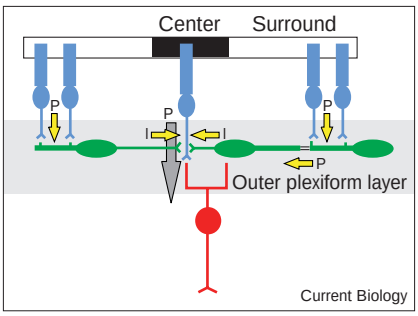
Figure 3



Generation of a center–surround receptive field in an OFF bipolar cell. The center is generated by direct connections (grey arrow) from photoreceptors (blue) to a bipolar cell (red). The synapse is sign-preserving (denoted by P), that is to say, a hyperpolarization in the pre-synaptic photoreceptor is transmitted as a hyperpolarization in the post-synaptic bipolar cell when light illuminates the center. The surround is generated by signal flow, shown by yellow arrows. When the surround is illuminated (as shown), photoreceptors cause horizontal cells (green) to hyperpolarize through sign-preserving synapses that release glutamate. Signals in horizontal cells spread laterally through gap junctions. Photoreceptors in the center receive inputs from horizontal cells but because the transmitter involved, GABA, is inhibitory, the synapse is sign-inverting (I). Photoreceptors in the center therefore depolarize when the surround is illuminated, antagonizing the effect of light in the center.

Photoreceptors, bipolar cells and horizontal cells in the outer retina do not fire action potentials; they generate relatively sustained voltage changes that are graded with light intensity. The signals in amacrine cells and ganglion cells in the inner retina differ in two ways: they generate action potentials, and many respond transiently when a light goes on or off. The receptive field of a ganglion cell is derived from the bipolar cells that provide its inputs. The time-course of the response is strongly affected by amacrine cells, which have a key role in signalling change and are very sensitive to moving stimuli. Amacrine cells release GABA onto the terminals of bipolar cells to control synaptic transmission to ganglion cells. Amacrine cells are electrically coupled, and these gap junctions, like those between horizontal cells, are modulated by dopamine.

Future directions

Most work on the retina, or any other neural circuit, has measured the output one neuron at the time, but it has recently become possible to record the responses of many ganglion cells simultaneously. These measurements reveal that ganglion cells do not respond independently of one another, and that information is contained in the pattern of spikes emerging from different cells. To understand vision, we must decipher this multi-neuronal code. To understand how this code is generated, we must understand how synapses transfer information, and how this transfer can be modulated.

Adaptive changes in the way the retina processes information will involve changes in the synaptic connections. Recently, it has become possible to make direct measurements of the processes regulating neurotransmitter release at the synapse of isolated retinal neurons, and in the future we can hope to understand these processes at the molecular level. Structural changes in synaptic connections are only just beginning to be characterized, and it will be fascinating to understand how these alter the flow of signals.

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Vacuolation in murine prion disease: an informative artifact

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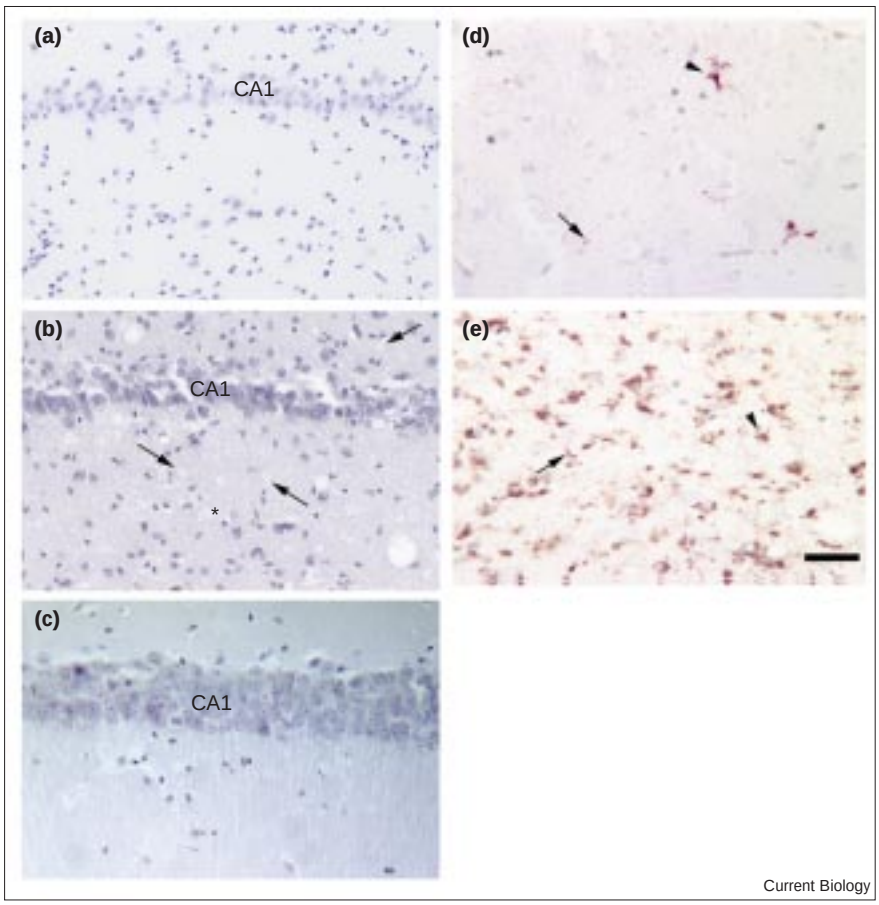
Vacuolation is the spongiform change in the brain that is regarded as one of the pathological hallmarks of the prion diseases. Other hallmarks include astrocytosis, amyloid deposition and loss of neurons. Vacuoles vary in size from 5 to 15 μm diameter and the consensus opinion is that they are located within dendrites and axons [1,2]. A description of the density and distribution of vacuoles is central to the definition of the 'lesion profile', which has been used to characterise the different strains of scrapie agent [3] and, more recently, to verify that the prion agent in bovine spongiform encephalopathy is similar to that found in the new-variant Creutzfeldt–Jakob disease [4]. The accumulation of scrapie infectivity has been shown to precede the development of vacuolation [5,6]. Although some studies have reported a direct correlation between the level of infectivity and the severity of vacuolation [7], others have not shown this [8]. The accumulation of the scrapie form of the prion protein, PrP^{Sc}, as shown by immunocytochemistry, has been reported to occur at an early stage of prion disease in mice, and has been shown to precede the development of vacuolation [9].

The molecular mechanisms responsible for vacuolation and the significance of vacuolar pathology in the prion diseases remain poorly defined. Jeffrey and colleagues [2] hypothesise that vacuolation is a non-specific spongiform change,

Figure 1



Brains from scrapie-affected and control mice were perfused and post-fixed with 10% formal saline, embedded in paraffin wax and cut into 10 µm sections on a microtome. A parallel group of scrapie-injected mice was used for the preparation of fresh frozen cryostat sections as previously described [14]. All brain sections were stained with cresyl violet. Indirect immunocytochemistry for Ig11 was performed using a previously described method [21]. **(a)** Fresh frozen brain section from a mouse with clinical signs of scrapie, showing neuronal loss in the CA1 region of the hippocampus. Note that there is no vacuolar pathology. **(b)** Paraffin-embedded brain section from a mouse with clinical signs of scrapie, showing neuronal loss in the CA1 region of the hippocampus and typical vacuoles (arrows). Note increase in parenchymal cellularity (asterisk). **(c)** Paraffin-embedded normal brain section. **(d)** Fresh frozen normal brain section showing the distribution of Ig11 staining. Note the diffuse staining of process-like structures (arrow) and the occasional cell-body staining (arrowhead). **(e)** Upregulation of Ig11 seen in scrapie-affected mouse brain. Note the marked staining of microglial processes (arrow) and the diffuse staining of cell bodies (arrowhead). The scale bar represents 50 µm.

occurring secondary to a disruption of axonal transport. It is also suggested that accumulation of PrP^{Sc} within the lysosomal compartment in neuronal processes is the precursor to spongiform change in the prion diseases [10–13].

We have been able to reproduce the predicted incubation period and all of the pathological hallmarks of mouse scrapie with the notable exception of vacuolation [14]. One of the differences between our work and previous work reporting vacuolar pathology [3] is in the way the brains were processed for histology. For technical purposes described in our previous study [14], we had used fresh frozen cryostat sections, whereas most researchers working in this field use fixed, paraffin-embedded brain tissue.

Vacuolation was a feature of mouse scrapie pathology only when the brains were perfusion-fixed and paraffin-embedded; fresh frozen cryostat sections did not show any vacuolar pathology (Figure 1a–c). It is possible that the vacuoles seen in paraffin-embedded tissue were once full of substance(s) that dissolved after the brains were put through the graded alcohols and Histoclear solvent before they are embedded in paraffin wax. There would then be no vacuolation on fresh frozen sections because the vacuoles would remain full and therefore invisible. A previous study has shown that there is considerable variation in the degree of vacuolation, depending on the method of tissue preparation, and that the accumulation of lipid in mouse scrapie does not correspond to the distribution of vacuoles [15]. We cannot, at this stage, report the presence of carbohydrate or lipid, using Periodic-acid Schiff and Oil red-O histochemical staining, respectively, within putative vacuoles on fresh frozen cryostat sections of brain. It is possible that PAS does not stain all carbohydrates. Furthermore, the Oil red-O reagent may not stain the abnormal glycolipids that may be present in this disease.

We therefore investigated whether the presence of vacuoles correlated with the distribution of lysosomes. We have shown that the size and distribution of vacuoles did not correspond to those of lysosomes as seen by immunocytochemistry using the antibody Ig11, which is directed against a lysosomal membrane antigen [16]. An upregulation of lysosomal staining was seen in cells with microglial morphology and also, more diffusely, within the neuropil of scrapie-affected brains (Figure 1e). This contrasted with the distribution of Ig11 staining in control brains, in

which occasional microglia were evident and neuronal staining was confined to the cell body (Figure 1d).

If the vacuoles do not correspond to a deposit of lipid or carbohydrate and are not lysosomal elements, then another possibility is that they are brought about by shrinkage or stress of the tissue during paraffin embedding and indicate a change in the extracellular matrix. We thus examined the extracellular matrix properties in the basement membrane of the major blood vessels and also the sizes of the major blood vessels. We found no differences in blood vessel size between normal brains and brains from mice with terminal prion disease. The extracellular matrix of the brain is, however, atypical [17] and we therefore examined whether there was an upregulation of the matrix degrading metalloproteinases that might disrupt this matrix, using competitive reverse-transcriptase PCR (RT–PCR) [18]. We found a 50-fold increase in macrophage metalloelastase expression, which may be an indication of microglia activation and/or macrophage infiltration. In contrast to previous observations in acute inflammatory models [18], there was also a 25-fold increase in stromelysin-1 expression. This may be in part secondary to the observed astrocytosis, but may also suggest that the vacuoles could arise as a consequence of an alteration in the brain's extracellular matrix. There was no upregulation in the other matrix metalloproteinases that were investigated: stromelysin-3, collagenase-3; the 72 kDa and 92 kDa gelatinases or matrilysin.

Recent studies indicate that the brain extracellular matrix is involved in kainic-acid-induced excitotoxicity [19], and that glycosaminoglycans may also play a significant role in neuronal excitability [20]. The vacuolation present in paraffin-embedded brain tissue of mice with prion disease serves to focus our attention on possible alterations in the brain's extracellular matrix that may, in turn, contribute to neuronal degeneration.

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