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HORMONES IMPACT STRESS, MEMORIES, AND UNDERSTANDING SOCIAL CUES

Research reveals new roles for estrogen and finds potential biomarker for maternal stress

SAN DIEGO — Research released today demonstrates unexpected roles that sex hormones may play in the cognitive function of females, including memory and interpreting social cues. Additionally, a chemical identified in pregnant mice may provide insight into developmental disorders, such as schizophrenia. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Today's new findings show that:

- Maternal stress can reduce levels of a chemical in the placenta that influences many other functions, such as development in mice. Additionally, the chemical could serve as a biomarker for maternal stress, a known risk factor for neurodevelopmental disorders like autism and schizophrenia (Tracy Bale, PhD, abstract 380.08, see attached summary).
- Estrogen replacement therapy in post-menopausal women may help prevent stress-related memory loss (Alexandra Ycaza, MA, abstract 376.1, see attached summary).

Other recent findings discussed show that:

- Tamoxifen, a drug used to treat breast cancer, may protect against cognitive loss in post-menopausal women (Paul Newhouse, MD, presentation 376.03, see attached speaker summary).
- Estrogens, commonly thought of as a female reproductive hormone, are produced in the brains of males and females. In songbirds, estrogen may help process auditory social cues in both sexes and visual cues in males (Luke Remage-Healey, PhD, presentation 204.06, see attached speaker summary).

"Researchers are recognizing there are more differences between male and female biologies than originally thought," said press conference moderator Catherine Woolley of Northwestern University, an expert on hormones such as estrogen. "These new studies help to show how sex differences and the actions of hormones in the brain affect how we develop, respond to the environment, and how we age. Through understanding sex differences, we can improve the way biology informs medicine."

This research was supported by national funding agencies such as the National Institutes of Health, as well as private and philanthropic organizations. Find more information about hormones and sex differences in the brain at *BrainFacts.org.*

Related Neuroscience 2013 Presentation:

Minisymposium: **Rethinking Estrogen Action in the Brain**
Sunday, Nov. 10, 1:30—4 p.m., Room 28A

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Abstract 380.08 Summary

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Maternal Stress Associated With Chemical Changes in the Placenta

Animal study could lead to new biomarker for developmental disorders like autism

Maternal stress changes a specific protein in the placenta that may have implications for brain development and stress in offspring, according to new mice research by Tracy Bale, PhD, of the University of Pennsylvania School of Medicine. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"The placenta is more than just a filter that passes nutrients and oxygen to the developing baby," said Bale. "Our studies have shown that maternal stress can be passed to a baby through changes in the placenta, and more specifically through a single chemical. These findings in animals suggest a potential new biomarker related to a range of neurodevelopmental disorders in children."

In the animal study, researchers looked for a biomarker that would indicate conditions of maternal stress, which is a known risk factor for disorders like autism and schizophrenia. They found an enzyme (O-linked-N-acetylglucosamine transferase or "OGT"), which modifies other proteins involved in a wide variety of funtions, including development.

Neurodevelopmental disorders are twice as common in males as in females, and the researchers showed that placentas associated with male mouse pups had lower levels of OGT than the placentas associated with female pups. These levels of OGT in the placenta were even lower when the pregnant mice were stressed.

The researchers then studied genetically altered mice with a specific reduction in levels of OGT in the placenta to test its importance. When males from genetically altered placentas became adults, they were smaller and more sensitive to stress, similar to males from stressed mothers. This suggests that OGT is an important chemical in the development of the offspring and a potential predictive biomarker of maternal stress. Low levels of OGT were found to have a specific effect on male offspring, a key finding for neurodevelopmental disorders.

Research results also showed that human placentas have similar OGT expression patterns, with placentas from boys exhibiting lower OGT than placentas from girls. Ongoing studies will examine stress during pregnancy as a factor in regulating OGT expression, and take into account the potential of OGT as an important predictive biomarker for maternal stress.

Research was supported with funds from the National Institute of Mental Health.

Scientific Presentation: Monday, Nov. 11, 11 a.m.–12 p.m., Halls B–H

380.08, OGT is a predictive placental biomarker of early maternal stress

*C. L. HOWERTON, **T. L. BALE**; Animal Biology, Sch. of Veterinarian Med., Univ. of Pennsylvania, Philadelphia, PA

TECHNICAL ABSTRACT: Neurodevelopmental disorders including autism and schizophrenia have been highly associated with parental factors including maternal stress. The mechanisms through which these influences may contribute to disease development are not well understood and likely involve very complex interactions between the maternal milieu and the developing placenta. We have identified a sensitive period of early gestation where maternal stress (EPS) produces sex-dependent epigenetic programming effects on offspring stress pathway neurodevelopment. In this mouse model, we identified O-linked N-acetylglucosamine transferase (OGT) as a sex-specific and stress-sensitive placental biomarker of maternal stress. OGT, a key intracellular glycotransferase, with over 800 identified target proteins including RNA polymerase II and all four core histones, has great potential to have broad impact on chromatin state and key cellular functions within the placenta. To link this placental gene with an effect in re-programming the developing hypothalamus, we examined gene expression patterns at PN2 in mice with a placental-specific targeted reduction or deletion in OGT. Females hemizygous for placental OGT showed a dramatic shift in hypothalamic gene expression patterns compared to control females, supporting a critical role for this gene in placental function important in neurodevelopment. Adult mice with reduced placental OGT recapitulated key features of our EPS phenotype, with male mice demonstrating a dysregulated HPA stress axis and showing reduced adult body weights. Mechanistically, to determine what functional change a reduction of placental OGT produces, placental microarray analyses revealed an overlapping regulatory role of both EPS and reduced OGT of a post-transcriptional tRNA modifying gene, Trmt11. This finding was strengthened by an EPS induced increase of O-GlcNAcylated proteins at the promoter using ChIP. Additionally, ChIP-Seq experiments for both trimethylated histone 3 lysine 4 (H3K4me3) and O-GlcNAcylation of chromatin related proteins have identified increased O-GlcNAcylation at the promoters of specific genes associated with

male placental steroidogenesis, including Hsd17b3. Concurrently, embryonic metabolomics in response to both EPS and reduced placental OGT support important perturbations in nutrient availability and placental transport from the maternal to fetal compartment during gestation. These studies provide valuable insight into novel placental mechanisms contributing to sex-biased disease vulnerability to maternal stress exposure impacting the developing brain.

Abstract 376.1 Summary

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Estrogen Therapy Post-Menopause May Safeguard Against Memory Loss Caused by Stress

Younger women may have natural protection from stress and cognitive impairment

Estrogen treatment may help reduce memory impairment associated with stress in postmenopausal women, according to new research presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Our research indicates that estrogen may be a built-in protection from the effects of stress in women," said lead author Alexandra Ycaza, MA, from The University of Southern California. "This study shows that giving postmenopausal women estrogen may make them resistant to stress and associated memory impairments."

Researchers investigated the impact of stress on a type of short-term memory in women, and examined whether estrogen might have a protective function. The type of short-term memory examined was working memory, or the ability to remember things for a short time while engaging in an unrelated task. Exposure to stress decreases working memory.

In this study, researchers worked with 42 postmenopausal women with very low levels of estrogen, from the ELITE clinical trial at the University of Southern California's Health Sciences Campus, where they had been taking long-term estrogen replacement therapy or placebo for approximately five years. The women were asked to submerge their hands in icy water for three minutes, as a cold stress. After 20 minutes, researchers tested their working memory, asking them to remember lists of words while simultaneously reading sentences and making decisions on whether sentences were grammatically correct.

Women with the highest estrogen levels were able to remember more words while reading unrelated sentences and made decisions on grammar more effectively than those with lowest estrogen levels. In addition, estrogen helped reduce levels of cortisol, a stress hormone, 15 minutes after the stress test itself, when cortisol levels begin peaking. The results suggest that estrogen offers a natural protection from stress for women, helping fight off negative effects on working memory.

"Stress, whether at home or at work, can have negative effects on the body and brain, hurting and killing neurons and producing problems with mood, memory, and sleep," said Ycaza. "Discovering factors that might protect people from stress could have huge impacts on how people perform in their daily lives, from work to relationships at home."

Research was supported with funds from the National Institute on Aging.

Scientific Presentation: Monday, Nov. 11, 9–10 a.m., Halls B–H

376.1, Estrogen treatment after menopause protects against stress-induced impairments in working memory
***A. E. YCAZA[1]**, H. N. HODIS[2], W. J. MACK[3], M. MATHER[4]; [1]Dept. of Psychology, [2]Atherosclerosis Res. Unit, [3]Dept. of Preventive Med., [4]Davis Sch. of Gerontology, USC, LOS ANGELES, CA

TECHNICAL ABSTRACT: Estrogen can reduce the magnitude of the stress response. This has important potential implications given the many negative effects of stress hormone exposure, in particular glucocorticoids (GC; e.g., cortisol). For example, exposure to GC can damage or kill neurons and impair cognitive performance. The estrogen-GC relationship has particularly important implications for aging human females who experience rapid declines in estrogen during menopause, yet how this interaction may affect cognition has received minimal attention. We hypothesized that women on estrogen replacement therapy would show fewer negative effects of stress exposure on cognitive performance compared with non-treated, post-menopausal women. Our study examined the effects of stress application on working memory (WM) in post-menopausal women who were randomly assigned to take estrogen or placebo for an average of 4.9 years in a randomized, double-blind, placebo-controlled trial (ELITE trial). Women completed WM tasks in two sessions (one stress and one control; order counterbalanced). During each session, women (n=42) were exposed to either a stressor (cold-pressor task) or a control task and 20 minutes later completed a WM task (i.e., sentence span task). Multiple saliva samples were collected during each session to determine estradiol (E2) and cortisol levels. Cortisol analyses revealed a stress x time interaction; whereas cortisol increased from baseline during stress sessions it decreased over time during control sessions. Further, the increase in cortisol during the stress sessions depended upon levels of E2; the cortisol increase in response to the stressor was significantly greater for women in

the lowest quartile of salivary E2 levels (Low-E2) than for women in the highest quartile of E2 levels (High-E2). Stress in turn affected WM; overall, WM performance was worse during the stress than during the control sessions. However, E2 protected against the negative effects of stress on WM. High-E2 women did not differ in their WM performance between the stress and control sessions, whereas Low-E2 women performed significantly worse during the stress session than during the control session. This study provides novel evidence for mitigation of the negative effects of stress on cognition with estrogen therapy in postmenopausal women. Given that perceived high work-home pressures are associated with higher cortisol levels in middle-aged women, estrogenic protection against GC exposure may prove beneficial to both cognition and the neural circuitry that maintains and propagates those cognitive faculties.

Speaker Summary (376.03)

Speaker: Paul Newhouse, MD (615) 936-0928
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Tamoxifen Produces Estrogen-Like Agonist Effects On Cholinergically-Mediated Learning In Postmenopausal Women

Monday, Nov. 11, 10–11 a.m., Halls B–H

Changes in memory and attention after menopause may be in part related to the loss of estrogen following the menopause transition. Previous research has suggested that estrogen treatment may maintain or improve some aspects of cognition in postmenopausal women. Our laboratory and others have provided evidence that estrogen acts in part through the cholinergic system to support cognition by showing that estrogen reduces the effects of anti-cholinergic drugs on attention, verbal learning, and memory in postmenopausal women. Degeneration of the cholinergic system is implicated in pathological cognitive aging. Estrogen may benefit cognition through neuroprotection and maintaining plasticity in brain areas that are responsive to cholinergic input (such as the hippocampus), and by directly supporting cells that produce acetylcholine.

However, there are controversies regarding estrogen treatment in women due to the increased risk for certain cancers and vascular disease. Selective estrogen receptor modulators (SERMs) are compounds that have different effects depending on the tissue type, and may provide cognitive benefits similar to estrogen with fewer peripheral side effects. Tamoxifen is a SERM that is commonly used to prevent breast cancer recurrence. Tamoxifen has anti-estrogen effects in breast tissue but may have pro-estrogen effects in brain. As women survive breast cancer and live into old age, it will be important to understand the cognitive effects of tamoxifen use in postmenopausal women. Additionally, tamoxifen or other SERMs may provide an alternative to estrogen for maintaining cognitive function as women age.

This study examined how tamoxifen affected cognitive function in postmenopausal women using a drug-induced model of the cognitive and brain changes that occur in late life, by blocking the effects of the neurotransmitter acetylcholine. We hypothesized that tamoxifen would act like estrogen. While being given drugs that block acetylcholine in the brain, women who had taken three months of tamoxifen (vs. placebo) would perform better on memory, attention, spatial, and learning tasks.

The participants in the study were healthy, postmenopausal women, who had not taken any form of hormone replacement within the year previous to study enrollment, and had no history of breast cancer or cognitive impairment. The women were genotyped for the APOE gene, of which one variant (APOE4) can increase the risk for Alzheimer's disease. Twenty-one postmenopausal women were randomly assigned to take three months of tamoxifen before or after they received three months of placebo. After each treatment phase, the women participated in drug challenges with agents (scopolamine and mecamylamine) that block receptors for acetylcholine in the brain or placebo. On each study day, the participants completed tasks that test memory, attention, learning, and spatial abilities.

The anti-cholinergic drugs impaired performance on all of the cognitive tasks as expected. However, after three months of tamoxifen treatment, women showed significantly smaller impairments on attention, verbal memory and spatial navigation than when they had taken three months of placebo. Tamoxifen thus protected against the negative effects of the anti-cholinergic drugs on memory and spatial navigation. In addition, the effect of tamoxifen was especially beneficial for women who were positive for the APOE4 allele.

The hippocampus is a part of the brain that is important for both memory and spatial performance, and has receptors for acetylcholine and estrogen. In previous studies, estrogen has been shown to support hippocampal function. This study provides evidence that tamoxifen may have effects similar to estrogen in protecting cognitive abilities that rely on the hippocampus in postmenopausal women. Further work is needed to determine the full cognitive effects of tamoxifen, however the results of this study suggest that tamoxifen or other SERMs may support the cholinergic system of the brain and provide cognitive protection in normal postmenopausal women.

Research was supported with funds from the National Institutes of Health.

Speaker Summary (204.06)

Speaker: Luke Remage-Healey, PhD (413) 545-0772
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Beyond Reproduction: Changing Brain Estrogens Rapidly Alter Sensorimotor Representations And Behaviors

Minisymposium: *Rethinking Estrogen Action in the Brain*
Sunday, Nov. 10, 1:30–4 p.m., Room 28A

Estrogens are no longer just reproductive hormones. When you get inside the brain, the old rules about estrogens being a 'female hormone' are tossed out the window. In fact, estrogens are synthesized by the brain itself, and our research shows that they can have rapid and local actions in males and females alike.

We have developed a new way to measure brain estrogens in vivo, and study their rapid and local fluctuations and actions. Some of our recent findings can help explain why estrogens have been associated with improved cognition, learning and memory. This technology has been developed in songbirds, which are particularly well-suited to neurobiological/hormone experiments.

Based on prior experience, we might expect that estrogen levels are much higher in the female brain than in the male brain. In fact, estrogen levels are approximately equal in males and females at baseline. What's more, the levels are changing in auditory cortex in response to stimuli in both sexes. When males and females hear socially-relevant sounds their estrogen levels increase. There is also sex-specificity: males alone exhibit rapid increases in brain estrogens when they are exposed to socially-relevant visual stimuli. In both sexes, estrogens have rapid actions on the patterning of neurons in sensory cortex and their ability to transmit information.

In summary, the role of estrogens as neuromodulators in brain circuits is just now emerging. Estrogens appear to fluctuate inside the brain, independent of plasma levels, and these fluctuations can occur in sex-specific ways. At the same time, our work shows that the role of estrogens to enhance sensory representations is evident in both males and females. It is possible that future estrogen therapies that are brain-specific could be useful as treatments for some neurological diseases. This work is supported by the National Institute of Neurological Disorders and Stroke and the University of Massachusetts.