National Institute on Aging

Bench to Bedside: Estrogen as a Case Study
September 28, 29 2004

The outlines of presentations for the workshop entitled: "Bench to Bedside: Estrogen as a Case Study" were prepared by the speakers. The topics under "specific points presented" were prepared from notes taken by Drs. Leon Thal and Marilyn Miller. The document containing this information was circulated to speakers and reviewed by them. Revisions provided by speakers were incorporated into the document provided below.

Meeting objectives:

1. To define and examine the discrepancies between the findings of the Women's Health Initiative on brain and cognitive function and the basic and longitudinal / epidemiological studies including different formulations, doses, duration of treatment, timing and mode of administration.
2. To examine the effects of estrogens on brain and cognitive function as they relate to aging.
3. To determine what is known and what information we would need to obtain that would determine whether additional hormone "interventions" could be developed.
4. To determine what lessons we have learned from studies on estrogen that will help in designing clinical trials for other classes of drugs.

Potential Conflict of Interest Issues:

The National Institute on Aging authorized the attendees with conflicts of interest to participate in the "National Institute on Aging 2004 Workshop entitled: "Bench to Bedside: Estrogen as a Case Study" based upon the fact that these individuals are among the pre-eminent experts in the field.

Participants stated their conflicts in both oral and written form. Participants stated that they had received or are receiving research funds from pharmaceutical companies: Abbot (2), Amgen (1), Bulex (1), Eli Lilly (3), Forest (3), Glaxo Smith Klein (1), Jansson (1), Merck (2), Pfizer (5), Proctor and Gamble (1), Smith Klein (1), Targacept (1), and Wyeth Ayerst (13). Participants stated that they had given expert advice to Clark, Thomas and Winters (1), Berles Holdings (1), and Galen Holdings (1). Participants stated that they hold 28 patents on compounds and procedures relevant to the workshop, and had 21 pending patents on compounds and procedures relevant to the workshop.

Welcome: Dr. Marcelle Morrison-Bogorad

Meeting Logistics: Dr. Marilyn Miller

Introduction: Dr. Bruce McEwen

Outline of presentation:

1. Women's Health Initiative (WHI) taught us we need to learn more about the actions of estrogen (E) and progesterone (P) in relation to the rest of the physiology of the body.
2. WHI was a success in that it changed the patterns of medical practice regarding the major form of hormone therapy (HT) in the US and it raised questions such as:
    a) critical windows for treatment;
    b) choice of the type of estrogen and progestin and dose and timing of treatment.
3. Basic science matters to clinical trials because it argues for an alternative approach to HT than Prem-Pro®. Moreover, basic science has produced evidence that E and P have beneficial effects on the brain but also revealed the complexities of E and P actions that were not anticipated even a decade ago.

I. BASIC SCIENCE: Effects of estrogen and progesterone on CNS cellular systems and in animal models.

A]. NEUROBIOLOGY OF ESTROGEN AND PROGESTERONE

1. **Dr. Roberta Brinton: "Estrogen Mechanisms of Action in Neurons: Factors that Determine Outcome *In Vitro* as Predictors of Efficacy *In Vivo*"**

Outline of presentation:

1. Overview of mechanisms of estrogen action in neurons
2. Timing of estrogen exposure matters: Estrogen therapy prior to - versus following - versus simultaneous - exposure to beta amyloid toxicity. Healthy cell bias of estrogen action
3. Dose matters: Dose response of 17 beta-estradiol vs conjugated equine estrogens (CEE); More 17 beta-estradiol is definitely not better
4. Formulation Matters: Estrogenic components; Progestin components
5. Strategies to optimize estrogen therapy for the brain.

Specific points presented:

- Hippocampal neurons contain multiple splice variants of the ERβ receptor including missing the nuclear receptor or the DNA binding domain.
- 17-β estradiol has an inverted U shaped dose response curve for hippocampal neuronal branching.
- In cell culture, with amyloid present, low dose 17 β-estradiol (10ng/ml) leads to protection, while high dose (200ng/ml) does not. Whether the estradiol is given continuously or not is not important.
- In cells without amyloid, all doses and modes of administration are protective.
- In hippocampal neurons, estrogens reduce tau hyperphosphorylation; addition of MPA reverses this effect.

- For protection from amyloid in cell culture, there is less and less protection from estrogen if given after the cells are exposed to amyloid.
- Hypothesis: hormone therapy might work at an earlier age when cells are healthier

2. **Dr. Dominique Toran-Allerand, "In defense of estradiol and progesterone"**

Outline of presentation:

1. The brain is a major target of the gonadal steroids
2. The biology of the gonadal steroids and of their receptors must be taken into any experimental design
3. All gonadal steroids are not equal: the type of hormone used is crucial
4. The method (timing) and route (oral vs transdermal) of replacement is crucial.

Specific points presented:

- Women's Health Initiative Memory Study (WHIMS) was not physiological.
- Studies are needed using pure estradiol 17β, not Premarin.
- Studies on cyclical therapy are needed.

DISCUSSION-BASIC SCIENCE:

- The healthy cell bias predicts that healthy neurons are protected by estrogen, but unhealthy cells are not. The healthy cell bias is consistent with epidemiological data.
- CEEs in culture are associated with low dendritic sprouting, while 17β estradiol produces abundant dendritic spines.
- Provera is not neuroprotective in culture.
- Women who started with cognitive health in WHIMS may have maintained it. Women who may have had pre-existing underlying and unidentified neuropathology may have been more vulnerable to neuron damage by the therapy provided in the study.

B]. COGNITIVE FUNCTION: Age-related changes in estrogen / progesterone levels and cognitive function

Dr. Robert Gibbs:"Hormone Therapy and Cognitive Performance – Reconciling Animal Studies with Clinical Data"

Outline of presentation:

1. Therapy for the brain must be tailored to the brain.
2. Results depend on dose and regimen, as well as on timing of hormone therapy with respect to age and loss of ovarian function.
3. Basal forebrain cholinergic projections play a very important role in mediating effects of estrogen replacement on cognitive performance.

Specific points presented:

- In rat models, 17-ß estradiol enhances learning and memory performance on multiple tasks. This effect can be blocked by immunotoxic lesions of septal cholinergic cells. Thus the effect is dependent upon these cells.

- Some effects of 17-ß estradiol on learning and memory correlate with effects on hippocampal plasticity which, in turn, are dependent upon basal forebrain cholinergic inputs.
- Hormone treatment can help prevent age-related cognitive impairment in rats; however, studies suggest there may be a window of time (<10 months) following ovariectomy during which hormone treatment must be initiated in order to be effective.
- Simultaneous and sustained E and P treatment has negative effects on basal forebrain cholinergic neurons in rats and in cynomologous monkeys, contrary to other treatment regimens.

Dr. Peter Rapp: "Estrogen Influences on Cognitive Aging in Monkeys "

Outline of presentation:

1. Are dose, schedule and timing critical for mediating the cognitive and neurobiological effects of estrogen?
2. Are the effects of estrogen replacement age-dependent?
3. Do we need to look outside the hippocampus to understand the neurobiological basis of estrogen effects on cognitive function?

Specific points presented:

- Estrogen replacement regimen used in all of the behavioral studies consisted of estradiol cypionate, 100μg/1ml sterile peanut oil, i.m., given in a single injection once every 3 three weeks.
- Estrogen administration substantially improved delayed response performance - a frontally mediated task - in aged ovariectomized monkeys relative to age-matched vehicle treated subjects.
- The effects of estrogen on recognition memory mediated by the medial temporal lobe were modest, and hormone treatment had no effect on simple object discrimination learning in aged ovariectomized monkeys.
- Preliminary data indicate that the cognitive effects of estrogen are qualitatively different in young monkeys.
- Conclusion: Estrogen influences on cognitive function are task and age dependent. The findings also establish a primate model for defining the neurobiological basis of ovarian hormone effects of cognitive aging (see Morrison presentation).

Dr. John Morrison: "Estrogen and the aging cortical synapse; implications for cognitive effects in aged monkeys"

Outline of Presentation:

1. What is the behavioral impact of the observed synaptic effects of estrogen?
2. In primates, the effects of estrogen on prefrontal cortex may be more important than those on hippocampus with respect to the cognitive effects.
3. In primates, how long does the aged cortical synapse continue to be responsive to estrogen? Is its responsiveness dependent on variables such as: a) length of time without estrogen prior to replacement; b) age of initiation of replacement; or c) schedule of replacement (e.g., continuous or cyclic)?

4. Aged cortical synapses may react differently to estrogen replacement than young synapses, though such differences may not be consistent across rats and nonhuman primates.

5. What are the molecular mechanisms of estrogen-induced spine/synapse enhancement and how are they affected by aging?

Specific points presented:

- Unopposed E, administered cyclically for 2.5 years, causes an enhancement of cognitive performance and an increase in spine counts in the prefrontal cortex of aged female rhesus monkeys.
- The behavioral effect was particularly pronounced on a Delayed Response task, a task that is dependent on prefrontal cortex.

Dr. MaryLou Voytko: Wrap up

Discussion on multiple processes involved in changes in cognitive function, appropriateness of the ovariectomized animal model to human menopause.

I. Important Issues for Cognitive Function:

Age of the animal matters

Timing of treatment: earlier the better in rats and monkeys

Route: Parenteral vs Oral

Regimen

Continuous vs cyclical

E alone vs E + P

Types of E and P

Dose: rats tested only; monkeys studies needed

II. Examples of Cognitive Testing in Memory and Attention with Hormone Therapy

1. Rats

- Morris Water Maze-spatial reference memory
- Radial Arm Maze - spatial working and reference memory
- Delayed Matching to Position in a T-maze spatial working memory

2. Monkeys

- Delayed Response - spatial working memory
- Delayed Nonmatching to Sample- visual working memory
- Delayed Recognition Span- visual or spatial working memory
- Discriminations & Reversals- associative learning & cognitive flexibility
- Visuospatial Cued Reaction Time- visuospatial attention

III. Ovarian Hormones Affect Brain Neurobiology

- Neurochemical systems: cholinergic, dopaminergic, serotonergic, noradrenergic, gabaergic, glutamatergic, neurotrophic
- Neuronal excitability
- Synaptogenesis/spines
- Glial cells
- Cerebral blood flow
- Glucose uptake
- Neurotoxicity

- Oxidative Stress

IV. Sites of Ovarian Hormone Actions in the Brain Relevant to Cognition
Hippocampus
Cerebral Cortex: Frontal Lobe, Parietal Lobe, Entorhinal Cortex

V. Differences Between Natural vs Surgical Menopause

1. *Natural*
- Gradual drop in hormones
- Androgen production by ovaries
- Mean age of 51 years

2. *Surgical*
- Abrupt drop in hormones
- Ovarian androgens absent
- Mean age of 45 years

Next Steps: Need better integrated communication between basic science and clinical investigators working on the issues of ovarian hormone therapy and its affects on cognition and the brain:

a. Clinical to Basic: basic scientists need to hear from clinicians regarding the animal and cell culture studies that are needed to best inform them about critical issues related to treating women for their cognitive and brain health

b. Basic to Clinical: clinicians need to use the information derived from basic science studies to develop strategies and make decisions on when and how to treat women.

DISCUSSION – COGNITIVE FUNCTION

- Earlier is better: waiting a long time before administering estrogen (10 years after loss of ovarian function) may result in the selective loss of function. This may be subtle and difficult to measure.
- Mode of administration: for continuous administration, most studies needed
- Integration of basic science findings into clinical studies, and clinical outcomes to drive new basic science studies
- Impaired glucose homeostasis is correlated with cognitive function decline.
  - Women who started with cognitive health in WHIMS may have maintained it. Women who may have had pre-existing underlying and unidentified neuropathology may have been more vulnerable to neuron damage by the therapy provided in the study.
  - The ovariectomized aging rodent animal model is relevant and somewhat comparable to the studies done by Barbara Sherwin. There are no other similar studies in human.
  - Differential sensitivity
    a. Sensitivity of brain to steroids is region-dependent. e.g. prefrontal cortex is steroid sensitive but medial temporal cortex is not. Some brain regions do not respond in either rodent or non-human primate.
    b. Some brain regions are less sensitive to age-related changes than are others.

c. Changes in memory function depend upon the modality tested. Some modalities are not sensitive to aging.

- Primate studies:
  a. Hippocampal dendritic spines in the primate are responsive to estrogen.
  b. Providing estrogen to ovx primate results in the induction of about 1 billion dendritic spines in specific brain regions (prefrontal cortex), but not others (visual cortex).
  c. There is no effect of estrogen on dendritic arbors, dendritic complexity, or neuron death.
- Reorganization of function with age: changes in neural networks underlying changes in cognitive function.
- Stroke and prefrontal tasks
- Metabolic syndrome, diabetes, CHS; E in combination with other treatments
- Interactive effects of E across systems and across brain regions

C]. NEUROPROTECTION: Summary of data that estrogen / progesterone are or are not neuroprotective.

Dr. James Simpkins: "Role of Non-feminizing Estrogens in Brain Protection from Cerebral Ischemia: An Animal Model of Alzheimer's Disease Neuropathology" Dr. Simpkins was unable to be present. Dr. McEwen summarized the work.

Talking points submitted by Dr. Simpkins in advance of the meeting:

1. The WHI reported side effects of continuous oral equine estrogens are primarily mediated by continuous stimulation of estrogen receptor alpha in reproductive tissues and the liver.
2. Briefly review approaches to eliminate these effects of estrogens.
3. Focus on studies of non-feminizing estrogens and their potent neuroprotective and anti-Alzheimer's neuropathological effects in rodents.

Dr. McEwen's summary of the work.

- Effects are mediated by known ERs (Alpha, beta, X). Non-feminizing Es-do not bind to ERs; and are protective in ischemic models; are anti-oxidative and free radical quenchers.
- Wise model-estrogen receptor knock out (ERKO): ER alpha has important neuroprotective effects (genomic or non-genomic); ER beta is less important
- Aromatization in the brain is activated by excitatory neurotransmitters; cholesterol side chain cleavage to pregnenolone (first step of estrogen synthesis) is activated by excitatory neurotransmission
- Endogenous E production is not sufficient for replacement, but may be a limited servo mechanism for protection
- Aromatase KO is susceptible to ischemic damage; add back E, then is protected.
- Calcium homeostasis is essential for neuroprotection

Dr. Caleb Finch: "Ovarian steroids, neuroinflammatory responses, and aging"

Outline of presentation:

1. Systemic estrogens and serum inflammatory markers: effects of mode of administration
2. Sex steroid effects on glia
3. Aging, glial activation, and neuronal functions

Specific points presented:

- Oral estrogens increase interleukin 6 (IL6) and C-reactive protein (CRP) at higher body mass index (BMI).
- CRP elevations may predict vascular events and dementia decades in advance of pathologic events.
- In a wound model in cell culture, progesterone but not medroxyprogesterone acetate (MPA) stimulates neuritic outgrowth.

DISCUSSION – NEUROPROTECTION

- Inflammatory proteins are found in plaques; complement deposits are associated with amyloid beta. Estrogen supports sprouting in vitro (wounding-in-a-dish model). However, sensitivity to estrogens changes with age, since astrocytes from old rat cortex lose responsiveness to estrogen. The age increase in astrocytic GFAP is implicated as a primary factor. GFAP levels can be down regulated with siRNA and this restores responses to estrogen in aging astrocytes.
- For anti-inflammatory treatment, in epidemiological studies, low dose is as effective as high dose.
- In young astrocytes progesterone can cause lengthening of processes, but this does not occur with MPA. MPA blocks the support function of E2.
- Progesterone by itself doesn't have much effect in the uterus, but in the brain it can be neuroprotective
- CEE treatment is associated with increased CRP
- Capillary density is increased in plaques where there appear to be local angiogenesis.
- Among rodents there are species differences such that ER alpha is present about 3X that found in rat.
- The Women's Estrogen for Stroke Trial (WEST), The Heart and Estrogen/Progestin Replacement Study (HERS), and WHI had poor vascular outcomes; we need to look at vascular markers.
- Targets for therapy must include early sets of events.
- Synaptic changes may be observed in the absence of neuron death.
- Changes in small vessels may be different than those occurring in large vessels.
- No data on the effects of E on the coagulation cascade
- Diet for monkey has been pellets laced with soy protein. Effect on neuroanatomy? Like the human?

II. CLINICAL STUDIES:

Dr. Thomas B. Clarkson: "Cardiovascular health and cognition: perspectives on using the primate as a model for human research"

Outline of presentation:

1. Use of nonhuman primates to elucidate the clinical implications of estrogen effects on early atherosclerosis vs. complicated plaques.
2. Use of nonhuman primates to investigate the pathobiologic mechanisms whereby postmenopausal estrogen treatment can provoke plaque instability in the later stages of coronary artery atherosclerosis progression.
3. Monkey models to explore the cardiovascular health and cognition across the menopausal transition (premenopausal → perimenopausal → menopausal → postmenopausal).
4. Overview of the Kronos Early Estrogen Prevention Study (KEEPS) study

Specific points presented:

- Coronary artery disease can progress from age 15 on.
- Hormone therapy has a beneficial effect age 35-55 but no benefit after age 55
- Women with CAD, on average, have lower levels of E2
- For surgically induced menopause in monkeys, estrogen begun immediately prevents CAD while estrogen late on(>5-6 yrs) does not
- KEEPS is underway to test this in women
- Estrogen: Good early, bad late

Dr. Barbara Sherwin: "Consequences of Surgical Menopause on Cognition and Mood"

Outline of presentation:

1. The drastic nature of the endocrine changes when surgery is undertaken in premenopausal women and whether the high incidence of symptoms is related to the abruptness of the hormonal changes.
2. Surgical menopause involves changes in androgen as well as in estrogen concentrations.
3. The possibility that surgically menopausal women, who are generally younger than naturally menopausal women when they are deprived of ovarian function, may be rendered more vulnerable to diseases of aging.
4. Possible adverse consequences of premenopausal ovarian ablation would also apply to treatment of women with non-estrogen sensitive cancers whose ovarian function is compromised by radiation and/or chemotherapy treatments.
5. The relationship between changes in mood and cognition in women.

Specific points presented:

- There are 600,000 hysterectomies/y in US and 300,000 ovariectomies
- 10 million surgically menopausal women in US
- Estradiol drops most between 50 and 52 then levels off by about age 57 at about 60-80 pmol/l
- The ovary also produces 25% of androsterone, 10% of dihydroepiandrosterone (DHEA); therefore, the levels of testosterone (T) are about ½ in ovariectomized (ovx) women.

- When ovx women are replaced with estrogen-alone, sex hormone binding globulin (SHBG) levels rise so that more of their already diminished levels of endogenous T is bound and biologically inactivated
- Beneficial effect of estrogen on verbal but not visual memory when replaced
- Beck scores decreased on E compared to an increase on P

Dr. Peter Schmidt: "CNS effects of hypogonadism and estrogen therapy in humans."
Outline of presentation:

1. Efficacy of short term estradiol therapy in perimenopause- (but not postmenopause)-related depression.
2. Evidence of an increased risk for the onset of depression within the 24 months surrounding the last menstrual period
3. Effects of GnRH agonist-induced ovarian suppression with and without estradiol and progesterone replacement on measures of mood, cognitive performance (e.g., verbal memory), cognition activated regional cerebral blood flow (positive electron tomography [PET]) and BOLD functional magnetic resonance imaging (fMRI) studies.

Specific points presented

- Estradiol decreases depression in the perimenopausal but not postmenopausal women
- Although the majority of women do not develop a depression during the menopausal transition, prospective longitudinal studies have identified the perimenopause as a time during which some women may be at an increased risk for the development of a depressive illness.
- Hypogonadism and estradiol or progesterone treatment will differentially alter the pattern of cognition-activated regional cerebral blood flow in the prefrontal cortex of the brain (as measured by O^{15} PET)

Dr. Neill Epperson: "The Differential Effects of Estrogen on Mood and Cognition in Peri- and Postmenopausal Women"
Outline of presentation:

1. The role of serotonin and aging in menopausal women.
2. The impact of estrogen on serotonergic function
3. The serotonin/estrogen interplay in mediating mood and cognition
4. The impact of tryptophan depletion on mood and cognition in menopausal women pre and post estrogen therapy

Specific points presented:
The tryptophan depletion paradigm can be used to study the effects of reduced brain serotonin on cognition and mood in humans

- Tryptophan depletion results in worsening of performance on the delayed paragraph recall and the paired associates subtasks of the Wechsler Memory Scale-Revised in both peri and postmenopausal women
- Estrogen improves performance on the delayed paragraph recall and the paired associates subtasks

In summary, estrogen appears to "protect" menopausal women from the detrimental effects that a reduction in brain serotonin has on certain aspects of cognitive function.

Dr. Natalie Rasgon: "Estrogen or no estrogen: Long-term follow-up."
Outline of presentation:

1. Brain metabolism in hypoestrogenic (postmenopausal) women with major depressive disorder
2. Brain metabolism in women with hypothyroidism
2. Brain metabolism in persons with familial and/or genetic risk for AD

Specific points presented:

- PET imaging
- No differences between estrogen users and non-users in memory at baseline
- Over 2 years, there was a slight increase in posterior cingulate metabolism when on estrogen not seen in non estrogen users

Dr. Vincent P. Clark, Ph.D. "Low dose estrogen, fMRI, and cognitive function."
Conclusions of the study:

- Low-dose 17 β-estradiol does not greatly alter behavioral responses in a simple reaction time task.
- Brain activation patterns in older women are consistent with findings in younger ones.
- FMRI responses in posterior parietal and lateral occpital-temporal brain areas were reduced with Low-dose 17 β-estradiol relative to placebo, suggesting greater efficiency of stimulus processing.
- However, because fMRI is an indirect measure of neural activity, these fMRI results may be due to either the neural or hemodynamic effects of 17 B-estradiol.

Specific points presented:

- Studied women on or off low dose E+P for 3 years, N=16 with fMRI No change in reaction time but some changes in fMRI BOLD signal

Dr. Susan Resnick: "Cognitive and Brain Aging: Using Imaging to Distinguish Potential Risks and Benefits of Estrogen"
Outline of presentation:

1. BLSA studies of hormone therapy and cognition
2. Women's Health Initiative Study of Cognitive Aging (WHISCA) findings on specific cognitive functions from the E + P subtrial of the WHI
3. Differential effects of HT on specific cognitive functions – competing risks and benefits
4. Imaging techniques to distinguish potential risks and benefits

Specific points presented:

BLSA observational studies indicate better verbal and figural memory in women using hormone therapy compared with never users

WHISCA study designed to investigate the effects of hormone therapy on rates of change over time in specific cognitive functions

- WHISCA E + P subtrial - about 700/group, well-balanced with respect to demographic characteristics and 3MS at WHI and WHISCA baselines
- Randomized and treated for about 3 years before first assessment

- One yr follow up for 92%, 43% had 2 yrs on treatment
- At baseline after 3 yrs treatment, no robust effects
- For rate of change over time, E+P women are worse on some aspects of memory but better on others.
- WHISCA CEE alone analysis is underway

Dr. Lew Kuller: "Vascular disease in the brain: An important cause of dementia?"
Outline of presentation:

1. Very high prevalence of vascular disease in the brain, i.e. high white matter grade and infarcts.
2. Vascular disease in the brain strongly associated with dementia.
3. Without MRI it is impossible to measure 'vascular disease in the brain' and type of dementia. Much dementia is likely a mixture of Alzheimer's disease type pathway and vascular disease.
4. Estrogen therapy increases risk of stroke. Risk of dementia may be a function of vascular disease in the brain. If so, there may be a need for preventive strategies for millions of women who were on estrogen or estrogen and progesterone therapy.

Specific points presented:

- Cardiovascular health study found that about 20-25% of men and women with no prior history of stroke have subclinical infarcts(N=~3000)
- Of 500 persons, about 50% AD, 35% MIX, 15% Vascular Dementia

Why don't estrogens work?

- Poor lipid lowering
- Increases thrombosis
- Differential effects –works when young, not old
- Metabolites of estrogens in blood
- Weight gain wipes out the benefit of HT

DISCUSSION – CLINICAL STUDIES

- Tasks that can differentiate hippocampus from the frontal cortex are needed: presently available-delayed matching and non-matching to sample.
- Need for analysis of life style changes

III. LONGITUDINAL AND EPIDEMIOLOGICAL STUDIES

Dr. Lenore Launer: "Levels of endogenous sex steroids and risk for brain aging"
Outline of presentation:

1. Endogenous levels of estrogen, MRI brain changes, and the risk for dementia in women-lessons from the Rotterdam study
2. Endogenous levels of estrogen, testosterone and the risk for MRI brain changes, dementia, and neuropathologic outcomes in men—lessons from the Honolulu Asian Aging Study (HAAS)
3. Changes in the association of hormone levels with stage of dementia
4. Both the HAAS and Rotterdam study results are more consistent with the trial data.

Specific points presented:

- Rotterdam study
    - An increase in reproductive years (menarche - menopause) predicted a higher incidence of dementia
    - On MRI, as the estradiol increased, hippocampal volume decreased
- HAAS-men
    - Slight increase in AD with higher estrogen levels

Dr. Claudia Kawas: "Estrogen therapy and risk of AD in the Baltimore Longitudinal Study of Aging" (BLSA)

Outline of presentation:

1. Key methods and results of the study
    a. prospective, observational study with long follow-up period (16 years).
    b. reduced risk ~50% if ET ever used, as compared to never used.
    c. no effect for duration of ET use
    d. most commonly used compound reported in the study was unopposed estrogens in the form of Premarin
    e. adjusted for education, but are there other unknown confounders?
2. Benefits and pitfalls of observational studies, case control studies, cohort studies, clinical trials, etc.
3. Each type of study provides important information, and each type of study also has it's pitfalls.
4. Implications of findings of this study for future studies in humans.

Specific points presented:

- BLSA, prospective study
    - Protective effect, ever vs never, RR 0.46, for protection from dementia
    - No dose effect
    - No duration effect
    - No route effect
- Leisure World
    - ~14,000 women
    - HT showed a risk reduction for dementia by about ½
    - Out of 5000 women, 2200 alive and >90
    - For mortality:
        - about a 6% reduction,
        - effect of duration (no reduction with < 3yrs of use),
        - last use effect present to about 15%

Dr. Peter Meyer: "A population-based longitudinal study of cognitive functioning in the menopausal transition (Study of Women's Health Across the Nation) [SWAN]."

Outline of presentation:

1. The Chicago cohort of the SWAN study is a population-based cohort of 868 women. Of these, 802 had valid cognitive assessments at one or more of their annual interviews from baseline through follow-up year 4.

2. Used Digit Span Backwards and the Symbol Digit Modality test as brief tests of Working Memory and Perceptual Speed.

3. Findings:

a. At baseline the proportion of women reporting problems with remembering things during the previous two weeks showed a clear association with age and with menopausal status.

b. There was no indication of a decline in Working Memory or in Perceptual Speed over the five years of the study related to age or menopausal status.

4. Implications of findings from the SWAN study for future studies in humans.

Specific points presented

- No real change in digit span and symbol digit modality test during menopause
- No difference in rate of change either

Dr. Victor Henderson: "Estrogen exposures in midlife, memory and dementia." Outline of presentation:

1. Midlife estrogen exposures do not have a substantial effect on episodic memory for women undergoing natural menopause.
2. Early exposure to menopausal hormone therapy (HT) may be associated with Alzheimer risk reduction.

Specific points presented:

- Melbourne Women's Midlife Health Project (n = 326 women; surgical menopause excluded)
- No difference on immediate or delayed word list task (10 items) by premenopausal, perimenopausal or post-menopausal status
- No relationship between endogenous estradiol and immediate or delayed recall
- No difference in immediate or delayed recall based on HT use (never, past users, current users)
- Post hoc analyses suggested better immediate recall for current HT users who initiated HT prior to their final menstrual period when compared to those who initiated HT after their final menstrual period
- Conclusion from Melbourne Women's Midlife Health Project: There is no evidence that menopausal status or other measures of midlife estrogen exposure affect verbal episodic memory as assessed by word list learning.
- Multi-Institutional Research in Alzheimer Genetic Epidemiology (MIRAGE) (n = 426 cases, 524 controls)
- HT was associated with a reduced risk of Alzheimer's disease (AD)
- There was a significant interaction with age
- In age tertiles, HT was significantly associated with reduced risk only in the youngest tertile
- Among other possibilities, findings raise question of whether early HT exposure might reduce AD risk

Dr. John Breitner: "Are epidemiologic studies worthless as an indication of trial results, or does "Nixon's Law" (timing is everything) prevail?"

Outline of presentation:

1. Review epidemiological literature suggesting protection with HT
2. Discuss possible reasons for contrast with trials data, e.g., inadequate control on SES
3. Another explanation: Timing of exposure in relation to onset of dementia, a late-stage phenomenon in the pathogenesis of AD. With proper consideration to this issue, Cache County data and the WHIMS data are entirely concordant.

Specific points presented:

- Cache County shows decreased risk of AD with HT which is time dependent on when hormones were used.
  - 0-10 yrs-HR 2.22
  - 3-10 yrs-HR 0.22
  - >10 yrs-HR 0.17
- Suggestion: HT is protective years before dementia but crosses over to RR of 0 at about 10 yrs before dementia

Dr. Diana Petitti: "Methodologic challenges in the non-experimental study of hormones and dementia"

Outline of presentation:

1. Proxy reports of estrogen use are unreliable (use is underreported)
2. Women with dementia/cognitive impairment do not accurately report their own current or past exposure to estrogen (use is under-reported)
3. Computer-stored prescription data can be used to mitigate these reporting biases in observational studies
4. Empiric results from a study based on computer stored data will be presented to illustrate studies consistent with data not only those that were different.

Specific points presented:

- 1944 women entered the study with estrogen use based on pharmacy records
- Classification based on telephone interview of cognitive status (TICS), and Dementia Questionnaire (DQ)
- Mean duration of E (29 yrs) or E+P (21 yrs), for women over 75
- Age at start for hormone use- 46-61 yrs of age
- Slightly higher relative risk (RR) for both types of therapy for all dementia:
  - E-1.26
  - E+P-1.46

DISCUSSION - LONGITUDINAL AND EPIDEMIOLOGICAL STUDIES

- Other trials that didn't work based on basic or epi studies: vitamin E, Beta carotene, low fat-high fiber;
- Socio-economic status (SES) must be considered in selection of subjects
- More observational studies are needed; Case-control studies may not answer the questions about dementia question

- Users of alcohol (1-3 drinks / day) have better memory
- Start ET early
- Long incubation time for dementia
- Underlying neuron health
- The question of oral contraceptive use is a black box: supraphysiologic levels of E in first pills commercially available
  - How can the findings of the Cache County study be reconciled with the Petitti Women's Memory Study?

IV. CLINICAL TRIALS

A] Prevention and treatment trials

Dr. Jacques Rossouw: "Hormones and CHD: Discrepancies between laboratory studies, observational studies, and clinical trials"

- Why the differences between observational data and clinical trials for cardiac events?
  - Healthier
  - During HT
    Compliance bias
    Those who take it are healthier
  - Early coronary heart disease (CHD) events are missed in cohort studies
- Primary prevention
  WHI
  WISDOM (Women's International Study of long Duration Oestrogen after Menopause)
  RUTH-raloxifene (Raloxifene [RLX] for the heart)
- Secondary prevention
  All negative for E, E+P
- Why the differences?
  Nurses Health Study, for example, were younger and were on cyclic MPA
- Why the difference between basic science and clinical studies?
  - Hormones may retard atherosclerosis if there is no or minimal atherosclerosis when women start therapy
  - Not helpful with raised lesions
  - Harmful with complicated lesions
- Conclusion: short term relief of menopausal symptoms will come at a small CAD or PE price
- Transdermal E2, unlike oral, does not increase triglycerides, etc.
- Future testing is likely to use:
  Transdermal E2
  Low dose

Dr. Steve Rapp: "Design and Analysis of the Women's Health Initiative Memory Study (WHIMS)"

Outline of presentation

1. Design and case ascertainment in WHIMS

2. Results of WHIMS for dementia, mild cognitive impairment and global cognitive functioning
3. What's next for WHIMS: The WHIMS-MRI study, Supplemental Case Ascertainment Protocol, Extended Follow-Up, mild cognitive impairment (MCI) Analysis
4. Critical analysis: MRI and supplemental case study

Specific points presented:

- Worse result for dementia and MCI on HT
- Modified mini mental status exam (MMSE) also worsened over time

Questions:

Why is there an increase in dementia?
Is the risk sustained over time?
What is the effect of withdrawal?

Dr. Sally Shumaker: "Implications of WHIMS"

Outline of presentation:

1. What do we know?
2. How did the design of WHIMS influence what was found.
3. What questions weren't answered.
4. What questions will be answered by studying other cohorts and what questions will never be answered.
5. What needs to be done?

Specific points presented:

- Further analyses
  - Refining mild cognitive impairment (MCI) category to amnestic
  - Does HT affect amnestic MCI?
- Supplemental case ascertainment protocol for follow-up on ~900 women who are deceased or not coming in using the Dementia Questionnaire
- Would standardize the instrument and allow phone collection of data
- MRI cross sectional study on 1450 women formerly in WHIMS to look for infarcts on HT
  - Secondary:
    Effect of different treatments on events
    Duration of treatment effect, etc
- Magnetic resonance imaging (MRI) scans to be done from 9/04-12/05
- WHIMS extension study-NHLBI funded
- Determine effect of cessation of HT on cognition and incidence of dementia and MCI
- Cognition in the study of tamoxifen [TAM] and raloxifen (Co-STAR), funded by NIA, still enrolling, uses same NP measures
- Testing the effects of HT on younger women using the simplified ascertainment.
- Weakness is the absence of a baseline measure
- Biases: selection
- Discussion:
  When do you randomize?

- Issues of dosage and timing.
- Optimization of design: genotypes and phenotypes- mood, menopause stage
- How far back do you go to find a cognitively normal woman?
- Use of neurologically intact cohort as a baseline
- Can't study the effects of HT and dementia in younger women-they don't have it.
- How do you know when women cross over into cognitive decline?
- HT exacerbated the underlying pre-existing DX
- What is the prevalence of subclinical dementia?
- What is the effect size expected for accelerating disease?

Dr. Mary Sano: "PREPARE: Preventing Postmenopausal Alzheimer's Disease with Replacement Estrogen"

Outline of presentation:

1. Justification of the trial, how it differs from WHIMS
2. Study design
3. Recruitment problems
4. Changes in protocol as a result of WHI/WHIMS
5. What answers does this study provide that other studies didn't / don't?
6. What is ongoing now?

Specific points presented:

- PREPARE
  - Like WHIMS but to prevent AD and memory decline in women 65 years or older with a positive family history of AD
- Current status;
  - All off meds
  - Statistical advisors and Data and Safety Monitoring Board (DSMB) recommended following them blinded for the full 5 yrs
- Possible results:
  - Tell us about incident MCI
  - Inform about stopping
- Data
  - N=466
  - MMSE 28.8
  - 29% apolipoprotein E (Apo E) 4 +
  - About 1/3 with hysterectomy
  - Age of onset of dementia in family members may predict onset of dementia

Dr. Ruth Mulnard: "Results of the ADCS Estrogen Treatment Trial for AD"

Outline of presentation:

1. Estrogen therapy was not effective in delaying progression of AD in hysterectomized women.

2. Estrogen therapy did not show stabilization or improvement of memory or any other specific cognitive domain.

3. An "unexplained" improvement in MMSE occurred in treated subjects at the 2-month time point, which was not sustained at the 12-month conclusion of the study.

4. Four cases of deep vein thrombosis occurred, representing significant risk for this subject pool, in the consideration of long-term estrogen administration.

Specific points presented:

- More decline on Global Clinical Dementia Rating (CDR) at either dose
- Slight separation on MMSE at 2 months for 0.625 mg dose
- No dose effect
- 4 deep vein thrombosis (DVT's) on E
- 4 baseline uteri missed

Dr. Sanjay Asthana: "Alzheimer's Disease: Efficacy of Transdermal 17 Beta-Estradiol"

Outline of presentation:

1. Discuss the biological advantages of using transdermal estradiol versus oral conjugated equine estrogen

2. Demonstrate that cognition-enhancing efficacy of estradiol is domain specific and propose sensitive neuropsychological tests for future studies

3. Discuss the potential impact of various progesterone preparations and cyclic versus continuous administration of HT on cognitive function of women with AD

Specific points presented:

- Patch uses 17 B estradiol (natural form of estrogen)
- Oral estradiol increases SHBG
- Ratio of estrone/estradiol goes from 1:1 to 5:1 with oral estrogens
- Estrone has much less affinity for the estrogen receptor
- In the post menopausal state, gonadotrophins are elevated CEE
- CEE contains
    - 45% estrone
    - Little 17β estradiol
- Of healthy aging studies, 86% on estradiol reported positive results in cognitive studies, 50% were positive on CEE
- Similar for AD studies
- Transdermal estradiol results in higher levels of plasma estradiol
- Oral estrogen increases prothrombotic activity
- Advantages of 17β estradiol
    - More potent
    - Lower incidence of DVT
- Better compliance
- Low dose study done (N=12)
    - Better on Stroop, better on Buschke
- High dose study:
    - Better on attention, Buschke, visual memory

Dr. Kris Yaffe: "The Effect of SERMs on Cognitive Function and Dementia"

Outline of presentation:

1. Review the basic mechanisms of Selective Estrogen Receptor Modulators (SERMs)
    - SERMS as estrogens and anti-estrogens
2. Present data on the effect of SERMs on cognitive function in older women
    - Prevention of MCI
3. Present data on the effect of SERMs on MCI and dementia in older women.
4. Ongoing studies with SERMS

Specific points presented:

- SERMS and cognition
- Raloxifene in MORE trial, (Multiple Outcomes of Raloxifen Evaluation)
    - No overall effect
    - For women over 70, better on TMT and Word list recall
    - For cognitive decline to 10th percentile, slight reduction in risk on TMTB and word list
- Raloxifene dementia study in MORE
    - 5386 women
    - 52 dementia
    - ~150 CIND
- On the 120 mg Raloxifene, but not the 60mg:
  33% dec in CIND
  48% dec in AD (NS)
  37% Dementia + CIND
- Lasofoxitne trial-negative so far
- Large RCT of arzoxifine
- Co-STAR
    - Tamoxifen (TAM) and Raloxifene (RLX) for dementia

Dr. Frank Miller: "Ethical framework for the design and conduct of clinical trials."
Outline of presentation:

A. Overview of an ethical framework for the design and conduct of clinical trials: 7 basic ethical requirements:

1. scientific/clinical value of the research;
2. scientific validity of research design;
3. fair selection of research subjects;
4. acceptable risk-benefit ratio;
5. independent ethics review;
6. informed consent;
7. protecting rights and welfare of subjects in the course of research.

B. Ethically significant differences between clinical trials and medical care

C. Issues relating to enrolling research subjects who may have diminished capacity to give informed consent.

DISCUSSION – CLINICAL TRIALS:

- Consider genotyping steroid receptor alleles; no data available on ER polymorphisms vs E levels; receptor polymorphisms that increase CRP, cytokines. Repositories of DNA for analysis
- NHLBI invites sharing of resources from funded studies: PIs should look for hypotheses arising from WHI. $35 M allocated to initiative.
- Consider the whole woman: bone, breast, uterus, adipose, CNS
- Consider combination of SERMS and E; TAM increase risk of stroke
- Consider dose and duration of treatment
- Consider neuroimaging component
- Were exercise and weight controlled for in clinical trials? There was no apparent interaction with BMI
- The WHI study groups need long term follow-up for durability of effect
- Conversion of E1 to E2; liver metabolism to cortisol
- Hypertension- no interactions with treatment
- Subgroups of large trials can be used to generate hypotheses
- Measure E levels; determine therapeutic doses and critical windows
- Proprietary information leads to clinical trials, but academic scientists do not have access to these data
- CEE- variability between lots can be a great as three fold
- Study aromatase inhibitors and cognition in monkey
- Can transgenic animal models of AD be used to advantage to study effects of E on neuropathology?
- Peripheral A beta and translational research-doesn't work well in human
- For multi-site MRI- the technology is not yet widely available and it is difficult to compare across sites

QUESTIONS ARISING FROM PRESENTATIONS AND PROBLEMS TO BE ADDRESSED:

1. Are plasma concentrations of steroids equivalent to concentrations found in brain tissue?
2. What are the windows of opportunity for administration of estrogen?
3. Oval versus parenteral administration have not been evaluated for efficacy of cognitive function.
4. Effects of cognition by various types of progesterone are not well studied.
5. The effects of varying doses of estrogen have only been studied in rodents, not in non-human primates.
6. More study needs to be done on the effects of steroids on attention as a measure of cognitive function.
7. Who would potentially stand to benefit (30 years from now!) from the early admin of hormone therapy?
8. What genetic factors may affect the way women respond to HT?
9. How can additional risk factors be analyzed with animal models to improve our ability to test factors that may impinge upon cognitive function (e.g. cardiovascular, diabetes, ischemia).
10. Should measures of stress be included in future analyses (e.g. cortisol)?

11. Are there early protective effects and are they enduring?
12. There are no data on the effects of estrogen on the coagulation cascade.
13. The WEST, HERS, and WHI had poor vascular outcomes; we need to look at vascular markers.'
14. Targets for therapy must include "critical windows"
15. The question of oral contraceptive use is a black box: supraphysiologic levels of E in first pills commercially available
16. How can the findings of the Cache County study be reconciled with the Petitti Women's Memory Study? Could both sets of data be right? Different populations, different exposures?

BROAD DISCUSSION OF THE MEETING AS A WHOLE

DATA and STUDIES NEEDED

- Mechanistic studies for why E+P causes harm
- **Not enough known to do a clinical trial now**
- Consider life styles in data acquisition (e.g. obesity)
- Do estrogens work only in healthy neurons?
- After identifying biomarkers in vitro and in vivo in animal models, can clinicians go to using surrogate and biomarkers in clinical trials
- Better delivery systems for E2
- Do studies need to be done using either cycling, infrequent and / or intermittent estrogen therapy?
- Is the difference due to the aging brain or menopause? Need to make young animals menopausal and study effects of hormones
- Need to confirm in additional models and humans whether E in young women and young rodents is helpful, but E in old women and old rats is harmful

DESIGNS

- Not ready for this yet

RESEARCH QUESTION, EFFECTS ON:

- Cognitive aging
- MCI
- Dementia

POPULATIONS TO STUDY

- Perimenopausal women
  - Parameters:
    MRI
    ? Pittsburgh compound-B (PIB) scan in a subset
    ? high risk women such as Apo E e4, other

POSSIBLE TREATMENTS

- Transdermal E + P

- Low dose E + P
- Testosterone + E
- E alone
- Blood brain barrier (BBB) crossing SERMS
- BBB non crossing SERMS + E

Recommendation that work groups be formed:

WORK GROUP Topics recommended:

MOLECULES

SUBJECTS

ENDPOINTS