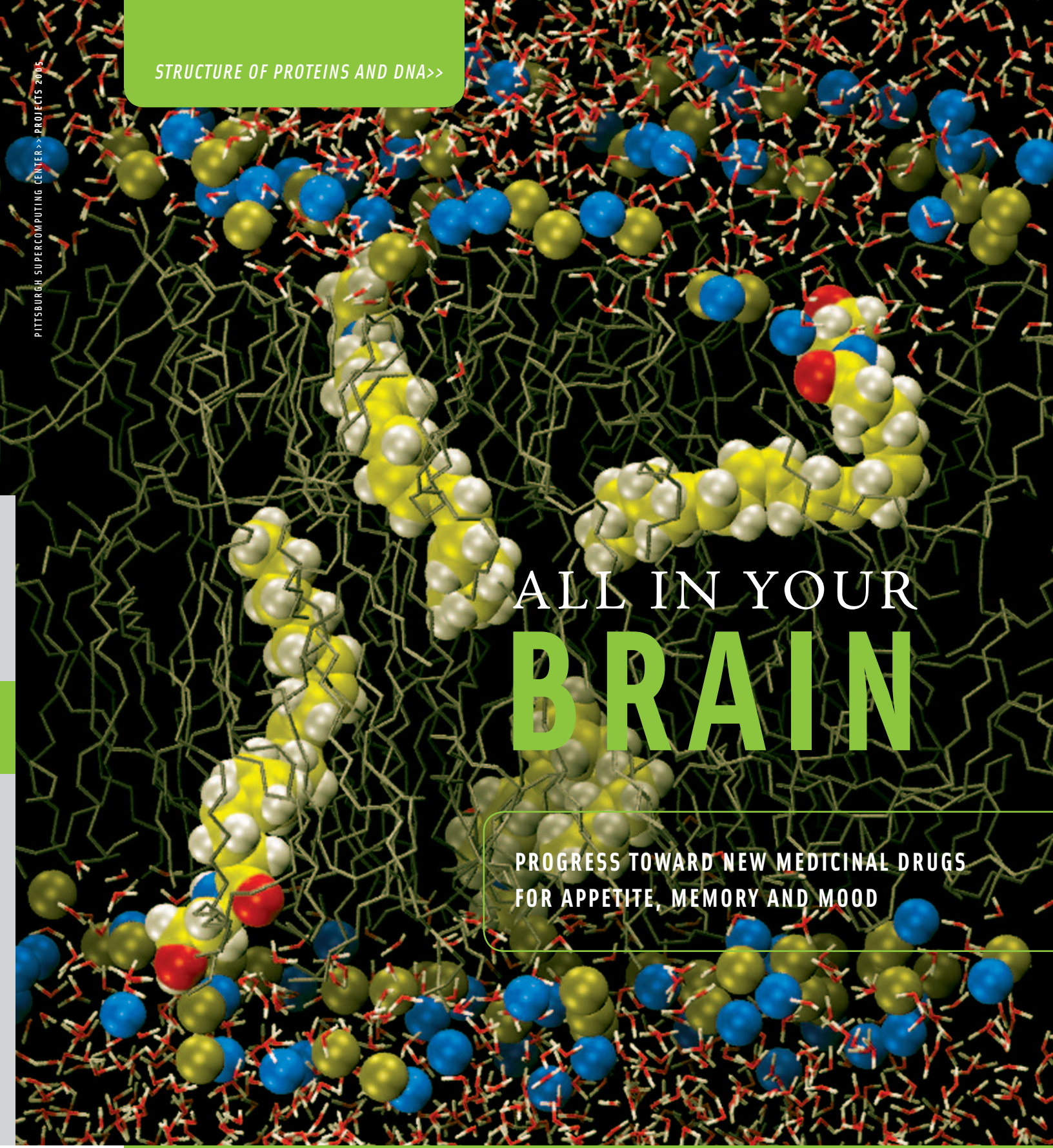


ALL IN YOUR BRAIN

PROGRESS TOWARD NEW MEDICINAL DRUGS FOR APPETITE, MEMORY AND MOOD

The model cell membrane for Lynch and Reggio's simulations was a bilayer sandwich of lipids – nitrogen (blue) and phosphorous (gold) heads facing outward on both sides of filamentary tails (gray). The membrane, embedded within water molecules (red & white), holds four anandamide molecules, with tails of carbon (yellow) and hydrogen (white) and heads containing oxygen (red). They used this model for preliminary simulations, without TMH6, to establish anandamide's arrangement, with its tail extended into the membrane core.

From left, **Patti Reggio**, University of North Carolina at Greensboro and **Diane Lynch**, Kennesaw State University

Your brain is loaded with receptors, billions of them. Each brain cell has thousands — molecules, usually proteins, like jigsaw puzzles with a missing piece. When another molecule of the shape and size to fit arrives, the receptor is activated, triggering a cascade of biochemical processes that, to name a few possibilities, can lift your mood, get rid of a headache or make you feel hungry.

The missing pieces, called ligands, can come from inside the body. Among these are the neurotransmitters — like the endorphins that produce "runner's high" or dopamine, which is involved in the so-called reward circuit associated with addiction. Other ligands, like nicotine or the active ingredients in aspirin, come from outside. Most therapeutic drugs have their effect by interacting with receptors, and much of drug research involves finding compounds of the right size and shape to activate receptors or to block them from other ligands.

Scientists postulated receptors in the first half of the twentieth century but didn't actually find them until 1972, when radioactive tagging led to finding the receptor for opium and related ligands. This spurred major efforts to find others, and in 1988 scientists found the brain's cannabinoid receptor — activated by tetrahydrocannabinol, the most active compound in marijuana, and called CB1.

Since the mid-80s, Patti Reggio, professor of chemistry and biochemistry at the University of North Carolina at Greensboro, has studied the cannabinoid receptor. When she started, scientists knew it existed and that it played a role in hunger, memory and appetite — making it a promising target for therapeutic drugs. But it wasn't until new tools came along — the ability to clone receptors — that scientists identified the amino-acid sequence of CB1. Even so, until recently scientists knew little about how ligands interact with CB1.

Reggio and her colleague Diane Lynch, a theoretical chemist at Kennesaw State University, used LeMieux, PSC's terascale system, to simulate part of CB1 interacting with a ligand called anandamide. Their results present the first picture of the atom-by-atom details of how a ligand initially becomes attached to CB1. This information, not available until now, is valuable to pharmacy companies searching for novel drugs that, for instance, could increase the appetite of chemotherapy patients or, as some have proposed, block painful memories associated with post-traumatic stress syndrome.

THE FIRST DETAILED PICTURE OF HOW THIS IMPORTANT RECEPTOR "GREETS" A LIGAND

A New Receptor Family

CB1 belongs to an important family of receptors, G-protein coupled receptors (GPCRs) — so named for the proteins with which they interact. "We now know," says Reggio, "that about 80 percent of all neurotransmitters and hormones work through GPCRs." Because GPCRs are involved in many pathological conditions — such as allergies, inflammation and depressed mood — they are the target of 40 to 50 percent of modern medicinal drugs.

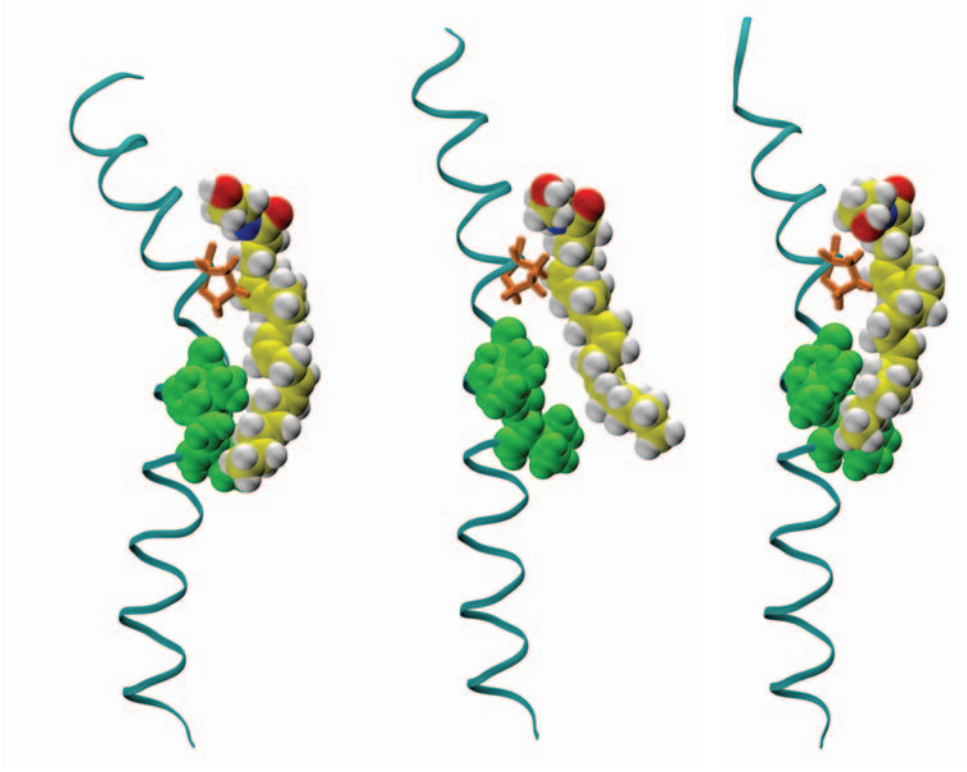
Densely clustered in areas of the brain associated with mood, memory, and appetite, CB1 is an unusual receptor in several ways. First, it's ancient on the evolutionary scale. A remarkably similar variation has been found in all organisms except insects. Second, it has the ability to interact with other brain receptors, dampening them by what Reggio calls a "mellowing effect." Although the mechanism isn't well understood, it's known, for instance, that CB1 activation can reduce the presence of dopamine. "CB1 as a direct drug target is interesting," says Reggio, "but as an indirect target it's even more interesting because it modulates other receptors."

CB1 is also unusual, even among GPCRs, in that its internal ligand, anandamide, is synthesized from lipid, a fat-like material in the cell membranes. Most ligands of GPCRs are water soluble and approach the receptor from outside the cell. The discovery of a lipid as the natural ligand for CB1 in 1992 added a fascinating new twist to CB1 research. "The whole lifetime of these ligands is connected to the lipid environment," says Lynch. "As a result, if you want to model CB1 and its ligands, you have to accurately describe lipids. That's a large computational task, hence the need for high-performance computing."

While CB1 in totality is a bundle of seven helices, the region that changes shape when CB1 is activated is in one helix, TMH6 (transmembrane helix 6). From a PSC biomedical workshop in 2001, Lynch learned to use software called VMD (Virtual Molecular Dynamics) to build a computational model. With VMD, she constructed the "lipid bilayer" sandwich that forms the cell membrane, added water molecules — to realistically represent the cellular environment — and inserted anandamide and TMH6.

With LeMieux, Lynch first "equilibrated" the model, nearly 16,000 atoms, with a series of calculations — allowing the molecules to adjust to each other. With a molecular dynamics (MD) program called NAMD2 — developed by Klaus Schulten's group at the University of Illinois Urbana-Champaign — she ran a series of MD simulations, which track the atoms as they move and how the molecules move and change shape as they interact with each other over time.

Lynch calculated the position and energies of each atom every femtosecond (a millionth of a billionth of a second). Each simulation covered six nanoseconds (six billionths of a second) of molecular activity — a long time in biological terms. Lynch repeated this simulation eight times, to track six different trajectories of the interaction and better close-in on a statistically meaningful picture. Using 64 LeMieux processors, it took two weeks of computing to generate 48 nanoseconds of data.



These three frames from the simulations show anandamide's hydrocarbon tail (white & yellow) inserted in the TMH6 groove, releasing and then reinserting, strong evidence that the two amino acids (isoleucine and valine, both green) which form a groove on the helix backbone (cyan) are CB1's "greeter" molecules, which initially recognize a ligand as it approaches.

In the Groove

The result — a short movie showing anandamide interacting with TMH6, short but loaded with information. In the opening scene, the tail of anandamide is recognized and "greeted" by two amino acids, side-chains on the TMH6 helical backbone, valine and isolecuine. They form a groove where anandamide briefly inserts, then breaks away, then reinserts — a sequence that appears to confirm that the initial recognition elements for anandamide/CB1 binding are in the TMH6 groove and that anandamide has the right shape to interact with this outward-facing lipid surface of CB1.

"This shows," says Reggio, "that anandamide is long enough in the membrane," says Reggio, "for its tail to reach the groove on the helix. It goes in and out of that groove and so is capable of touching the area of the two amino-acids and interacting with it." From this result, a collaborator of Lynch and Reggio — Zhao-Hui Song at the University of Louisville — did laboratory studies to confirm that the valine/isoleucine groove is the gateway by which anandamide and other cannabinoids attach to CB1 from within the cell membrane.

Perhaps more importantly, the movie suggests the signaling mechanism of the activation process. When anandamide interacts with TMH6, the helix "kicks out" to the side. "If you were sitting inside the cell and looking up at the full receptor," says Reggio, "you would see the receptor widen as a result. When it does this, it opens space for the G protein, which is sitting very nearby, to insert a segment into the bottom." The G protein changes shape and pieces of it come into contact with enzymes and other cellular components, sending a signal that starts the biochemical cascade.

A drug about to come on the market targets CB1. Sanofi-Aventis, the third-largest pharmaceutical company in the world, has developed Accomplia, a drug that reduces cravings for food and cigarettes by turning off CB1 in the hypothalamus. In the last stage of clinical trials, Accomplia may reach the market in 2006. Deeper knowledge of how CB1 interacts with ligands, such as provided by Lynch and Reggio's findings, will no doubt lead to further drugs.

For the next stage of their work with LeMieux, Reggio and Lynch plan to repeat their simulations with the entire seven-helix CB1. They also plan to look at how anandamide is destroyed in the cell membrane after it activates CB1. This will involve building a simulation cell containing fatty-acid amide hydrolase — the protein, says Lynch, that "chews up and spits out anandamide" — and will require five to ten times the number of atoms of the anandamide-TMH6 simulations, a much more demanding job for LeMieux.

"We're trying to figure out how something works that's going on in your brain right now," says Reggio. "That figuring out is fun for its own sake, and once you understand it, you can use that information to improve someone's life, to help them with a disease for which there's no drug at this point that works." (TP)

>>More information: **http://www.psc.edu/science/2005/brain**