Estrogen Stakes Claim to Cognition

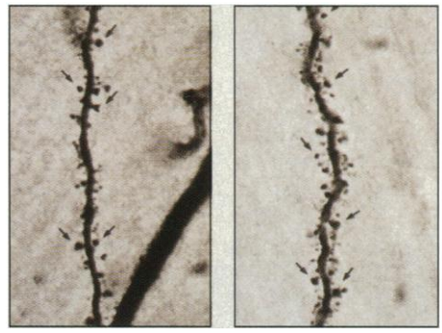
Recent work is showing that the female hormone estrogen has many effects on brain neurons that could give it the ability to improve such higher mental functions as learning and memory

Over the years, the so-called "female" sex hormones have come in for their share of scorn. One notorious example was in 1970 when Edgar Berman, personal physician to former Vice President Hubert Humphrey, declared that women were unfit for many jobs because of their "raging hormonal imbalances." But recently one female hormone—estrogen—has begun to reveal some unsuspected talents: an apparent ability to preserve and even improve some of the brain's highest functions. "We used to think [estrogens] influenced only sexual behavior; now we know they also influence learning and memory," says one pioneer of the work, neuroendocrinologist Victoria Luine of Hunter College of the City University of New York.

Estrogen has won this new respect through a wealth of cellular and molecular studies showing that the hormone can empower brain cells involved in thinking in many ways: It boosts the cells' chemical function, spurs their growth, and even keeps them alive by shielding them from toxins. Earlier work had shown that estrogen stimulates nerve-cell growth in the brains of developing embryos, but until recently, no one realized that the hormone could exert similar power over cognitive portions of the adult brain. Now, says Phyllis Wise, a reproductive endocrinologist at the University of Kentucky in Lexington, "There are clear-cut, basic science data showing a biochemical substrate for a memory effect."

What's more, recent work suggests that estrogen's neuronal effects have functional consequences. Human epidemiological studies and small clinical trials indicate that the hormone improves memory in both healthy women and female patients with Alzheimer's disease, and may even stave off that disease if given to women after menopause (see sidebar). If confirmed, such findings will not only help secure estrogen's status as a memory molecule, but also may lead to better treatments and prophylactics for Alzheimer's disease and possibly normal, age-related memory loss as well. And even men may benefit if researchers can design appropriate drugs that don't cause feminization or other unwanted side effects. Studies suggest that the male brain is sensitive to estrogen, which a brain enzyme in men synthesizes from testosterone.

The first hints that estrogen might affect cognition came 2 decades ago, although the investigators didn't realize it at the time. While studying how the hormone might control reproductive behavior, Luine, then working with Bruce McEwen at Rockefeller University in New York City, gave estrogen to female rats in which the ovaries had been removed, then looked for changes in brain areas thought to govern reproduction. Among other things, the researchers saw an increase in levels of an enzyme called choline acetyltransferase (ChAT) in certain neurons of the basal forebrain.



WOOLLEY *ET AL.*, THE JOURNAL OF NEUROSCIENCE 17 (5), 1848 (1997)

Building better neurons. Estrogen-deprived brain neurons *(left)* have fewer synapse-forming spines than do neurons from animals that have received estrogen treatment.

Because ChAT makes acetylcholine, the chemical those neurons use to communicate with other nerve cells, it looked like estrogen might be revving up activity in the basal forebrain cells. But because the part of the basal forebrain Luine and McEwen examined was not widely known to be involved in learning and memory, the researchers didn't make the connection to cognition.

Then, in the early 1980s, Luine stumbled across a review article describing a massive loss of acetylcholine-releasing neurons in the basal forebrains of patients with Alzheimer's disease. She realized that these neurons must play a role in cognition—and that estrogen might have a therapeutic effect in Alzheimer's disease.

What's more, Luine did further animal studies that linked estrogen changes to two brain areas more commonly associated with memory and learning: the hippocampus and cerebral cortex. Because basal forebrain neurons send long projections called axons to both areas, she reasoned that the extra ChAT produced in the basal forebrain in response to estrogen could reach the hippocampus and cortex through the basal forebrain axons. And in fact, Luine found that ovariectomized female rats given estrogen did have more ChAT enzyme in the hippocampus and frontal cortex than control animals did.

Molding the brain

Within a few years, Catherine Woolley, Elizabeth Gould, McEwen, and their Rockefeller colleagues uncovered another way in which estrogen might act on neurons involved in learning and memory: by helping to build and maintain synapses, the specialized structures through which one neuron communicates with another.

Synapses form at points of contact between axon endings and tiny branches, called spines, that jut out from the shorter neuronal projections known as dendrites on the target cell. In the 1970s, Dominique Toran-Allerand, of the Columbia College of Physicians and Surgeons in New York, had shown that estrogen stimulates the sprouting of axons and dendrites from developing mouse neurons in cell culture, but it was not supposed to affect adult neurons.

Yet, the Rockefeller team found that depleting adult female rats of estrogen by removing their ovaries caused a loss of spines from certain hippocampal cells. By contrast, ovariectomized rats that received estrogen injections had hippocampal cells with almost the same number of spines that rats with ovaries had. The study "opened up our thinking about what hormones could do in an adult animal," says Luine.

Two new studies now have suggested that the estrogen-induced dendritic changes actually affect neuron function, by linking them to a molecule that plays an important role in cognition. This is the NMDA receptor, a membrane protein that detects incoming signals from the neurotransmitter glutamate. In the first study, completed late last year, John Morrison and Adam Gazzaley at Mount Sinai School of Medicine in New York, working with Nancy Weiland and McEwen at Rockefeller, found 30% more of the protein in certain hippocampal neurons from ovariectomized female rats treated with estrogen than in the cells of untreated animals. The increase was concentrated in the same hippocampal region where the Rockefeller team had found an increase in spines.

And just last month, Woolley and Philip

Estrogen: A New Weapon Against Alzheimer's?

Back in the mid-1980s, a pioneering young doctor named Howard Fillit unwittingly rekindled true love between a poet and his girlfriend—an 80-year-old woman named Elsa who had Alzheimer's disease. Before Fillit treated her, Elsa was quiet, apathetic, and unable to learn pairs of words, even after seeing them dozens of times. Afterward, she was much more alert, talkative, and able to remember the words after just a few trials. The treatment that brought on this transformation was estrogen, and its effects won this woman not only a new mind but also a marriage proposal from her boyfriend.

Fillit, who was then at Rockefeller University in New York City, had begun a pilot study of estrogen after one of his colleagues there, Victoria Luine, found laboratory evidence that the hormone protects the neurons that deteriorate in Alzheimer's. He found that besides Elsa, two of the six other women in the study also showed gains in their cognitive skills. But when Fillit applied for funding for further studies of estrogen's efficacy in Alzheimer's disease, several agencies, including the National Institutes of Health, roundly refused—on the grounds, he recalls, that his applications had "no scientific merit." Now, barely a decade later, the possibility that estrogen might help Alzheimer's patients has become one of the hottest topics in the field.

At least five small treatment trials have replicated Fillit's findings, and other studies suggest that the hormone might prevent or delay the onset of Alzheimer's in women. Men also could benefit, if analogs of the hormone that lack its feminizing properties can be developed. And while experts caution that these human studies aren't definitive, a flood of other work has shown that estrogen has neuronal effects consistent with a role in cognition (see main text). As a result, Alzheimer's researchers are becoming "cautiously optimistic" about estrogen's ability both to treat and stave off dementia, says Victor Henderson, a neurologist at the University of Southern California School of Medicine.

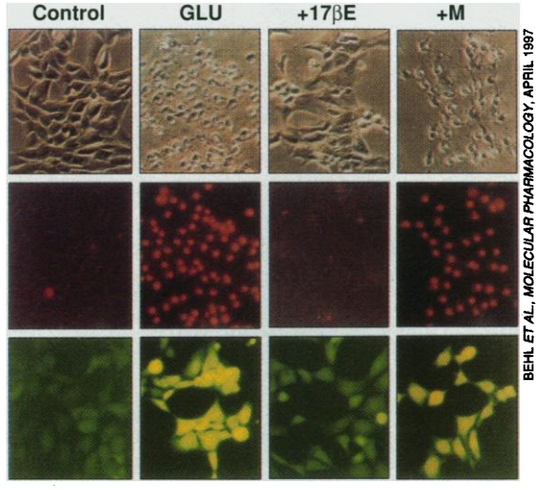
Among the recent clinical trials engendering this optimism is the first double-blind, controlled study in which Alzheimer's patients received the standard estrogen dose given to postmenopausal women in the United States. Study leader Sanjay Asthana, of the Veterans Affairs Medical Center in Tacoma, Washington, reported the following at last year's annual meeting of the Society for Neuroscience: He and his colleagues found that five of six women who had mild Alzheimer's disease and wore an estrogen patch for 2 months showed noteworthy improvements in verbal memory and attention. For example, from a list presented to them 20 minutes earlier, they could remember an average of twice as many words as they could at the start of the study. "We found a direct relationship between the level of estrogen in the blood and improvement in memory," says Asthana. The effect disappeared when the treated women went off estrogen, however.

Schwartzkroin, now both at the University of Washington, Seattle, along with Rockefeller's Weiland and McEwen, determined that the additional NMDA receptors fostered by estrogen are active in transmitting neuronal signals. After first confirming that estrogen replacement induces a 30% rise in both NMDA receptors and spines in the hippocampuses of ovariectomized female rats, the researchers electrically stimulated hippocampal neurons under conditions in which only NMDA receptors are active. They found that neurons from estrogen-treated rats responded to this stimulation with larger currents than did neurons from control rats. "That says that the new spine synapses are mainly NMDA-type synapses," McEwen notes.

The growth-factor theory

But how might estrogen induce the nerve cell growth needed for such synapse formation? Work done over the past few years by Toran-Allerand's group suggests one possibility: The hormone may cooperate with neurotrophins, potent stimulators of nerve-cell growth, such as nerve growth factor (NGF). In 1992, for example, Toran-Allerand and her colleagues found receptors for both estrogen and the neurotrophins on the same neurons in the rodent basal forebrain. A year later, they found the same receptor pairing in neurons in the cerebral cortex and hippocampus.

But the functional significance of this finding didn't become clear until 1994, when the Columbia team discovered that estrogen increases the expression of NGF receptors in cultured rat cells and, conversely, that NGF enhances the binding of estrogen to the same cells. This hinted that each type of molecule may act in the cell nucleus to boost the expression of the other's receptor, allowing estrogen and NGF to amplify each other's growth responses.



BEHL *ET AL., MOLECULAR PHARMACOLOGY*, APRIL 1997

Combating oxidative damage. Treating cultured neurons with glutamate damages them, as indicated by the large number of dying cells (red stain) and free-radical buildup (yellow stain). Adding an estrogen (17-βE), but not a derivative (M) lacking the hydroxyl on the steroid ring, protects against the glutamate-induced damage.

More recently, Toran-Allerand has taken this work further with results suggesting a possible new mode of action for estrogen. The hormone is supposed to exert its effects by forming a complex with its receptor, which then helps turn on certain genes in the cell nucleus. Such a mechanism could account for the hormone's ability to increase the synthesis of the NGF receptor and also that of the ChAT enzyme.

But at last year's Society for Neuroscience meeting, Toran-Allerand's group presented evidence indicating that estrogen also co-opts NGF's own growth-stimulatory pathway. In tissue slices from the cerebral cortexes of developing rats, the researchers found that estrogen activates a key class of molecules in the NGF signaling pathway: cytoplasmic enzymes called extracellular-signal regulated kinases that help relay the NGF signal from its receptor to the nucleus. In that way, estrogen could regulate many more genes than anyone thought it could. But even though this flies in the face of the current dogma on estrogen action, neuroscientists, including James Simpkins of the University of Florida, Gainesville, are warming up to the idea. "It's clear that many effects of estrogen do not involve what we learned in school about the estrogen receptor," he says.

While Asthana's results are encouraging, experts say they need to be confirmed in larger studies, such as a multicenter trial sponsored by the National Institute on Aging, which will include 120 women with Alzheimer's disease. Those results are expected in 1999. Meanwhile, other studies are strengthening the case that the hormone can also slow the development of Alzheimer's.

VETERANS AFFAIRS MEDICAL CENTER

Will estrogen help? An Alzheimer's patient takes a cognition test.

That case began building in 1994, when an epidemiological study by Henderson and his colleagues suggested that estrogen use by women may lower their risk of Alzheimer's disease by at least 45%. These results were open to question because they relied upon death certificates for the diagnosis of Alzheimer's; however, in the past year, at least two large trials in which dementia was diagnosed by experts during the trial have tied estrogen use to a diminishing risk of Alzheimer's.

One of these comes from a team led by Richard Mayeux of the Columbia College of Physicians and Surgeons in New York City. At the start of this study, the researchers questioned 1124 elderly women, all of them mentally healthy at the time, about their estrogen use. The women were then followed for 1 to 5 years. At the study's end, only 6% of the 156 estrogen users had developed Alzheimer's disease, compared to 16% of the 968 women who had never taken the hormone. Moreover, estrogen users who did develop dementia did so significantly later. The researchers calculated that taking estrogen for more than a year would lower a woman's risk of developing Alzheimer's by up to 5% annually. "From the newest data, it looks like estrogen might benefit women by delaying things," says epidemiologist Walter Kukull of the University of Washington, Seattle.

Still, to be confident about that, experts would like to see similar results from more prospective studies such as Mayeux's. Better yet would be a positive outcome from the first randomized intervention study of estrogen's ability to stave off Alzheimer's disease. This effort, part of the government-sponsored Women's Health Initiative, will test the mental acuity of 8000 elderly women each year for 6 to 9 years, to find out whether the Alzheimer's incidence is lower in women given estrogen than in those given a placebo.

If it is, there will be a push to devise a treatment that can mimic estrogen's properties in the brain without producing growth elsewhere—an action thought to lead to reproductive cancers in women and feminizing effects in men. In the meantime, doctors may recommend estrogen to postmenopausal women who don't want to risk cancers to bolster their bones or their hearts—but will take that chance to safeguard their brains. **–I.W.**

Chemical shield

Indeed, estrogen seems to have yet another trick up its sleeve. Through a mechanism involving neither its own receptor nor the growth factor–signaling pathways, the hormone can directly protect brain cells from toxins. The first hints of this shielding effect emerged from experiments that Simpkins and his Florida colleagues performed in 1994. Normally, 80% to 90% of human neuroblastoma cells, which were derived from a cancer of the peripheral nervous system, die within 2 days when placed in culture fluids lacking blood serum, which contains factors essential for their growth. But the Simpkins team found that estrogen prevents this cell death.

What's more, the protective effect seemed to have little to do with the estrogen receptor: It occurred in other cell types that lack the receptor, and various forms of estrogen—which have different affinities for the estrogen receptor—were equally good at keeping the neurons alive.

So how might estrogen be protecting nerve cells? Recent data show it can act as an antioxidant, soaking up highly reactive molecules called free radicals, which can kill a cell by fracturing its membrane lipids, proteins, and DNA. In 1995, Christian Behl and his colleagues at the Max Planck Institute of Psychiatry in Munich, Germany, reported that high estrogen concentrations reduce the neuron-killing effects of several toxins that boost production of free radicals. Among these are glutamate, a neurotransmitter that is toxic to cells in high concentrations, and β amyloid, a protein that accumulates in the brains of Alzheimer's patients and is thought by some to be a cause of their neuronal degeneration.

Simpkins's team has confirmed Behl's results, using lower estrogen concentrations similar to those found in the body. "Our data clearly indicate that estrogens, at physiologically relevant concentrations, eliminate most of the oxidation" in a cell that results from its exposure to β amyloid, says Simpkins.

Memory medicine?

Researchers are now amassing evidence that estrogen's cellular effects improve mental function. Experiments in female rats and monkeys have linked high blood levels of estrogen to better performance on cognitive tasks thought to involve the hippocampus, where the hormone stimulates synapse formation. And a few studies in people have also supported the estrogen-cognition link.

For example, psychologist Barbara Sherwin of McGill University in Montreal studied 18 women in their 30s who were being treated for fibroid tumors of the uterus with a medication that suppresses estrogen production. The women's verbal memory scores dropped after they began taking the drug. The decline was then reversed in women who received 8 weeks of estrogen-replacement therapy, but not in those who got a placebo. The results, Sherwin wrote last year in the *Journal of Endocrinology*, "strongly suggest that estrogen serves to maintain verbal memory in women."

Such results, together with the mounting data on estrogen's beneficial effects in Alzheimer's, are spurring investigators to develop drugs that might bolster brain function without promoting reproductive cancers in women (one side effect of estrogen therapy) or feminine characteristics in men. Indeed, the Behl and Simpkins teams have already discovered a clue that may aid in the design of drugs that imitate estrogen's antioxidant effect.

In the April 1997 issue of *Molecular Pharmacology*, Behl's group reports that rodent hippocampal neurons exposed to estrogens and various related steroids were protected against β amyloid and glutamate only when the steroid had a hydroxyl group dangling from a particular place on one of its molecular rings. Simpkins independently reported similar results at last year's meeting of the Society for Neuroscience and is already working with medicinal chemists to synthesize neuroprotective estrogens that lack the hormone's other effects.

Finding a new estrogenlike drug that works well in the body, however, may require an even more detailed understanding of estrogen's maneuverings through neurons. It's still not clear, for example, precisely how estrogen helps neurons skirt death by oxidation, or exactly what molecular steps it takes to induce neuronal sprouting, or even which other molecules might influence its encounter with its nuclear receptor. "We're very

early in the game, even on a basic research level," Simpkins warns.

Still, many researchers believe that estrogen will soon reveal its other mind games, given the army of investigators assigned to its case. "It's going to happen very rapidly," says McEwen. And as it does, a hormone once considered a key only to reproduction may open new doors to our brains and keep us mentally sharp beyond our reproductive years.

–Ingrid Wickelgren

Additional Reading

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CELL BIOLOGY

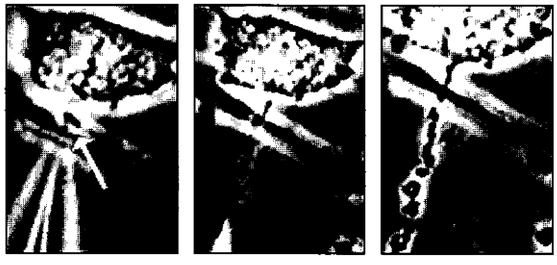
Force-Carrying Web Pervades Living Cell

"Don't fight forces; use them." The words of the engineer and architect R. Buckminster Fuller might turn out to be a motto for the living cell. Investigators have traditionally pictured the cell's cytoskeleton of protein fibers as mainly a supporting mechanism. Recent findings, however, have hinted that mechanical forces on the cell can affect everything from the way proteins bind to DNA to whether a malignant cell develops into a full-blown tumor. And now a team of cell biologists—inspired, in part, by Fuller's structural ideas—has demonstrated that mammalian cells are densely "hard-wired" with force-carrying connections that reach all the way from the membrane through the cytoskeleton to the genome.

The team, at Harvard Medical School and Children's Hospital in Boston, combined micromanipulation, video microscopy, and highly specific molecular "adhesives" to show that tugging on particular receptors at the surface of a living cell triggers nearly instantaneous rearrangements in the nucleus. The experiment, by Andrew Maniotis, Donald Ingber, and their collaborators, is being hailed as a triumph that required, among other things, "a really masterful use of reagents that have become available only in the last 5 years," says Stuart Newman of the New York Medical College in Valhalla.

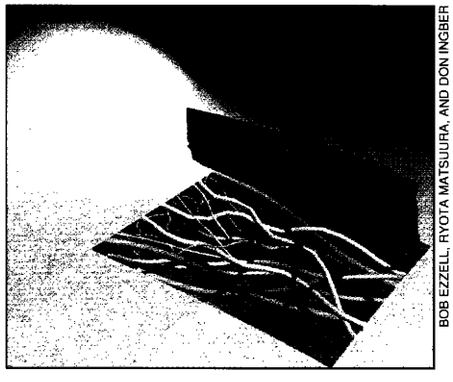
More important, he adds, "It puts the cytoskeleton in a new light: as a mechanism for signal transduction rather than just as a supporting mechanism." Although various researchers have suggested that cytoskeletal connections could transmit regulatory information through the cell, this direct demonstration "really puts [that conjecture] on the map," according to Newman. The mechanical communications system, if that's what it is, even extends through the nucleus, as the team found in a follow-up experiment in which it reached directly into the nucleus and plucked out structures such as individual chromosomes. They too were linked—by elastic strands of DNA. "It would suggest that everything in the nucleus is in fact connected," says Jeffrey Nickerson of the University of Massachusetts Medical Center in Worcester. "From my point of view, that's remarkable, and it's wonderful."

Other cell biologists, such as Zena Werb at the University of California, San Francisco, say that to establish the significance of what it has seen, the Harvard group still must show whether the connections are important in, say, regulating specific genes. But few researchers doubt that the results will raise the profile of mechanical forces in the cell. They are also likely to draw attention to the concept that inspired the experiments: the idea that the cell owes its shape and many of its properties to a "tensegrity" structure—a design principle described by Fuller.



MANIOTIS *ET AL.*

Making connections. A force-carrying network *(below)* extends from the cell membrane into the nucleus, where a micropipette pulls out linked chromosomes *(above).*



BOB EZZELL, RYOTA MATSUURA, AND DON INGBER

Tensegrity (tensional integrity) structures gain shape and strength by combining elements that resist compression with a network of other elements under tension, creating a "prestressed" system, explains Ingber. A bow used to shoot an arrow is one example, as are Fuller's own geodesic domes and the gravity-defying, strut-and-cable sculptures of the artist Kenneth Snelson. Ingber argues that the cell's internal skeleton shares properties with these structures, because it combines structural elements that resist compression, called microtubules, with others that are strong under tension—the actin microfilaments and the intermediate filaments.

Because tensegrity structures act as a force-carrying network, the model predicts that forces applied to cell surface receptors anchored to the cytoskeleton will quickly propagate into the cell interior. Using live human and cow endothelial cells, which line blood vessels, Maniotis, Ingber, and Christopher Chen set out to test this hypothesis.

First, they coated 4.5-micrometer beads with fibronectin, a protein that binds only to integrin receptors—cell surface structures moored to the cytoskeleton through the cell membrane. With a manually operated micromanipulation device, Maniotis then used a micropipette "like a golf club" to move the beads about 10 micrometers a second, while monitoring the cell with the video microscope.

As the group reported in the 4 February issue of the *Proceedings of the National Academy of Sciences*, the video microscope captured almost instantaneous movements and realignments of nuclear structures—dense structures called nucleoli suddenly lining up, for example, or moving toward the edge of the nucleus. Pulling on other membrane receptors that aren't linked to the cytoskeleton had no such effect, suggesting that the rearrangements were not caused by a "sausage-casing" effect of tensing the membrane.

"These studies are compatible with a prestressed cytoskeletal system, [which is] part of the tensegrity model proposed by Ingber," says Avri Ben-Ze'ev of the department of molecular cell biology at the Weizmann In-