The dendrimeric backbone contains a large number of aromatic benzene rings, each of which can be excited into a stretching vibration by an infrared photon. With low light intensity, no one ring gets excited by many photons, but the absorption of a single photon by several rings puts enough energy in the dendrimer to make an isomerization possible. Intramolecular funnelling of the energy to the reaction site appears to be the rate-limiting factor. This is reminiscent of thermally activated unimolecular reactions at low pressures, where intermolecular activation determines the rate¹².

No attempt is made to coherently pump the energy, and the absorption of light is strictly linear. The role of the dendrimeric structure is twofold. First, it serves as a light-harvesting device that collects many excitations close to the reactive site. Second, it maintains these excitations for a long time by slowing the escape of vibrational energy to the surrounding medium. In a dendrimeric geometry the density of atoms increases rapidly with size (or branching generation) and the atoms at the boundary become closely packed, hindering the motions that lead to the loss of energy. The trapped excitations then have plenty of time to explore phase space (that is, to be randomly redistributed between bonds) and eventually combine in the desired region of the molecule where the reaction takes place.

This picture of an unconstrained interior protected by a rigid boundary is supported by nuclear magnetic resonance measurements. (The degree of rigidity may depend on the type of dendrimer and the solvent's quality, as shown by molecular dynamics simulations¹³.) Further evidence for this process is provided by the absence of reactivity when the reactive site is on the outside rather than the inside of the dendrimer. Moreover, the increase in reactivity is observed only in high-generation dendrimers, where the exterior is closely packed and therefore stiff.

The reported rate scales with the fifth power of light intensity. This can be explained as follows: if each dendrimer has N benzene rings and if a fraction s of the rings has been excited (on average, among many molecules), then the probability that a particular dendrimer will carry n vibrational quanta is the poissonian $P(n) = e^{(-Ns)} (Ns)^n / n!$. Because $s \ll 1$, only a small fraction of the molecules will acquire the necessary energy; but all these molecules will eventually react, as the energy does not dissipate. The fraction s is proportional to the light intensity and the s^n factor therefore implies n th power dependence on the light intensity. The azobenzene system needs five photons to induce the reaction, which explains the $n = 5$ observed dependence on incident power. (In the multiphoton laser experiments, the nonlinear dependence on incident power reflects the nonlinear absorption of light; here the

absorption is strictly linear, and the non-linearity comes from the need to create many excitations in the same molecule.)

Similar funnelling of energy occurs naturally in photosynthesis, where assemblies of chlorophyll antennae absorb energy and direct it to a reaction centre, where a primary electron transfer takes place. Dendrimeric geometry is very attractive as a possible artificial antenna¹⁴ — the absorbing units can be closely packed without much support structure, and a simple chemical change of the central group may induce a large conformational rearrangement of the dendrimer¹⁵. So this new-found double role of dendrimers, as light-harvesting antennas as well as prisons for vibrational energy, may allow new forms of supramolecular photochemistry. □

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Neurobiology

Long-distance long-term depression

Roger A. Nicoll and Robert C. Malenka

Those who study learning and memory generally agree that many forms of these processes are due to long-lasting, use-dependent changes in the strength of synapses in the brain. Depending on the pattern of synaptic activation, synapses can show either a long-term potentiation (LTP) or a long-term depression (LTD). Both forms of plasticity are spatially restricted — that is, they seem to be limited only to those synapses in which the plasticity has been induced. This synapse-specificity has been used in neural-network models that store information, and it suggests that the signals responsible for the changes in synaptic strength are highly localized.

Now, an article by Fitzsimonds *et al.*¹ (page 439 of this issue) indicates that a form of LTD may spread over hitherto unimagined distances. Using simultaneous recordings from three interconnected neurons in culture, the authors show that LTD is not limited to the synapses at which it is induced, but that it spreads to other synapses in the microcircuit. They propose that this spread involves signals travelling retrogradely across the synapse and, in addition, signals that travel intracellularly for considerable distances. Moreover, although the changes are widespread, specific rules determine which synapses are affected (Fig. 1, overleaf).

The idea that LTD^{2–6} and LTP^{7–9} may not be strictly synapse-specific is not entirely new. So why is the paper by Fitzsimonds *et al.*¹ so provocative? First, it challenges the classic view — as originally put forward by Cajal — that the neuron is 'dynamically polarized'; that is, the information received by the dendrites is passed forward through

the axon to the synapses on the next cell. The newly discovered backward-propagation of LTD indicates that there is also an intracellular, retrograde, axon-terminal-to-dendritic flow of information (on a much slower time-scale). In this way, the input of the neuron is 'told' the state of the output of the neuron. So dendrites not only integrate synaptic potentials, but they also integrate retrograde signals from various axonal outputs of the cell. This has important implications for models of learning that are based on back-propagation algorithms¹⁰.

Second, the cell-biological mechanisms that must be proposed to account for many of the observations go far beyond those previously considered. Earlier work^{2–5,7–9} indicates that synaptic plasticity may spread laterally via a signal in the extracellular space. But Fitzsimonds *et al.* suggest that a retrograde signal (at synapse 2 in Fig. 1) generates a secondary intracellular signal in the presynaptic cell. This signal must travel all the way back into the dendrites (to depress synapse 1) and also throughout the presynaptic axonal arbour (to depress synapse 5). So this signal can depress synapses, whether it reaches the synapse from the presynaptic or postsynaptic side. The same, or a different, signal produced by excitatory or inhibitory synapses spreads LTD through the dendrites of the postsynaptic cell to other inputs that converge onto the cell (to depress synapse 4), yet it is incapable of forward propagation down the axon (to synapse 3). All in all, this is a level of complexity that few would have ever considered.

What remains to be done? As is the case with most provocative studies, far more questions are raised than answered. First, how far

Figure 1 The spread of LTD (long-term depression) in a neural network in culture, as proposed by Fitzsimonds *et al.*¹. If LTD is induced at excitatory synapse 2 (circled synapse on cell B), LTD travels back through cell A to depress transmission at synapse 1. It also travels laterally within both the presynaptic cell (to synapse 5 on cell C) and the postsynaptic cell (to synapse 4 on cell B). If synapse 2 is inhibitory, LTD travels only laterally within the same postsynaptic cell (to synapse 4). Importantly, LTD induced at synapse 2 — whether it is excitatory or inhibitory — does not travel forward to affect synapse 3. Furthermore, the synapses that are affected by the propagated LTD can be either excitatory or inhibitory.

does the LTD spread? The authors provide a hint that synapses close to that in which the LTD is induced are subjected to more LTD than those further away. But whether there is a limit to the spread of LTD remains one of the key issues. Second, might LTD of a single synapse be enough to generate the propagating signal(s), or are a number of synapses needed to generate sufficient signal? The inputs in the study by Fitzsimonds *et al.* were large (hundreds of pA), indicating that each input consisted of a large number of synapses. This is in striking contrast to the intact nervous system, where single inputs generally make less than a dozen synapses. Third, is the propagated LTD graded or all-or-none? Fourth, because the propagated LTD requires the spread of a signal, a delay might be expected in its onset compared with the site of LTD induction — an issue that is not considered by Fitzsimonds *et al.* Fifth, what is the mechanism for the induction of LTD at inhibitory synapses? The LTD is induced while the membrane potential is voltage-clamped, so there is no obvious route for calcium entry, which is a normal requirement for plasticity. However, the LTD is clearly induced postsynaptically, because it is blocked if the membrane potential is held at hyperpolarized levels.

Finally, do the new findings in culture reflect what is going on in the intact nervous system? Many of the predictions have not been tested in the slice preparation because of the difficulty in identifying individual neurons in a circuit. One aspect that has been addressed by other investigators is the postsynaptic spread of LTD. When similar induction protocols are used, LTD seems to be limited to the activated synapses. In addition, many excitatory neurons in the central nervous system are projection neurons which send their axons great distances. This

anatomy is asking a lot of a back-propagation signal, yet if this mechanism is to be used by the brain it must find its way back to the dendrites. Lastly, there is little evidence that monosynaptic inhibitory inputs in the hippocampus show LTD or that they

transmit LTD to excitatory synapses.

The new work of Fitzsimonds *et al.* shouts out two very loud marching orders to scientists working in the synaptic-plasticity field. First, they need to find the underlying mechanisms and signals that are involved in this propagated synaptic plasticity in cell culture. Second will come the search for similar phenomena in the intact nervous system. Given the provocative nature of the present findings, we will not have to wait long for answers. □

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V(D)J recombination

From RAGs to stitches

David T. Weaver and Fredrick W. Alt

DNA double-strand breaks (DSBs) can be formed by ionizing radiation and, if unrepaired, they are lethal to the cell. DSBs are also made as an initial step of the programmed V(D)J recombination pathway. This is used by lymphocytes to assemble the variable (V), diversity (D) and joining (J) gene segments which encode the variable regions of antigen receptors.

How do cells join double-strand breaks in their chromosomal DNA? Genetic evidence has shown that to rejoin the site-specific DSBs generated during V(D)J recombination, some of the proteins that are used in all cells to rejoin other DSBs are required. However, the mechanisms by which DSB-repair proteins effect the repair reaction is unknown. Now, papers by Ramsden *et al.*¹, Grawunder *et al.*² and Wilson *et al.*³ on pages 488, 492 and 495 of this issue, respectively, along with studies elsewhere^{4–6}, provide new insights into the joining phase of V(D)J recombination, and identify a related end-joining reaction in the yeast *Saccharomyces cerevisiae*.

The lymphoid-specific recombination-activating gene proteins 1 and 2 (RAG1 and RAG2) initiate V(D)J recombination by generating DSBs at the conserved recombination signal sequences (RSSs) that flank all antigen-

receptor variable-region gene segments. This initiation reaction has been carried out *in vitro*, and it has been shown to generate hairpin coding ends and blunt RSS ends⁷ (Fig. 1a). To complete the reaction the RSS ends are precisely joined, whereas the coding ends undergo further processing (for example, opening of the hairpin or the loss and gain of nucleotides) before they are joined (Fig. 1b). In a significant advance, Ramsden *et al.*¹ and others^{4,5} have now coupled the RAG1/RAG2-dependent initiation step with one or the other joining reactions in a cell-free system.

The most striking new finding is that if RAG1/RAG2 are retained after the initiation step, the formation of coding-junction products is stimulated. Coupling initiation to joining would allow a RAG1/RAG2 complex to direct the joining of appropriate ends. It is not known whether RAG1 and RAG2 physically interact with coding ends to promote joining. However, RAG1 and RAG2 are associated with RSS ends after cleavage⁸, and this may contribute to the difficulty in observing RSS joining *in vitro*^{1,5}. Because the efficiency of V(D)J joining reactions seems to be quite low¹, other factors or altered conditions may be required. Further studies are needed to show how accurately the *in vitro* reactions mimic true