International Society for Neurochemistry
Committee for Aid and Education in Neurochemistry
(ISN-CAEN)
Final Report Award Category 1A (August 2017 Round)

Project title: Hormonal effects on mitochondrial DNA repair processes in the brain

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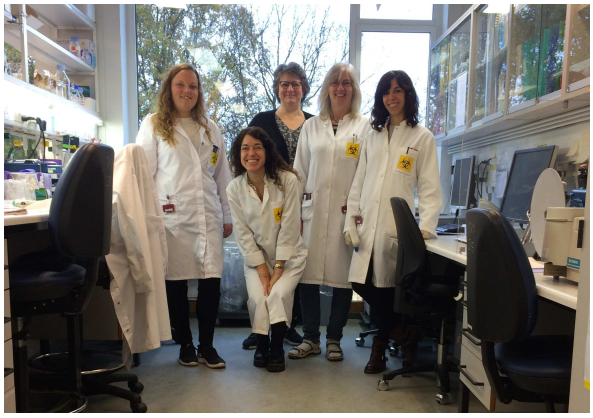
Principal investigator at host laboratory: Tinna V. Stevnsner, PhD.

Overview and Outcomes

In August 2017 I was awarded an ISN-CAEN Category 1A grant to visit the laboratory of Dr. Tinna V. Stevnsner at the University of Aarhus, in Denmark for two months. This stay was a follow-up of a previous collaborative work which started last year, when we obtained preliminary results on the effects of ovarian hormone deprivation in mitochondrial DNA repair mechanisms in the brain. This visit let us continue with this study by assessing the effects of hormonal treatments on these processes and strengthened our collaborative relationship. This visit to Dr. Stevnsner's lab was a wonderful opportunity not only to get hands-on experience in techniques that I do not have access to in my home lab but also to share ideas and experience in the field of DNA repair mechanisms and the aging process. During my stay, I actively participated in weekly journal clubs and lab meetings, where all lab members in turn exposed current projects and discussed present and future experiments. The results obtained during this stay were part of an oral presentation I gave in the Symposium *"Genomic instability & DNA repair: lessons from cancer, neurodegeneration and aging"* at the Joint Meeting of Bioscience Societies, an international meeting held in Buenos Aires last November. Also, during my stay we were able to write a scientific review, which has just been accepted for publication (Zárate S, Stevnsner T and Gredilla R (2017) Role of Estrogen and Other Sex Hormones in Brain Aging. Neuroprotection and DNA Repair. Front. Aging Neurosci. 9:430. doi:10.3389/fnagi.2017.00430). All in all, this visit supported by the ISN-CAEN grant greatly contributed to my professional and personal growth. We are currently analyzing data and performing additional experiments with the aim to write an article in the near future.



At Dr. Stevnsner's lab: from left to right; Camilla Holst, Sandra Zárate, Tinna Stevnsner, Ulla Henriksen and Inés Sánchez-Román Rojas.

A walk on the beach: from left to right; Tinna Stevnsner, Helene Mortensen, Camilla Holst, Sandra Zárate, Beatriz Ferrando-Fores, Sofie Lautrup and Ulla Henriksen.

At Journal Club: all members of the TVS group.

The Department of Molecular Biology and Genetics at Aarhus University.

Project background

Aging is an inevitable biological process characterized by a progressive decline in physiological function and increased susceptibility to disease. Normal aging and several aged-related diseases are associated to mitochondrial dysfunction[1-3]. This organelle is central in ATP generation and it is also the primary site of cellular reactive oxygen species (ROS) production. As an organ with a high demand of energy and low antioxidant capacity, the brain is particular susceptible to mitochondria dysfunction and oxidative stress[1]. These two interdependent mechanisms play a central role in brain aging[4]. ROS can cause oxidative damage to different macromolecules, among which mitochondrial DNA (mtDNA) is particularly vulnerable in part due to its close proximity to the source of ROS production. Accumulative damage in mtDNA over time, if not properly repaired, leads to mitochondrial dysfunction and disease. Studies on human tissues and animal models have demonstrated the importance of mtDNA integrity for normal cell function[5,6] and ample evidence suggests that accumulation of mutated mtDNA correlates with normal aging and neurodegenerative diseases[7-9]. Therefore, mtDNA repair mechanisms appear as potential targets to counteract age-related diseases and promote a healthy lifespan. Base excision repair (BER) is the main mtDNA repair pathway to remove oxidative lesions and constitutes an important mechanism to avoid accumulation of mtDNA mutations[1,8,10]. This repair system includes several enzymatic steps: (i) the recognition and excision of the damaged base by a DNA glycosylase; (ii) the incision of the DNA backbone in the abasic site by AP-endonuclease 1; (iii) the 5'-dRP excision and gap filling by a DNA polymerase and (iv) the sealing of the remaining DNA nick by a DNA ligase[10]. Exposure and defense against oxidative stress varies among brain regions, among which the hippocampus emerges as a highly vulnerable area[11].

Ovarian hormones, particularly estrogens, possess potent antioxidant properties and play important roles in maintaining normal reproductive and non-reproductive functions[12]. They exert neuroprotective actions in part by positively affecting mitochondrial function and their loss during reproductive senescence is associated with mitochondrial dysfunction, synaptic decline, cognitive impairment and increased risk of age-related disorders[12-14]. Estrogen anti-aging and neuroprotective mechanisms are currently an area of intense study. However, data is still limited on the relation between ovarian hormones and mtDNA repair mechanisms. We have recently reported that chronic ovarian hormone deprivation induces structural and functional changes in hippocampal mitochondria comparable to an aging phenotype[15]; and preliminary results obtained from collaborative work with the host laboratory show regional specific regulation of mitochondrial BER pathway regarding hormonal status and suggest differential capabilities of brain regions to respond to mtDNA damage[16].

Hypothesis and general aim

Lack of ovarian hormones may alter mtDNA repair mechanisms in brain areas highly vulnerable to aging and hormone actions. These repair pathways may be differentially altered in different brain areas, and early hormone replacement treatments may exert a protective effect on mtDNA repair machinery before the brain becomes unresponsive to ovarian hormone actions.

We aim to study the effect of long-term ovarian hormone deprivation as well as estrogen and progestin replacement treatments upon mitochondrial BER mechanisms in mitochondria from rat cortex and hippocampus, areas primarily affected in age-related disorders and highly responsive to ovarian hormones.

Results

Hippocampal and cortical mitochondria from the different experimental groups (ovariectomized (OVX), cycling (SHAM), estradiol-treated (E2), progesterone-treated (P4) or E2 and P4-treated (E2+P4) rats) were isolated by differential centrifugation according to[18]. As a control for proper mitochondria isolation without nuclear contamination, western blotting for mitochondrial outer membrane protein Voltage Dependent Anion Channel 1 (VDAC1) and nuclear protein Ku-86 were performed (Fig 1).

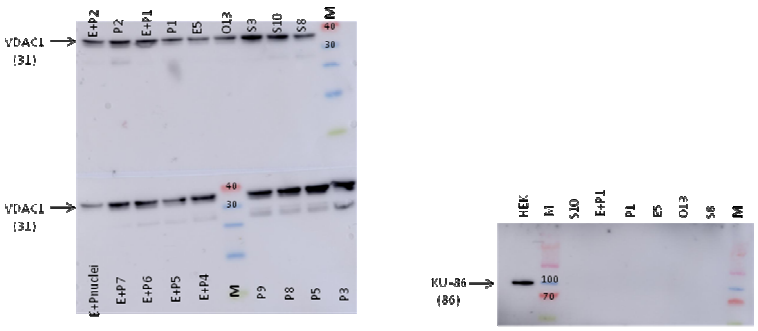


Fig. 1. Representative western blotting for mitochondrial protein VDAC1 and nuclear protein Ku-86 in isolated mitochondria samples from rat hippocampus. HEK cell homogenate was included as a positive control for Ku-86. O: ovariectomized rat sample; E: estrogen-treated rat sample; P: progesterone-treated rat sample; E+P: estrogen plus progesterone-treated rat sample; S: sham-operated (cycling) rat sample; M: MW protein marker. The same procedure was performed in mitochondrial extracts from cortex (not shown).

The activity of specific enzymes catalyzing single steps in the mitochondrial BER pathway was studied by incision assays using specific ^{32}P-labelled oligonucleotides as described in[18]. Briefly, enzyme activities were determined by quantification of the damage-specific cleavage product normalized to VDAC1 protein content in the corresponding fraction.

Considering that mtDNA is particularly susceptible to oxidative damage, we first evaluated the effect of long-term ovarian hormone deprivation in the activity of DNA glycosylases that specifically recognize and excise oxidized bases: 8OHG DNA glycosylase (OGG1, responsible for removal of oxidized deoxyguanosine) and Nei-like homolog 2 (NEIL2, removing oxidative pyrimidine lesions). Our data show that the activities of both enzymes were lower in hippocampal mitochondria from OVX rats. On the contrary, both OGG1 and NEIL2 activities were higher in cortical mitochondria from OVX rats (Fig. 2).

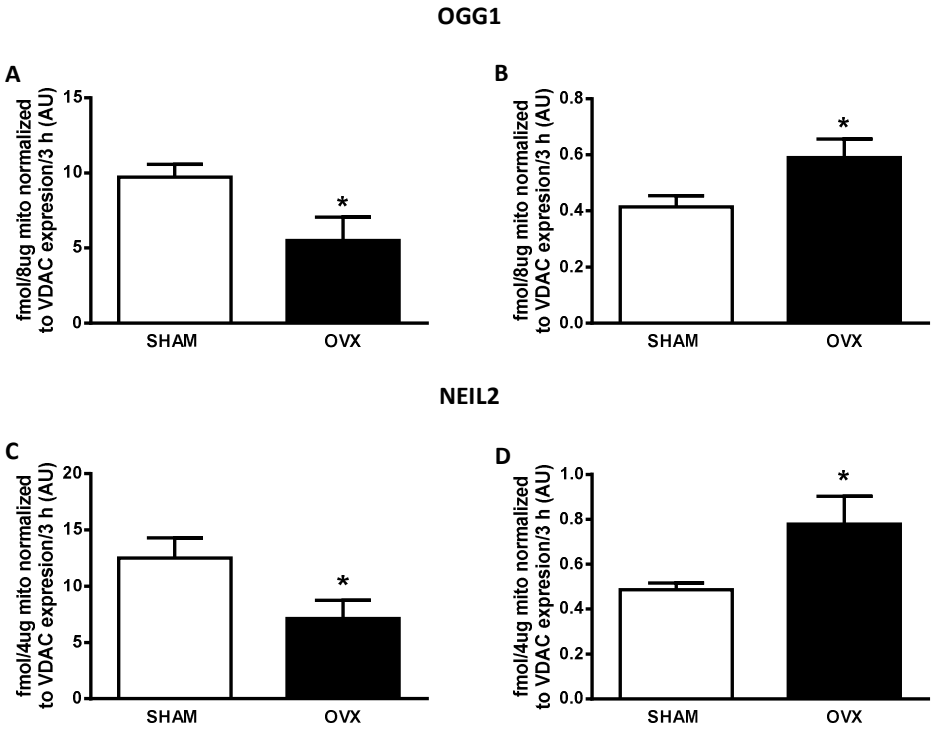


Fig. 2. OGG1 and NEIL2 glycosylase activity in (A, C) hippocampal and (B, D) cortical mitochondria from SHAM and OVX rats. Each column represents the mean ± SEM of OGG1 or NEIL2 enzymatic activity (AU) in (A, C) hippocampal or (B, D) cortical mitochondria normalized to VDAC1 expression. (n= 5-6 animals/group). *p<0.05, Student's t test.

The lower capability of the hippocampus to respond to damage in the mtDNA (at the level of glycosylases) seems to be specific for oxidative lesions, since chronic ovariectomy increased the activity of uracil DNA glycosylase (UNG1, responsible for the recognition and removal of deoxy-uracil from DNA) in mitochondria from both brain regions (Fig. 3).

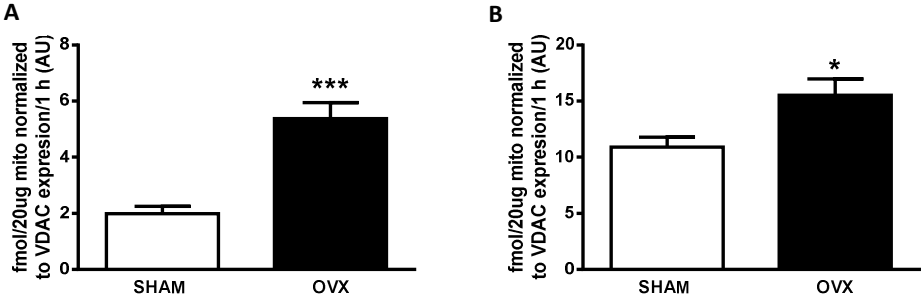


Fig. 3. UNG1 glycosylase activity in (A) hippocampal and (B) cortical mitochondria from SHAM and OVX rats. Each column represents the mean ± SEM of UNG1 enzymatic activity (AU) in (A) hippocampal or (B) cortical mitochondria normalized to VDAC1 expression. (n= 4-6 animals/group). *p<0.05, Student's t test.

Also, we evaluated the next step in the pathway, i. e. the processing of the abasic site by mitochondrial apurinic/apyrimidinic endonuclease 1 (mtAPE1). Similar to what was observed for OGG1 and NEIL2, chronic ovariectomy decreased the activity of mtAPE1 in the hippocampus whereas it increased its activity in the cortex (Fig. 4).

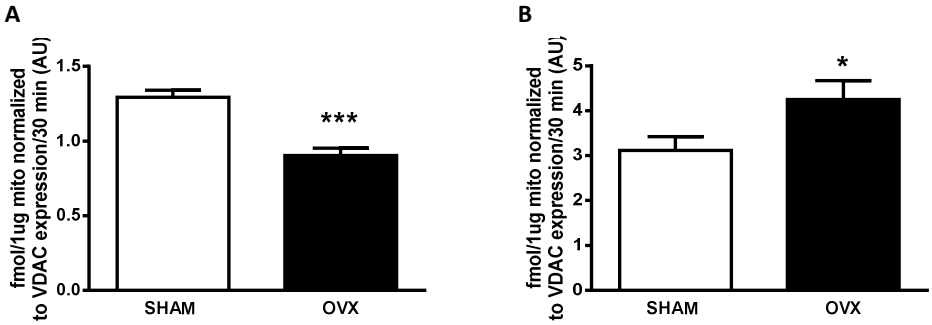


Fig. 4. mAPE1 endonuclease activity in (A) hippocampal and (B) cortical mitochondria from SHAM and OVX rats. Each column represents the mean ± SEM of mAPE1 enzymatic activity (AU) in (A) hippocampal or (B) cortical mitochondria normalized to VDAC1 expression. (n= 4-6 animals/group). *p<0.05, Student's t test.

We then studied the effect of hormone-replacement treatments in the activity of the same enzymes of the BER pathway. We observed that, in hippocampal mitochondria, estrogen increased the activity of glycosylases that remove oxidative lesions, reaching levels similar to the SHAM group, while progesterone either decreased this activity (for OGG1) or did not have an effect (for NEIL2). On the other hand, in cortical mitochondria, estrogen either decreased the enzymatic activity, reaching levels similar to the SHAM group (for OGG1),

or had the same effect as ovariectomy (for NEIL2). Progesterone either alone or when combined with estrogen, decreased the activity of both glycosylases in this brain region(Fig. 5).

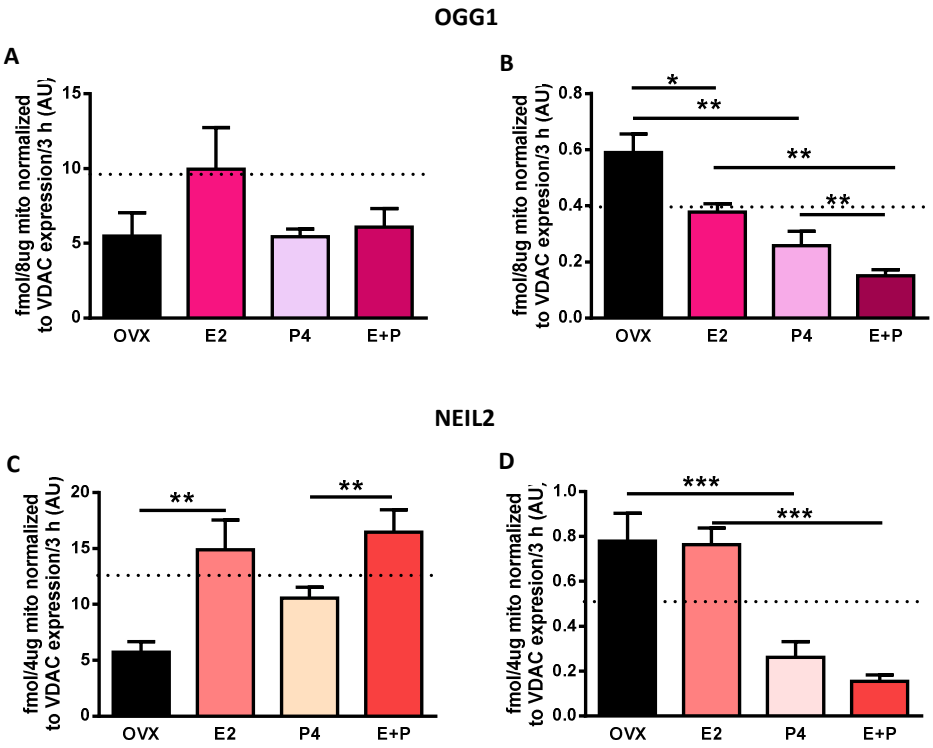


Fig. 5. OGG1 and NEIL2 glycosylase activity in (A, C) hippocampal and (B, D) cortical mitochondria from rats under hormone-replacement treatment. Each column represents the mean ± SEM of OGG1 or NEIL2 enzymatic activity (AU) in (A, C) hippocampal or (B, D) cortical mitochondria normalized to VDAC1 expression. The dotted line represents the activity of each enzyme in the SHAM group (n= 4-7 animals/group). *p<0.05, **p<0.01, ***p<0.001, ANOVA.

When evaluating the activity of UNG1, we observed a similar pattern for mitochondria from the hippocampus and cortex, estrogen having the same effect as ovariectomy while progesterone either alone or when combined with estrogen, decreasing its activity (Fig. 6).

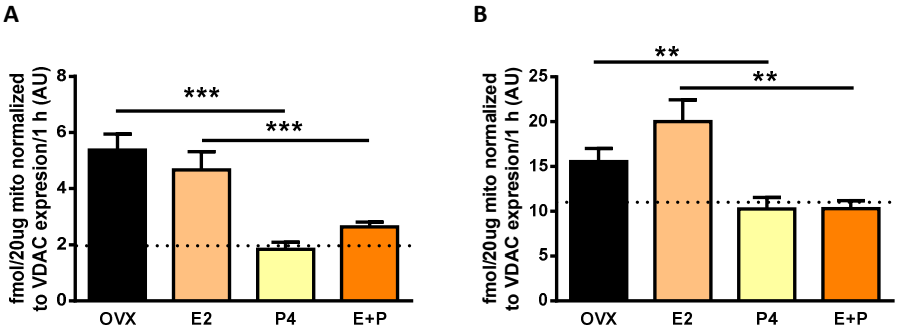


Fig. 6. UNG1 glycosylase activity in (A) hippocampal and (B) cortical mitochondria from rats under hormone-replacement treatment. Each column represents the mean ± SEM of UNG1 enzymatic activity (AU) in (A) hippocampal or (B) cortical mitochondria normalized to VDAC1 expression. The dotted line represents the activity of each enzyme in the SHAM group (n= 4-6 animals/group). **p<0.01, ***p<0.01, ANOVA.

Regarding mAPE1 activity, we observed that estrogen had the same effect as ovariectomy while progesterone alone or when combined with estrogen either increased or decreased its activity in the hippocampus and the cortex, respectively, reaching levels similar to the SHAM group in both cases (Fig. 7).

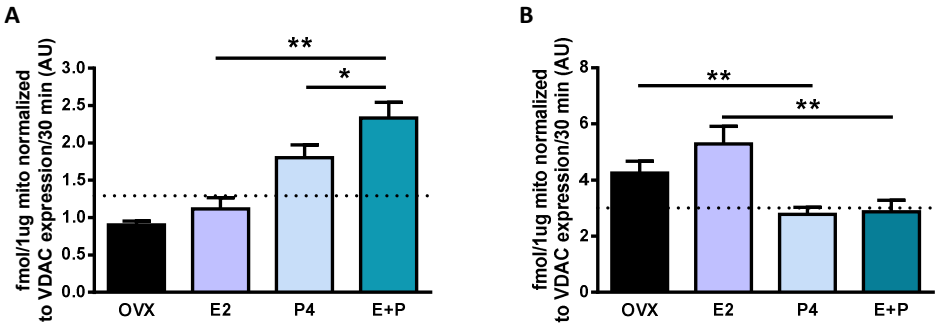


Fig. 7. mAPE1 endonuclease activity in (A) hippocampal and (B) cortical mitochondria from rats under hormone-replacement treatment. Each column represents the mean ± SEM of mAPE1 enzymatic activity (AU) in (A) hippocampal or (B) cortical mitochondria normalized to VDAC1 expression. The dotted line represents the activity of each enzyme in the SHAM group (n= 4-6 animals/group). *p<0.05, **p<0.01, ANOVA.

Our results show regional specific regulation of mitochondrial BER pathway regarding hormonal status and suggest differential capabilities of brain regions to respond to mtDNA damage. In the hippocampus, the decrease in the activities of NEIL1 and OGG1 and mAPE1 induced by ovariectomy suggests that this brain area has lower capability of removing oxidative lesions after ovarian hormone loss, which may contribute to higher mtDNA instability and higher vulnerability to functional decline after natural or surgical

menopause. On the other hand, the increase in the activity of these glycosylases induced by ovariectomy in the cortex suggests a compensatory mechanism to avoid the accumulation of oxidative lesions in this brain area.
Hormone-replacement treatments have a differential effect on BER pathway regarding enzyme and brain region. Further studies are underway to elucidate the mechanisms involved in the regulation of the BER pathway by ovarian hormones.
Considering the importance of preserving mtDNA integrity for normal cell function, this study provides insights into the role of early hormone replacement therapy in mtDNA repair capacity and could help find new therapeutic targets to promote a healthier lifespan for women after natural or surgical menopause.

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