New application for an old drug: using nimodipine (Nimotop™, an FDA-approved vasodilator) to maintain brain cell functions and cognitive performance after menopause

Wendy W. Wu, Ph.D., Oregon Health & Science University, Division MFM.

Summary. Estrogen is an ovarian hormone that affects cognitive performance in women and female animals. Estrogen does so by directly acting on brain regions important for learning and memory, such as the hippocampus. During the reproductive years, learning and memory functions mediated by the hippocampus fluctuate with the circulating level of estrogen in women and female animals alike. Following menopause, a period marked by a permanent drop in the circulating estrogen level, many women report experiencing impaired memory and attentional processes. Similarly in animals, sustained loss of estrogen leads to learning and memory impairment. These converging results from human and animal studies show that estrogen not only regulates, but also maintains normal cognitive functions.

Currently cognitive symptoms resulting from menopause are addressed with estrogen-based hormone replacement therapy (HRT), which although effective, is non-specific and has the side effects of affecting multiple systems at once. HRT is contraindicated in the population of women who are at risk for cancer. Furthermore, exactly how long HRT can safely be administered to peri- and postmenopausal women has not been determined. For these reasons, developing an alternative treatment that does not require estrogen to maintain cognitive performance following menopause is an important and pressing area of research that will benefit all women. A prerequisite for developing such a treatment is an understanding of the cellular changes induced by sustained estrogen loss that result in cognitive deficits.

Since my appointment by the Dept. of OB/GYN, my research has focused on determining changes in brain cells induced by uncompensated loss of estrogen that lead to cognitive deficits. Because female rats respond to estrogen very similarly as humans do, we use adult female rats with ovaries surgically removed as an animal model of menopause. This rat model is a good starting point for our research, because it allows us to easily and precisely manipulate the level of circulating estrogen and unambiguously determine the consequences of estrogen loss on learning and memory processes at both the cellular and behavioral levels. We found that after just a 5-month period of sustained estrogen loss, adult female rats become significantly impaired at learning hippocampus-dependent memory tasks (see “Preliminary Studies” #1 in “Research Appendix”). This learning impairment is associated with a marked reduction in the activity of cells in the hippocampus (“Preliminary Studies” #2) - a change that has also been observed in hippocampal cells from rodent models of Alzheimer's disease and very advanced stage of brain aging. We further found that the cause for this reduced activity is an enhanced function of proteins called Ca\textsubscript{v}1 Ca\textsuperscript{2+} channels in hippocampal cells from estrogen-deficient rats. Under the normal circumstance, Ca\textsubscript{v}1 channels function as a part of an inhibitory negative feedback system that prevents brain cells from generating epileptic activity. However, the dramatically enhanced function of Ca\textsubscript{v}1 channels induced by sustained estrogen loss places too much inhibitory constraints on these cells and drives their activity to levels far below the normal operating range. Altogether, our research strongly suggests that this cellular change - the reduced activity in hippocampal cells caused by aberrant protein function of Ca\textsubscript{v}1 channels - is responsible for hippocampus-dependent learning and memory impairment exhibited by our rat model of menopause (See “Summary Diagram” on pg. 2). It is well-established that the effects of estrogen and estrogen loss on brain functions are consistent in humans and animals including rats. Thus, our findings are very likely to be applicable to women.

The ability of brain to perform complex cognitive functions ultimately relies on the normal activity of its constituent cells. Thus, we hypothesize that restoring the activity of hippocampal cells from estrogen-deficient rats to the normal level will rescue hippocampal function and reverse learning and memory deficits exhibited by these rats. The function of Ca\textsubscript{v}1 channels can be selectively suppressed with a drug called nimodipine. In a set of recent in vitro experiments, we found that treating hippocampal cells from estrogen-deficient rats with nimodipine fully restored their activity to the level seen in control rats. For this application, we propose to evaluate the therapeutic potential of in vivo nimodipine treatment on rescuing hippocampus-dependent learning and memory functions in estrogen-deficient rats. Important for this application, nimodipine (marked as Nimotop™) is already

[1]
FDA-approved for blood vessel dilation and is clinically used to treat conditions unrelated to menopause. By testing the possibility of extending the application of nimodipine to manage menopause-related cognitive symptoms, the outcomes of this proposal may have a direct and immediate translational impact for women’s mental health.

**Background & Significance.** Normal brain aging is associated with a gradual and progressive decline in selective cognitive functions, such as that mediated by the hippocampus. Specifically, the hippocampus is required for explicit memory, which allows us to consciously recall past experiences and learned information. We use explicit memory in our daily lives, for example, to remember our appointment schedules and conversations with people. This basic hippocampal function is well-conserved across species, including humans, non-human primates, and rodents such as rats. Additionally, humans and animals exhibit parallel decline in hippocampal function during normal brain aging. Thus, animals including rats are valid models for studying age-related changes in cognitive functions mediated by the hippocampus.

Estrogen, an ovarian steroid produced far more abundantly in women than in men, acts directly on the hippocampus to influence learning and memory functions performed by this brain region. In fact, in women, female non-human primates, and rodents such as rats, hippocampus-dependent learning and memory functions fluctuate with cyclic changes in the level of circulating estrogen. In contrast, hippocampal function does not fluctuate in men and male animals. Even though the hippocampus performs the same cognitive function and exhibit age-related decline in men and women (and in male and female animals), the underlying biological processes driving these brain changes undoubtedly differ between the two sexes. For women, the cycling estrogen production during the reproductive years is replaced by a very low, constant level of estrogen during and following the period of menopausal transition. Therefore, around that time the process of normal brain aging in women also converges with a changing neuroendocrine background. To understand how the brain ages in women, we must therefore understand the impact of sustained estrogen loss on the physiology of brain cells especially in regions important for learning and memory functions. Determining how brain cells change in the absence of estrogen has been my research focus since my appointment by the Dept. of OB/GYN.

Given our current understanding of how the brain functions, there are only two possible ways by which sustained loss of estrogen could produce a detectable cognitive change in women and in animals. First is the widespread cell death in brain regions involved in learning and memory. However, this does not occur in postmenopausal women or in animals that are chronically estrogen-deficient. Second is a dramatic change in the way brain cells communicate with one another - the basis of all cognitive functions. A change in the way that brain cells talk to each other can be measured directly in terms of their activity, and this is the expertise that I have acquired over the past 15 years. Using a special microscope and a very technical set-up, we are able to visualize individual live cells within a freshly dissected but intact hippocampus and unambiguously measure their activity. Using a technique called single-cell electrophysiology, we found that activity of hippocampal cells from estrogen-deficient rats is profoundly reduced in comparison with activity from cells of control rats (Preliminary Studies #2). In fact, the maximal activity level of hippocampal cells from estrogen-deficient rats was only 50% of that in control rats. We recently published this work, which forms the basis of this application (See reference 4, “Research Appendix”).

We further found that the reduced activity in hippocampal cells from estrogen-deficient rats is mediated by an enhanced function of proteins called CaV1 Ca²⁺ channels. Normally, these proteins play an important role in preventing epileptic activity from developing by limiting the activity level of hippocampal cells. However, when CaV1 channel function is dramatically enhanced as we have observed in cells from estrogen-deficient rats, hippocampal cells become so inactive that normal cognitive functions are
impaired. Shown on the right is a schematic diagram summarizing the cellular changes (orange) that contribute to learning and memory deficits (green) in our rat model of menopause. Because rats respond to estrogen very similarly as humans do, these results should apply to humans as well.

The function of Ca,v1 channels can be selectively suppressed with a drug called nimodipine. We have recently acquired preliminary results ("Preliminary Studies" #3) showing that nimodipine treatment fully restores the activity of hippocampal cells from estrogen-deficient rats in vitro. Thus, nimodipine may be a viable treatment strategy for managing menopause-related cognitive symptoms. Using our rat animal model of menopause, we propose to test the therapeutic potential of nimodipine on reversing hippocampus-dependent learning and memory deficits in estrogen-deficient rats. Nimodipine (marketed as Nimotop\textsuperscript{TM}) is an FDA-approved vasodilator clinically used to manage symptoms unrelated to menopause. When used as directed, nimodipine is safe with minimal side effects. Considering that hypertension is often observed in postmenopausal women, the vasodilating effect of nimodipine may have added benefit to treat, at once, cognitive and peripheral symptoms associated estrogen loss following menopause.

**Methods (brief overview):** Our preliminary data show that nimodipine fully reversed cellular changes induced by sustained estrogen loss in our rat model of menopause. Our main goal is to test the hypothesis that nimodipine treatment in vivo rescues hippocampus-dependent learning and memory in estrogen-deficient rats. We will use a battery of behavioral tasks that require the hippocampus and we will determine whether administering nimodipine normalizes the behavioral performance of estrogen-deficient rats to the level we found for control rats.

**Outcomes.** The goal of this application is to generate preclinical data evaluating the therapeutic potential of nimodipine treatment to maintain cognitive performance following sustained estrogen loss in a rat model of menopause. Targeting ion channels as a strategy to reverse aberrant activity associated with disease states has long been used to successfully manage disorders of the blood vessels, the heart, and the brain. In fact, nimodipine, marketed as Nimotop\textsuperscript{TM}, is an FDA-approved vasodilator used to improve neurological outcome after subarachnoid hemorrhage. However, this treatment strategy is yet to be applied to cognitive symptoms resulting from estrogen loss following menopause. Our experimental approach is thus innovative in the areas of basic and clinical neuroendocrinology, where menopausal symptoms are still largely addressed with estrogen-based HRT. As mentioned above, HRT is non-specific, affects many systems at once, and cannot be prescribed to women at risk for cancer. Additionally, exactly how long HRT can be safely given to post-menopausal women has not been determined. These fundamental problems associated with HRT can be circumvented with a selective, non-steroidal treatment strategy as proposed in this application. At the very least, the outcomes of the proposed studies will provide a useful framework for the future design of non-steroidal therapies to manage menopausal symptoms related to brain functions. By attempting to restore cognitive function with nimodipine that is FDA-approved for certain disorders, the outcomes of this proposal may have a direct and immediate translational impact.

**Budget.** The Circle of Giving Fund will be used to: 1) support the salaries/fringe benefits of the principal investigator ($83,000) and a part-time technician ($25,000), 2) purchase and maintain the rat colonies for the proposed experiments ($12,000), 3) pay for blood-level nimodipine analysis performed by the OHSU Core for correlation with the behavioral data ($2,000), 4) cover for the manuscript submission and publication fees ($1,500), and 5) cover for the travel expense and meeting registration fee for presenting the outcomes of this proposal at the Annual Society for Neuroscience meeting ($1,500).
RESEARCH APPENDIX

**Specific Aim:** Restoring intrinsic membrane excitability (IE) of hippocampal CA1 pyramidal neurons of long-term estrogen-deficient (OVX<sub>LT</sub>) rats with nimodipine rescues hippocampal function and reverses hippocampus-dependent learning and memory deficits. We found that long-term (5 mo.) loss of estrogen leads to an impaired contextual fear learning in OVX<sub>LT</sub> rats. For this proposal, we will replicate and extend these behavioral studies by using two additional behavioral paradigms to assess hippocampus-dependent non-spatial and spatial recognition memories in control and OVX<sub>LT</sub> rats. The reason we want to use multiple behavioral paradigms that depend upon the hippocampus to characterize our rat model of menopause is to ensure that consistent differences in behavioral performance are indicative of changes in hippocampal function rather than some more general, task-specific effects. Then, using contextual fear conditioning and both non-spatial and spatial recognition memory tests, we will evaluate the efficacy of nimodipine treatment on rescuing hippocampus-dependent learning and memory functions in OVX<sub>LT</sub> rats.

**Preliminary studies:**

1. **OVX<sub>LT</sub> rats exhibit significant hippocampus-dependent contextual fear learning deficit.** Control and OVX<sub>LT</sub> rats were trained in a fear conditioning paradigm. In this task, rats learn to associate the context (training chamber) and a tone conditioning stimulus (CS) with an aversive footshock. Successful learning is indicated by rats exhibiting fear response (freezing) when returned to the context or presented with the CS alone. Contextual learning is hippocampus-dependent<sup>1,2</sup>; CS learning, amygdala-dependent<sup>3</sup>. OVX<sub>LT</sub> rats (gray bars) showed less freezing than control rats (black bars) when returned to the context where fear conditioning took place, demonstrating hippocampal dysfunction. No difference was found in freezing to the CS or baseline activity, suggesting no overt change in amygdalar function or motor function/alertness in OVX<sub>LT</sub> rats.

![Graph showing freezing behavior](image)

2. **IE is significantly reduced in hippocampal CA1 pyramidal neurons from OVX<sub>LT</sub> rats.** "Neuronal activity" referred throughout the “Main Application” refers to action potential (AP) firing. The propensity to generate APs (a property called IE) is significantly reduced in OVX<sub>LT</sub> rats relative to control rats. Passive membrane properties including input resistances and resting membrane potentials were similar. The degree of IE of CA1 pyramidal neurons is positively related to the ability of animals to learn hippocampus-dependent tasks<sup>5,6</sup>. Thus, reduced IE is a likely cellular mechanism that contributes to contextual fear learning deficit in OVX<sub>LT</sub> rats.

**Fig 2.** **A)** Representative traces for a control and an OVX<sub>LT</sub> neuron, evoked with 50-250 pA and 100-500 pA current steps, respectively. Scales: 40 mV, 200 ms. **B)** An IE summary plot of evoked APs for each current step. Control and OVX<sub>LT</sub> neurons differ significantly at all overlapping current steps tested.

3. **Nimodipine restores IE of OVX<sub>LT</sub> neurons to the control level.** Nimodipine significantly increased the number of APs generated by OVX<sub>LT</sub> neurons at all current steps (A). Although preliminary, these data show that nimodipine fully restored IE of OVX<sub>LT</sub> neurons to the control level (B).

**Fig 5.** **A)** Traces are from an OVX<sub>LT</sub> neuron. APs were evoked with a 250 pA, 1-s current step, in aCSF and in nimodipine. Scales: 40 mV, 200 ms. **B)** Summary plots of IE.
Research design & Methods:

**Rat model of menopause.** Female rats (7 and 12 mo. old) will be ovariectomized (OVX) according to our published protocol. Following OVX, 12 mo. old rats will recover in their home cages for 7 days (Control rats; 7 days = 1-2 estrus cycles), while 7 mo. old rats will recover in their home cages for 5 months (OVX_LT rats). A third group of 12 mo. old rats will receive sham surgery (Intact cycling rats). For this rat model of menopause, we have demonstrated that it is the duration of estrogen deficiency, and not the age at OVX, which affects IE of CA1 pyramidal neurons. Further, there is no difference in IE between neurons from control rats and sham-operated, intact cycling rats.

**Behavioral paradigms.**

1. **Fear conditioning.** Training and testing for cued and contextual fear conditioning will be conducted in rectangular conditioning chambers situated in sound-attenuating cubicles. An 85 dB white noise CS (cue) will be presented through a sound generator, and a 0.35 mA footshock US will be administered through the stainless steel rod floor with a shock scrambler/generator. We will examine the degree of learning (e.g. freezing in response to context and cue) following 2 presentations of CS-US pairings in control and OVX_LT rats. Training protocol. Each CS-US pairing will be separated by a 90-s inter-trial interval (ITI). Behavioral testing. To test for contextual learning, 24 hr following training, rats will be returned to the same training chamber, and freezing, defined as the absence of all movement except for respiration, will be assessed for 5 min. To test for cued learning, 48 hr following training rats will be placed in the training chamber altered with an opaque Plexiglas sheet covering the stainless steel rod floor. Generalized freezing will be assessed for 3 min, followed by two 3 min-long CS presentations (3-min ITI), and then a 3 min post-CS period. Freezing will be assessed across the entire session, and the degree of freezing in the absence of the CS (pre-CS, ITI, and post-CS) will be combined to indicate baseline freezing, and the degree of freezing during the 2 CS presentations will be combined. Freezing will be assessed for 1-s at 10-s intervals by an observer blind to the condition and treatment given to each rat.

2. **Recognition memory tests.** Rats show an innate preference for novelty, and this preference is used to study novelty discrimination (or recognition memory). We will compare non-spatial object recognition memory (ORM) and spatial object location memory (OLM) between control and OVX_LT rats. Training and testing sessions will be recorded with a video camera for analysis. These protocols are shown on the right. Training. Rats will be placed in a white open field (enclosed by tall dark walls) and allowed to explore two identical sample objects placed in adjacent corners of the field for 5 min. Testing. 24 hours after training, rats will be placed back in the same open field with one sample and one novel object (ORM test), or the same pair of sample objects with one occupying a new location (OLM test). Rats will be allowed to explore these objects for 3 min. An increase in % time spent exploring the novel object or sample object in a new location as a function of the total time spent exploring both objects is a discrimination index for recognition memory. This index will be plotted in 1-min bins. The total time interacting with both objects during testing will be used to compare the level of motivation and motor coordination between control and OVX_LT rats.

**Nimodipine treatment.** Nimodipine (5 mg/kg) or sesame oil (vehicle) will be injected subcutaneously into the nape of control and OVX_LT rats 2 hours prior to training. In rats, this dose and the route of delivery have been shown to facilitate complex maze learning and prevent the amnesic effects of scopolamine and alcohol on object memory without side effects. We will impose a 2 hr delay following nimodipine injection to initiate behavioral training, because that is the amount of time for nimodipine to reach peak plasma level. Importantly, the level of nimodipine measured in the plasma is comparable to that measured in the hippocampus. The plasma level of nimodipine achieved with this protocol will remain at the peak value for at least 6 hrs following injection, and will still be significantly elevated 24 hrs later. Following training, ~250 mL blood sample will be collected from the tail vein of each rat for plasma nimodipine quantification using HPLC-ESI-MS. Nimodipine quantification will be performed by the OHSU Bioanalytical/Pharmacokinetics Core Service. We will correlate the level of nimodipine in the plasma with behavioral performance for each rat.
References.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Wendy W. Wu

eRA COMMONS USER NAME (credential, e.g., agency login)
wuwend

POSITION TITLE
Assistant Professor, OB/GYN

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington University, St. Louis, MO</td>
<td>B.A.</td>
<td>1998</td>
<td>Honors Chemistry/Biochemistry</td>
</tr>
<tr>
<td>Northwestern University Institute for Neuroscience</td>
<td>Ph.D.</td>
<td>2005</td>
<td>Neuroscience</td>
</tr>
<tr>
<td>Oregon Health &amp; Science University</td>
<td>Postdoc</td>
<td>2005-2008</td>
<td>Neuroscience</td>
</tr>
</tbody>
</table>

A. Personal Statement.
I specialize in cellular and cognitive neuroscience. As shown in my publication record, I have been using a variety of in vitro electrophysiological techniques to evaluate brain functions and central nervous system disorders since 1998. Recently we showed that electrical signaling by rat hippocampal CA1 pyramidal neurons important for learning and memory is drastically altered following premature and prolonged loss of ovarian hormones. The current application builds on this publication and our new preliminary data, and is designed to investigate the therapeutic potential of modulating Ca$_{v1}$ channels for correcting the aberrant electrical signaling in CA1 pyramidal neurons and hippocampus-dependent learning and memory deficits in long-term ovarian hormone-deficient rats. The proposed one-year project utilizes behavioral pharmacology, which is within our expertise and specific areas of training. We can carry out the proposed experiments successfully.

B. Positions and Honors.

Positions
2005-2008 Postdoctoral Fellow, Oregon Health & Science University, Portland, OR
2008- Assistant Professor, Dept. of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR

Professional Membership
1998- Society for Neuroscience

Honors & Awards
1997 Howard Hughes Summer Research Fellowship, Washington University, St. Louis, MO
1998 Academic Honors in Chemistry, Washington University, St. Louis, MO
1998-1999 University Scholar, Northwestern University, Evanston, IL
2001-2004 Individual National Research Service Award, (Predoctoral NRSA Fellowship MH 012858)
2004-2005 NIH Training Program in Neurobiology of Information Storage Award (Institutional NRSA F31 MH 067564)
2005-2006 NIH Training Program in Signaling Cascade Award (Institutional NRSA T32 NS 007381)
2005-2006 Tartar Trust Fellowship, Oregon Health & Science University
2008-2009 Tartar Trust Fellowship, Oregon Health & Science University
2008-2010 NIH Mentored Research Scientist Development Program Award (K12 BIRCWH Award; 5K12 HD043488-07)
2012-2013 OHSU OB/GYN Mission Support Award

C. Selected Peer-reviewed Publications (in chronological order).


---

**D. Research Support**

**Ongoing**

R01 MH081860-03 Maylie (PI) 07/01/2008-01/31/2013

SK channels: Roles and mechanisms for dendritic excitability and plasticity

The major goal of this study is to test the hypothesis that SK channels in hippocampal CA1 dendrites limit electrotonic propagation of dendritic EPSPs, reducing the fidelity of EPSP-spike (E-S) coupling, and limit retrograde back-propagating action potentials (b-APs), thereby affecting the induction of synaptic plasticity due to theta-burst pairing (TBP) of Schaffer collateral inputs with postsynaptic b-APs.

Role: Co-I.

OHSU OB/GYN Mission Support 03/01/2012-02/28/2013

Cloning the sAHP channels as a strategy to identify novel therapeutic targets for cognitive protection following surgical menopause

Role: PI.

**Completed**

5 K12 HD043488-07 (PI: Dr. Lesley Hallick) 09/26/2002-07/31/2012

Scholars in Women's Health Research Across the Lifespan (NICHD)

The NIH-funded Oregon BIRCWH K12 program is intended to advance mentored research career development of junior faculty who will be engaged in interdisciplinary basic, behavioral, clinical, or health services research in women’s health.

Role: BIRCWH Scholar.