-/-}$ mice, and decreased apoptosis in CA1 in *Neil2*$^{-/-}$ three hours post HI (Paper II). This difference was not present six hours after HI. This is in contrast to the findings of Canugovi *et al*. (2012), demonstrating increased apoptosis in 9-13 months old mice lacking NEIL1 48 hours after MCAO. Our results suggest a delayed damage response in the *Neil1*$^{-/-}$ mice. Moreover, the decrease in apoptosis of *Neil2*$^{-/-}$ mice three hours after HI in CA1 may contribute to the reduction in infarct volumes.

The base lesions removed in the initial step of the BER pathway are not as cytotoxic as the glycosylase products; AP-sites and single strand breaks (Alseth *et al.*, 2004; Meira *et al.*, 2009; Roth *et al.*, 2002). DNA glycosylases, especially bifunctional glycosylases such as NEIL1 and NEIL2, can potentially increase sensitivity to oxidative stress by production of AP-sites and SSBs. γ-H2A.x is primarily known as a marker for DSBs, but has been argued to have alternative roles in DNA repair as well (Ismail *et al.*, 2008). MUTYH-deficient MEFs have been shown to have decreased levels of γ-H2A.x (Grasso *et al.,* 2015). When investigating the number of strand breaks after HI, we found a reduced number of γ-H2A.x foci three hours, but not six hours, post HI in *Neil1*$^{-/-}$ mice (Paper II). We also found a reduced number of foci in *Mutyh*$^{-/-}$ mice six hours after HI (Paper I). No differences were observed in the other genotypes. These results may indicate less accumulation of strand brakes at an early stage after injury or a delayed stress response in NEIL1- and MUTYH-deficient brain.

To further investigate the presence of BER intermediates, we examined PARP1 and XRCC1 levels after HI. PARP1 binds specifically to SSBs, such as nicked AP sites, and recruits downstream BER enzymes by catalyzing poly-ADP ribosylation of cellular proteins, a process requiring NAD^+ (D'Amours *et al.*, 1999; de Murcia *et al.*, 1994). Inhibition of PARP activity has been shown to be neuroprotective after transient brain ischemia in mice (Eliasson *et al.,* 1997; Endres *et al.,* 2004), but does not affect the amount of DNA damage (Giovannelli *et al.,* 2002). We did not find any significant difference in PARP1 protein levels. Noren Hooten *et al.* (2011) demonstrated that in response to DNA damage, there were decreased levels of poly(ADP-ribose) in *Ogg1*$^{-/-}$ cells compared with wild type cells. However, they did not find any difference in PARP1 levels, and their results are thus consistent with ours. XRCC1 coordinates enzymatic steps in BER and is involved in repair of SSBs (Thompson *et al.*, 2000). XRCC1 has been shown to interact with NEIL2 and OGG1, as well as several other downstream components of the BER pathway (Campalans *et al.,* 2005; Hanssen-Bauer *et al.,* 2012; Marsin *et al.,* 2003). We did not observe any significant differences in XRCC1 levels between any of the genotypes after HI. Collectively, the lack of significant alterations in levels of BER intermediates implies that the differences in extent of damage after HI in mice lacking DNA glycosylases are not solely mediated through lack of canonical repair.

Lack of OGG1 has been suggested to increase the level of 8-oxoG in genomic DNA (Minowa *et al.,* 2000). Cerebral cortical neurons in OGG1-deficient mice exhibit increased vulnerability to ischemia caused by permanent MCAO compared with wild type mice (Liu *et al.,* 2011). However, more FaPyG lesions rather than 8-oxoG accumulate after ischemic stroke in the same mice (Liu *et al.,* 2011). Ruggieri *et al.* (2013) showed accumulation of 8-oxoG in a human biallelic mutant of MUTYH. Further, both NEIL1 and NEIL2 participates in the maintenance of the mitochondrial genome (Canugovi *et al.,* 2012; Hu *et al.,* 2005; Sampath *et al.,* 2011; Vartanian *et al.,* 2006) and accumulation of FaPyA lesions and mitochondrial DNA damage has been shown in the brains of *Neil1*$^{-/-}$ mice (Chan *et al.,* 2009; Vartanian *et al.,* 2006). Recently, Chakraborty *et al.* (2015) demonstrated accumulation of oxidative DNA damage primarily in transcribed genes in aged *Neil2*$^{-/-}$ mice. We investigated global accumulation of nDNA or mtDNA damage in the various genotypes three and six hours after HI (Paper I and II).

Surprisingly, we did not detect any significant effect of HI in either nDNA or mtDNA in wild type, *Neil1*$^{-/-}$ and *Neil2*$^{-/-}$ mice (Paper II). No differences in nDNA damage accumulation were observed in OGG1 and/or MUTYH mice either (Paper I). In mtDNA however, there was a significant increase only seen in *Mutyh*$^{-/-}$ when comparing the ipsilateral side to the contralateral side (Paper I). To further elucidate the damage profile, we measured 8-oxoG (Paper I) and 5ohC (Paper II) levels by LC-MS/MS. We could not detect any differences in DNA damage levels between any of the genotypes investigated (Paper I and II), except for a time-dependent increase in 8-oxoG in *Ogg1*$^{-/-}$ mice compared to wild type. However, 8-oxoG is a non-lethal damage and a limited increase is unlikely to cause increased acute cell death.

In response to HI, a number of genes, like stress-response genes, pro-apoptotic genes, and genes involved in glutamergic signaling, are differentially regulated (Cox-Limpens *et al.,* 2014). In order to reach a better understanding of the molecular mechanisms underlying the observed difference in stroke volumes in the various genotypes, we examined the transcription profiles of the HC of mice subjected to HI. We showed that the majority of the DEGs in both *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice after HI were upregulated, and that there was a striking 50% overlap between the two knockouts (Paper I). Further, by performing IPA for the DEGs, we found that most of the upregulated genes are involved in inflammation. The only commonly down-regulated gene was CD59a, a complement regulatory molecule that mediates neuroprotection after acute injury (Amantea *et al.,* 2015). We also found necrotic cell death to be an enriched process in both *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice, suggesting necrotic cell death to be the reason for increased tissue loss in the acute phase. OGG1 has previously been proposed to be involved in transcriptional activation of pro-inflammatory genes after being exposed to ROS in a mouse model of airway inflammation (Ba *et al.,* 2014). MUTYH has also been associated with inflammation in a mouse model of ulcerative colitis (Casorelli *et al.,* 2010). Subdued activation of the immune response has been connected to ischemic tolerance (Amantea *et al.,* 2015). Our results revealed a role of both OGG1 and MUTYH in inflammation of the brain and we postulate that the increased inflammatory response seen in the knockouts contribute to the increase in cerebral damage after HI.

Inflammation, being a necessary, but potential harmful event, is under tight transcriptional control. One of the ways for the cell to control inflammation is histone methylation (Ishii *et al.*, 2009; Saccani *et al.*, 2002), both in activation and resolution of the inflammatory process. Polycomb group (PcG) proteins are epigenetic chromatin marks that function within multiprotein complexes, called polycomb repressive complexes (PRC). They catalyze methylation of histones and silence target genes as well as induce chromatin compaction (Di Croce *et al.*, 2013). Methylation of different lysines on histone 3 may cause repression or activation, depending on which lysine is being methylated. Methylation of lysine 9 and 27 has been shown to silence or repress genes, while methylation on lysine 4, 36 and 79 has been shown to activate genes (Sims, III *et al.*, 2003). The importance of methylation of histones in transcriptional control of inflammation has been highlighted in recent studies (Ishii *et al.*, 2009; Saccani *et al.*, 2002). Demethylation of H3K27me3 is important for activation of inflammation (De Santa *et al.*, 2007), while PRCs that contain PcG proteins trimethylate H3K27 which leads to gene silencing (Di Croce *et al.*, 2013). In addition, genes may carry a bivalent mark of both the repressive H3K27me3 and the activator histone 3 lysine 4 trimethylation (H3K4me3), keeping the gene in a poised state. We found that there was an enrichment of common DEGs from naïve *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ HC that are targets of PRCs and PcG proteins (Paper I). Intriguingly, these DEGs displayed a significant elevated expression three hours post HI, suggesting that OGG1 and MUTYH may be associated with epigenetic modifiers to suppress the inflammatory reaction. In the *Mutyh*$^{-/-}$ and *Ogg1*$^{-/-}$ mice pro-inflammatory genes are upregulated following HI, and one hypothesis is that this may be due to interference with PRCs to avoid repression of inflammation. Further, microglia are activated in the acute inflammation following HI (Ivacko *et al.,* 1996). The activated microglia may enhance or limit the inflammation and tissue injury depending on the phenotype of the differentiated macrophage (Amantea *et al.,* 2015). Following HI, microglia assume a protective M2 phenotype initially, but then differentiate into the pro-inflammatory M1 phenotype (Hu *et al.,* 2012). We see a significant increase in both occurrence and activation of microglia in *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice.

Interestingly, we also find altered transcriptional regulation of genes involved in the inflammatory response after HI in NEIL1- and NEIL2-deficient mice. Chakraborty *et al.* (2015)

observed an increased level of oxidative damage in transcribed genes, but not in untranscribed genes in *Neil2*$^{-/-}$ mice. The same report demonstrated a pathological development of the innate inflammation in *Neil2*$^{-/-}$ mice. We show that the most significantly affected pathway in *Neil1*$^{-/-}$ and *Neil2*$^{-/-}$ mice both three and six hours after HI was inflammation and that the inflammatory response function was highly enriched in DEGs from the HC of *Neil1*$^{-/-}$ and *Neil2*$^{-/-}$ mice already at three hours after HI (Paper II). Interestingly, six hours after HI inflammatory mediators were the most enriched in DEGs from *Neil1*$^{-/-}$ mice. Further, in line with our post-HI tissue damage results and apoptosis analysis, *Neil2*$^{-/-}$ DEGs three hours post HI were enriched in cell death and survival pathways. More specifically, cell survival was predicted to be activated and apoptosis was predicted to be inhibited. Thus, although both NEIL1 and NEIL2 influence inflammatory and cells death pathways, there seems to be a crucial difference in the timing of the immediate transcriptional response concerning regulation of inflammation, cell survival and apoptosis that ultimately renders *Neil1*$^{-/-}$ mice more susceptible and *Neil2*$^{-/-}$ mice more resistant to HI damage.

MUTYH and OGG1 in learning and memory

Neurogenesis, synaptoplasticity and cell death all contribute to the development and maintenance of cognitive functions (Bredesen *et al.,* 2006; Sander *et al.,* 2009). Accumulation of oxidative damage has been connected to cognitive decline and brain aging (Bishop *et al.,* 2010). During the normal aging process, oxidative DNA damage accumulates in neurons, which may lead to decreased expression of proteins important for synaptic plasticity, learning and memory (Lu *et al.,* 2004). Expression of different glycosylases has been proven to differ in various neurological diseases. Especially OGG1, but also NEIL1 has been shown to be differentially regulated in Alzheimer's disease (Bucholtz *et al.*, 2013; Canugovi *et al.*, 2014). 8-oxoG levels were significantly increased in the DNA of patients suffering from neurodegenerative disorders and altered expression levels of OGG1 and MUTYH have been found in neurons exposed to chronic oxidative stress in vivo (Arai *et al.,* 2002; Fukae *et al.,* 2005; Polidori *et al.,* 1999; Shimura-Miura *et al.,* 1999). Further, Canugovi *et al.* (2012) reported decreased memory, but no alteration in activity level, in aged *Neil1*$^{-/-}$ mice.

Behavioral studies are of great importance in order to characterize the effect of oxidative damage, including the importance of the oxidative DNA damage repair, on cognitive abilities. Decreased spontaneous locomotor behavior has been reported in old (26 months), but not young (three months) *Ogg1*$^{-/-}$ mice compared to wild type (Cardozo-Pelaez *et al.,* 2012). No change in voluntary movement in 12 weeks old *Ogg1*$^{-/-}$ mice was published by Sampath *et al.* (2012). Consistent with this, we showed that our four months old *Ogg1*$^{-/-}$ mice behaved similarly to the wild type mice in the open field and zero maze tests (Paper III). The *Ogg1*$^{-/-}$*Mutyh*$^{-/-}$ mice, on the other hand, were more active and displayed decreased anxiety-like behavior both in the Open Field test and the Zero Maze compared to wild type and single knockout mice. The *Ogg1*$^{-/-}$ *Mutyh*$^{-/-}$ mice weighed less than the other genotypes and weight has been shown to affect the activity level (Crawley *et al.*, 1997). However, we do not know whether the decreased weight is due to increased activity, or if the increased activity is due to decreased body weight. An increase in bodyweight of young *Ogg1*$^{-/-}$ mice on a high fat diet and aged (12-15 months old) chow-fed *Ogg1*$^{-/-}$ mice has previously been demonstrated (Sampath *et al.,* 2012). There was, however, no alteration in body weight of young chow-fed *Ogg1*$^{-/-}$ mice, and the results are thus consistent with ours.

Regnell *et al.* (2012) showed a clear impairment in spatial learning and memory in mice lacking the DNA glycosylase NEIL3. In addition, *Neil1*$^{-/-}$ mice displayed decreased ability to retain long-term memory (Canugovi *et al.,* 2012). We tested learning and memory in the MWM (Paper III). *Mutyh*$^{-/-}$ mice showed improved learning compared to the other genotypes tested. Intriguingly, the double knockout displayed a slow learning curve, but memory was not affected. Thus, we see a reduction in learning ability when removing two, but not one, DNA glycosylase. The ability to retain memory was not affected in any of the genotypes.

As mentioned, decline in cognitive performance and neurodegeneration is associated with advanced age, and this deterioration of the brain function is hypothesized to be caused by accumulation of oxidative damage in macromolecules. This process has been termed the “free radical theory of aging” (Barja 2004; HARMAN 1956; Harman 1981). There is compelling evidence for an age-dependent accumulation of DNA damage in organs with limited proliferative

capacity such as the brain (Moller *et al.,* 2010). DNA damage accumulates in promoter regions with increasing age, and is associated with reduced gene expression and impaired DNA repair in neurons (Lu *et al.,* 2004). However, a causal relationship between oxidative DNA damage and cognitive decline remains to be established. Replication of DNA is essential for accumulation of mutations in both nDNA and mtDNA. Neurons are for the most part terminally differentiated, and only mtDNA, but not nDNA, is replicated. Incorporation of A opposite 8-oxoG is also dependent on replication. Therefore, it has been proposed that 8-oxoG primarily accumulates in mtDNA, causing SSBs during oxidative stress (Sheng *et al.,* 2012). However, it has been demonstrated that lack of the DNA glycosylases MUYTH and OGG1 does not increase the mtDNA mutagenesis in the brain tissue of neither one month old nor six month old mice (Halsne *et al.,* 2012). Further, a review by Moller *et al*. (2010), suggested accumulation of 8-oxoG in aged animals and animals lacking glycosylases, particularly in tissues with low cell proliferation. In Paper III, we aimed to determine whether the differences seen in behavior and cognitive abilities could be explained by accumulation of 8-oxoG. In line with the results from Halsne *et al*. (2012), LC-MS/MS analysis of the HC and hypothalamus did not reveal any differences in 8-oxoG levels. This may be due to the relatively young age of the animals (six months). Nonetheless, we cannot attribute the behavior changes seen in mice lacking OGG1 and MUTYH to bulk accumulation of 8-oxoG in HC and hypothalamus.

To investigate whether the cognitive effects seen in the behavioral trials could be caused by alterations in the genome-wide transcriptional profiles, we applied RNA sequencing analysis of the HC from wild type and mice lacking OGG1 and MUTYH. We found that ~90% of the DEGs in the HC of mice deficient of OGG1 and/or MUTYH were upregulated. The vast majority of the genes were upregulated in both OGG1- and MUTYH-deficient animals, and a large group of the DEGs was commonly upregulated in both single knockouts and the double knockout. A subset of DEGs unique for the double knockout is strongly associated with anxiety-like behavior, supporting the overlapping function of these enzymes in regulating distinct pathways modulating hippocampal activity. More specifically, these pathways include the corticotropin releasing factor receptor signaling pathway and the gonadotropin releasing hormone receptor pathway that

have previously been reported to be top candidate pathways for anxiety (Le-Niculescu *et al.,* 2011).

Alternative mechanisms of DNA glycosylases

It has been suggested that the substantial overlap of substrate specificities between DNA glycosylases to some extent eliminate the effect of single DNA glycosylase defects. This theory has been partly validated by the construction of double knockout mice (Larsen *et al.,* 2007; Robertson *et al.,* 2009). We demonstrate that it is not as clear-cut in reality. MUTYH is neuroprotective during acute oxidative stress (Paper I). NEIL2 on the other hand seems to have a detrimental effect, as removing NEIL2 renders the brain more resistant to acute damage, an effect not seen in the other genotypes (Paper II). While we found a significant increase in infarction size in OGG1 and NEIL1 single knockouts, the effect of knocking out MUTYH was much greater. It is however not clear from our results whether any of these findings are due to impaired canonical DNA repair. Multiple previous reports have demonstrated an increase in vulnerability to oxidative stress when BER of endogenous lesions in nDNA and mtDNA is defective (Canugovi *et al.,* 2012; Endres *et al.,* 2004; Liu *et al.,* 2011; Vasko *et al.,* 2005). We were not able to demonstrate any changes in DNA damage accumulation in any of the genotypes neither in the newborn exposed to HI, nor in older animals. Rather, there seem to be a regulatory shift in mice lacking the various glycosylases. Reduced brain damage has previously been proposed to be due to transcriptional interference (Formisano *et al.,* 2013). In the *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice, there is an imbalance in timing of the pro-inflammatory response as seen by RNA sequencing three hours after injury. We also observed a marked increase in activated microglia three hours after cerebral injury in *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice. Many inflammatory genes were differentially regulated. Regarding NEIL1 and NEIL2, there seems to be a delayed damage response in mice lacking NEIL1, as suggested by the γH2Ax, transcriptome and apoptosis data (Paper II). Moreover, the transcriptome of *Neil2*$^{-/-}$ HC may suggest an accelerated response to HI as compared to wild type. We propose that NEIL1 and NEIL2 have different roles in regulating the early stress response to brain injury.

Increasing evidence suggests a role of oxidative DNA damage and DNA glycosylase-initiated repair in the regulation of cell function and epigenetic modifications. Meissner *et al.* (2008) has demonstrated a better correlation between DNA methylation patterns and histone methylation patterns than between DNA methylation pattern and the underlying genome sequence context. OGG1 has been proved to be involved in histone modification-mediated transcription (Perillo *et al.,* 2008). Further, OGG1 has been suggested, along with other glycosylases, to have a role in epigenetic regulation by involvement in active demethylation (Spruijt *et al.,* 2013). Active demethylation may cause increased levels of 5hmC, which upregulate transcription levels. Oxidative damage, like 8-oxoG, has been reported to contribute to the epigenetic status of the cell. 8-oxoG at an unmethylated CpG-site inhibits binding of transcription factors (Ghosh *et al.*, 1999), whilst 8-oxoG at a methylated CpG site inhibits binding of methyl-CpG binding protein (Valinluck *et al.,* 2004). Recently, Sassa *et al.* (2014) proposed that the presence of 8-oxoG in a CpG dinucleotide can reduce the TDG-initiated BER and thereby reduce the active DNA demethylation process. This is in line with the suggestion that ROS may inhibit DNA methylation by oxidizing G at a CpG site, and thereby influence the ability of DNA methyltransferases (DNMTs) to recognize the DNA sequence as a substrate (Maltseva *et al.,* 2009; Valinluck *et al.,* 2004; Weitzman *et al.,* 1994) . Recently, Zarakowska *et al.* (2014) reported a selective accumulation of 8-oxoG in transcriptionally active chromatin suggesting a role of 8-oxoG and DNA glycosylases in gene regulation. Zhou *et al.* (2016) has proposed OGG1 to be essential for oxidative stress-induced DNA demethylation by recruiting TET. In Paper III, we performed gene set enrichment analysis (http://www.broadinstitute.org/gsea) and identified significant overlap between the DEGs from our *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ and two Meissner brain high-CpG-density promoter gene sets. The genes of the overlapping gene sets carried the bivalent repressive histone mark H3K27Me3/H3K4Me3, and represented therefore genes that are generally repressed, but which may readily be activated (Meissner *et al.,* 2008). Interestingly, all of the overlapping genes are upregulated in all the mutants in Paper III. We also found an upregulation of enriched DEGs in genes carrying either the repressive H3K27Me3 mark alone, or the bivalent H3K27Me3/H3K4Me3 mark in their promoters in the HC of OGG1- and MUTYH-deficient mice (Paper I).

The high percentage of commonly upregulated genes in the *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice compared to wild type, suggests an involvement in regulation of gene expression (Paper I and Paper III). Our results imply that there is a regulatory function of the glycosylases that might be equally or more important than the role in canonical repair. The transcriptome profile we have uncovered does not by itself explain the phenotypes observed in Paper III, but that is not surprising, as the pathways in learning and memory are not fully discovered. We can still postulate that the DNA glycosylases either have a direct or indirect function in learning and memory. However, the molecular mechanisms linking oxidative DNA damage and the repair machinery to behavior are not yet revealed. We hypothesize that accumulation of oxidative lesions or epigenetic modifications at specific gene regulatory regions not found when performing whole genome analysis might cause dysregulation of transcription in neuronal cells responsible for the cognitive phenotypes seen in mice lacking OGG1 and MUTYH.

Methodological Considerations

Hypoxia-Ischemia

HI was produced in postnatal day 8 (P8) mice by permanent occlusion of the left common carotid artery followed by systemic hypoxia as described in (LEVINE 1960; Vannucci *et al.,* 2005) with some modifications. HI resembles asphyxia, which is deprivation of oxygen in the newborn. We chose to initiate HI in P8 mice because the mouse brain at this age is comparable to the term human brain (Semple *et al.,* 2013). The operated pups were put in a heating chamber (36°C) until all pups were operated, then allowed to recover with the dam for 60 minutes. It is important that the recovery time is not too short, as it may prevent the pups from suckle equally, and not too long, as compensatory mechanisms to the occluded artery are initiated and the damage caused by HI will be less severe (Dwyer *et al.*, 1988; Vannucci *et al.*, 2005). Brain edema accompanies HI in immature rodents. To minimize the size of edema, the duration of hypoxia is limited to one hour (Vannucci *et al.*, 2005). One of the most important variables in this model is the body temperature of the pups during hypoxia. Hypothermia is neuroprotective, and small alterations in the body temperature may affect the size of the brain injury greatly (Vannucci *et al.*, 2005; Zhu *et al.*, 2006). To make sure the temperature was stable, we kept the pups in petridishes, between 1-4 pups in each dish, positioned at the same distance from the chamber heater. The body temperature was measured by laser at the beginning of hypoxia, as well as 30 and 55 minutes into hypoxia. In spite of these variables, this model is considered to be the best for imitating human perinatal HI brain damage in rodents.

Behavior analysis

There are numerous different mazes to test behavioral traits in mice. We chose to test the mice using the open field test, the elevated zero maze, and the MWM. The open field test is used to test locomotor activity, assess novel environment exploration, and to screen for anxiety-related behavior in mice. Two factors influencing anxiety-like behavior in this test is separation from litter mates while performing the test, and the stress created by the unprotected, brightly lit, novel test environment (Prut *et al.*, 2003). The elevated zero maze is based on the elevated plus maze

model and is used to measure anxiety. The basis of the test is the exploratory behavior that comes natural to mice in novel environments as well as the natural aversion against open and elevated areas (Komada *et al.,* 2008). The MWM is a model to accurately and reproducibly examine spatial learning and memory. The basis of the test is that the animal learns to find a platform to escape from the water through a number of training trials (D'Hooge *et al.,* 2001; Morris 1984; Vorhees *et al.,* 2006). When planning behavioral analysis several factors need to be taken into account including sex, age, nutrition, stress, apparatus, training procedures and quantification. Because there are gender and age differences in physical strength, hormonal influence and cognitive function, we only tested males of four-six months of age. The training procedure was standardized and equal for all animals, and quantification was carried out by using the ANY-maze computer program.

An alternative to the MWM is the T-maze. This is a T-shaped maze consisting of only one choice point with two alternatives. The principle of the test is built on the instinct of the mouse to choose the opposite arm of the most recently visited. The tests can be performed either in a free or a forced manner, and a positive reinforcement may be placed in one of the arms to reward alternation behavior in food deprived animals (Deacon *et al.*, 2006). It is a test used to assess spatial memory and is the easiest way to assess working spatial memory in that the maze is simple, no extra equipment is needed, and the results are highly reproducible (Sharma *et al.,* 2010). The major disadvantage with the MWM is the stress induced by placing the mice in water (de Quervain *et al.,* 1998; Holscher 1999). Although this stress is avoided in the T-maze, stress induced by food deprivation is present instead. The advantages of the MWM is that there is almost 100% task completion, close to 100% of the mice master the task, minimal training is required, and it is a time-efficient test in that fewer test days are needed than other learning tests. Finally, water remains a strong motivator throughout the test day whereas food rewards become less motivating during the course of trials (Vorhees *et al.*, 2014).

Conclusions

From our findings in Paper I and II, we conclude that MUTYH, OGG1, and NEIL1 have a neuroprotective effect, while NEIL2 has a harmful effect during cerebral hypoxic-ischemic encephalopathy. These effects do not seem to be caused by accumulation of oxidative lesions, but rather mediated by an altered transcription profile. We discovered a dysregulation of the inflammatory response by whole genome transcription analysis, as well as increased IL-1β-expressing activated microglia in the HC of OGG1- and MUTYH-deficient mice. While OGG1 and MUTYH seem to act synergistically in neuroinflammation, NEIL1 and NEIL2 most likely regulate the early stress response to HI brain injury differently.

In Paper III, we conclude that behavior and cognition are affected in mice lacking OGG1 and/or MUTYH. As for the HI studies, there was no apparent accumulation of 8-oxoG in brain regions essential for cognition and anxiety. Transcriptome analysis revealed a high percentage of overlap in DEGs associated with pathways for anxiety, learning and memory in OGG1- and/or MUTYH-deficient HC.

Collectively, our findings suggest a role of oxidative DNA glycosylases in gene regulation, which merits further investigation of the molecular mechanisms underlying the DNA glycosylase function in acute brain damage in infancy as well as cognition.

Future Perspectives

We want to further elucidate the role of DNA glycosylases in higher cognitive functions, and are in progress of investigating the role of NEIL1 and NEIL2 in learning and memory by testing knockout mice in the Open Field Maze, the elevated Zero Maze and the MWM. To address the impact of NEIL1 and NEIL2 on cognition we aim to analyze the transcription and mutation profiles of mutant brains by RNA sequencing and whole genome deep sequencing, respectively.

In Paper II, we found NEIL2 to be a potential target in treating HI encephalopathy. We are in the process of developing inhibitors of NEIL enzymes to investigate in particular NEIL2 as a potential therapeutic target during HI.

We uncovered altered transcription profiles as a potential explanation for our findings in response to cerebral HI and the altered cognitive functions, and we would like to pursue these findings. From the results presented in this thesis, we speculate if the DNA glycosylases are involved in the fine-tuning of tissue- and cell-specific processes through recognition of small changes in the DNA. More research is needed to prove this hypothesis. The next step will be to reveal the molecular mechanisms underlying the observed differences between the various knockouts.

Although none of the DNA glycosylases has been shown to possess activity for 5mC or 5hmC, OGG1 has been suggested to be important for recruiting TET when 5hmC is opposite 8-oxoG (Zhou *et al.,* 2016). TDG has been proven to be essential for embryonic development due to association with active C demethylation (Cortazar *et al.,* 2011), and NEIL DNA glycosylases cooperate with TDG during DNA methylation (Schomacher *et al.,* 2016). Both OGG1 (Zhou *et al.,* 2016) and UNG2 (Xue *et al.,* 2016) have been proposed to be involved in TET-mediated demethylation of DNA. CpG sequences containing oxidized G have been shown to disrupt DNA methylation by DNMTs (Maltseva *et al.,* 2009; Valinluck *et al.,* 2004; Weitzman *et al.,* 1994). The presence and/or repair of these lesions might serve as modulators of the epigenetic landscape. We want to further characterize the biochemical properties and sequence dependency

of purified DNA glycosylases on substrates containing epigenetic methylation and oxidative lesion by activity assays and quantification of cleavage products.

As discussed earlier, we found an overlap between overexpressed genes and two Meissner high-CpG-density promoter gene sets in OGG1- and MUTYH-deficient mice (Paper III). We also demonstrated altered histone methylation patterns in promoters of DEGs in the HC of *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ mice. Promoter-associated CpG-islands show an overall low DNA methylation that tend to increase with age, while intergenic non-island CpGs with high methylation tend to lose methylation with age (reviewed in Jones *et al.* (2015)). High throughput screening of epigenetic readers and erasers demonstrate that NEIL DNA glycosylases and OGG1 are associated with epigenetic DNA (Spruijt *et al.,* 2013). We hypothesize that there is an accumulation of epigenetic modifications at specific gene regulatory regions causing a dysregulation of transcription. To test this hypothesis, we are planning to investigate the methylation profile in mice, both the total genome profile as well as the methylation profile of the promoter regions by newly developed single base resolution sequencing methods for 5mC, 5hmC, 5fC, and 5caC. We would like to compare the epigenomes, transcriptomes and mutation profiles of brains of naïve young animals deficient of various DNA glycosylases to both aged animals and animals subjected to HI damage to address the role of the DNA glycosylases in epigenetic DNA methylation and gene regulation, and consequently, their function in cognition and neuroinflammation.

Reference List

Abe T, Shimamura M, Jackman K, Kurinami H, Anrather J, Zhou P, and Iadecola C (2010) Key role of CD36 in Toll-like receptor 2 signaling in cerebral ischemia. Stroke *41,* 898-904.

Adibhatla RM and Hatcher JF (2010) Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid.Redox.Signal. *12,* 125-169.

Adwan L and Zawia NH (2013) Epigenetics: a novel therapeutic approach for the treatment of Alzheimer's disease. Pharmacol.Ther. *139,* 41-50.

Aguilera-Aguirre L, Bacsi A, Radak Z, Hazra TK, Mitra S, Sur S, Brasier AR, Ba X, and Boldogh I (2014) Innate inflammation induced by the 8-oxoguanine DNA glycosylase-1-KRAS-NF-kappaB pathway. J.Immunol. *193,* 4643-4653.

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

Ali C, Nicole O, Docagne F, Lesne S, MacKenzie ET, Nouvelot A, Buisson A, and Vivien D (2000) Ischemia-induced interleukin-6 as a potential endogenous neuroprotective cytokine against NMDA receptor-mediated excitotoxicity in the brain. J.Cereb.Blood Flow Metab *20,* 956-966.

Alonso J, Angermeyer MC, Bernert S, Bruffaerts R, Brugha TS, Bryson H, de GG, Graaf R, Demyttenaere K, Gasquet I, Haro JM, Katz SJ, Kessler RC, Kovess V, Lepine JP, Ormel J, Polidori G, Russo LJ, Vilagut G, Almansa J, Arbabzadeh-Bouchez S, Autonell J, Bernal M, Buist-Bouwman MA, Codony M, Domingo-Salvany A, Ferrer M, Joo SS, Martinez-Alonso M, Matschinger H, Mazzi F, Morgan Z, Morosini P, Palacin C, Romera B, Taub N, and Vollebergh WA (2004) Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. Acta Psychiatr.Scand.Suppl21-27.

Alseth I, Korvald H, Osman F, Seeberg E, and Bjoras M (2004) A general role of the DNA glycosylase Nth1 in the abasic sites cleavage step of base excision repair in Schizosaccharomyces pombe. Nucleic Acids Res. *32,* 5119-5125.

Altieri F, Grillo C, Maceroni M, and Chichiarelli S (2008) DNA damage and repair: from molecular mechanisms to health implications. Antioxid.Redox.Signal. *10,* 891-937.

Amantea D, Bagetta G, Tassorelli C, Mercuri NB, and Corasaniti MT (2010) Identification of distinct cellular pools of interleukin-1beta during the evolution of the neuroinflammatory response induced by transient middle cerebral artery occlusion in the brain of rat. Brain Res. *1313,* 259-269.

Amantea D, Micieli G, Tassorelli C, Cuartero MI, Ballesteros I, Certo M, Moro MA, Lizasoain I, and Bagetta G (2015) Rational modulation of the innate immune system for neuroprotection in ischemic stroke. Front Neurosci. *9:147. doi: 10.3389/fnins.2015.00147. eCollection;%2015.,* 147.

Ames BN, Shigenaga MK, and Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc.Natl.Acad.Sci.U.S.A *90,* 7915-7922.

Andersen S, Ericsson M, Dai HY, Pena-Diaz J, Slupphaug G, Nilsen H, Aarset H, and Krokan HE (2005) Monoclonal B-cell hyperplasia and leukocyte imbalance precede development of B-cell malignancies in uracil-DNA glycosylase deficient mice. DNA Repair (Amst) *4,* 1432-1441.

Arai T, Kelly VP, Minowa O, Noda T, and Nishimura S (2002) High accumulation of oxidative DNA damage, 8-hydroxyguanine, in Mmh/Ogg1 deficient mice by chronic oxidative stress. Carcinogenesis *23,* 2005-2010.

Arana ME and Kunkel TA (2010) Mutator phenotypes due to DNA replication infidelity. Semin.Cancer Biol. *20,* 304-311.

Azzam EI, Jay-Gerin JP, and Pain D (2012) Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett. *327,* 48-60.

Ba X, Bacsi A, Luo J, Aguilera-Aguirre L, Zeng X, Radak Z, Brasier AR, and Boldogh I (2014) 8-oxoguanine DNA glycosylase-1 augments proinflammatory gene expression by facilitating the recruitment of site-specific transcription factors. J.Immunol. *192,* 2384-2394.

Bading H, Ginty DD, and Greenberg ME (1993) Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science *260,* 181-186.

Bandaru V, Sunkara S, Wallace SS, and Bond JP (2002) A novel human DNA glycosylase that removes oxidative DNA damage and is homologous to Escherichia coli endonuclease VIII. DNA Repair (Amst) *1,* 517-529.

Banerjee D, Mandal SM, Das A, Hegde ML, Das S, Bhakat KK, Boldogh I, Sarkar PS, Mitra S, and Hazra TK (2011) Preferential repair of oxidized base damage in the transcribed genes of mammalian cells. J.Biol.Chem. *286,* 6006-6016.

Barja G (2004) Free radicals and aging. Trends in Neurosciences *27,* 595-600.

Barnes DE and Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu.Rev.Genet. *38,* 445-476.

Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG, and Feuerstein GZ (1997) Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. Stroke *28,* 1233-1244.

Barzilai A (2007) The contribution of the DNA damage response to neuronal viability. Antioxid.Redox.Signal. *9,* 211-218.

Battaglia M (2013) Gene-environment interaction in panic disorder and posttraumatic stress disorder. Can.J.Psychiatry *58,* 69-75.

Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, and Bartkowski HM (1986) Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke *17,* 1304-1308.

Beilharz EJ, Williams CE, Dragunow M, Sirimanne ES, and Gluckman PD (1995) Mechanisms of delayed cell death following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss. Brain Res.Mol.Brain Res. *29,* 1-14.

Bergman Y and Cedar H (2013) DNA methylation dynamics in health and disease. Nat.Struct.Mol.Biol. *20,* 274-281.

Berti R, Williams AJ, Moffett JR, Hale SL, Velarde LC, Elliott PJ, Yao C, Dave JR, and Tortella FC (2002) Quantitative real-time RT-PCR analysis of inflammatory gene expression associated with ischemia-reperfusion brain injury. J.Cereb.Blood Flow Metab *22,* 1068-1079.

Bishop NA, Lu T, and Yankner BA (2010) Neural mechanisms of ageing and cognitive decline. Nature. *464,* 529-535.

Bjoras M, Luna L, Johnsen B, Hoff E, Haug T, Rognes T, and 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.

Bliss TV and Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J.Physiol *232,* 357-374.

Blundred RM and Stewart GS (2011) DNA double-strand break repair, immunodeficiency and the RIDDLE syndrome. Expert.Rev.Clin.Immunol. *7,* 169-185.

Boiteux S and Radicella JP (2000) The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch.Biochem.Biophys. *377,* 1-8.

Bologna S and Ferrari S (2013) It takes two to tango: Ubiquitin and SUMO in the DNA damage response. Front Genet. *4,* 106.

Booth MJ, Branco MR, Ficz G, Oxley D, Krueger F, Reik W, and Balasubramanian S (2012) Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. Science *336,* 934-937.

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, and Rothwell NJ (2001) Role of IL-1alpha and IL-1beta in ischemic brain damage. J.Neurosci. *21,* 5528-5534.

Bredesen DE, Rao RV, and Mehlen P (2006) Cell death in the nervous system. Nature *443,* 796-802.

Brierley DJ and Martin SA (2013) Oxidative stress and the DNA mismatch repair pathway. Antioxid.Redox.Signal. *18,* 2420-2428.

Brooks SC, Adhikary S, Rubinson EH, and Eichman BF (2013) Recent advances in the structural mechanisms of DNA glycosylases. Biochim.Biophys.Acta *1834,* 247-271.

Brown JA, Pack LR, Sanman LE, and Suo Z (2011) Efficiency and fidelity of human DNA polymerases lambda and beta during gap-filling DNA synthesis. DNA Repair (Amst) *10,* 24-33.

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, and Mattson MP (1996) Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat.Med. *2,* 788-794.

Bucholtz N and Demuth I (2013) DNA-repair in mild cognitive impairment and Alzheimer's disease. DNA Repair (Amst) *12,* 811-816.

Butterfield DA and Kanski J (2002) Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide 1-42. Peptides *23,* 1299-1309.

Cai Z, Guo W, Chen H, Tao J, Cao L, Sun W, and Wang Y (2012) Base excision repair gene polymorphisms are associated with inflammation in patients undergoing chronic hemodialysis. Biochem.Biophys.Res.Commun. *424,* 611-615.

Campalans A, Marsin S, Nakabeppu Y, O'connor TR, Boiteux S, and Radicella JP (2005) XRCC1 interactions with multiple DNA glycosylases: a model for its recruitment to base excision repair. DNA Repair (Amst) *4,* 826-835.

Canugovi C, Shamanna RA, Croteau DL, and Bohr VA (2014) Base excision DNA repair levels in mitochondrial lysates of Alzheimer's disease. Neurobiol.Aging *35,* 1293-1300.

Canugovi C, Yoon JS, Feldman NH, Croteau DL, Mattson MP, and 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.

Cardozo-Pelaez F, Sanchez-Contreras M, and Nevin AB (2012) Ogg1 null mice exhibit age-associated loss of the nigrostriatal pathway and increased sensitivity to MPTP. Neurochem.Int. *61,* 721-730.

Carney JM, Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF, and Floyd RA (1991) Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-alpha-phenylnitrone. Proc.Natl.Acad.Sci.U.S.A *88,* 3633-3636.

Casorelli I, Pannellini T, De LG, Degan P, Chiera F, Iavarone I, Giuliani A, Butera A, Boirivant M, Musiani P, and Bignami M (2010) The Mutyh base excision repair gene influences the inflammatory response in a mouse model of ulcerative colitis. PLoS.One. *5,* e12070.

Chakraborty A, Wakamiya M, Venkova-Canova T, Pandita RK, Aguilera-Aguirre L, Sarker AH, Singh DK, Hosoki K, Wood TG, Sharma G, Cardenas V, Sarkar PS, Sur S, Pandita TK, Boldogh I, and Hazra TK (2015) Neil2-null Mice Accumulate Oxidized DNA Bases in the Transcriptionally Active Sequences of the Genome and are Susceptible to Innate Inflammation. J.Biol.Chem.

Chan MK, Ocampo-Hafalla MT, Vartanian V, Jaruga P, Kirkali G, Koenig KL, Brown S, Lloyd RS, Dizdaroglu M, and Teebor GW (2009) Targeted deletion of the genes encoding NTH1 and NEIL1 DNA N-glycosylases reveals the existence of novel carcinogenic oxidative damage to DNA. DNA Repair (Amst) *8,* 786-794.

Chen H, Dzitoyeva S, and Manev H (2012) Effect of aging on 5-hydroxymethylcytosine in the mouse hippocampus. Restor.Neurol.Neurosci. *30,* 237-245.

Chen J, Jin K, Chen M, Pei W, Kawaguchi K, Greenberg DA, and Simon RP (1997) Early detection of DNA strand breaks in the brain after transient focal ischemia: implications for the role of DNA damage in apoptosis and neuronal cell death. J.Neurochem. *69,* 232-245.

Chiba T and Umegaki K (2013) Pivotal roles of monocytes/macrophages in stroke. Mediators.Inflamm. *2013,* 759103.

Clausen BH, Lambertsen KL, Babcock AA, Holm TH, Dagnaes-Hansen F, and Finsen B (2008) Interleukin-1beta and tumor necrosis factor-alpha are expressed by different subsets of microglia and macrophages after ischemic stroke in mice. J.Neuroinflammation. *5,* 46.

Cortazar D, Kunz C, Selfridge J, Lettieri T, Saito Y, MacDougall E, Wirz A, Schuermann D, Jacobs AL, Siegrist F, Steinacher R, Jiricny J, Bird A, and Schar P (2011) Embryonic lethal phenotype reveals a function of TDG in maintaining epigenetic stability. Nature *470,* 419-423.

Cortellino S, Xu J, Sannai M, Moore R, Caretti E, Cigliano A, Le CM, Devarajan K, Wessels A, Soprano D, Abramowitz LK, Bartolomei MS, Rambow F, Bassi MR, Bruno T, Fanciulli M, Renner C, Klein-Szanto AJ, Matsumoto Y, Kobi D, Davidson I, Alberti C, Larue L, and Bellacosa A (2011) Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. Cell *146,* 67-79.

Cox-Limpens KE, Gavilanes AW, Zimmermann LJ, and Vles JS (2014) Endogenous brain protection: what the cerebral transcriptome teaches us. Brain Res. *1564,* 85-100.

Crawley JN and Paylor R (1997) A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm.Behav. *31,* 197-211.

D'Amours D, Desnoyers S, D'Silva I, and Poirier GG (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem.J. *342 (Pt 2),* 249-268.

D'Hooge R and De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res.Brain Res.Rev. *36,* 60-90.

Dalhus B, Laerdahl JK, Backe PH, and Bjoras M (2009) DNA base repair--recognition and initiation of catalysis. FEMS Microbiol.Rev. *33,* 1044-1078.

Das A, Rajagopalan L, Mathura VS, Rigby SJ, Mitra S, and Hazra TK (2004) Identification of a zinc finger domain in the human NEIL2 (Nei-like-2) protein. J.Biol.Chem. *279,* 47132-47138.

de Lange T (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. *19,* 2100-2110.

de Murcia G and Menissier de Murcia J (1994) Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem.Sci. *19,* 172-176.

de Quervain DJ, Roozendaal B, and McGaugh JL (1998) Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature *394,* 787-790.

De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, and Natoli G (2007) The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell. *130,* 1083-1094.

Deacon RM and Rawlins JN (2006) T-maze alternation in the rodent. Nat.Protoc. *1,* 7-12.

Deb P, Sharma S, and Hassan KM (2010) Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. Pathophysiology. *17,* 197-218.

Deng W, Saxe MD, Gallina IS, and Gage FH (2009) Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. J.Neurosci. *29,* 13532-13542.

Devasagayam TP, Steenken S, Obendorf MS, Schulz WA, and Sies H (1991) Formation of 8-hydroxy(deoxy)guanosine and generation of strand breaks at guanine residues in DNA by singlet oxygen. Biochemistry *30,* 6283-6289.

Di Croce L and Helin K (2013) Transcriptional regulation by Polycomb group proteins. Nat.Struct.Mol.Biol. *20,* 1147-1155.

Diaz M and Casali P (2002) Somatic immunoglobulin hypermutation. Curr.Opin.Immunol. *14,* 235-240.

Domschke K and Deckert J (2012a) Genetics of anxiety disorders - status quo and quo vadis. Curr.Pharm.Des *18,* 5691-5698.

Domschke K and Maron E (2013a) Genetic factors in anxiety disorders. Mod.Trends Pharmacopsychiatri. *29,* 24-46.

Domschke K, Tidow N, Kuithan H, Schwarte K, Klauke B, Ambree O, Reif A, Schmidt H, Arolt V, Kersting A, Zwanzger P, and Deckert J (2012b) Monoamine oxidase A gene DNA hypomethylation - a risk factor for panic disorder? Int.J.Neuropsychopharmacol. *15,* 1217-1228.

Domschke K, Tidow N, Schrempf M, Schwarte K, Klauke B, Reif A, Kersting A, Arolt V, Zwanzger P, and Deckert J (2013b) Epigenetic signature of panic disorder: a role of glutamate decarboxylase 1 (GAD1) DNA hypomethylation? Prog.Neuropsychopharmacol.Biol.Psychiatry *46,* 189-196.

Domschke K, Tidow N, Schwarte K, Ziegler C, Lesch KP, Deckert J, Arolt V, Zwanzger P, and Baune BT (2015) Pharmacoepigenetics of depression: no major influence of MAO-A DNA methylation on treatment response. J.Neural Transm. *122,* 99-108.

Dou H, Mitra S, and Hazra TK (2003) Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. J.Biol.Chem. *278,* 49679-49684.

Dumont M, Wille E, Stack C, Calingasan NY, Beal MF, and Lin MT (2009) Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic mouse model of Alzheimer's disease. FASEB J. *23,* 2459-2466.

Dwyer BE, Nishimura RN, and Fujikawa DG (1988) Cerebral hypoxia-ischemia in immature rats: methodological considerations. Exp.Neurol. *99,* 772-777.

Ebrahimkhani MR, Daneshmand A, Mazumder A, Allocca M, Calvo JA, Abolhassani N, Jhun I, Muthupalani S, Ayata C, and Samson LD (2014) Aag-initiated base excision repair promotes

ischemia reperfusion injury in liver, brain, and kidney. Proc.Natl.Acad.Sci.U.S.A. *111,* E4878-E4886.

Edinger AL and Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. Curr.Opin.Cell Biol. *16,* 663-669.

Eker AP, Quayle C, Chaves I, and van der Horst GT (2009) DNA repair in mammalian cells: Direct DNA damage reversal: elegant solutions for nasty problems. Cell Mol.Life Sci. *66,* 968-980.

Eliasson MJ, Sampei K, Mandir AS, Hurn PD, Traystman RJ, Bao J, Pieper A, Wang ZQ, Dawson TM, Snyder SH, and Dawson VL (1997) Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat.Med. *3,* 1089-1095.

Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ, and Tyrrell PJ (2005) A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. J.Neurol.Neurosurg.Psychiatry *76,* 1366-1372.

Endres M, Biniszkiewicz D, Sobol RW, Harms C, Ahmadi M, Lipski A, Katchanov J, Mergenthaler P, Dirnagl U, Wilson SH, Meisel A, and Jaenisch R (2004) Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase. J.Clin.Invest *113,* 1711-1721.

Evans MD, Dizdaroglu M, and Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutat.Res. *567,* 1-61.

Fan G, Beard C, Chen RZ, Csankovszki G, Sun Y, Siniaia M, Biniszkiewicz D, Bates B, Lee PP, Kuhn R, Trumpp A, Poon C, Wilson CB, and Jaenisch R (2001) DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. J.Neurosci. *21,* 788-797.

Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, and Fan G (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat.Neurosci. *13,* 423-430.

Feng Q, Wang YI, and Yang Y (2015) Neuroprotective effect of interleukin-6 in a rat model of cerebral ischemia. Exp.Ther.Med. *9,* 1695-1701.

Finkel T (2012) Signal transduction by mitochondrial oxidants. J.Biol.Chem. *287,* 4434-4440.

Fontes FL, Pinheiro DM, Oliveira AH, Oliveira RK, Lajus TB, and Agnez-Lima LF (2015) Role of DNA repair in host immune response and inflammation. Mutat.Res.Rev.Mutat.Res. *763,* 246-257.

Formisano L, Guida N, Valsecchi V, Pignataro G, Vinciguerra A, Pannaccione A, Secondo A, Boscia F, Molinaro P, Sisalli MJ, Sirabella R, Casamassa A, Canzoniero LM, Di RG, and

Annunziato L (2013) NCX1 is a new rest target gene: role in cerebral ischemia. Neurobiol.Dis. *50,* 76-85.

Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, and Sohal RS (1996) Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc.Natl.Acad.Sci.U.S.A *93,* 4765-4769.

Fortini P and Dogliotti E (2007) Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. DNA Repair (Amst) *6,* 398-409.

Fromme JC, Banerjee A, Huang SJ, and Verdine GL (2004a) Structural basis for removal of adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. Nature *427,* 652-656.

Fromme JC, Banerjee A, and Verdine GL (2004b) DNA glycosylase recognition and catalysis. Curr.Opin.Struct.Biol. *14,* 43-49.

Fromme JC and Verdine GL (2004c) Base excision repair. Adv.Protein Chem. *69,* 1-41.

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

Fukui K, Onodera K, Shinkai T, Suzuki S, and Urano S (2001) Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. Ann.N.Y.Acad.Sci. *928,* 168-175.

Gao Y, Katyal S, Lee Y, Zhao J, Rehg JE, Russell HR, and McKinnon PJ (2011) DNA ligase III is critical for mtDNA integrity but not Xrcc1-mediated nuclear DNA repair. Nature *471,* 240-244.

Gary DS, Bruce-Keller AJ, Kindy MS, and Mattson MP (1998) Ischemic and excitotoxic brain injury is enhanced in mice lacking the p55 tumor necrosis factor receptor. J.Cereb.Blood Flow Metab *18,* 1283-1287.

Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, and Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature *439,* 589-593.

Ghosh R and Mitchell DL (1999) Effect of oxidative DNA damage in promoter elements on transcription factor binding. Nucleic Acids Res. *27,* 3213-3218.

Ghosh S, Canugovi C, Yoon JS, Wilson DM, III, Croteau DL, Mattson MP, and Bohr VA (2015) Partial loss of the DNA repair scaffolding protein, Xrcc1, results in increased brain damage and reduced recovery from ischemic stroke in mice. Neurobiol.Aging *36,* 2319-2330.

Ginet V, Puyal J, Clarke PG, and Truttmann AC (2009) Enhancement of autophagic flux after neonatal cerebral hypoxia-ischemia and its region-specific relationship to apoptotic mechanisms. Am.J.Pathol. *175,* 1962-1974.

Giovannelli L, Cozzi A, Guarnieri I, Dolara P, and Moroni F (2002) Comet assay as a novel approach for studying DNA damage in focal cerebral ischemia: differential effects of NMDA receptor antagonists and poly(ADP-ribose) polymerase inhibitors. J.Cereb.Blood Flow Metab *22,* 697-704.

Globisch D, Munzel M, Muller M, Michalakis S, Wagner M, Koch S, Bruckl T, Biel M, and Carell T (2010) Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. PLoS.One. *5,* e15367.

Goelet P, Castellucci VF, Schacher S, and Kandel ER (1986) The long and the short of long-term memory--a molecular framework. Nature *322,* 419-422.

Gong G, Bai S, Wu W, Hu L, Liu Y, Niu J, Dai X, Yin L, and Wang X (2014) Lrg participates in lipopolysaccharide preconditioning-induced brain ischemia injury via TLR4 signaling pathway. J.Mol.Neurosci. *54,* 20-26.

Grasso F, Ruggieri V, De LG, Leopardi P, Mancuso MT, Casorelli I, Pichierri P, Karran P, and Bignami M (2015) MUTYH mediates the toxicity of combined DNA 6-thioguanine and UVA radiation. Oncotarget. *6,* 7481-7492.

Grin IR and Zharkov DO (2011) Eukaryotic endonuclease VIII-like proteins: new components of the base excision DNA repair system. Biochemistry (Mosc.) *76,* 80-93.

Groves JO, Leslie I, Huang GJ, McHugh SB, Taylor A, Mott R, Munafo M, Bannerman DM, and Flint J (2013) Ablating adult neurogenesis in the rat has no effect on spatial processing: evidence from a novel pharmacogenetic model. PLoS.Genet. *9,* e1003718.

Guerrero-Bosagna C and Skinner MK (2014) Environmentally induced epigenetic transgenerational inheritance of male infertility. Curr.Opin.Genet.Dev. *26,* 79-88.

Guo JU, Su Y, Zhong C, Ming GL, and Song H (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell *145,* 423-434.

Gutteridge JM and Halliwell B (2010) Antioxidants: Molecules, medicines, and myths. Biochem.Biophys.Res.Commun. *393,* 561-564.

Hailer MK, Slade PG, Martin BD, Rosenquist TA, and Sugden KD (2005) Recognition of the oxidized lesions spiroiminodihydantoin and guanidinohydantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2. DNA Repair (Amst) *4,* 41-50.

Hakem R (2008) DNA-damage repair; the good, the bad, and the ugly. EMBO J. *27,* 589-605.

Halsne R, Esbensen Y, Wang W, Scheffler K, Suganthan R, Bjoras M, and 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.

Hamanaka RB and Chandel NS (2009) Mitochondrial reactive oxygen species regulate hypoxic signaling. Curr.Opin.Cell Biol. *21,* 894-899.

Hanssen-Bauer A, Solvang-Garten K, Akbari M, and Otterlei M (2012) X-ray repair cross complementing protein 1 in base excision repair. Int.J.Mol.Sci. *13,* 17210-17229.

Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, and Moskowitz MA (1997) Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc.Natl.Acad.Sci.U.S.A *94,* 2007-2012.

Hardeland U, Bentele M, Jiricny J, and Schar P (2003) The versatile thymine DNA-glycosylase: a comparative characterization of the human, Drosophila and fission yeast orthologs. Nucleic Acids Res. *31,* 2261-2271.

HARMAN D (1956) Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology *11,* 298-300.

Harman D (1981) The aging process. Proc.Natl.Acad.Sci.U.S.A *78,* 7124-7128.

Harper JW and Elledge SJ (2007) The DNA damage response: ten years after. Mol.Cell *28,* 739-745.

Hashimoto H, Hong S, Bhagwat AS, Zhang X, and Cheng X (2012) Excision of 5-hydroxymethyluracil and 5-carboxylcytosine by the thymine DNA glycosylase domain: its structural basis and implications for active DNA demethylation. Nucleic Acids Res. *40,* 10203-10214.

Hazra TK, Hill JW, Izumi T, and Mitra S (2001) Multiple DNA glycosylases for repair of 8-oxoguanine and their potential in vivo functions. Prog.Nucleic Acid Res.Mol.Biol. *68,* 193-205.

Hazra TK, Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M, and Mitra S (2002a) Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. Proc.Natl.Acad.Sci.U.S.A *99,* 3523-3528.

Hazra TK, Kow YW, Hatahet Z, Imhoff B, Boldogh I, Mokkapati SK, Mitra S, and Izumi T (2002b) Identification and characterization of a novel human DNA glycosylase for repair of cytosine-derived lesions. J.Biol.Chem. *277,* 30417-30420.

Hazra TK and Mitra S (2006) Purification and characterization of NEIL1 and NEIL2, members of a distinct family of mammalian DNA glycosylases for repair of oxidized bases. Methods Enzymol. *408,* 33-48.

He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, Ding J, Jia Y, Chen Z, Li L, Sun Y, Li X, Dai Q, Song CX, Zhang K, He C, and Xu GL (2011) Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science *333,* 1303-1307.

Hegde ML, Hazra TK, and Mitra S (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. *18,* 27-47.

Hegde ML, Hegde PM, Bellot LJ, Mandal SM, Hazra TK, Li GM, Boldogh I, Tomkinson AE, and Mitra S (2013) Prereplicative repair of oxidized bases in the human genome is mediated by NEIL1 DNA glycosylase together with replication proteins. Proc.Natl.Acad.Sci.U.S.A *110,* E3090-E3099.

Heinrich PC, Castell JV, and Andus T (1990) Interleukin-6 and the acute phase response. Biochem.J. *265,* 621-636.

Henikoff S and Matzke MA (1997) Exploring and explaining epigenetic effects. Trends Genet. *13,* 293-295.

Hettema JM, Neale MC, and Kendler KS (2001) A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am.J.Psychiatry *158,* 1568-1578.

Hildrestrand GA, Rolseth V, Bjoras M, and Luna L (2007) Human NEIL1 localizes with the centrosomes and condensed chromosomes during mitosis. DNA Repair (Amst) *6,* 1425-1433.

Hirano T, Taga T, Yasukawa K, Nakajima K, Nakano N, Takatsuki F, Shimizu M, Murashima A, Tsunasawa S, Sakiyama F, and . (1987) Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc.Natl.Acad.Sci.U.S.A *84,* 228-231.

Ho VM, Lee JA, and Martin KC (2011) The cell biology of synaptic plasticity. Science *334,* 623-628.

Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. Nature *411,* 366-374.

Holscher C (1999) Stress impairs performance in spatial water maze learning tasks. Behav.Brain Res. *100,* 225-235.

Hovatta I and Barlow C (2008) Molecular genetics of anxiety in mice and men. Ann.Med. *40,* 92-109.

Hu D, Serrano F, Oury TD, and Klann E (2006) Aging-dependent alterations in synaptic plasticity and memory in mice that overexpress extracellular superoxide dismutase. J.Neurosci. *26,* 3933-3941.

Hu J, de Souza-Pinto NC, Haraguchi K, Hogue BA, Jaruga P, Greenberg MM, Dizdaroglu M, and 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.

Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, and Chen J (2012) Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. Stroke. *43,* 3063-3070.

Huen MS and Chen J (2008) The DNA damage response pathways: at the crossroad of protein modifications. Cell Res. *18,* 8-16.

Iadecola C and Anrather J (2011) The immunology of stroke: from mechanisms to translation. Nat.Med. *17,* 796-808.

Ikehata H and Ono T (2011) The mechanisms of UV mutagenesis. J.Radiat.Res. *52,* 115-125.

Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, Yel L, Forveille M, Kavli B, Krokan HE, Ochs HD, Fischer A, and Durandy A (2003) Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat.Immunol. *4,* 1023-1028.

Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itohara S, and Kageyama R (2008) Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat.Neurosci. *11,* 1153-1161.

Inder TE and Volpe JJ (2000) Mechanisms of perinatal brain injury. Semin.Neonatol. *5,* 3-16.

Ishii M, Wen H, Corsa CA, Liu T, Coelho AL, Allen RM, Carson WF, Cavassani KA, Li X, Lukacs NW, Hogaboam CM, Dou Y, and Kunkel SL (2009) Epigenetic regulation of the alternatively activated macrophage phenotype. Blood. *114,* 3244-3254.

Iskandar BJ, Rizk E, Meier B, Hariharan N, Bottiglieri T, Finnell RH, Jarrard DF, Banerjee RV, Skene JH, Nelson A, Patel N, Gherasim C, Simon K, Cook TD, and Hogan KJ (2010) Folate regulation of axonal regeneration in the rodent central nervous system through DNA methylation. J.Clin.Invest *120,* 1603-1616.

Ismail IH and Hendzel MJ (2008) The gamma-H2A.X: is it just a surrogate marker of double-strand breaks or much more? Environ.Mol.Mutagen. *49,* 73-82.

Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, and Zhang Y (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science *333,* 1300-1303.

Ivacko JA, Sun R, and Silverstein FS (1996) Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. Pediatr.Res. *39,* 39-47.

Iyama T and Wilson DM, III (2013) DNA repair mechanisms in dividing and non-dividing cells. DNA Repair (Amst) *12,* 620-636.

Jackson SP and Bartek J (2009) The DNA-damage response in human biology and disease. Nature *461,* 1071-1078.

Jacobs AL and Schar P (2012) DNA glycosylases: in DNA repair and beyond. Chromosoma *121,* 1-20.

Jaruga P, Xiao Y, Vartanian V, Lloyd RS, and Dizdaroglu M (2010) Evidence for the involvement of DNA repair enzyme NEIL1 in nucleotide excision repair of (5'R)- and (5'S)-8,5'-cyclo-2'-deoxyadenosines. Biochemistry *49,* 1053-1055.

Jessberger S and Gage FH (2008) Stem-cell-associated structural and functional plasticity in the aging hippocampus. Psychol.Aging *23,* 684-691.

Jin SG, Wu X, Li AX, and Pfeifer GP (2011) Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Res. *39,* 5015-5024.

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

Jones MJ, Goodman SJ, and Kobor MS (2015) DNA methylation and healthy human aging. Aging Cell. *14,* 924-932.

Kanaar R, Wyman C, and Rothstein R (2008) Quality control of DNA break metabolism: in the 'end', it's a good thing. EMBO J. *27,* 581-588.

Kang D, Nishida J, Iyama A, Nakabeppu Y, Furuichi M, Fujiwara T, Sekiguchi M, and Takeshige K (1995) Intracellular localization of 8-oxo-dGTPase in human cells, with special reference to the role of the enzyme in mitochondria. J.Biol.Chem. *270,* 14659-14665.

Karahalil B, de Souza-Pinto NC, Parsons JL, Elder RH, and Bohr VA (2003) Compromised incision of oxidized pyrimidines in liver mitochondria of mice deficient in NTH1 and OGG1 glycosylases. J.Biol.Chem. *278,* 33701-33707.

Kee N, Teixeira CM, Wang AH, and Frankland PW (2007) Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat.Neurosci. *10,* 355-362.

Kemmerich K, Dingler FA, Rada C, and Neuberger MS (2012) Germline ablation of SMUG1 DNA glycosylase causes loss of 5-hydroxymethyluracil- and UNG-backup uracil-excision activities and increases cancer predisposition of Ung-/-Msh2-/- mice. Nucleic Acids Res. *40,* 6016-6025.

Kempermann G, Gast D, and Gage FH (2002) Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann.Neurol. *52,* 135-143.

Kessler RC, Chiu WT, Demler O, Merikangas KR, and Walters EE (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch.Gen.Psychiatry *62,* 617-627.

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, and Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J.Neurosci. *29,* 13435-13444.

Kinslow CJ, El-Zein RA, Rondelli CM, Hill CE, Wickliffe JK, and Abdel-Rahman SZ (2010) Regulatory regions responsive to oxidative stress in the promoter of the human DNA glycosylase gene NEIL2. Mutagenesis *25,* 171-177.

Klann E (1998) Cell-permeable scavengers of superoxide prevent long-term potentiation in hippocampal area CA1. J.Neurophysiol. *80,* 452-457.

Klatzo I (1987) Pathophysiological aspects of brain edema. Acta Neuropathol. *72,* 236-239.

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, and Binder EB (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat.Neurosci. *16,* 33-41.

Klose RJ and Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem.Sci. *31,* 89-97.

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

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, and 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.

Kolb B and Gibb R (2011) Brain plasticity and behaviour in the developing brain. J.Can.Acad.Child Adolesc.Psychiatry *20,* 265-276.

Komada M, Takao K, and Miyakawa T (2008) Elevated plus maze for mice. J.Vis.Exp.

Kriaucionis S and Heintz N (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science *324,* 929-930.

Krishnamurthy N, Zhao X, Burrows CJ, and David SS (2008) Superior removal of hydantoin lesions relative to other oxidized bases by the human DNA glycosylase hNEIL1. Biochemistry *47,* 7137-7146.

Kroemer G and Levine B (2008) Autophagic cell death: the story of a misnomer. Nat.Rev.Mol.Cell Biol. *9,* 1004-1010.

Krokan HE and Bjoras M (2013) Base excision repair. Cold Spring Harb Perspect Biol. *5,* a012583.

Krokeide SZ, Laerdahl JK, Salah M, Luna L, Cederkvist FH, Fleming AM, Burrows CJ, Dalhus B, and Bjoras M (2013) Human NEIL3 is mainly a monofunctional DNA glycosylase removing spiroimindiohydantoin and guanidinohydantoin. DNA Repair (Amst) *12,* 1159-1164.

Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, and Lindahl T (1996) Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. EMBO J. *15,* 6662-6670.

Kumar R, Horikoshi N, Singh M, Gupta A, Misra HS, Albuquerque K, Hunt CR, and Pandita TK (2012) Chromatin modifications and the DNA damage response to ionizing radiation. Front Oncol. *2,* 214.

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

Kuo CY, Shieh C, Cai F, and Ann DK (2011) Coordinate to guard: crosstalk of phosphorylation, sumoylation, and ubiquitylation in DNA damage response. Front Oncol. *1,* 61.

Lagouge M and Larsson NG (2013) The role of mitochondrial DNA mutations and free radicals in disease and ageing. J.Intern.Med. *273,* 529-543.

Lalancette-Hebert M, Swarup V, Beaulieu JM, Bohacek I, Abdelhamid E, Weng YC, Sato S, and Kriz J (2012) Galectin-3 is required for resident microglia activation and proliferation in response to ischemic injury. J.Neurosci. *32,* 10383-10395.

Lambertsen KL, Biber K, and Finsen B (2012) Inflammatory cytokines in experimental and human stroke. J.Cereb.Blood Flow Metab *32,* 1677-1698.

Lan J, Li W, Zhang F, Sun FY, Nagayama T, O'Horo C, and Chen J (2003) Inducible repair of oxidative DNA lesions in the rat brain after transient focal ischemia and reperfusion. J.Cereb.Blood Flow Metab *23,* 1324-1339.

Lardenoije R, Iatrou A, Kenis G, Kompotis K, Steinbusch HW, Mastroeni D, Coleman P, Lemere CA, Hof PR, van den Hove DL, and Rutten BP (2015) The epigenetics of aging and neurodegeneration. Prog.Neurobiol.

Larsen E, Gran C, Saether BE, Seeberg E, and Klungland A (2003) Proliferation failure and gamma radiation sensitivity of Fen1 null mutant mice at the blastocyst stage. Mol.Cell Biol. *23,* 5346-5353.

Larsen E, Meza TJ, Kleppa L, and Klungland A (2007) Organ and cell specificity of base excision repair mutants in mice. Mutat.Res. *614,* 56-68.

Lavine SD, Hofman FM, and Zlokovic BV (1998) Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury. J.Cereb.Blood Flow Metab *18,* 52-58.

Le-Niculescu H, Balaraman Y, Patel SD, Ayalew M, Gupta J, Kuczenski R, Shekhar A, Schork N, Geyer MA, and Niculescu AB (2011) Convergent functional genomics of anxiety disorders: translational identification of genes, biomarkers, pathways and mechanisms. Transl.Psychiatry *1,* e9.

Leibeling D, Laspe P, and Emmert S (2006) Nucleotide excision repair and cancer. J.Mol.Histol. *37,* 225-238.

Lerea LS and McNamara JO (1993) Ionotropic glutamate receptor subtypes activate c-fos transcription by distinct calcium-requiring intracellular signaling pathways. Neuron *10,* 31-41.

LEVINE S (1960) Anoxic-ischemic encephalopathy in rats. Am.J.Pathol. *36,* 1-17.

Li P, Hu X, Gan Y, Gao Y, Liang W, and Chan J (2011) Mechanistic insight into DNA damage and repair in ischemic stroke: exploiting the base excition repair pathway as a model of neuroprotection. Antioxid.Redox.Signal. *14,* 1905-1918

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature *362,* 709-715.

Liu D, Croteau DL, Souza-Pinto N, Pitta M, Tian J, Wu C, Jiang H, Mustafa K, Keijzers G, Bohr VA, and 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 M, Bandaru V, Bond JP, Jaruga P, Zhao X, Christov PP, Burrows CJ, Rizzo CJ, Dizdaroglu M, and 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 PK, Hsu CY, Dizdaroglu M, Floyd RA, Kow YW, Karakaya A, Rabow LE, and Cui JK (1996) Damage, repair, and mutagenesis in nuclear genes after mouse forebrain ischemia-reperfusion. J.Neurosci. *16,* 6795-6806.

Liu R, Liu IY, Bi X, Thompson RF, Doctrow SR, Malfroy B, and Baudry M (2003) Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. Proc.Natl.Acad.Sci.U.S.A *100,* 8526-8531.

Loddick SA and Rothwell NJ (1996) Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. J.Cereb.Blood Flow Metab *16,* 932-940.

Loddick SA, Turnbull AV, and Rothwell NJ (1998) Cerebral interleukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. J.Cereb.Blood Flow Metab *18,* 176-179.

Lord CJ and Ashworth A (2012) The DNA damage response and cancer therapy. Nature *481,* 287-294.

Love, S (1996) Oxidative stress in brain ischemia. Brain Pathol *9*, 119-131

Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, and Yankner BA (2004) Gene regulation and DNA damage in the ageing human brain. Nature. *429,* 883-891.

Luheshi NM, Kovacs KJ, Lopez-Castejon G, Brough D, and Denes A (2011) Interleukin-1alpha expression precedes IL-1beta after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. J.Neuroinflammation. *8,* 186.

Luo Y, Ji X, Ling F, Li W, Zhang F, Cao G, and Chen J (2007) Impaired DNA repair via the base-excision repair pathway after focal ischemic brain injury: a protein phosphorylation-dependent mechanism reversed by hypothermic neuroprotection. Front Biosci. *12,* 1852-1862.

Mabley JG, Pacher P, Deb A, Wallace R, Elder RH, and Szabo C (2005) Potential role for 8-oxoguanine DNA glycosylase in regulating inflammation. FASEB J. *19,* 290-292.

Macrez R, Ali C, Toutirais O, Le MB, Defer G, Dirnagl U, and Vivien D (2011) Stroke and the immune system: from pathophysiology to new therapeutic strategies. Lancet Neurol. *10,* 471-480.

Madabhushi R, Gao F, Pfenning AR, Pan L, Yamakawa S, Seo J, Rueda R, Phan TX, Yamakawa H, Pao PC, Stott RT, Gjoneska E, Nott A, Cho S, Kellis M, and Tsai LH (2015) Activity-Induced DNA Breaks Govern the Expression of Neuronal Early-Response Genes. Cell *161,* 1592-1605.

Maier W, Lichtermann D, Minges J, Oehrlein A, and Franke P (1993) A controlled family study in panic disorder. J.Psychiatr.Res. *27 Suppl 1,* 79-87.

Maltseva DV, Baykov AA, Jeltsch A, and Gromova ES (2009) Impact of 7,8-dihydro-8-oxoguanine on methylation of the CpG site by Dnmt3a. Biochemistry *48,* 1361-1368.

Mandal SM, Hegde ML, Chatterjee A, Hegde PM, Szczesny B, Banerjee D, Boldogh I, Gao R, Falkenberg M, Gustafsson CM, Sarkar PS, and Hazra TK (2012) Role of human DNA glycosylase Nei-like 2 (NEIL2) and single strand break repair protein polynucleotide kinase 3'-phosphatase in maintenance of mitochondrial genome. J.Biol.Chem. *287,* 2819-2829.

Marsin S, Vidal AE, Sossou M, Menissier-de MJ, Le PF, Boiteux S, de MG, and Radicella JP (2003) Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. J.Biol.Chem. *278,* 44068-44074.

Massaad CA and Klann E (2011) Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid.Redox.Signal. *14,* 2013-2054.

Massaad CA, Washington TM, Pautler RG, and Klann E (2009) Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. Proc.Natl.Acad.Sci.U.S.A *106,* 13576-13581.

Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD, and Rogers J (2010) Epigenetic changes in Alzheimer's disease: decrements in DNA methylation. Neurobiol.Aging *31,* 2025-2037.

Matsubara M, Tanaka T, Terato H, Ohmae E, Izumi S, Katayanagi K, and Ide H (2004) Mutational analysis of the damage-recognition and catalytic mechanism of human SMUG1 DNA glycosylase. Nucleic Acids Res. *32,* 5291-5302.

Mattson MP (2003) Methylation and acetylation in nervous system development and neurodegenerative disorders. Ageing Res.Rev. *2,* 329-342.

McGahon BM, Martin DS, Horrobin DF, and Lynch MA (1999) Age-related changes in LTP and antioxidant defenses are reversed by an alpha-lipoic acid-enriched diet. Neurobiol.Aging *20,* 655-664.

Mecocci P, MacGarvey U, Kaufman AE, Koontz D, Shoffner JM, Wallace DC, and Beal MF (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. Ann.Neurol. *34,* 609-616.

Meira LB, Moroski-Erkul CA, Green SL, Calvo JA, Bronson RT, Shah D, and Samson LD (2009) Aag-initiated base excision repair drives alkylation-induced retinal degeneration in mice. Proc.Natl.Acad.Sci.U.S.A *106,* 888-893.

Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, and Lander ES (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. *454,* 766-770.

Michaels ML, Cruz C, Grollman AP, and Miller JH (1992) Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. Proc.Natl.Acad.Sci.U.S.A *89,* 7022-7025.

Miller CA and Sweatt JD (2007) Covalent modification of DNA regulates memory formation. Neuron *53,* 857-869.

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, and 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.

Moller P, Lohr M, Folkmann JK, Mikkelsen L, and Loft S (2010) Aging and oxidatively damaged nuclear DNA in animal organs. Free Radic Biol Med. *48,* 1275-1285.

Mori H, Ouchida R, Hijikata A, Kitamura H, Ohara O, Li Y, Gao X, Yasui A, Lloyd RS, and Wang JY (2009) Deficiency of the oxidative damage-specific DNA glycosylase NEIL1 leads to reduced germinal center B cell expansion. DNA Repair (Amst) *8,* 1328-1332.

Moriya M, Ou C, Bodepudi V, Johnson F, Takeshita M, and Grollman AP (1991) Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in E. coli. Mutat.Res. *254,* 281-288.

Morland I, Rolseth V, Luna L, Rognes T, Bjoras M, and Seeberg E (2002) Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. Nucleic Acids Res. *30,* 4926-4936.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J.Neurosci.Methods *11,* 47-60.

Moser EI, Kropff E, and Moser MB (2008) Place cells, grid cells, and the brain's spatial representation system. Annu.Rev.Neurosci. *31,* 69-89.

Muller U, Bauer C, Siegl M, Rottach A, and Leonhardt H (2014) TET-mediated oxidation of methylcytosine causes TDG or NEIL glycosylase dependent gene reactivation. Nucleic Acids Res. *42,* 8592-8604.

Murray CA and Lynch MA (1998) Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J.Neurosci. *18,* 2974-2981.

Nakabeppu Y, Kajitani K, Sakamoto K, Yamaguchi H, and Tsuchimoto D (2006) MTH1, an oxidized purine nucleoside triphosphatase, prevents the cytotoxicity and neurotoxicity of oxidized purine nucleotides. DNA Repair (Amst) *5,* 761-772.

Nakabeppu Y, Oka S, Sheng Z, Tsuchimoto D, and Sakumi K (2010) Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase. Mutat.Res. *703,* 51-58.

Nakamura J, Walker VE, Upton PB, Chiang SY, Kow YW, and Swenberg JA (1998) Highly sensitive apurinic/apyrimidinic site assay can detect spontaneous and chemically induced depurination under physiological conditions. Cancer Res. *58,* 222-225.

Neumann H, Kotter MR, and Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain *132,* 288-295.

Neurauter CG, Luna L, and Bjoras M (2012) Release from quiescence stimulates the expression of human NEIL3 under the control of the Ras dependent ERK-MAP kinase pathway. DNA Repair (Amst) *11,* 401-409.

Neves G, Cooke SF, and Bliss TV (2008) Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat.Rev.Neurosci. *9,* 65-75.

Nilsen H, Stamp G, Andersen S, Hrivnak G, Krokan HE, Lindahl T, and Barnes DE (2003) Gene-targeted mice lacking the Ung uracil-DNA glycosylase develop B-cell lymphomas. Oncogene *22,* 5381-5386.

Nishimoto N, Kanakura Y, Aozasa K, Johkoh T, Nakamura M, Nakano S, Nakano N, Ikeda Y, Sasaki T, Nishioka K, Hara M, Taguchi H, Kimura Y, Kato Y, Asaoku H, Kumagai S, Kodama F, Nakahara H, Hagihara K, Yoshizaki K, and Kishimoto T (2005) Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. Blood *106,* 2627-2632.

Noren Hooten N., Kompaniez K, Barnes J, Lohani A, and Evans MK (2011) Poly(ADP-ribose) polymerase 1 (PARP-1) binds to 8-oxoguanine-DNA glycosylase (OGG1). J.Biol.Chem. *286,* 44679-44690.

Northington FJ, Chavez-Valdez R, and Martin LJ (2011) Neuronal cell death in neonatal hypoxia-ischemia. Ann.Neurol. *69,* 743-758.

Nugent NR, Tyrka AR, Carpenter LL, and Price LH (2011) Gene-environment interactions: early life stress and risk for depressive and anxiety disorders. Psychopharmacology (Berl) *214,* 175-196.

Nyberg KA, Michelson RJ, Putnam CW, and Weinert TA (2002) Toward maintaining the genome: DNA damage and replication checkpoints. Annu.Rev.Genet. *36,* 617-656.

Odell ID, Newick K, Heintz NH, Wallace SS, and Pederson DS (2010) Non-specific DNA binding interferes with the efficient excision of oxidative lesions from chromatin by the human DNA glycosylase, NEIL1. DNA Repair (Amst) *9,* 134-143.

Ohtsubo T, Nishioka K, Imaiso Y, Iwai S, Shimokawa H, Oda H, Fujiwara T, and Nakabeppu Y (2000) Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. Nucleic Acids Res. *28,* 1355-1364.

Oka S, Ohno M, Tsuchimoto D, Sakumi K, Furuichi M, and Nakabeppu Y (2008) Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. EMBO Journal *27,* 421-432.

Okereafor A, Allsop J, Counsell SJ, Fitzpatrick J, Azzopardi D, Rutherford MA, and Cowan FM (2008) Patterns of brain injury in neonates exposed to perinatal sentinel events. Pediatrics *121,* 906-914.

Oler JA, Fox AS, Shelton SE, Rogers J, Dyer TD, Davidson RJ, Shelledy W, Oakes TR, Blangero J, and Kalin NH (2010) Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. Nature *466,* 864-868.

Onizuka K, Yeo J, David SS, and Beal PA (2012) NEIL1 binding to DNA containing 2'-fluorothymidine glycol stereoisomers and the effect of editing. Chembiochem. *13,* 1338-1348.

Orr BA, Haffner MC, Nelson WG, Yegnasubramanian S, and Eberhart CG (2012) Decreased 5-hydroxymethylcytosine is associated with neural progenitor phenotype in normal brain and shorter survival in malignant glioma. PLoS.One. *7,* e41036.

Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, and Bao JK (2012) Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif. *45,* 487-498.

Parada E, Egea J, Buendia I, Negredo P, Cunha AC, Cardoso S, Soares MP, and Lopez MG (2013) The microglial alpha7-acetylcholine nicotinic receptor is a key element in promoting neuroprotection by inducing heme oxygenase-1 via nuclear factor erythroid-2-related factor 2. Antioxid.Redox.Signal. *19,* 1135-1148.

Parsons JL, Kavli B, Slupphaug G, and Dianov GL (2007) NEIL1 is the major DNA glycosylase that processes 5-hydroxyuracil in the proximity of a DNA single-strand break. Biochemistry *46,* 4158-4163.

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

Perillo B, Ombra MN, Bertoni A, Cuozzo C, Sacchetti S, Sasso A, Chiariotti L, Malorni A, Abbondanza C, and Avvedimento EV (2008) DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science. *319,* 202-206.

Perry VH, Nicoll JA, and Holmes C (2010) Microglia in neurodegenerative disease. Nat.Rev.Neurol. *6,* 193-201.

Petermann E, Ziegler M, and Oei SL (2003) ATP-dependent selection between single nucleotide and long patch base excision repair. DNA Repair (Amst) *2,* 1101-1114.

Pfeifer GP, You YH, and Besaratinia A (2005) Mutations induced by ultraviolet light. Mutat.Res. *571,* 19-31.

Polidori MC, Mecocci P, Browne SE, Senin U, and Beal MF (1999) Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. Neurosci Lett. *272,* 53-56.

Poon HF, Calabrese V, Scapagnini G, and Butterfield DA (2004) Free radicals and brain aging. Clin.Geriatr.Med. *20,* 329-359.

Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, Jacobsen SE, and Reik W (2010) Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature *463,* 1101-1105.

Prut L and Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur.J.Pharmacol. *463,* 3-33.

Razin A and Riggs AD (1980) DNA methylation and gene function. Science *210,* 604-610.

Redrejo-Rodriguez M, Saint-Pierre C, Couve S, Mazouzi A, Ishchenko AA, Gasparutto D, and Saparbaev M (2011) New insights in the removal of the hydantoins, oxidation product of pyrimidines, via the base excision and nucleotide incision repair pathways. PLoS.One. *6,* e21039.

Regnell CE, Hildrestrand GA, Sejersted Y, Medin T, Moldestad O, Rolseth V, Krokeide SZ, Suganthan R, Luna L, Bjoras M, and 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 JE, III, Vannucci RC, and Brierley JB (1981) The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann.Neurol. *9,* 131-141.

Robertson AB, Klungland A, Rognes T, and Leiros I (2009) DNA repair in mammalian cells: Base excision repair: the long and short of it. Cell Mol.Life Sci. *66,* 981-993.

Rolseth V, Runden-Pran E, Luna L, McMurray C, Bjoras M, and Ottersen OP (2008) Widespread distribution of DNA glycosylases removing oxidative DNA lesions in human and rodent brains. DNA Repair (Amst) *7,* 1578-1588.

Roth RB and Samson LD (2002) 3-Methyladenine DNA glycosylase-deficient Aag null mice display unexpected bone marrow alkylation resistance. Cancer Res. *62,* 656-660.

Ruggieri V, Pin E, Russo MT, Barone F, Degan P, Sanchez M, Quaia M, Minoprio A, Turco E, Mazzei F, Viel A, and Bignami M (2013) Loss of MUTYH function in human cells leads to accumulation of oxidative damage and genetic instability. Oncogene *32,* 4500-4508.

Russo MT, De LG, Degan P, and Bignami M (2007) Different DNA repair strategies to combat the threat from 8-oxoguanine. Mutat.Res. *614,* 69-76.

Sabatini BL, Maravall M, and Svoboda K (2001) Ca(2+) signaling in dendritic spines. Curr.Opin.Neurobiol. *11,* 349-356.

Saccani S and Natoli G (2002) Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. Genes Dev. *16,* 2219-2224.

Sakamoto K, Tominaga Y, Yamauchi K, Nakatsu Y, Sakumi K, Yoshiyama K, Egashira A, Kura S, Yao T, Tsuneyoshi M, Maki H, Nakabeppu Y, and Tsuzuki T (2007) MUTYH-null mice are susceptible to spontaneous and oxidative stress induced intestinal tumorigenesis. Cancer Res. *67,* 6599-6604.

Sampath H, Batra AK, Vartanian V, Carmical JR, Prusak D, King IB, Lowell B, Earley LF, Wood TG, Marks DL, McCullough AK, and Stephen R (2011) Variable penetrance of metabolic phenotypes and development of high-fat diet-induced adiposity in NEIL1-deficient mice. Am.J.Physiol Endocrinol.Metab *300,* E724-E734.

Sampath H, Vartanian V, Rollins MR, Sakumi K, Nakabeppu Y, and Lloyd RS (2012) 8-Oxoguanine DNA glycosylase (OGG1) deficiency increases susceptibility to obesity and metabolic dysfunction. PLoS.One. *7,* e51697.

Sander M, Bergersen LH, and Storm-Mathisen J (2009) Molecular approaches to understanding neural network plasticity and memory: the Kavli Prize Inaugural Symposium on Neuroscience. Neuroscience *163,* 965-976.

Sassa A, Caglayan M, Dyrkheeva NS, Beard WA, and Wilson SH (2014) Base excision repair of tandem modifications in a methylated CpG dinucleotide. J.Biol.Chem. *289,* 13996-14008.

Schomacher L, Han D, Musheev MU, Arab K, Kienhofer S, von SA, and Niehrs C (2016) Neil DNA glycosylases promote substrate turnover by Tdg during DNA demethylation. Nat.Struct.Mol.Biol. *23,* 116-124.

Seeberg E, Eide L, and Bjoras M (1995) The base excision repair pathway. Trends Biochem.Sci. *20,* 391-397.

Sejersted Y (2012) Neil3 DNA Glycosylase in Mainenance and Repair of the Mammalian Brain. Series of dissertations submitted to the Faculty of Medicine, University of Oslo *1430,*

Sejersted Y, Hildrestrand GA, Kunke D, Rolseth V, Krokeide SZ, Neurauter CG, Suganthan R, Atneosen-Asegg M, Fleming AM, Saugstad OD, Burrows CJ, Luna L, and Bjoras M (2011) Endonuclease VIII-like 3 (Neil3) DNA glycosylase promotes neurogenesis induced by hypoxia-ischemia. Proc.Natl.Acad.Sci.U.S.A *108,* 18802-18807.

Semple BD, Blomgren K, Gimlin K, Ferriero DM, and Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog.Neurobiol. *106-107,* 1-16.

Serada S, Fujimoto M, Mihara M, Koike N, Ohsugi Y, Nomura S, Yoshida H, Nishikawa T, Terabe F, Ohkawara T, Takahashi T, Ripley B, Kimura A, Kishimoto T, and Naka T (2008) IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. Proc.Natl.Acad.Sci.U.S.A *105,* 9041-9046.

Sharma S, Rakoczy S, and Brown-Borg H (2010) Assessment of spatial memory in mice. Life Sci. *87,* 521-536.

Sheldon RA, Sedik C, and Ferriero DM (1998) Strain-related brain injury in neonatal mice subjected to hypoxia-ischemia. Brain Res. *810,* 114-122.

Sheng Z, Oka S, Tsuchimoto D, Abolhassani N, Nomaru H, Sakumi K, Yamada H, and Nakabeppu Y (2012) 8-Oxoguanine causes neurodegeneration during MUTYH-mediated DNA base excision repair. J.Clin.Invest *122,* 4344-4361.

Shimura-Miura H, Hattori N, Kang D, Miyako K, Nakabeppu Y, and Mizuno Y (1999) Increased 8-oxo-dGTPase in the mitochondria of substantia nigral neurons in Parkinson's disease. Ann Neurol. *46,* 920-924.

Shin JA, Park EM, Choi JS, Seo SM, Kang JL, Lee KE, and Cho S (2009) Ischemic preconditioning-induced neuroprotection is associated with differential expression of IL-1beta and IL-1 receptor antagonist in the ischemic cortex. J.Neuroimmunol. *217,* 14-19.

Sims RJ, III, Nishioka K, and Reinberg D (2003) Histone lysine methylation: a signature for chromatin function. Trends Genet. *19,* 629-639.

Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis AK, Van HB, Shuman S, McKinnon PJ, and Jasin M (2011) Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. Nature *471,* 245-248.

Smaga I, Niedzielska E, Gawlik M, Moniczewski A, Krzek J, Przegalinski E, Pera J, and Filip M (2015) Oxidative stress as an etiological factor and a potential treatment target of psychiatric disorders. Part 2. Depression, anxiety, schizophrenia and autism. Pharmacol.Rep. *67,* 569-580.

Smith CJ, Emsley HC, Gavin CM, Georgiou RF, Vail A, Barberan EM, del Zoppo GJ, Hallenbeck JM, Rothwell NJ, Hopkins SJ, and Tyrrell PJ (2004) Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. BMC.Neurol. *4,* 2.

Song CX, Szulwach KE, Fu Y, Dai Q, Yi C, Li X, Li Y, Chen CH, Zhang W, Jian X, Wang J, Zhang L, Looney TJ, Zhang B, Godley LA, Hicks LM, Lahn BT, Jin P, and He C (2011) Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat.Biotechnol. *29,* 68-72.

Spruijt CG, Gnerlich F, Smits AH, Pfaffeneder T, Jansen PW, Bauer C, Munzel M, Wagner M, Muller M, Khan F, Eberl HC, Mensinga A, Brinkman AB, Lephikov K, Muller U, Walter J, Boelens R, van I, Leonhardt H, Carell T, and Vermeulen M (2013) Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. Cell. *152,* 1146-1159.

Squire LR, Stark CE, and Clark RE (2004) The medial temporal lobe. Annual Review of Neuroscience *27,* 279-306.

Stone SS, Teixeira CM, Zaslavsky K, Wheeler AL, Martinez-Canabal A, Wang AH, Sakaguchi M, Lozano AM, and Frankland PW (2011) Functional convergence of developmentally and adult-generated granule cells in dentate gyrus circuits supporting hippocampus-dependent memory. Hippocampus *21,* 1348-1362.

Stracker TH and Petrini JH (2011) The MRE11 complex: starting from the ends. Nat.Rev.Mol.Cell Biol. *12,* 90-103.

Sun C, Chen H, Guo W, Zhang K, Qi Q, Gu X, Zhu D, and Wang Y (2010) A common mutation of the MYH gene is associated with increased DNA oxidation and age-related diseases. Free Radic.Biol.Med. *48,* 430-436.

Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, and Rao A (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science *324,* 930-935.

Takao M, Kanno S, Shiromoto T, Hasegawa R, Ide H, Ikeda S, Sarker AH, Seki S, Xing JZ, Le XC, Weinfeld M, Kobayashi K, Miyazaki J, Muijtjens M, Hoeijmakers JH, van der Horst G, and Yasui A (2002) Novel nuclear and mitochondrial glycosylases revealed by disruption of the mouse Nth1 gene encoding an endonuclease III homolog for repair of thymine glycols. EMBO J. *21,* 3486-3493.

Takao M, Zhang QM, Yonei S, and Yasui A (1999) Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. Nucleic Acids Research *27,* 3638-3644.

Taoufik E, Valable S, Muller GJ, Roberts ML, Divoux D, Tinel A, Voulgari-Kokota A, Tseveleki V, Altruda F, Lassmann H, Petit E, and Probert L (2007) FLIP(L) protects neurons against in vivo ischemia and in vitro glucose deprivation-induced cell death. J.Neurosci. *27,* 6633-6646.

Taylor RA and Sansing LH (2013) Microglial responses after ischemic stroke and intracerebral hemorrhage. Clin.Dev.Immunol. *2013,* 746068.

Taylor RC, Cullen SP, and Martin SJ (2008) Apoptosis: controlled demolition at the cellular level. Nat.Rev.Mol.Cell Biol. *9,* 231-241.

Thomas WE (1992) Brain macrophages: evaluation of microglia and their functions. Brain Res.Brain Res.Rev. *17,* 61-74.

Thompson LH and West MG (2000) XRCC1 keeps DNA from getting stranded. Mutat.Res. *459,* 1-18.

Torisu K, Tsuchimoto D, Ohnishi Y, and Nakabeppu Y (2005) Hematopoietic tissue-specific expression of mouse Neil3 for endonuclease VIII-like protein. J.Biochem. *138,* 763-772.

Ueda H and Fujita R (2004) Cell death mode switch from necrosis to apoptosis in brain. Biol.Pharm.Bull. *27,* 950-955.

Valinluck V, Tsai HH, Rogstad DK, Burdzy A, Bird A, and Sowers LC (2004) Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). Nucleic Acids Res. *32,* 4100-4108.

van Loon B, Markkanen E, and Hubscher U (2010) Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine. DNA Repair (Amst) *9,* 604-616.

Vannucci RC and Vannucci SJ (2005) Perinatal hypoxic-ischemic brain damage: evolution of an animal model. Dev.Neurosci. *27,* 81-86.

Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F, Cross MK, Williams BA, Stamatoyannopoulos JA, Crawford GE, Absher DM, Wold BJ, and Myers RM (2013) Dynamic DNA methylation across diverse human cell lines and tissues. Genome Res. *23,* 555-567.

Vartanian V, Lowell B, Minko IG, Wood TG, Ceci JD, George S, Ballinger SW, Corless CL, McCullough AK, and Lloyd RS (2006) The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase. Proc.Natl.Acad.Sci.U.S.A *103,* 1864-1869.

Vasko MR, Guo C, and Kelley MR (2005) The multifunctional DNA repair/redox enzyme Ape1/Ref-1 promotes survival of neurons after oxidative stress. DNA Repair (Amst) *4,* 367-379.

Vicencio JM, Galluzzi L, Tajeddine N, Ortiz C, Criollo A, Tasdemir E, Morselli E, Ben YA, Maiuri MC, Lavandero S, and Kroemer G (2008) Senescence, apoptosis or autophagy? When a damaged cell must decide its path--a mini-review. Gerontology *54,* 92-99.

Vorhees CV and Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. *1,* 848-858.

Vorhees CV and Williams MT (2014) Assessing spatial learning and memory in rodents. ILAR.J. *55,* 310-332.

Waje-Andreassen U, Krakenes J, Ulvestad E, Thomassen L, Myhr KM, Aarseth J, and Vedeler CA (2005) IL-6: an early marker for outcome in acute ischemic stroke. Acta Neurol.Scand. *111,* 360-365.

Wang X, Li X, Currie RW, Willette RN, Barone FC, and Feuerstein GZ (2000) Application of real-time polymerase chain reaction to quantitate induced expression of interleukin-1beta mRNA in ischemic brain tolerance. J.Neurosci.Res. *59,* 238-246.

Warmerdam DO and Kanaar R (2010) Dealing with DNA damage: relationships between checkpoint and repair pathways. Mutat.Res. *704,* 2-11.

Weitzman SA, Turk PW, Milkowski DH, and Kozlowski K (1994) Free radical adducts induce alterations in DNA cytosine methylation. Proc.Natl.Acad.Sci.U.S.A *91,* 1261-1264.

West AE and Greenberg ME (2011) Neuronal activity-regulated gene transcription in synapse development and cognitive function. Cold Spring Harb.Perspect.Biol. *3,*

West AE, Griffith EC, and Greenberg ME (2002) Regulation of transcription factors by neuronal activity. Nat.Rev.Neurosci. *3,* 921-931.

Wilson CB, McLaughlin LD, Nair A, Ebenezer PJ, Dange R, and Francis J (2013) Inflammation and oxidative stress are elevated in the brain, blood, and adrenal glands during the progression of post-traumatic stress disorder in a predator exposure animal model. PLoS.One. *8,* e76146.

Wojtowicz JM, Askew ML, and Winocur G (2008) The effects of running and of inhibiting adult neurogenesis on learning and memory in rats. European Journal of Neuroscience *27,* 1494-1502.

Xie W, Barr CL, Kim A, Yue F, Lee AY, Eubanks J, Dempster EL, and Ren B (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. Cell *148,* 816-831.

Xie Y, Yang H, Cunanan C, Okamoto K, Shibata D, Pan J, Barnes DE, Lindahl T, McIlhatton M, Fishel R, and 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.

Xu X and Jiang Y (2014) The Yin and Yang of innate immunity in stroke. Biomed.Res.Int. *2014,* 807978.

Xue JH, Xu GF, Gu TP, Chen GD, Han BB, Xu ZM, Bjoras M, Krokan HE, Xu GL, and Du YR (2016) Uracil-DNA Glycosylase UNG Promotes Tet-mediated DNA Demethylation. J.Biol.Chem. *291,* 731-738.

Yang F, Wang Z, Wei X, Han H, Meng X, Zhang Y, Shi W, Li F, Xin T, Pang Q, and Yi F (2014a) NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke. J.Cereb.Blood Flow Metab *34,* 660-667.

Yang GY, Gong C, Qin Z, Ye W, Mao Y, and Bertz AL (1998) Inhibition of TNFalpha attenuates infarct volume and ICAM-1 expression in ischemic mouse brain. Neuroreport *9,* 2131-2134.

Yang Z, Zhao T, Zou Y, Zhang JH, and Feng H (2014b) Curcumin inhibits microglia inflammation and confers neuroprotection in intracerebral hemorrhage. Immunol.Lett. *160,* 89-95.

Yenari MA, Kauppinen TM, and Swanson RA (2010) Microglial activation in stroke: therapeutic targets. Neurotherapeutics. *7,* 378-391.

Zarakowska E, Gackowski D, Foksinski M, and Olinski R (2014) Are 8-oxoguanine (8-oxoGua) and 5-hydroxymethyluracil (5-hmUra) oxidatively damaged DNA bases or transcription (epigenetic) marks? Mutat Res Genet Toxicol Environ Mutagen. *764-765,* 58-63.

Zhao X, Krishnamurthy N, Burrows CJ, and David SS (2010) Mutation versus repair: NEIL1 removal of hydantoin lesions in single-stranded, bulge, bubble, and duplex DNA contexts. Biochemistry *49,* 1658-1666.

Zhou X, Zhuang Z, Wang W, He L, Wu H, Cao Y, Pan F, Zhao J, Hu Z, Sekhar C, and Guo Z (2016) OGG1 is essential in oxidative stress induced DNA demethylation. Cell Signal. *28,* 1163-1171.

Zhu C, Wang X, Xu F, Bahr BA, Shibata M, Uchiyama Y, Hagberg H, and Blomgren K (2005) The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia. Cell Death.Differ. *12,* 162-176.

Zhu C, Wang X, Xu F, Qiu L, Cheng X, Simbruner G, and Blomgren K (2006) Intraischemic mild hypothermia prevents neuronal cell death and tissue loss after neonatal cerebral hypoxia-ischemia. European Journal of Neuroscience *23,* 387-393.