amples include models of measles dynamics (*4*) and of boreal rodents (*5*). The drawback, of course, is that the predictions may be highly model-dependent, an outcome that is hard to identify if validation is purely through comparison with limited time series data obtained by observation. Ideally one would like to use the model to design manipulative experiments for the field, but this has so far proven elusive.

So, if not in the field, what about the laboratory? Population ecologists are often ambivalent about moving indoors, pejoratively referring to what Kareiva (*6*) has dubbed "bottle experiments" and to their use as analog computers. Yet many of the fundamental concepts underlying modern population ecology were formulated after studies of laboratory populations of blow flies (*7*), flour beetles (*8*), and ciliates (*9*) in the 1930s and 1940s. The work of Costantino *et al.* forcibly argues for the continuing value of bottle experiments.

They studied the flour beetle, *T. castaneum*, a classic laboratory model insect. *Tribolium* can be kept in milk bottles supplied with flour or bran; eggs are laid in the medium, the insect taking about 2 weeks to reach the pupal stage and a further fortnight to become a reproductive adult. *Tribolium* is cannibalistic: older larvae eat smaller larvae and adults consume larvae and pupae. Cannibalism is the main feedback mechanism limiting population growth.

In previous studies (*10*, *11*) the group had found that the dynamics of the system could be approximated by a straightforward model consisting of three coupled difference equations representing the change in densities of larvae, pupae, and adults over a 2-week period. Three nonlinear terms represented the effects of cannibalism. To parameterize the model, they converted the deterministic skeleton into a stochastic model through the addition of multivariate normal noise on a logarithmic scale. The parameters and noise variance-covariance matrix were then estimated with maximum-likelihood techniques.

The model predicts that the dynamics of the system depends critically on parameters such as the adult mortality rate and the fraction of pupae that mature into adults. In a previous study, Costantino *et al.* manipulated adult mortality. The model predicted that as mortality increased the beetle dynamics should move from stable equilibrium through cycles to a second region of stable equilibrium, to quasiperiodicity. Quasiperiodicity occurs when trajectories move over the surface of an attractor and never exactly repeat each other (think of a rotational transformation with an angle that is an irrational multiple of π). Quasiperiodicity is different from chaos, because it does not show extreme sensitivity to initial conditions (the Liapunov exponent is zero). However, in bifurcation studies, quasiperiodicity frequently presages chaos and is an alternative to the more familiar period doubling route to chaos. The results of the experiments provided impressive confirmation of the model predictions.

In the new study, Costantino *et al.* manipulate recruitment to the adult stage. As the severity of adult cannibalism of pupae increases, the system moves from stable equilibrium to quasiperiodicity, the aperiodic cycles becoming phase-locked to give regular cycles, followed by chaos. For even higher levels of cannibalism, regular cycles are again observed and there is also a region of multiple attractors. Again, the results of the experiments are impressively consistent with the model.

Without doubt, the experiments on *Tribolium* are the clearest experimental demonstration of quasiperiodicity and chaos. These results will encourage similar studies in other systems. Some problems remain, however. First, the identification of the complex dynamic behavior in the different time series is largely through inspection. We need better statistical techniques to allow more objective characterization. Second, the *Tribolium* system is extremely artificial and extrapolation to other systems difficult. Cannibalism is an extremely potent feedback mechanism, which predisposes the system to show complex dynamics. The absence of a spatial dimension, other species, or of biological evolution (all individuals were periodically replaced by the same number of beetles from stock cultures) may all have acted to make complex dynamics more (or less) likely. But these caveats aside, ecologists at last have a convincing example of chaos that they can use as a base to understand better complex dynamics in other laboratory systems and, more importantly, in the field.

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NEUROSCIENCE

Synapse Elimination: For Nerves It's All or Nothing

Eric Frank

In muscles of adult vertebrates, each muscle fiber is innervated by a single motoneuron. This is not true at birth, however, when several motoneurons innervate each fiber. Then over the next 2 weeks, in a process called synapse elimination, motoneurons give up their connections with most of these muscle fibers so that each fiber is left with a single input. The experiments described by Colman *et al.* (*1*) on page 356 of this issue provide the first account of the physiological changes in synaptic transmission that occur during synapse elimination.

Colman *et al.* studied these changes by recording intracellularly from muscle fibers, while separately stimulating two motoneurons that innervated a single site on the fiber. They determined the strength of each input by measuring the number of quantal packets of the neurotransmitter acetycholine (ACh) each motoneuron released. The change from multiple to single inputs occurs progressively rather than suddenly. Shortly after birth, when most muscle fibers are still multiply innervated, the inputs usually differ in strength by less than a factor of 2, as indicated in panel A of the figure. After a week, however, the strength of the two inputs diverges, with one-third of the remaining multiply-innervated fibers having inputs that differ by more than fourfold. The subsequent panels of the figure illustrate this process: the input from axon 2 becomes progressively stronger (indicated by the increase in the

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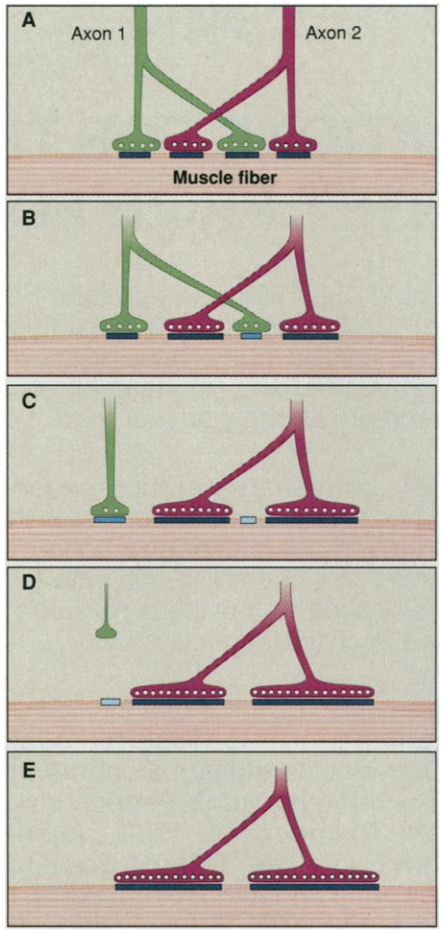
size of its presynaptic terminals and the number of synaptic vesicles), whereas that from axon 1 becomes progressively smaller. At the end of this process, the last remaining input from axon 1 is lost and the muscle fiber is singly innervated (panels D and E).

The distribution of ACh receptors (AChRs) in subregions of developing neuromuscular junctions changes over time (*2*). By lightly labeling AChRs with fluorescent α-bungarotoxin (α-BTX) in neonatal muscles, Balice-Gordon and Lichtman (*3*) monitored this distribution during synapse elimination, visualizing the same junction repeatedly in the living mouse. At small patches within the junction, AChR density decreases over a period of several days, either by receptor migration or internalization. This receptor loss occurs despite the continued presence of a nerve terminal precisely over the patch, suggesting that transmitter released from such a terminal would find fewer AChRs to bind to and would be less effective in producing a response in the muscle. The new experiments bear out this suggestion. At synapses where there is a large disparity in the strength of the two inputs, the quanta released from the weaker axon produce synaptic potentials of various sizes. Some quanta have normal efficacy; they produce responses just as large as those from the stronger input (panel B, left-hand green terminal). But other quanta produce responses with small amplitudes, which often disappear into the noise of the recording (panel B, right-hand green terminal). An indirect argument, based on the rise times of the small responses, suggests that their small size is caused by low AChR density rather than a reduced amount of ACh per quantum or a larger diffusion distance between nerve and muscle.

Although the mechanisms responsible for synapse elimination are unknown, patterns of electrical activity in both nerve and muscle are crucially important. Neonatal blockade of action potentials in nerve prolongs the period of elimination, whereas extra stimulation can accelerate the process (*4*). If the AChRs in a small region of an adult junction are blocked by a focal application of α-BTX, these AChRs disappear over the next several days, and the nerve terminal overlying the blocked receptors is withdrawn (*5*), a process remarkably similar to normal synapse elimination. Importantly, if AChRs are blocked over the entire junction, they do not disappear, and nerve terminals are not withdrawn. Evidently, when pre- and postsynaptic activity are not temporally correlated, the synapse is destabilized and is eventually lost. This result explains how initially unequal inputs could result in eventual elimination of the weaker input. The stronger input often produces postsynaptic responses when the weaker input is inactive, whereas activation of the weaker input is often insufficient to produce a postsynaptic response. This temporal mismatch of activity at the weaker synapse further weakens it, and ultimately it is lost altogether.



Schematic diagram of the process of synapse elimination. A single neuromuscular junction undergoing synapse elimination at various stages in the process (**A** through **E**), from innervation by both red and green axons at the time of birth (A) to single innervation by the red axon 1 to 2 weeks later (E). Patches of ACh receptor (blue rectangles) lie within the postsynaptic membrane of the muscle fiber. The density of receptors within each patch is indicated by the density of blue color: the darkest color represents the highest density. In (D), all green terminals have been withdrawn, but a tiny axon tipped by a retraction bulb is still visible in the vicinity of the synapse.

Synapse elimination at the neuromuscular junction may provide a useful model for synaptic rearrangements in the central nervous system (CNS). There are only a few instances in the CNS where the situation is strictly analogous—that is, where all but a single input are eliminated. But synaptic rearrangements are common, both during development and in adults. A common theme is that temporally correlated pre- and postsynaptic activity produces a strengthening of synaptic connections. An extension of this idea is that uncorrelated activity results in synaptic weakening. The novel implication of the results by Colman *et al.* is that a change in synaptic strength, as measured by a change in quantal content and quantal efficacy, is followed by a permanent anatomical change—physical removal of the weakened input. If alterations in synaptic strength within the CNS also result in eventual withdrawal of ineffective inputs, it would provide a mechanism of consolidating physiological changes permanently. An example is the development of ocular dominance columns in the visual cortex, where axons carrying input from one eye physically retreat from cortical areas that are dominated by the other eye (*6*). Terminal withdrawal might also consolidate alterations in synaptic strength in the adult brain, such as those produced by long-term potentiation and depression.

Our understanding of synapse elimination is now somewhat analogous to that of the action potential, another classic example of the "all-or-nothing" behavior of nerve, after its experimental description by Hodgkin and Huxley in the early 1950s. Initial inequalities in the strength of two inputs result in reduced correlation of pre- and postsynaptic activity at the weaker synapse. ACh receptors begin to disperse at these synapses, reducing synaptic strength still further. The process is regenerative, and the ineffective terminals are eventually completely withdrawn. As for the action potential in the 1950s, we do not yet know the molecular mechanisms underlying the process of synapse elimination. How do local mismatches in pre- and postsynaptic activity cause a local decline of AChR density? And how do postsynaptic changes, such as lower AChR density, result in physical withdrawal of the overlying nerve terminal? Given the excellent experimental accessibility of the neuromuscular junction and the powerful molecular techniques now available, answers to these questions should be coming soon.

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