A Low-Fat Theory of Anesthesia

Specific membrane proteins, rather than membrane lipids, may be the targets through which general anesthetics exert their effects

ALMOST 150 YEARS HAVE PASSED SINCE American dentist William Morton demonstrated that ether could be used to put patients gently—and reversibly—to sleep before surgery. Surgery without anesthesia quickly became unthinkable, but, surprisingly, researchers have not been able to work out a detailed explanation of how general anesthetics such as ether might work. The compounds were supposed to act nonspecifically by dissolving in the fatty membranes of nerve cells, thereby interfering with the cells' normal responses to stimuli. Now come biophysicists Nick Franks and Bill Lieb of the Imperial College of Science, Technology, and Medicine in London, with evidence promising a different, much more precise, idea of anesthetic action.

Their work suggests that general anesthetics operate not indiscriminately on membrane lipids, but instead on certain sensitive membrane proteins, perhaps those that form the ion channels that govern the responses of nerve cells. If Franks and Lieb are correct—and not all anesthesia researchers are convinced that they are—it would mean that anesthetic action is much more specific than previously thought. And that might open the way to the development of improved "designer anesthetics."

Improved versions are needed because today's anesthetics, while safe and effective, are difficult to use, says Franks, whose work is backed by anesthetic maker Anaquest of Murray Hill, New Jersey, part of the international BOC Group. "The overwhelming problem today is that anesthetics have to be very carefully controlled; they have very low margins of error," he explains. "They affect much more than just consciousness. The respiratory and cardiovascular systems are depressed, too. It would be nice to have compounds a lot less delicate to handle." If the anesthetics interact with ion channels or other well-defined membrane proteins, then the identification of those proteins might help researchers design new anesthetics with fewer hazardous side effects.

Back in the 1970s, when Franks and Lieb first began working on anesthetics, they had no intention of challenging the well-entrenched "lipid hypothesis." Instead, they hoped to flesh it out. The hypothesis had its origins in work done in Germany in the 1890s by chemists Hans Meyer and Ernst Overton, who showed that the potency of anesthetics correlates with their fat solubility, as measured by their ability to dissolve in olive oil. This finding led later researchers to argue that the agents work by dissolving in nerve cell membranes, which are rich in fatty components, somehow modifying nerve cell behavior.

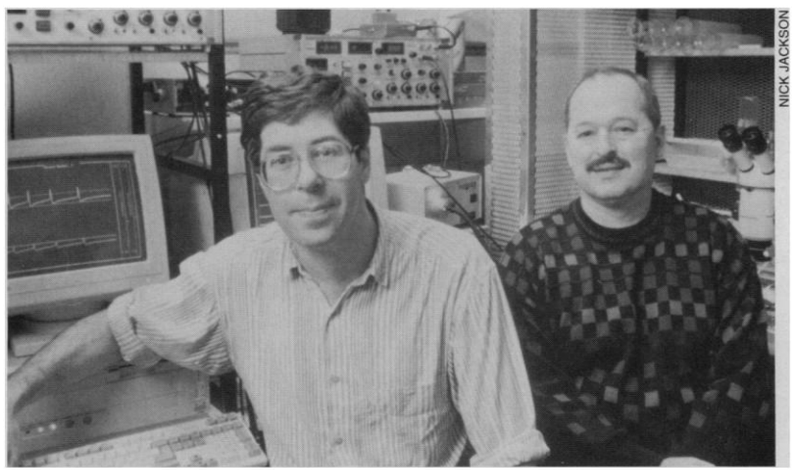
Findings by several groups did indicate that anesthetics affect the operation of the channels that control the flow of ions across the nerve cell membrane in response to stimulation. But the general view was that the compounds do so nonspecifically, perhaps by making membrane lipids more fluid than they normally are. Other than that, few efforts were made to pin down anesthetic action. Indeed, the idea that anesthetics work nonspecifically may have discouraged researchers from probing further. In addition, studying the agents' effects on the lipid membranes of living cells was technically difficult.

During a coffee break discussion in the mid-1970s, however, Franks and Lieb decided that they could make some inroads into the morass with the help of advances in techniques, including x-ray and neutron diffraction, used for analyzing the structures of complex biological materials. As a former student of chemist Maurice Wilkins, who had shared a Nobel Prize with James Watson and Francis Crick for solving the structure of DNA by x-ray crystallography, Franks was well acquainted with the power of that technique. But when the Imperial College researchers attempted to use the two diffraction techniques to find out how both cellular and artificial lipids were altered by exposure to nitrous oxide and a range of other anesthetics, things took a decidedly unexpected turn.

"We got an awful shock from our very first diffraction pattern," recalls Franks. "We couldn't tell the difference between the controls and those exposed to nitrous oxide." At first, the pair blamed their experimental set-up. But after checking, it dawned on them that the fault might lie not with their experiment but with the lipid hypothesis itself. Then the question was, If anesthetics didn't act on membrane lipids, where did they act? One obvious possibility was the membrane proteins.

Franks and Lieb were not the first to get this idea. In experiments performed in the 1940s, Frank Johnson and Henry Eyring at Princeton University showed that certain soluble bacterial enzymes are inhibited by anesthetics. This led them to propose that a similar enzyme inhibition might hold the key to general anesthesia in animals. The proposal was, however, widely ignored, mainly because most soluble enzymes proved to work perfectly normally even when exposed to lethal anesthetic concentrations.

One of the enzymes shown by Johnson and Eyring to be inhibited by anesthetics nevertheless proved key to the further development of Franks and Lieb's work. This was bacterial luciferase, a light-emit-

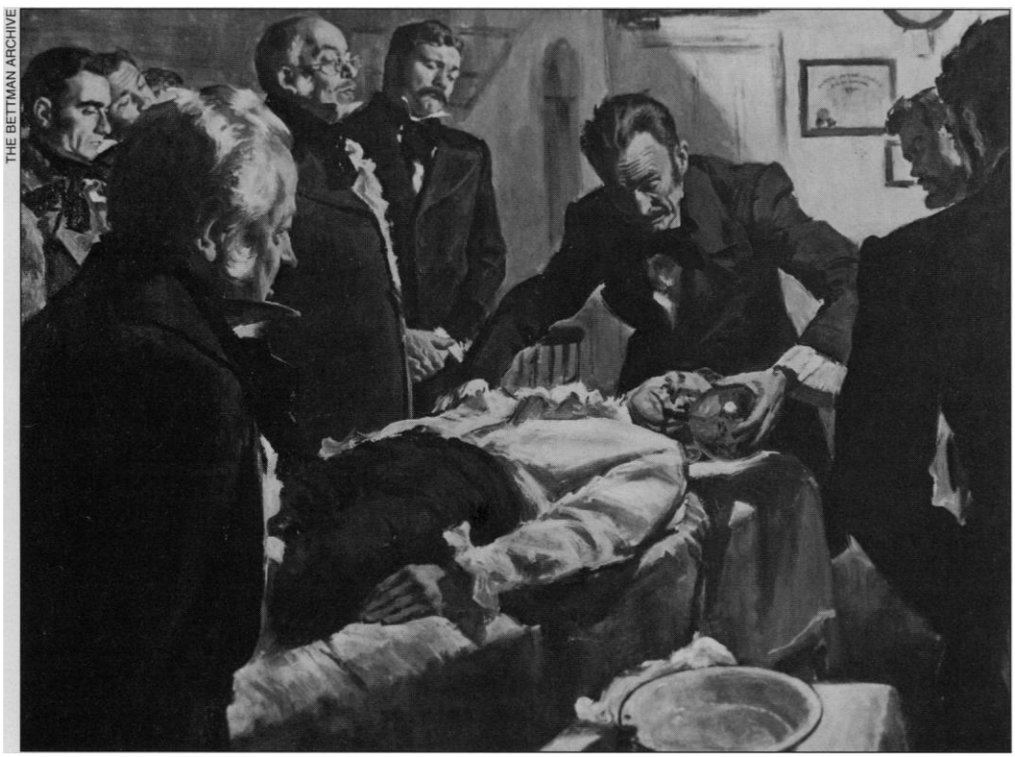


NICK JACKSON

Lipid theory skeptics. *Nick Franks* (left) *and Bill Lieb think it's the membrane proteins that are important for anesthetic action.*

ting enzyme that is very sensitive to anesthetic action. Indeed, it is inhibited by concentrations as low as those used in surgery. In contrast, huge—even lethal—concentrations generally have to be used to induce changes in the properties of cell membranes. To Franks and Lieb, the anesthetic effects on luciferase provided substantial support for the idea that anesthetics could act on specific proteins.

By the mid-1980s, they had gone on to produce the protein equivalent of Meyer and Overton's olive oil rule. They examined the effects of a score of anesthetics on luciferase and found that the drugs' potency correlates extremely well with their ability to inhibit the enzyme. "What they did was expand the range of anesthetics covered by the luciferase relationship," says Brian Smith, a longtime anesthesia researcher at Oxford University. "It put the protein hypothesis back on the map again."



THE BETTMAN ARCHIVE

Going under. *William Morton demonstrates to a skeptical band of surgeons that ether can be used to render a patient unconscious for surgery.*

It was far from definitive proof of that hypothesis, however. "The luciferase work clearly shows that proteins can be sensitive to anesthetics, but the problem is that luciferase is not *the* protein that's sensitive in the brain," says pharmacologist Adron Harris of the University of Colorado's Health Sciences Center in Denver. But in the past few months, Franks and Lieb have brought out what they think is altogether more convincing evidence for the protein hypothesis.

A few years ago, they showed that isoflurane, a widely used general anesthetic, activates an outward flow of potassium ions in certain nerve cells from the great pond snail *Lymnaea stagnalis*. Isoflurane happens to come in two versions, or isomers, that are mirror images of each other but are otherwise chemically identical. For that reason they are very hard to separate, and the early work with isoflurane was done with a mixture of the two isomers.

Franks and Lieb realized that if the two isomers could be isolated, they could be used to test the two rival theories. If isoflurane's effect on potassium ion flow was due simply to its ability to dissolve in membrane lipids, the isomer used shouldn't make any difference. But if the anesthetic interacts with a specific membrane protein, then a difference would be expected. One of the two isomers would preferentially fit the binding site on the putative protein target.

Recently Anaquest scientists were able to purify the isomers, and in the current work Franks and Lieb compared their effects on the anesthetic-sensitive snail neurons (see *Science*, 18 October 1991, p. 427). The result: One of the isoflurane isomers had about twice the effect on the potassium current as the other. Yet both had the same effect on the fluidity of the nerve cell membrane.

Those results, taken with Franks and Lieb's previous findings, provide impressive support for the protein theory, says Oxford's Smith. "They have a powerful academic approach. They've added a lot to the development of a theory I think has been self-evident for a very long time," he remarks. "Most people in the field were continuing to think black was white because they had made a major investment in the lipid theory. The great thing is that Franks and Lieb were people who converted themselves."

But others claim the jury is still out. "It's still too early to write off the lipid theory," maintains anesthesia expert Keith Miller of Harvard Medical School and Massachusetts General Hospital. He says, for example, that lipid solubility does a better job of predicting an anesthetic's potency than does its effect on the luciferase reaction, but, nonetheless, he thinks that the final theory could well turn out to be an eclectic mix of the two rivals. "There's not going to be a brick wall where the protein theory ends and the lipid theory begins," he remarks.

Colorado's Harris agrees that proteins play some role in anesthetic action but does not yet find the evidence completely convincing. "None of the experiments are absolute proof," he says. "We're frustrated with trying to get definitive evidence."

That may change if Franks and Lieb's next set of experiments pans out. They aim to find the brain's anesthetic-sensitive proteins. The recent snail work suggests, they say, that the most likely candidates can be found among the proteins forming potassium ion channels in nerve cells. So they are taking the genes encoding such proteins from rats and introducing them into unfertilized frog eggs, where the ion channel proteins will be made and incorporated into the egg-cell membrane. The eggs should then serve as an easily studied system in which the effect of anesthetics on ion channel proteins can be measured directly. "If the target proteins can be identified this way, the structure of the anesthetic-binding sites might then be characterized," explains Franks. "It is then at least possible that one could design anesthetics that lock onto that very site specifically." The hope is that these will have fewer hazardous side effects than current general anesthetics.

Still, Lieb cautions that there's no guarantee the final result will be this neat. "If you didn't think so, though, you'd give up immediately," he says. ■ ROBERT MATTHEWS

Robert Matthews is an Oxford-based journalist who covers science for the Sunday Telegraph *in London.*