THE NEUROMUSCULAR JUNCTION

The Supple Synapse: An Affair That Remembers

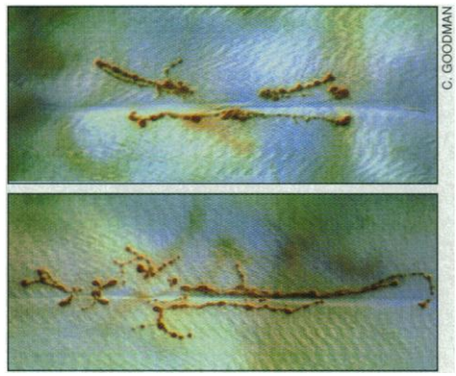
As in a rocky marriage, the link between a muscle fiber and its controlling nerve endings is perpetually in flux, alternately gaining and giving up strength in the face of shifting demands. Some of these couplings must grow in size and power to keep up with muscle growth in young animals, for example, while others naturally shrivel and disappear when no longer needed. But while the sources of marital ups and downs among humans are all too obvious, scientists are still struggling to make out the ingredients of the remarkable—and advantageous—plasticity of the neuromuscular synapse. "There have to be very tight molecular controls" behind the synapse's ability to wax and wane in strength, says University of California, Berkeley, neuroscientist Graeme Davis, who studies the phenomenon in fruit flies. "We're doing our best to understand that."

Understanding the ups and downs of the neuromuscular synapse, after all, could lead to a bigger prize: insight into the workings of our own brains. Neuroscientists believe that our long-term memories are deposited in the brain in the form of physical connections between neurons that—like the neuromuscular junctions in fruit flies—wax and wane in strength. And strong behavioral and anatomical similarities between fruit fly synapses and nerve connections in the mammalian brain have led some investigators to believe that decoding plasticity in the fly could lead to a better understanding of learning and memory. As Berkeley developmental neuroscientist Corey Goodman puts it, "We think of the fly neuromuscular junction as more or less like the central synapses of the vertebrate brain."

In a package of recent experiments described in last month's issue of the journal *Neuron*, his group and others have now identified some of the genes and proteins involved in remodeling fly synapses—and shed light on broader puzzles in synaptic plasticity. One set of experiments, by Davis and colleagues in Goodman's laboratory, shows that synapse remodeling in molting fly larvae is highly reminiscent of the early development of the nervous system. Both make use, for example, of a protein called fasciclin II (Fas II), which helps hold bundles of growing nerve axons together until they reach their final destinations in the embryo. Neuroscientists have long speculated that postembryonic plasticity is an extension of developmental plasticity. But, says neurobiologist Mu-Ming Poo of the University of California, San Diego, "it's very exciting to be able to find out some of the molecular bases for these processes."

The Goodman team may also have come up with a solution to one of the central mysteries of plasticity: how changes in gene expression in a neuron that has many axons and many synaptic connections can alter the strength of only some of its synapses. Their studies suggest that the neuron nucleus oversees the assembly of the synaptic machinery while local factors in individual axons determine where in the nerve cell this machinery gets installed. And other researchers have shown how the cells on the other side of the synapses—in this case, muscle cells—help complete synaptic renovations.

FAScinating rhythm. Goodman and his colleagues took their first steps toward the current work when they were using the fruit fly (*Drosophila melanogaster*) to unravel a different puzzle: how the nervous system is wired up during development. In work begun about 9 years ago, they had shown that Fas II, an adhesive protein, is needed to keep long-distance axons stuck together in bundles, or "fascicles," while they grow toward their target muscles. But in 1993, Columbia University neuroscientist Eric Kandel and coworkers made an observation that suggested Fas II might also come in handy during synapse remodeling.



Free to flourish. In muscles from flies with decreased Fas II protein *(bottom)*, enlarged synapses bear many more boutons *(brown dots)* than do normal synapses *(top)*.

In studies of the sea snail *Aplysia* begun more than 2 decades earlier, Kandel's lab showed that changes in electrical activity can lead to long-term structural changes in the neuromuscular synapse. Kandel and colleague Sam Schacher then found they could re-create those activity-dependent changes in a tissue-culture dish by combining the neurons and muscle cells that bring about the gill retraction reflex in *Aplysia* and exposing them to tiny puffs of serotonin, the neurotransmitter that activates the reflex. Multiple puffs, they found, caused the neurons to sprout new axons and form more synapses with the muscle cells. This strengthening of neuronal connections, known as "sensitization," is a crude form of learning and memory.

The serotonin puffs, Kandel and coworkers later showed, work by activating an intracellular messenger in the neurons called cyclic AMP (cAMP). This, they found, has both local and general effects on a membrane-bound protein called *Aplysia* cell adhesion molecule, or apCAM. At active synapses, one of cAMP's jobs is to cause the cell to retrieve the protein from its membrane. Immediately after this change, the neurons sprout new axons and form more synapses. The timing suggests, Kandel says, that "these CAMs usually serve to inhibit synapse growth by zipping [nerve extensions] together. One of the prerequisites for growth may be to allow them to come apart."

That idea intrigued Goodman, because Fas II is apCAM's structural equivalent in the fruit fly. If Fas II was also apCAM's functional equivalent in regulating synapse formation, he hypothesized, then decreased production of Fas II or its removal from the cell surface would strengthen synaptic connections.

But where Kandel's studies had merely shown a correlation between reduced cell adhesion and sprouting, the Berkeley researchers could use *Drosophila*'s powerful genetics to show direct causation. Goodman and *Neuron* co-authors Davis, Christoph Schuster, and Richard Fetter compared the synapses of normal fruit flies to those of a mutant strain that produces Fas II at only half the normal level. The mutant fly, they found, had about a 50% increase in the number of "boutons," the nodules containing neurotransmitters and their release machinery, at the synaptic connections with a body-wall muscle. This indicates that the decreased production of the protein did in fact foster synapse growth. In contrast, engineering the fly genome to express Fas II at high levels caused a sharp reduction in bouton number. These results "provided the strongest evidence yet that changes in the level of an adhesion molecule at the synapse really are regulating growth and remodeling," says Goodman.

But another mutant strain called *dunce*, which has abnormally high cAMP levels, suggested there was more to the story than just Fas II. When the Berkeley group examined the neuromuscular synapses in the *dunce* mutants, they found—as Kandel's studies predict—that like the Fas II mutants,

they bore less Fas II than normal and had more boutons. But the researchers also found a change not seen in the Fas II mutants: The *dunce* synapses were functionally stronger, putting out more neurotransmitter per bouton when stimulated. In contrast, the average neurotransmitter output of the boutons in Fas II mutants was actually lower than normal. "We were a little surprised at that point," says Goodman, for the result seemed to show that a reduction in Fas II could accomplish only part of the job of synaptic strengthening. One possibility was that cAMP strengthens synapses by affecting not just Fas II but another protein as well.

And that hypothesis led Goodman and his colleagues to yet another protein: the cAMP response element–binding protein (CREB), a Janus-faced molecule that reacts to increased cAMP levels by either repressing or activating the expression of certain genes in the nucleus. Work in Kandel's lab had already shown that CREB is essential for long-term changes in synapse structure in *Aplysia*. Moreover, behavioral geneticists Tim Tully and Jerry Yin at Cold Spring Harbor Laboratory on Long Island had shown that doping fly neurons with CREB's activator form greatly increases the speed with which flies learn a simple task. Davis, Schuster, and Goodman wondered whether CREB's role in plasticity might be to strengthen synapses by carrying cAMP's second signal to the nucleus and triggering the construction of new neurotransmitter release machinery.

To test the new hypothesis, the researchers created transgenic fly larvae carrying both the flawed Fas II gene and an extra CREB gene that they could turn on artificially. The results were "beautiful," says Goodman. "When we put the two together—increased CREB activator at the nucleus and decreased Fas II at the synapse—we got a bigger, stronger synapse that was indistinguishable from those of the *dunce* mutants." The researchers had reconstituted two of the major biochemical pathways that strengthen a synapse. Increased neuronal activity activates cAMP, which triggers both the removal of Fas II at the synapse—allowing physical expansion—and CREB-mediated construction of new neurotransmitter machinery, providing extra firepower.

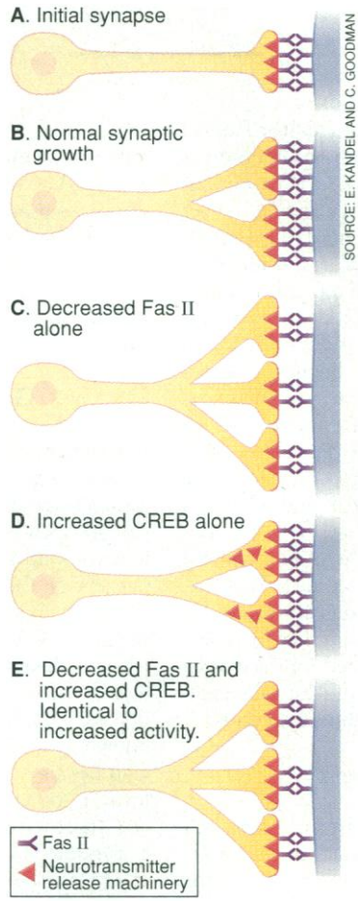
As Kandel and Columbia colleague Kelsey Martin argue in a review article in the same issue of *Neuron*, the Berkeley group's findings also suggest a possible solution to a problem that has puzzled neuroscientists ever since the early experiments on sensitization, cAMP, and CREB. In the vertebrate brain, a single neuron may make synaptic contact with as many as 1000 other neurons, but the changes in synapse size and strength that encode any particular long-term memory may occur in only a few of these synapses. If changes in gene expression are crucial to learning and memory, how can these changes affect only a few of a neuron's many synapses? Kandel proposes that the synapse enlargement and other changes triggered by the down-regulation of Fas II in specific axons pave the way for the installation of new neurotransmitter release machinery produced at the direction of the nucleus. Axons still gummy with Fas II, on the other hand, have no room for such reinforcements. That way, the cellwide effects of the changes in gene expression can be targeted to just a few synapses.

Care to dance? Intriguing as they are, though, the Berkeley group's findings tell only one side of the plasticity story. Added neurotransmitter output by neurons counts for nothing unless muscle cells are equipped with enough neurotransmitter receptors to detect this amplified signal, and results described in the October *Neuron* by another team point to an important role for another fly protein, called discs-large (DLG), in ensuring that there is enough acreage in the muscle membrane around each synaptic bouton for the needed neurotransmitter receptors.

DLG is made by the neurons, but it crosses the synapse and accumulates mainly in the subsynaptic reticulum (SSR), a convoluted part of the muscle cell membrane that surrounds each bouton and harbors the receptors. Flies in which the DLG gene is mutated have shrunken SSRs, and that plus DLG's cellular location led neuroscientists Vivian Budnik, Michael Gorczyca, and coworkers at the University of Massachusetts, Amherst, to suspect that the protein might be involved in synapse development and plasticity. Consistent with that idea, Budnik's group discovered that in the mutant flies, in what may be an effort to compensate for that deficiency, the electrical output of each axon is much greater than normal.

What finally persuaded the Massachusetts team that DLG is the neurons' tool for coordinating SSR size—a muscle cell's "receptive area"—with the neurotransmitter output of the adjacent neuron was their finding that artificially expressing the protein in mutant neurons both restored the electrical output of the boutons to normal and corrected the SSR defects. In both development and plasticity, says Budnik, "there has to be some sort of adjustment between the pre- and postsynaptic sites. Perhaps DLG is involved in that matching."

While the Budnik and Goodman studies have concentrated on the neuron's role in plasticity, a third team's findings show that the neuron doesn't always lead in its complicated dance with its muscle-cell partner. Neurobiologists Michael Bate, Andreas Prokop, and colleagues at Cambridge University studied mutant fly larvae whose embryonic muscle tissue never differentiates into individual cells. They report in October's *Neuron* that axons in these mutants find their way to the correct target muscle precursors, but they then mistakenly form synapses facing supporting tissues, body fluids, and each other—but never muscle. The message, says Bate, is that "you actually need something from the muscle for the axon's synaptic apparatus to be properly localized"—an as-yet-unknown molecule that's missing from muscle tissue whose development is derailed by the mutation.



Axon addition. In the Goodman group's new model, neural activity both reduces cell adhesion locally (C) and increases production of neurotransmitter release machinery (D), creating bigger, stronger synapses (E).

With all the new proteins and genes they have seen taking part in synapse formation and remodeling, neuroscientists now have a number of leads they can follow as they attempt to solve the molecular mystery of plasticity at the neuron-muscle junction. And those investigations, in turn, promise to take them closer to their ultimate goal—the brain. The only surprise, say Goodman and other researchers, would be if the fine-tuning of neuromuscular synaptic connections and mechanisms for learning didn't have molecular steps in common. Says Kandel: "Whenever you convert a short-term change in activity to a long-term change involving growth, it may require a similar program. This may be only the tip of the iceberg."

–Wade Roush