PERSPECTIVES: NEUROBIOLOGY

The Constant Junction

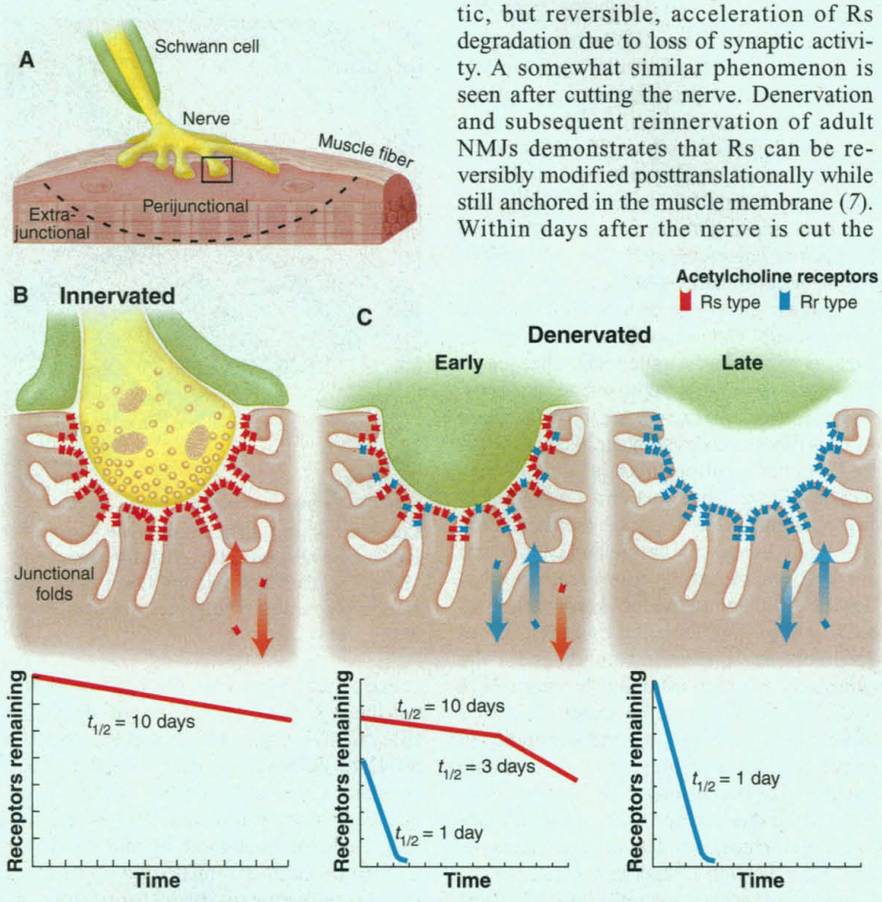
Miriam M. Salpeter

For synapses (the locations in the nervous system where nerves communicate) to function correctly, neurotransmitters must be released from presynaptic nerve terminals and then must bind to receptors on the plasma membranes of postsynaptic cells. In synapses that have to generate action potentials within microseconds of neurotransmitter release (that is, fast synapses) the receptors must be clustered in the postsynaptic membrane at high density, close to where the neurotransmitter is released. The neuromuscular junction (NMJ)—the synapse in the peripheral nervous system where a nerve cell meets muscle—has just such an organization. The principal neurotransmitter at the NMJ is acetylcholine, which is released from the presynaptic nerve terminal within 50 nm of the postsynaptic muscle membrane that contains densely arrayed acetylcholine receptors (~10,000 AChRs/μm^2). There is a steady turnover of AChRs, with newly synthesized receptors replacing those that are periodically degraded. However, the location on the muscle membrane where the AChRs are internalized as well as the regulatory mechanisms involved remain unclear. Now, on page 503 of this issue, Akaaboune *et al.* report an elegant study in which they visualize individual NMJs and study them repeatedly over time (*1*). Their demonstration that the muscle membrane surrounding the NMJ (the perijunctional membrane) is involved in receptor turnover and that muscle contraction is essential for regulating AChR degradation helps to explain how AChR abundance is precisely controlled.

One factor mediating the AChR's stability is its molecular composition. The NMJ has two populations of receptors that differ in structure, physiology, and degradation, and that are independently regulated (*2*). Fetal AChRs (Rr) are composed of α_2, β, γ, and δ subunits. During muscle development, Rr are replaced by adult AChRs (Rs) in which an ε subunit replaces the γ subunit. Prior to innervation of embryonic muscle by nerve cells, Rr are dispersed throughout the muscle membrane at a very low density (<<500/μm^2), and are turned over very quickly [half life ($t_{1/2}$) ~ 1 day]. After innervation, these mobile Rr receptors migrate to the postsynaptic muscle membrane where they become anchored at high density. Eventually they are replaced by the adult Rs, which are very stable and are turned over only very slowly ($t_{1/2}$ ~10 days). If the connection between the nerve and muscle is severed, the reverse occurs and the slowly degraded Rs are replaced by Rr. The separate identities of Rs and Rr become clear immediately after denervation when the two types of receptors coexist in the muscle membrane, each being degraded at its own rate (see the figure) (*3*).

How the γ and ε subunits are involved in receptor degradation is not yet established. Whereas Rs and Rr normally have degradation half lives of about 10 days and 1 day, respectively, their degradation rates can be altered drastically. Receptor stability and degradation are controlled by activity at nerve terminals, and by chemicals that alter the levels of signaling molecules in muscle cells (*2*, *3*). These agents may posttranslationally modify AChR subunits or alter proteins that bind to the receptors providing sites for them to anchor to the postsynaptic muscle membrane (*5*, *6*). Such flexibility allows the NMJ to switch from one type of receptor to the other while maintaining a constant high receptor density, which is necessary for a rapid response to neurotransmitter release.

The Akaaboune study now shows that full inactivation of NMJs produces a drastic, but reversible, acceleration of Rs degradation due to loss of synaptic activity. A somewhat similar phenomenon is seen after cutting the nerve. Denervation and subsequent reinnervation of adult NMJs demonstrates that Rs can be reversibly modified posttranslationally while still anchored in the muscle membrane (*7*). Within days after the nerve is cut the



Up the junction. (**A**) The neuromuscular junction in a single teased muscle fiber, as visualized by light microscopy. The nerve fiber (enshrouded by a Schwann cell) terminates on the muscle. The perijunctional and extrajunctional regions of the muscle fiber are shown. (**B** and **C**) A nerve terminal, as visualized by electron microscopy. (B) A nerve terminal (enveloped in a Schwann cell) sits in a trough of the muscle fiber whose postsynaptic membrane is deeply folded. The adult Rs acetylcholine receptors (red) are anchored at the crests of the junctional folds. Exponential degradation ($t_{1/2}$ ~ 10 days) is characteristic of Rs receptors. Arrows show the balance of Rs insertion and degradation, which maintains a constant AChR density. (C) After denervation the degradation of Rs (red) initially continues with a $t_{1/2}$ ~ 10 days and then accelerates to a $t_{1/2}$ ~ 3 days. As the Rs degrade they are replaced by the fetal Rr ($t_{1/2}$ ~ 1 day; blue arrows show insertion and degradation). The Rr (blue) are interspersed with degrading Rs. At late stages of denervation (right side) only the Rr remain (thus maintaining the pre-denervation receptor concentration constant) until reinnervation reverses the process.

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degradation rate of Rs accelerates to a $t_{1/2}$ of 3 days (see the figure) but restabilizes after reinnervation. Accelerated degradation of Rs is also seen in dystrophin-deficient *mdx* mice, which are a model for muscular dystrophy in humans (*8*). Thus instability of Rs may be a general feature of various pathologic conditions.

AChRs are internalized and degraded via the lysosomal pathway (*9*) but the membrane route that AChRs take has been a matter of speculation. The perijunctional region of the NMJ has long been known to have a higher receptor density than the rest of the muscle membrane (*10*). An early model suggested that receptors at the NMJ cannot be degraded while anchored in the muscle membrane and that the degradation rate depends on the affinity of the receptors for these anchoring sites. Once detached from their anchors, the receptors are free to diffuse in the membrane and can move to the perijunctional region where they are internalized (*7*). This model allows for receptors with different degradation rates to reside side by side in the membrane, which is in fact the case at the NMJ. Akaaboune *et al.* lend support to this hypothesis by showing that the perijunctional region serves as a way-station in normal AChR turnover receiving both new receptors and those destined for degradation.

After AChRs are saturated with the snake venom α-bungarotoxin, there is an initial, rapid ($t_{1/2}$ < 1 day) increase in degradation rate. While one hypothesis to explain this phenomenon—that Rs are degraded rapidly when first inserted into the postsynaptic muscle membrane but then are stabilized by nerve activity—has been disproved (*11*), no alternate satisfactory mechanism has been proposed. Now Akaaboune *et al.* solve this long-standing puzzle by showing that it is the full inactivation of AChRs that is the cause of accelerated Rs degradation. Electrical stimulation of the muscle prevents this and also prevents the acceleration of Rs degradation after denervation (*12*). Thus, the nerve can regulate AChR stability by activating muscle contraction.

What are the functional consequences of maintaining a high density of stable receptors at the NMJ? A high AChR concentration close to the nerve terminal enables acetylcholine to act over a small distance at saturating concentrations, providing efficient binding of neurotransmitter to AChRs and the initiation of muscle contraction. The location of the enzyme acetylcholine esterase close to the receptors enables quick termination of acetylcholine activity, thus allowing the muscle to be activated repeatedly (*13*).

Much regarding the regulation of AChR turnover still remains to be clarified. Why is receptor degradation after prolonged denervation (when Schwann cells no longer release acetylcholine) slower ($t_{1/2}$ ~ 3 to 4 days) than that induced by complete inactivation of AChRs ($t_{1/2}$ << 1 day) by α-bungarotoxin? How does direct stimulation of muscle maintain receptor stability and how is calcium involved? How do the mechanisms involved in maintaining a high receptor density at the NMJ adapt to transitions, such as those in development or during reinnervation? If during such periods, rapidly degrading receptors are removed before they are fully replaced by receptors with a slower turnover, the decrease in receptor number could reduce the ability of the muscle to respond to nerve stimulation. Finally, fast synapses in the brain, where similarities with the NMJ are already emerging (*14*), also necessitate high receptor densities in the postsynaptic neuronal membrane. The similarities and differences in regulation of these two synaptic organizations will be the focus of studies for many years to come.

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PERSPECTIVES: ASTROPHYSICS

The Complexity of Stellar Death

Yervant Terzian

Before the Hubble Space Telescope (HST), with its superior angular resolution, sensitivity, and dynamic range, began sending back images of stars in various stages of their life cycles, the death of a small star—such as our sun—was believed to be accompanied by formation of a smoothly expanding nebula. Now, dozens of spectacular images of planetary and protoplanetary nebulae provided by HST (*1*) have revealed morphological aspects completely unsuspected before (*2*). The newly found complexity represents a challenge for researchers trying to understand the mechanisms of stellar death.

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When a sunlike star, with a mass of up to a few solar masses, reaches the last stages of its evolution, it expands and becomes a cool (about 2500 K) red giant, with a size so large that its outer perimeter could include the orbit of Mars. This asymptotic giant branch (AGB) star loses mass in the form of stellar wind, followed by a more intense mass loss as a result of what is known as a superwind. The star hereby loses a substantial fraction of its mass, and the ejected material forms a planetary nebula. The stellar core contracts and becomes a white dwarf star (about the size of Earth, with a density of ~10^7 g/cm^3) with surface temperatures reaching about 10^5 K.

The material ejected from the star mostly initially consists of atomic and molecular gas, which partly coalesces shortly after ejection to form warm dust particles. Molecular species such as H_2, OH, CO, and SiO have been detected around many proto–planetary nebulae and in the envelopes of AGB stars (*3*). Dust surrounding the star absorbs starlight, and after reprocessing it emits strongly in the infrared.

Eventually, the hot white dwarf's ultraviolet radiation ionizes the nebula, which begins to emit strongly in the recombination lines of hydrogen and helium and spectral lines characteristic for doubly ionized oxygen, singly ionized nitrogen, and other ionized species. Within a few tens of thousands of years, the expanding planetary nebula diffuses into the interstellar medium with a velocity of ~ 20 km/s.

These stellar outbursts play an important role in the chemical evolution of our galaxy. It is estimated that they contribute at least 20 solar masses per century of stellar material to the interstellar medium; a comparable amount is provided by the more violent supernova explosions. The

References and Notes

[1] **Rapid and Reversible Effects of Activity on Acetylcholine Receptor Density at the Neuromuscular Junction In vivo**
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