Morphology of physiologically identified neurons

Michael J. Friedlander and S. Murray Sherman

Individual neurons can be classified in terms of their physiological properties or in terms of their structure and interconnections with other neurons. When both types of information are available for a defined neuronal population, relationships can be inferred between the structure and function of single neurons. This, in turn, leads to inferences about functional connectivity among neurons. However, in many parts of the nervous system, the heterogeneity of neural structure and function limits the usefulness of this approach. We have applied a technique that allows us to directly determine the structure of individual, physiologically characterized neurons.

Intracellular staining

Recent methodological advances permit neuroscientists to compare structure and function directly for the same neuron. One can record functional activity intracellularly with a fine, hollow micropipette and then eject a marker substance from the micropipette into that same neuron[6,11,14]. We have used this approach, with horseradish peroxidase (HRP) as the marker substance (fluorescent dyes have also been used as a marker in other laboratories), to study neurons in the cat's lateral geniculate nucleus (LGN[6,7]). The LGN is a major component of the cat's central visual pathways.

For a number of reasons, HRP has proved to be an excellent marker in these studies. It rapidly fills the entire cytoplasm of the injected neuron, including all of the dendritic and all or most of the axonal processes, without leaking out of the neuron. One thus obtains Golgi-like filling of the functionally identified neuron which can readily be viewed with conventional microscopy after one of several histochemical reactions. These reaction products tend to be quite stable over time, and at least one (e.g. with 3-3′ diaminobenzidine) provides a preparation suitable for electron microscopy[14]. This technique is becoming increasingly common in a number of laboratories and is being directed at many neural regions, including the cerebral cortex, brainstem, and spinal cord.

Three parallel pathways

We used this method to determine the morphological features of each of the three functional subgroups of geniculate neurons. These subgroups are termed W-, X-, and Y-cells. Each class has receptive field properties similar to those of W-, X-, and Y-cells found in the retina. In fact, a geniculate W-, X-, or Y-cell, respectively, receives input only from retinal W-, X-, or Y-cells. As illustrated in Fig. 1, these cell groups seem to represent three separate, parallel pathways from retina through the lateral geniculate nucleus to visual cortex (see Ref. 15; for a more detailed review, see Refs 12, 17). Presumably, each pathway is involved in the neural analysis of different aspects of a visual scene.

W-cells are distinguished by their poor responsiveness and low sensitivity to visual targets, and they have the slowest conducting axons in the central visual pathways. However, our understanding of these neurons is still quite preliminary. X- and Y-cells are much more sensitive to visual stimuli, and so we know considerably more about the response properties of these cells[15]. Compared to Y-cells, X-cells have slower conducting axons (but faster than those of W-cells) smaller receptive fields, more linear spatial summation, and respond tonically to appropriate stationary targets. Furthermore, X-cells are relatively sensitive to the higher spatial frequencies of a visual stimulus, therefore they can extract fine spatial details from a visual scene such as texture. Y-cells, however, are much more sensitive to the lower spatial frequencies and can thus convey information about larger, more basic forms.

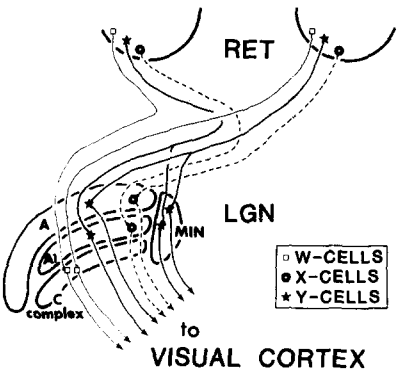

Fig. 1. Schematic summary diagram of the cat's retinogeniculo-cortical pathways. W-, X-, and Y-cells and the LGN divisions are shown. 'C complex' refers to the C laminae, including C and C2 which are innervated by the contraleteral eye, and C1 and C3 which receive inputs from the ipsilateral eye. In addition to the distributions of cell types shown for the LGN, Y-cells are also located in lamina C, and some W- and X-cells have been reported for MIN.

Morphology of geniculate neurons

Indirect attempts have been made to establish morphological descriptions of these cell types in the retina[4] and LGN[13,20]. In the LGN, for instance, laminae A and A1 contain only X- and Y-cells. The top of lamina C contains mostly Y-cells and the rest of the C laminae are comprised mostly of W-cells. The medial interlaminar nucleus (MIN) is mainly a Y-cell population with some W- and X-cells (see Fig. 1). Knowledge of the different morphological types found in each of these regions has led to proposals concerning specific structure/function relationships at the single cell level[13,20].

Our more direct approach, using intracellular HRP staining of functionally identified neurons in the A and C laminae, has confirmed only some of these proposals[6,7,16]. We found that each group of W-, X-, and Y-cells includes a rather heterogeneous collection of morphological features, but nonetheless distinct structural characteristics are presented by each group (see Fig. 2). Our data base presently includes over 70 W-, X-, and Y-cells well characterized both physiologically and morphologically.

W-cells. These were found only in the C laminae. They have small to intermediate sized somata (75–322 μm^2 in cross sectional area; mean = 188 μm^2) and fine dendrites, which for some W-cells have numerous stalked appendages. Other W-cells have virtually no dendritic appendages. The dendritic arbors of these cells tend to be rich and are oriented parallel to the geniculate laminae.

X-cells. These were found only in the A laminae. They have small to medium sized somata (68–420 μm^2; mean = 219 μm^2) and fine, sinuous dendrites oriented perpendicular to the geniculate laminae. The entire dendritic arbor of each X-cell is contained within a single geniculate lamina, and for some X-cells, the arbor extends dorsoventrally across the entire lamina. Most X-cells are further characterized by complex, stalked appendages along the dendrites, although a few of these neurons

0378 – 5912/81/0000 – 0000/$02.50

are virtually devoid of dendritic appendages. In many ways, X-cells appear morphologically similar to W-cells rotated by 90° around the anteroposterior axis.

Of particular interest is our discovery that some X-cells confirmed as relay neurons (i.e. with geniculocortical axons) have morphological features characteristic of the 'class 3' neuron described by Guillery[9]. These class 3 cells have been generally and confidently equated with interneurons[5,13]. Although we cannot rule out the possibility that many interneurons exist with class 3 morphology, the assumption that class 3 morphology is isomorphic with interneurons should be discarded. Unfortunately, the identification of any such interneurons in the LGN is difficult, since negative evidence can be hard to interpret. For instance, one could imagine circumstances under which a relay neuron would fail to be retrogradely labelled after a cortical injection of HRP or would fail to be activated antidromically by electrical stimulation of cortex (see Ref. 7 for a detailed discussion of this).

Y-cells. These were found in the A and C laminae. They have the largest somata among our HRP filled sample (238–935 μm^2; mean = 490 μm^2). The dendrites are relatively thick and straight, and the dendritic arbors have a fairly cruciate appearance. Few dendritic appendages are typically seen. The dendritic arbors are more or less spherically symmetrical, and every Y-cell identified as a relay neuron has some dendrites that cross laminar borders. In fact, one Y-cell with a soma in lamina A and some dendrites extending into lamina A1 had an excitatory receptive field only for the 'ipsilateral' eye. In other words, it appears that the cell's dominant optic tract input arrived via the peripheral segments of these translaminar dendrites.

Axonal features. The axons of these W-, X-, and Y-cells are usually filled with HRP and can be traced over considerable distances (several mm) into the optic radiations. W-cell axons are thinnest, Y-cell axons are thickest, and X-cell axons are intermediate in diameter. This correlates well with conduction velocity measurements, since W-cells conduct most slowly, Y-cells most quickly, and X-cells with an intermediate conduction velocity[12,17].

Fine axon collaterals with numerous swellings were also routinely observed, the swellings probably being synaptic terminals[19]. Occasionally, such collateral branches were seen within the LGN. Much more common were fine axon collaterals in the perigeniculate nucleus, located immediately dorsal to lamina A. Many W- and X-cells and practically all Y-cells issue perigeniculate collaterals. These collaterals branch from the parent axon as it proceeds into the optic radiations. Perigeniculate neurons thus receive collateral input from geniculate relay cells, and the axons of these perigeniculate cells, in turn, project back to the LGN laminae[1,2].

The perigeniculate nucleus, then, seems to act as an interneuronal pool in a feedback circuit, and it receives information from each of the W-, X,- and Y-cell pathways. This could provide much or all of the interneuronal properties suggested for the LGN and often presumed to require intrageniculate interneurons.

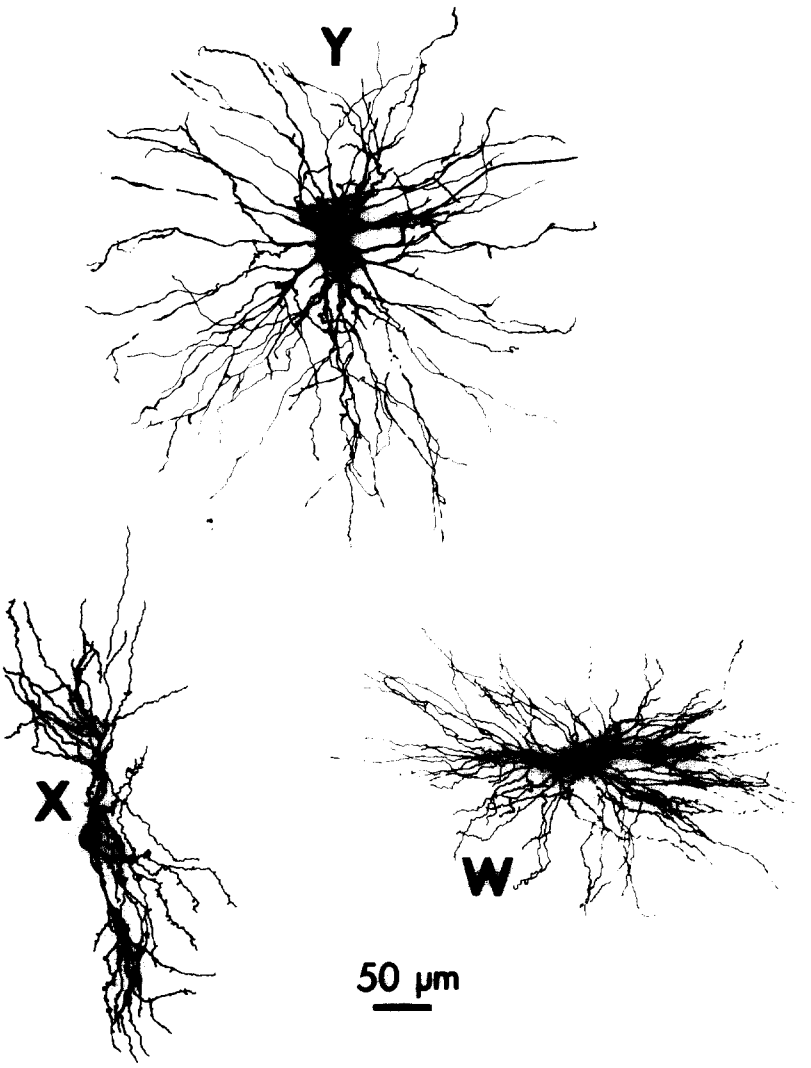

Fig. 2. Camera lucida drawings of representative W-, X-, and Y-cells from the lateral geniculate nucleus. Each of these was physiologically identified during intracellular recording and morphologically characterized from the intracellularly injected HRP. The scale is oriented parallel to the geniculate laminae.

Relative proportions of X- and Y-cells

Another issue that we can address, albeit very indirectly, is the relative distribution and proportion of the functional cell types. However, our data are presently adequate to analyse this only for X- and Y-cells in the A laminae. Past estimates of these proportions were based upon electrophysiological recording and suffered from the vagaries of uncontrolled electrode sampling biases. For instance, it is generally assumed that microelectrodes are more likely to sample larger somata than smaller ones, and that sampling probability might even be related to soma volume[10]. The observation that X- and Y-cells are recorded in roughly equal numbers in the A laminae is often interpreted to reflect strong biases in favor of the large Y-cell somata. It has been commonly and reasonably concluded that the actual geniculate X- to Y-cell ratio is similar to that suggested for retinal ganglion

cells (i.e. roughly 5–10 to 1).

For these reasons, we expected to penetrate and inject HRP into cells with larger somata more frequently than into those with smaller somata. This did not seem to happen. The soma size distribution of our HRP filled cells is virtually identical to what is actually available, based upon the sizes of adjacent somata stained for Nissl substance in the same tissue. The expected skewing of the distribution to larger somata did not occur. In any case, we were able to infer the relative proportion of X- and Y-cells by comparing the soma size distribution of our HRP filled and identified neurons with the soma size distribution of adjacent, Nissl stained cells[7]. We conclude from this that the X- to Y-cell ration in the A laminae is less than 2 to 1. Because Y-cells are relatively numerous in the top of lamina C and in MIN while X-cells are mostly confined to the A laminae, the X- to Y-cell ratio across the entire LGN probably approaches 1 to 1.

We have no evidence for any electrode sampling bias based upon soma size, at least for the geniculate X- and Y-cells in the A laminae. Presumably, factors other than soma size are rather important in determining these biases (see Ref. 7 for a more detailed discussion of this).

Retinogeniculate circuitry

This suggestion of a relatively low X- to Y-cell ratio for the LGN is surprising in the context of the much higher ratio deduced for retina (see above). If these estimates are correct, and it must be emphasized that they rest on indirect and limited data, then we can draw certain conclusions regarding retinogeniculate circuitry. Fig. 3 summarizes this and also takes into account that there are roughly 4–5 geniculate neurons for each retinal ganglion cell.

As shown, the large decrease in the X- to Y-cell ratio that results from retinogeniculate circuitry requires much more divergence along the Y-cell pathway than along the X-cell pathway. In very rough terms, each X-cell optic tract axon innervates four geniculate X-cells on average, and for Y-cells this number is roughly 20–30 geniculate neurons per optic tract axon (see Ref. 7 for details). Bowling and Michael[3] were able to inject single Y-cell optic tract axons with HRP, and they described branching patterns of single retinogeniculate axons not unlike that predicted by Fig. 3.

We concluded that retinogeniculate circuitry serves to amplify Y-cell numbers relative to X-cell numbers. This may continue central to the LGN, since geniculate Y-cells innervate many areas of visual cortex (e.g. areas 17, 18, 19 and the lateral suprasylvian cortex), often by branching axons, while geniculate X-cells project solely to cortical area 17[12,17]. Therefore, relatively few retinal ganglion Y-cells influence large areas of visual cortex.

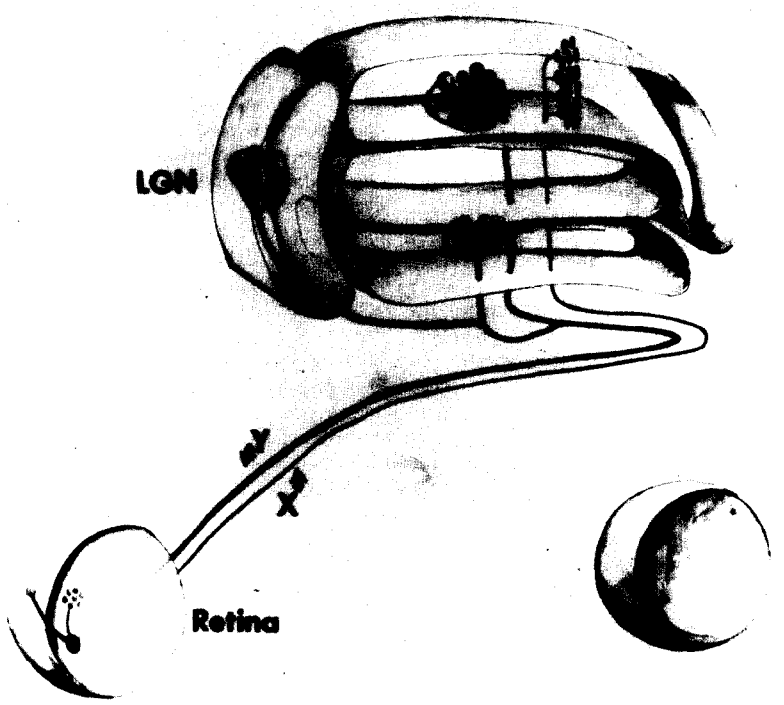

Fig. 3. Schematic and hypothetical drawing of retinogeniculate circuitry for X- and Y-cells. For simplicity, only circuitry from the contralateral nasal retina is represented. We suggest that each retinal X-cell axon innervates only about four geniculate neurons within a single limited zone and that each retinal Y-cell axon innervates 20–30 geniculate neurons within several relatively extensive zones.

Other uses of the intracellular HRP method

The above discussion illustrates some of the preliminary approaches one can take with the intracellular HRP technique. This is just scratching the surface. Many other approaches are possible, and we have begun to explore two of these.

- *Electron microscopy.* First, it is possible to carry this to the ultrastructural level. The HRP filling offers the advantage that the entire, functionally identified neuron, including the complete dendritic arbor and most or all of the axon, can be analysed via the electron microscope without the need for impractical or tedious serial sectioning procedures[14,19].

We have just begun to characterize parametric differences in the ultrastructural features of geniculate X- and Y-cells, including the distribution and types of synaptic input to these neurons[19]. For instance, preliminary data indicate that the distribution of optic tract terminals and F terminals (F terminals have flattened vesicles and are thought to be inhibitory) are similarly arranged onto X- and Y-cells, with optic tract input concentrated on proximal dendrites and F terminals distributed throughout the dendritic arbor. However, the ratio of F to optic tract terminals is much higher for X-cells than for Y-cells.

- *Abnormal structure/function relationships.* Another approach involves using the intracellular HRP technique to study the morphological concomitants of abnormal function at the single cell level. For instance, cats reared with monocular eyelid suture develop abnormalities in the central visual pathways, including the LGN (see Ref. 15). Such cats, incidentally, are often considered to be useful animal models for the study of clinical disorders of vision, such as amblyopia *ex anopsia*. Our preliminary studies have revealed very abnormal structure/function relationships for some geniculate neurons in laminae that receive retinal input from the sutured eye[8].
- *Further uses.* Once one can identify for morphological analysis a neuron from which electrophysiological data are available, many other kinds of issues can be considered. For instance, it might be possible to obtain chemical or pharmacological information from such neurons with appropriate methods, and this would further broaden the multidisciplinary

approach to the study of single neurons. Also, one might be able to relate detailed biophysical data to the dendritic geometry and synaptic input locations for individual neurons[18]. Many other possibilities could become available through this intracellular HRP methodology.

Our studies of geniculate neurons serve to illustrate only some of the most preliminary kinds of new information and suggestions made feasible by the intracellular staining of physiologically identified cells with HRP. For the first time, we can unequivocally describe the morphological characteristics of geniculate W-, X-, and Y-cells. In some favorable cases, we can follow their axon collaterals to describe local circuits for functionally identified cells. Our data also allow us to at least question some cherished tenets held by many neuroscientists, such as the ability to identify interneurons on purely morphological grounds and the notion that electrode sampling reliably favors larger somata over smaller somata. We can use the technique to study ultrastructural features of physiologically identified cells and to describe morphological characteristics of functionally abnormal neurons.

The extension of these sorts of studies of geniculate neurons might eventually separate morphological features that seem to relate to specific functional parameters from those that are epiphenomena. Many laboratories are pursuing the same approach for neurons in various parts of the neuraxis, and an overview of these data may allow us to generalize about relevant structure/function relationships. Finally, this approach can potentially be wedded to pharmacological, biochemical, biophysical, and other methodologies to obtain a wide variety of correlated multidisciplinary information about individual, identified neurons.

Acknowledgement

This research was supported by USPHS Grant EY-03038.

Reading list

1 Ahlsen, G. and Lindstrom, S. (1978) *Neurosci. Lett. Suppl.* 1, S367
2 Ahlsen, G., Lindstrom, S. and Sybirska, E. (1978) *Brain Res.* 156, 106–109
3 Bowling, D. B. and Michael, C. R. (1980) *Nature (London)* 286, 899–902
4 Boycott, B. B. and Wassle, H. (1974) *J. Physiol. (London)* 240, 397–419
5 Famiglietti, W. V. and Peters, A. (1972) *J. Comp. Neurol.* 144, 285–334
6 Friedlander, M. J., Lin, C.-S. and Sherman, S. M. (1979) *Science*, 204, 1114–1117
7 Friedlander, M. J., Lin, C.-S., Stanford, L. R. and Sherman, S. M. (1981) *J. Neurophysiol.* 46, 80–129
8 Friedlander, M. J., Stanford, L. R. and Sherman, S. M. (1980) *Soc. Neurosci. Abstr.* 6, 789
9 Guillery, R. W. (1966) *J. Comp. Neurol.* 238, 21–50
10 Humphrey, D. R. and Corrie, W. S. (1978) *J. Neurophysiol.* 41, 216–243
11 Kitai, S. T., Kocsis J. D., Preston, R. J. and Sugimori, M. (1976) *Brain Res.* 109, 601–606
12 Lennie, P. (1980) *Vision Res.* 20, 561–594
13 Le Vay, S. and Ferster, D. (1977) *J. Comp. Neurol.* 172, 563–584
14 Muller, K. J. and McMahan, U. J. (1976) *Proc. R. Soc. London, Ser. B* 194, 481–499
15 Sherman, S. M. (1979) *Trends NeuroSci.* 2, 192–195
16 Standord, L. R., Friedlander, M. J. and Sherman, S. M. (1981) *J. Neurosci.* 1, 578–584
17 Stone, J., Dreher, B. and Leventhal, A. G. (1979) *Brain Res. Revs* 1, 345–394
18 Turner, D. A. and Schwartzkroin, P. A. (1980) *J. Neurophysiol.* 44, 184–199
19 Wilson, J. R., Friedlander, M. J. and Sherman, S. M. (1980) *Soc. Neurosci. Abstr.* 6, 583
20 Wilson, P. D., Rowe, M. H. and Stone, J. (1976) *J. Neurophysiol.* 39, 1193–1209

Michael J. Friedlander is Assistant Professor of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294, U.S.A. and S. Murray Sherman is Professor of Neurobiology and Behavior, State University of New York at Stony Brook, Long Island, NY 11794, U.S.A. Reprint requests, correspondence, etc. should be sent to Dr Sherman.

Molecular mechanisms of visual transduction

M. Deric Bownds

Photoexcitation of a single visual pigment molecule can influence many ion channels. With the discovery that this excitation causes large and rapid changes in cyclic GMP and calcium metabolism the way has been opened for biochemical studies of the amplification stages of visual transduction.

No one can fail to be amazed at the ability of our visual system to function both in dim moonlight and bright sunlight, a range of light intensity spanning 8–10 log units. Much of this great adaptability is accounted for by the properties of individual photoreceptors, with rod cells responding to low light levels and cone cells predominating at higher intensities. In dim light our rod photoreceptors can signal the absorption of single photons by transiently decreasing the conductance of the outer segment's plasma membrane, but as the ambient light intensity increases, their sensitivity is drastically reduced. In biochemical terms this translates into the statement that absorption of a single photon by a visual pigment molecule triggers the closing of 100–1000 sodium channels; and further, that absorption of only a few photons sets in motion mechanisms which diminish the sensitivity of the system to further illumination. Recent experiments indicate that both calcium and cyclic nucleotides play a central role in these processes*.

Work on the biochemistry which regulates phototransduction began over 100 years ago with the discovery of rhodopsin. Studies between 1930 and 1970 showed rhodopsin to consist of a membrane protein, opsin, bound to the 11-*cis* isomer of retinal (vitamin A aldehyde). The only known action of light in vision is to photoisomerize this isomer mainly to the all-*trans* configuration. A series of colored intermediates then form as protein conformation changes occur, all-*trans* retinal detaches from opsin, and eventually rhodopsin is regenerated. Until 10 years ago, work on the chemistry of transduction centered almost entirely on this rhodopsin cycle and the retinal isomers.

This period ended with the discovery of two new light triggered reactions in vertebrate rod photoreceptor membranes: a phosphorylation of rhodopsin, and activation of enzymes regulating cyclic nucleotide metabolism. The list of known light-initiated reactions has now grown longer, and many studies are shifting away from direct emphasis on the visual pigment rhodopsin to concentrate on the proteins through which rhodopsin regulates calcium and cyclic nucleotide metabolism. Progress in the past 2 years has been so rapid that many electrophysiological studies have failed to keep pace with the new biochemical information becoming available.

The preparation most useful for correlating both electro-physiological and biochemical studies has been the outer segment of the amphibian rod cell (Fig. 1). This large cylindrical structure (6 μm diameter, 60–70 μm length) is a modified cilium which consists of a plasma mem-

* Recent work on transduction is summarized in the October 1980 issue of *Photochemistry and Photobiology* (Vol. 32, No. 4) which contains the proceedings of a meeting held at Ohtsu, Japan in December 1979, and also in Vol. 15 of *Current Topics in Membranes and Transport* (Academic Press, 1981), resulting from a recent lecture series at Yale University. Two earlier reviews should also be consulted for references covering much of the information presented here[7,12].

0378 – 5912/81/0000 – 0000/$02.50