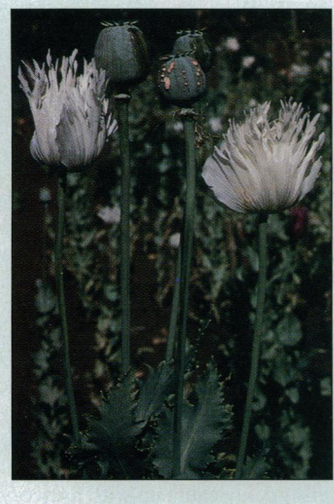
Pot, Heroin Unlock New Areas for Neuroscience

In today's world of crack babies and swaggering teenage drug lords cradling their automatic weapons, the drugs of the Swinging Sixties—marijuana for rebellious middle-class youth, heroin for hard-core addicts—seem almost quaint. But as the make-love-not-war era fades from memory, marijuana and heroin continue to hold an up-to-date place in the world of brain research. That's because, unlike cocaine and other drugs that act by interrupting brain processes, marijuana and heroin have a more intriguing modus operandi: Like keys that happen to fit locks they weren't designed to open, the active ingredients of these drugs trigger specific receptors in the brain.



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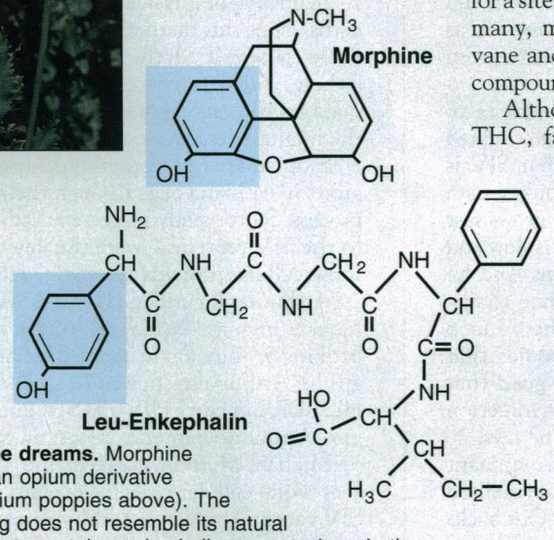


Pipe dreams. Morphine is an opium derivative (opium poppies above). The drug does not resemble its natural counterpart, leu-enkephalin, except where both bind the same receptor *(color)*.

Of course, those receptors didn't evolve millions of years ago just to wait around for someone to get high. But, then, what is their normal role? And what are the molecules that bind to them during ordinary brain function? This issue of *Science* contains papers that represent big steps forward in answering those questions for marijuana and opiate drugs, which include heroin and morphine. On page 1946 William Devane and Raphael Mechoulam of the Hebrew University of Jerusalem and their colleagues report the identity and structure of a natural brain molecule that binds the marijuana receptor. And two groups report this week that they have cloned the long-sought opioid-receptor gene. Christopher Evans, Robert Edwards, and their collaborators at the University of California, Los Angeles (UCLA), present their results on page 1952 of this issue of *Science*, and Brigitte Kieffer and her colleagues at the Ecole Superieure de Biotechnologie, in Strasbourg, France, report similar results in the current issue of the *Proceedings of the National Academy of Sciences*.

"Both of these findings are really important. They will lead to some very interesting science in the next half-decade," says Michael Brownstein of the National Institute of Mental Health (NIMH), a member of the team that in 1990 cloned the brain's receptor for tetrahydrocannabinol (THC), the active ingredient in marijuana. Drug researchers have high hopes that the two findings will help fulfill their wish lists, which include a more complete inventory of receptors for the two drugs, a better understanding of the roles the receptors play in normal brain, the ways they change during drug dependence, and how to target them with new therapeutic drugs.

To make that wish list come true, researchers need at least two tools: the gene for the receptor and the identity of the natural brain chemical, known as the endogenous ligand, that binds the receptor. In both the opiate and marijuana stories, one was missing. The endogenous ligands for the opioid receptor (a family of peptides known as enkephalins and endorphins) have been known for 15 years, but the gene for the receptor proved elusive. In the marijuana case, the THC receptor was cloned 2 years ago, but the endogenous ligand remained at large. With this week's discoveries, both gaps appear to have been filled.

Finding the cannabinoid ligand in the brain is "an enormous breakthrough," says neuroscientist Solomon Snyder of Johns Hopkins University, whose lab had a role in discovering the opioid receptor. "This has greater implications than just the study of marijuana," adds cannabinoid researcher Billy Martin of Virginia Commonwealth University. "We will be learning about an entirely new neurochemical system."

The first clue that such a system had to exist came when the cannabinoid receptor was identified in 1988. Before that, many researchers thought THC, a fat-soluble molecule, might not work through a receptor at all, but might insert itself directly into cell membranes. The discovery of the receptor nixed that idea and made it clear that there must be natural brain molecules that trigger the receptor.

Devane's involvement with the cannabinoid system began in 1988, when, as a graduate student with Allyn Howlett at St. Louis University, he proved the existence of the cannabinoid receptor. Not long after that, he started a postdoc with Mechoulam (who in 1964 had characterized THC as the active ingredient in marijuana) and started looking for the endogenous ligand. Since THC is fat-soluble, Devane decided to look for the ligand among fat-soluble compounds in the brain. He extracted brain chemicals and found one batch that had the ability to block a synthetic, THC-like molecule from binding the cannabinoid receptor—suggesting that this fraction contained an endogenous ligand, which was competing with the synthetic one for a site on the receptor. Two years—and many, many chemical steps—later, Devane and his colleagues had purified the compound enough to find its structure.

Although the natural ligand is, like THC, fat-soluble, the similarity stops there. THC has a complex ring structure; the endogenous ligand is a simpler molecule derived from arachidonic acid, a fatty acid common in cell membranes. Devane christened the new find "anandamide," from a Sanskrit word meaning—appropriately—internal bliss.

But is anandamide really our internal equivalent of THC? The dissimilarity of the two chemicals is not of major concern, says Devane, given the precedent set by the alkaloid chemicals heroin and morphine, which are worlds apart chemically from the endogenous opiate peptides. Still, it remained to be shown that anandamide not only blocks binding to the THC receptor, but in fact mimics the action of THC. To get such evidence, Devane and Mechoulam sent some anandamide to Roger Pertwee and colleagues at the University of Aberdeen, who found that it relaxes the smooth muscle of the mouse vas deferens, something THC is known to do. That's encouraging, notes Snyder, but "not totally ironclad," since other drugs are known to have similar effects.

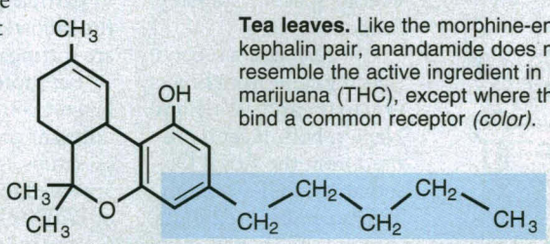
More recently, Devane got better evidence from Chris Felder and Eileen Briley at the NIMH, where Devane is doing another postdoc in Julius Axelrod's group. Felder and

Briley found that anandamide binds to cells that express the cloned cannabinoid receptor and inhibits cyclic AMP production in those cells—but has no effect on identical cells lacking the receptor. That has Snyder calling anandamide "the real McCoy."

But there might be other McCoys. Two other groups—Howlett's group at St. Louis University, and Steven Childers and his collaborators at Bowman Gray School of Medicine in Winston-Salem, North Carolina—are also hot on the trail of endogenous cannabinoids, and though neither group has identified its compound, it doesn't look like either is anandamide, since these compounds seem to be water-soluble, and anandamide is not. Most researchers say it would be remarkable if there are several endogenous cannabinoids that are chemically unrelated to one another.

While Howlett and Childers pursue those leads, the field is poised to run with anandamide. "What this opens up is the opportunity to try to figure out what [anandamide-making] neurons do in the central nervous system," says Brownstein. Until now, he says, people have been able to study the receptor, but they have had no way of finding the neurons that make the molecule that triggers it.

The discovery of anandamide also promises to infuse new energy into the search for drugs that have the therapeutic effects of marijuana (painkilling, antihypertensive, and antinausea actions, and the ability to lower eye pressure in glaucoma) without causing a high. "Pfizer had a very intensive program for cannabinoids, but ...we couldn't separate the pharmacological effects," says Lawrence Melvin, director of medicinal chemistry at Pfizer pharmaceutical company in Groton, Connecticut. The effects may truly be inseparable, if there is only one type of cannabinoid receptor, which causes them all. But many in the field believe there are other forms of the receptor and that new, synthetic cannabinoids modeled on anandamide may help to uncover the other receptors and develop drugs to target them.



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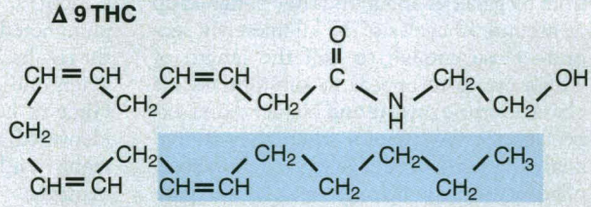
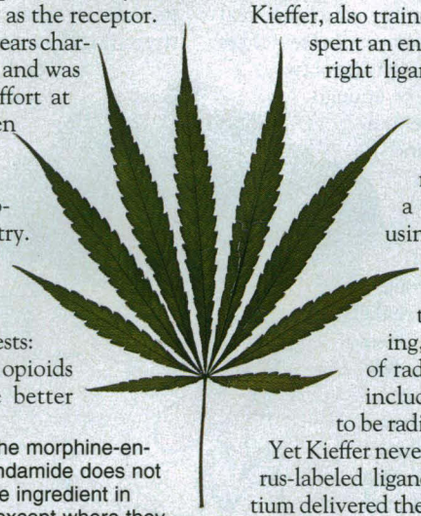


Anandamide

Similar hopes for drugs with selective action have driven opioid research, and those hopes are drawing new life from the long-awaited cloning of the opioid receptor. Since the discovery of the receptor and its endogenous ligands in the 1970s, studies of opioid binding and action have revealed that there are at least three types of opioid receptor, that some are involved in dulling pain, and that they belong to a family of cellular receptors that work through interactions with regulatory proteins known as G-proteins. With so much known about the opioid receptors, everyone expected they would be among the first G-protein-coupled receptors to be cloned.

But that's not the way the story turned out. Other receptors in the G-protein-coupled family were cloned in the early 1980s. Then researchers used the sequences of those genes to find related receptor genes, and by the end of the decade the number of G-protein-linked receptors that had been cloned exceeded 100. Among that number were receptors for well-known neurotransmitters and also some "orphan" receptors, whose ligands were not known. Conspicuously absent was the opioid receptor. "Everybody wondered where it was," says Robert Lefkowitz, who studies G-protein-coupled receptors at Duke University. And while they wondered, there were false alarms, including a variety of clones whose protein products bound opioids weakly, but not enough to mark them as the receptor.

Chris Evans had spent years characterizing opioid peptides and was a veteran of one failed effort at cloning the receptor when he teamed up with research associate Duane Keith and molecular biologist Edwards for another try. But where other teams had come up empty-handed, this group pulled out a clone that passed all tests: Its protein not only bound opioids tightly, but bound some better than others, with a selectivity that identified it as the receptor they had set out to clone—the delta-opioid receptor. Upon sequencing the gene, they found it had the hallmarks of a G-protein-linked receptor. To clinch the case, they showed that the binding of an opioid ligand to the cloned receptor triggers one expected change inside the cell: an inhibition of the enzyme that makes the cellular messenger cyclic AMP.



RUNK/SCHOENGERGER/GRANT HEILMAN

Tea leaves. Like the morphine-enkephalin pair, anandamide does not resemble the active ingredient in marijuana (THC), except where they bind a common receptor *(color)*.

Meanwhile, a similar story was unfolding in Strasbourg, where Kieffer, working with Christian Hirth, had independently settled on a strategy similar to that of the UCLA group, and, with colleagues Katia Befort and Claire Gaveriaux-Ruff, had come up with a clone for the same receptor.

In explaining their success where others had failed, both teams say a key element was the choice of radioactively labeled ligand for identifying cells expressing the receptor gene—and the fact that the choice of a ligand made it possible to overcome key technical problems. The technique requires a radioactive opioid peptide, but the process of labeling the peptides with radioactive phosphorus or iodine atoms often diminishes their ability to bind receptor. But "Chris is quite a good peptide chemist," says Edwards, who adds that Evans used his experience to choose a ligand that could be tagged with iodine and still bind well.

Kieffer, also trained as a chemist, says she spent an entire year developing the right ligand. She came up with two schemes, she says, one using a carefully engineered phosphorus-labeled peptide, and a long-shot alternative using a peptide labeled with tritium, the radioactive form of hydrogen. Tritium doesn't affect binding, but it has lower levels of radioactivity, and no one, including Kieffer, expected it to be radioactive enough to work.

Yet Kieffer never got to use her phosphorus-labeled ligand, she says, because tritium delivered the goods the first time out.

Kieffer and Evans haven't compared DNA sequences yet, but they are likely to be the same, since both groups started with a cell line that makes only the delta-opioid receptor. That sequence is "going to set off a stampede," says NIMH's Brownstein, whose lab was in the race to clone the receptor. "As soon as this sequence is published, people will compare the sequence to all their orphans. And anybody who's got one that looks pretty similar is going to test right away to see if it is an opioid receptor." And, he says, there is a good chance people will discover new receptor sub-types that had not been identified by the binding studies.

Then the hunt will begin to see where the different receptor types are expressed in the brain, what functions they regulate, and which ligands they bind best. On the biochemical front, researchers will begin mutating the receptors to understand what parts of their structure are crucial for their function. And that information could spur drug companies to re-open the quest for a nonaddictive, opiate-derived painkiller. "There is still a fantasy out there that you could make a

nonaddictive analgesic," says Brownstein. "If you had the entire family of receptors, and if you could learn which ones of those are really involved in mediating pain sensation...you could imagine trying to target [those] with a set of drugs very specific for them."

The cloning of the receptor also holds promise for progress on drug dependence. "Most biological systems adapt to the constant presence of a drug," says Evans, and after such adaptation, withdrawal is a painful process. Indeed, some theories suggest that drug addiction is merely the avoidance of withdrawal. Researchers have been trying for years to discover the changes in cells that account for adaptation to opiates—but with only limited success. The receptor clone will be an important aid in that search, enabling researchers to probe the receptor for chemical changes that may alter its behavior. Understanding those changes, Evans adds, may lead to better means of helping addicts cope with withdrawal.

Clinical payoffs like that will have to wait a while. Still, this week's findings in cannabinoid and opiate research have left researchers in both fields feeling not only optimistic about the future, but also in a state that can only be described as being, well, high.

–Marcia Barinaga

PHYSICAL SCIENCE

Survival of the Fittest in 1992's Physics and Astronomy Bestiary

Out on the frontiers of physics and astronomy some strange beasts pop in and out of sight. At first nobody knows which are real and which are just the mirages of researchers thirsty for discoveries. A sighting or two, or even a footprint, can be enough to get these objects or phenomena into the journals and conference presentations, but even then they remain in the shadows until some decisive experiment either dispels the cloud of doubt or makes them real.

Sometimes a claim is so outlandish that only a few other researchers will take the time to check it out for themselves. Other times scientists leap to the task: The sighting is vivid, or strongly predicted by theory. Even so, there's no guarantee that the beast—a supermassive neutrino or a missing quark—will make it into the physics bestiary; even after several confirmations by independent teams, these eagerly sought creatures can fade off into the mists again.

In 1992, a handful of not-quite-real creatures got a closer look. Some vanished, others solidified into real science, and a few remain in limbo, still embraced by their discoverers and ignored by everyone else.

A quantized stairway to heaven?

If University of Arizona astronomer William Tifft is right and galactic red shifts follow a "quantized" distribution, every textbook in physics and astronomy may end up in the trash. But astronomers have seen such revolutionary findings come and go, so they didn't react with much surprise when, earlier this year, a research group in Scotland backed up Tifft's claim and garnered some print in *The New York Times* and *Scientific American*.

Tifft first detected this periodic spacing of red shifts from galaxies back in the 1970s. The red shift of light from an object indicates its velocity away from Earth, the recession stretching the wavelengths toward the longer, red end of the spectrum. Unless something weird is going on, a big enough sample of galaxies should give a random distribution of red shifts. But Tifft noticed a bunching around values corresponding to multiples of 72 kilometers a second. If true, it implies that galaxies are arranged in some sort of evenly spaced, stairstep fashion.

This year a second sighting came from Bruce Guthrie and William Napier, both recently retired from the Royal Observatory in Edinburgh. They examined red shifts from 89 galaxies and found they bunched up in around 30 cycles of 37 kilometers a second—close enough to half the length of Tifft's cycles to appear to substantiate his claim. Guthrie says he and Napier didn't expect to see any pattern when they started analyzing a catalogue of galaxy red shifts compiled by various radio telescopes. "It was quite a surprise when we found it difficult to reject Tifft's hypothesis," says Guthrie. "It's a very strong effect," he adds; the probability that such a pattern would crop up by chance, he says, is around one in 10,000.

Prominent astronomers scoff at the suggestion. "It's just noisy data," says Joseph Silk of the University of California, Berkeley. The sample is too small, he says, to determine if a pattern like this is real. James Gunn of Princeton agrees. "The measurements aren't terribly good," says Gunn of the work. "If you look at a large enough body of data you will find strange things. There is a strong propensity to find what you want to find."

Guthrie agrees about that danger and calls for better data. But Gunn, Silk, and most other astronomers aren't planning to go looking for it. "We have [a lot of] crank science in our field," says Gunn. "It's easy to pooh-pooh this because there's so much of this kind of thing going on." Sure, he says, he could investigate, "but there are only 24 hours in the day." He says his strongest skepticism stems from the radical nature of the claim. "If Tifft is right, physics is wrong," he says. And if he were a betting man, he says, he'd go with physics.

The 17-kilovolt mistake

A year ago, particle physicists were flocking to any talk that promised news about a possible new particle known as the 17-kilovolt neutrino. With 1000 times more mass than anyone had reported before for the neutrino, the new particle threatened a major shakeup in particle physics and cosmology. But now the monster neutrino is dead, and physicists are sorting out why it once seemed so alive.

For more than a year, after all, it was the biggest controversy in particle physics. Four different groups, using different experimental setups, had sighted the same thing—what appeared to be a particle with a mass-energy of 17,000 electron volts (17 keV). In the last 6 months, though, the particle's credibility plummeted as other teams failed to duplicate the results. The fatal blow came last October, when one of its strongest backers, Andrew Hime of Los Alamos National Laboratory, identified a mistake in his original experiment that had led him to see a nonexistent neutrino.

Hime's original evidence came from the radioactive decay of sulfur-35; when he measured the energy of the decay products, he found they were missing a consistent 17,000 electron volts of energy, as if some unknown particle was carrying it away. That wasn't the first glimpse of a massive neutrino; John Simpson of the University of Guelph, in Canada, had spotted the same gap in energy spectra in 1985. But Hime's result brought quick confirmations by other workers, and by early 1991 the 17-kilovolt neutrino was all the rage.

ILLUSTRATIONS BY TERRY E. SMITH