Microcircuitry and functional architecture of the cat retina

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Neurons in cat retina belong to specific types. Each type is characterized by a specific correspondence between morphology and physiology and forms a regular array that connects lawfully to the arrays of certain other types. Two circuits have been traced quantitatively through these arrays from photoreceptors to alpha- and beta-ganglion cells. The 'cone-bipolar circuit' appears to convey the centre-surround receptive field to ganglion cells, using cones in daylight and rods (via gap junctions to cones) in twilight. A 'rod-bipolar circuit' appears to convey the quantal signal and the pure center receptive field to the ganglion cells in starlight.

Identified cell types

The first hint that the ganglion cell population in cat retina is composed of identified cell types was provided in 1974 by Boycott and Wassle1. Their crucial contribution was to compare neurons at the same retinal position, and in doing so found two fundamental anatomical categories, termed alpha and beta (Fig. 1). The alpha cell has a large soma and sparsely branched dendrites that form a wide field, whereas the beta cell at the same retinal position has a medium-sized soma and more densely branched dendrites forming a narrower field. Boycott and Wassle suggested a correspondence between alpha- and physiological Y-cells, and also between beta- and physiological X-cells (Fig. 1). Cleland and colleagues' went on to support this conjecture for Y-cells by mapping all the Y-cells in a small patch of living retina and then mapping all the alpha-cells in an anatomical preparation of the same tissue. The two maps corresponded closely.

Meanwhile Famiglietti and Kolb7 had observed that the dendrites of alpha- and beta-cells stratify sharply in the...
inner plexiform layer. They proposed that ganglion cells branching high in the inner plexiform layer (sublamina a) are off-center and those branching low (sublamina b) are on-center. Nelson and they soon confirmed this by recording from ON and OFF ganglion cells and then filling them with fluorescent dye. Recently, direct proof of the correspondence between the four morphological and the four physiological categories has been accomplished in several laboratories by intracellular recording and dye injection. Taken as a whole, these studies provide good evidence that ganglion cells in the mammalian retina are of 'identified types'. They also show that a particular cell type can be characterized by the distinctive co-variation of its structural features, such as cell size, branching pattern and stratification, with its physiological features such as receptive field size and structure, time course of response, and conduction velocity.

There were two important corollaries to defining ganglion cell types. First, if the form and function of a ganglion cell is defined, it seemed likely that the neurons providing its input would be similarly well defined. This has proved to be the case; there is now evidence that every neuron in cat retina belongs to an identified type and that there are roughly 60 types in all. Second, the synaptic connections between an identified cell type and its input neurons must also be well defined, otherwise form and function at its output would not consistently match. This corollary is also richly supported by recent studies that demonstrate specific and remarkably regular patterns of synaptic connection between specific neuron types.

Structure of identified neural pathways

Identification of the actual synaptic connections in the chain of neurons linking photoreceptors to ganglion cells was an important next step. Neuroanatomy since Cajal had largely satisfied itself with inferring these connections from light microscopic evidence of contiguity between presynaptic and postsynaptic structures. Such inferences proved accurate for the simplest cases, where only two types of...
process are contiguous. Cajal noticed, for example, that the dendrites of only one form of bipolar neuron reach the layer of rod axon terminals (Fig. 2e). Assuming that only this bipolar could receive rod input, he called the cell a 'rod bipolar', and in this he was proved correct by electron microscopy.

However, this approach is useless where there is contiguity between many types of process. Thus, Cajal noted that the end of the rod bipolar axon was contiguous with ganglion cell bodies (Fig. 2). Again inferring the presence of a synaptic connection, he concluded that rods connect to ganglion cells via a three-neuron arc. Here, however, he was quite wrong. Electron microscopy showed that in cat retina the rod bipolar connects only to amacrine processes and that ganglion cell bodies are devoid of synapses. Cajal's error is not surprising because the processes of many cell types intermingle at the level of the ganglion bodies, so his inference could hardly have been better than a guess.

Studies in the 1960s identified the fine structure of synapses between the major classes of retinal neuron. These observations were gleaned largely from single, ultrathin sections and short series of consecutive sections, but because there was no three-dimensional information, it was impossible to tell which types of bipolar, amacrine, and ganglion cell were involved. This difficulty was overcome in the 1970s by electron microscopy of Golgi-impregnated or HRP-filled cells and by reconstruction from long series of ultrathin sections.

Cone pathways to ganglion cells

Both of these methods confirmed that the neurons Cajal had called 'cone bipolars' do in fact receive cone input. They are a diverse group, with as many as 10 distinct cell types. It was also found that the cone bipolars form specific pathways.

Two types connect to the ON-beta ganglion cell, and the connection is a strong one, each type providing as many as 50 synapses to the bushy dendrites of a single ON-beta cell (Fig. 3). These cone bipolars (CB) were designated b1 and b2 in our terminology because their axons terminate in sublamina b of the inner plexiform layer. Two further types connect to the OFF-beta ganglion cell in sublamina a, and so were designated CBA and CBB (Ref. 11). At least one member of each pair also connects to the corresponding alpha ganglion cell (Fig. 3).
Rod pathways

Two rod pathways to ganglion cells have been discovered. One route is from the rod axon terminal cones, via gap junctions, to the fine basal processes that Cajal had observed to extend from the cone axon terminal (Fig. 2). Thus, one pathway for rod transmission to the ganglion cell is through the cone terminal, and so through the cone-bipolar pathways described above. Nelson showed that rod signals in the moderately dark-adapted state do in fact take this path. The other route from the rod axon terminal is to rod bipolar via chemical synapses upon their dendrites. The rod bipolar axon is directed at a type of amacrine cell called AII (Ref. 20). This neuron is remarkable in having a bifunctional output. In the ON sublamina, the AII cell forms numerous, large gap junctions with the axon terminal of the CBβ1 cone bipolar, and in the OFF sublamina it forms numerous chemical synapses with the dendrites of the OFF-beta and OFF-alpha ganglion cells. Thus, a second possible pathway for the rod signal is shown below.

rod → rod bipolar → AII → CBβ1 (axon) → ON-beta, ON-alpha
→ OFF-beta, OFF-alpha

Function of identified neural pathways: cone pathways

The membrane potentials of the two types of cone bipolar that innervate the ON-beta cell are driven by light in opposite directions. Type CBβ1 depolarizes and CBβ2, hyperpolarizes to the same stimulus in a transient and sustained manner. One may infer that upon illumination, transmitter release increases in the first cell in a transient and sustained manner and decreases in the second cell. It is then logical to suppose that the transient and sustained response of the ON-beta cell is caused by increased excitation from the first cone bipolar and decreased inhibition from the second one (see centrefold).

Such complementary action of the cone bipolar pair resembles the mechanism of a push–pull amplifier. The advantage of this over simple excitation or disinhibition is that it can provide a linear signal to the ganglion cell over a wider dynamic range. Also, it can provide more gain than a single-ended mechanism, which may contribute to the cell's brisk response to changing contrast. The cone bipolar pair interconnecting the OFF-beta cell is thought to act similarly, but in a reverse manner, so that center excitation and disinhibition occur when the light is switched off. The model is the simplest way to unify what is known of the anatomy and physiology of the pathways, however some circumvention is necessary in that there is as yet no direct evidence that any cone bipolar neuron employs an inhibitory transmitter.

Rod pathways in twilight and starlight

It did not occur to Cajal that even if the anatomical pathways for rods did merge with those of cones, their signals would mix only over a narrow range of light intensity. He might have reasoned, since perception of color fails in twilight, that cones cease to function in dim light and that rods, which he presumed were for 'black and white', take over. If so, the cones and rods might just as well share a common anatomical pathway. Since it is the cone axon terminal that drives the push–pull mechanism, the source of the signal that controls transmitter release by this terminal is not very important. In daylight, the signal is supplied by transduction in the cone outer segment. In twilight it is supplied by transduction in the ganglion cell change drastically. The surround antagonism weakens, and the center itself becomes so extremely sensitive that it can register and linearly sum the single quantum events that arise in individual rods. Thus, in low intensity levels equivalent to that of starlight the ganglion cell changes from a detector of local contrast to a spatial summator of single photons.

The rod bipolar pathway is the obvious candidate to serve this latter function. Our calculations based on an electrotonic model suggest that a current of one picoampere arising in a rod outer segment due to the absorption of a single photon would be dissipated if it were injected into the rod–cone electrical network. Furthermore, the calculations suggest that if the rod–cone gap junctions were to close after prolonged dark adaptation, the signal would be concentrated at one rod axon terminal where it would be effective in changing the membrane potential and thus in altering transmitter release at the chemical synapse onto the rod bipolar. The third-order neuron in this pathway, the AII amacrine cell, directs the rod bipolar signal into an excitatory pathway for the ON ganglion cell and into an inhibitory pathway for the OFF ganglion cell. The antagonistic surround effectively drops out in this state presumably because it belongs to the functional organization of the cone bipolar and not of the rod bipolar pathway (see centrefold).

Identified neural circuits

It is useful to distinguish the idea of a neural 'circuit' from that of a neural 'pathway'. In our terminology, pathway denotes a linear sequence of connections in one dimension and is not...
Cone bipolar circuit for the ON-beta cell

Near the center of the area centralis there are about 26,000 cones, 6500 CBb₁ cone bipolars, and 2000 ON-beta ganglion cells per square millimeter (Fig. 4). Convergence of successive arrays is quite limited; serial reconstructions show that the beta-cell receives input from only four CBb₁'s, and these in turn each receive input from only about four cones (Fig. 5). Thus the spatial diameter of the beta-cell's receptive field apparently derives from the receptive fields of only a few CBb₁'s. The fields of the latter are essentially concentric because their centers are wide (70 μm diameter) whereas their spacing is narrow (12 μm) (see centrefold). It is obvious that something is missing in this circuit that would account for the size of the CBb₁ receptive field. This is so because the CBb₁ dendritic field, of 15 μm diameter, is less than one-quarter the diameter of its receptive field center. There must be additional neural elements to collect cone signals for the bipolar over a wider area.

The obvious candidates are the two identified types of horizontal cell. The type-B horizontal cell spans about 60 μm in the area centralis and its dendritic processes innervate the axon terminals of most of the cones within this field. It is our hypothesis that the chemical transmitter of the cone and the type-B horizontal cell are mutually excitatory. When a cone hyperpolarizes to light, it would hyperpolarize the type-B horizontal cell as well. This cell would in turn hyperpolarize all the cones to which it is connected. Such a mechanism could account for the size of the cone bipolar's receptive field center (see centrefold).

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**Fig. 6.** Actual convergence and divergence through successive arrays along the rod bipolar pathway. In this circuit the actual connections between rods, rod bipolars, and AII amacines are close to the potential connections; the actual connections between AII's, CBb₁'s, and ON-beta ganglion cells are narrower than the potential connections. See text for structure-function relationships of this circuit.
The type-A horizontal cell extends about 80 μm in the area centralis and its dendritic processes also innervate the cones. This span is not by itself great enough to account for the cone bipolar surround; however, the type-A cell has extremely thick dendrites that are connected via large gap junctions to dendrites of adjacent type-A cells. Thus, they seem well suited to conduct electrotonic signals over wide expanses. We believe that chemical transmission from the cone to the type-A horizontal cell is excitatory and that transmission from the type-A horizontal cell to the cone is inhibitory. When a cone hyperpolarizes, it would hyperpolarize the A horizontal cell by disfacilitation. The type-A horizontal cell would then depolarize by disinhibition, all of the cones to which it is connected, and thus antagonize their intrinsic responses to light. This mechanism could explain the wide cone bipolar surround (see centrefold).

The overall circuit proposed here, including the type-B and type-A horizontal cells, can account for the size of both the cone bipolar center and its surround. It would function in daylight when driven by cone outer segments, and in twilight when driven by rods through the rod–cone gap junctions.

**Conic bipolar circuit for the ON-alpha cell**

The pathway cone → CBb₁ → ganglion cell is the same for the ON-alpha ganglion cell as for the ON-beta cell, but the circuit is quite different. The ON-alpha cell's dendritic field in the tangential plane is broad (150 μm in diameter). Serial reconstructions show that it is penetrated by the regularly-spaced axons of approximately 180 CBb₁ cells, each of which provides a few synaptic contacts. Thus, whereas a beta cell collects about 50 contacts from each of four CBb₁ cells, an alpha cell collects two to four contacts from nearly 200 CBb₁s (Fig. 3). This wide convergence accounts for the alpha cell's characteristically wide receptive field center (see centrefold).

This circuit may explain, at least in part, the reason why the response evoked from the alpha cell's receptive field center is typically transient. At every point in the center, a stimulus provides excitation to the alpha cell by activating the centers of a few CBb₁s. The same stimulus also falls on the surrounds of many other CBb₁s, tending to suppress their excitation of the alpha cell. This disfacilitation, which occurs with some delay, would tend to cut short excitation to the alpha cell, thereby contributing to the characteristically transient response.

The circuit might also explain why there is no null position for reversing the contrast of a periodic stimulus in the alpha cell receptive field center. The responses of a CBb₁ to dark and light bars in its receptive field center are nearly equal but of opposite polarity. Thus, they would tend to cancel in the alpha cell. However, the responses of the CBb₁ to dark and light bars in its receptive field center are of the same polarity and would tend not to cancel in the alpha cell. Therefore, the contribution of many, overlapping CBb₁s to the alpha cell's receptive field center would cause the alpha cell to respond to contrast reversal. Whether the CBb₁ circuitry can account quantitatively for the alpha cell's transient response and lack of a null position remains to be established.

**Rod bipolar circuit for the ON-beta cell**

Near the center of the area centralis there are about 450,000 rods, 30,000 rod bipolar. 4100 AII amacrines, 6500 CBb₁ axons, and 2000 ON-beta ganglion cells per square millimeter (Fig. 4). Through these successive arrays, about 1500 rods converge on a beta ganglion cell. Most of the convergence occurs in the first two stages, where 30 rods contact each rod bipolar and 30 rod bipolar contacts each AII amacrine cell (Fig. 4). The AII cell contacts the CBb₁ axon without convergence. And the latter, providing a minor convergence of about 4:1 on the beta cell, serves as the final common path for both the rod bipolar and the cone bipolar circuits. The cone bipolar circuit involves about the same number of rods as the rod bipolar circuit. This must be so because the receptive field centers established by the two circuits are approximately the same size. However, the rod bipolar circuit accomplishes this stepwise through the agency of many narrow-field bipolar neurons, whereas the cone bipolar circuit merges the receptor signals at the first synaptic stage in broad-field horizontal cells.

This rod bipolar circuit connecting 1500 rods to the ON-beta ganglion cell in the area centralis accounts for the size of what is in effect a pure center receptive field in the fully dark-adapted state. Furthermore, it explains the ON-beta cell's maintained discharge in the dark. This discharge, about 15 spikes per second, has been attributed to quantum 'dark events' arising from thermal isomerization of single rhodopsin molecules, which are transmitted through the circuit with a gain of 2-3 spikes per event. The number of these events in the receptive field of a ganglion cell has been estimated at about 6 per second. Fifteen hundred rods converging on a ganglion cell would carry this many dark events if their rate of occurrence were about 0.004 per second per rod, and this figure is close to what has been measured recently in monkey rods.

These considerations may also explain why the number of rods connected to the beta-cell is 1500 and not 15,000 or 150. If 15,000 rods were connected to the beta cell, the maintained discharge due to quantal dark events would be on the order of 150 spikes per second, and against this high background discharge the incremental 2-3 spikes caused by photon absorption would tend to be obscured. Thus the upper limit on the number of rods that can be usefully connected at high gain to a cat ganglion cell may be limited by the thermal stability of rhodopsin at 37°C. On the other hand if only 150 rods were connected, quantal events would be rare in the ganglion cell (less than one per second) and the dynamic range of the ganglion cell (0-800 spikes per second) would not be exploited.

There also appears to be a reason why the convergence of 1500 rods is accomplished in two stages (rod→rod bipolar→AII amacrine) rather than directly (rod→rod bipolar→ganglion cell) as Cajal supposed. The quantal signal rises above the continuous noise in the rod outer segment only modestly, by a factor of about five. This noise will tend to increase at each subsequent synaptic stage by the square root of the number of elements converging. If it were not removed by some form of non-linear signal processing, the quantal signal would certainly be swamped by the continuous noise and could not be transmitted to the ganglion cell. It is likely, therefore, that the reason for breaking the rod convergence into two steps is to permit suppression of the continuous noise at each step by a thresholding mechanism.

The last feature of the rod bipolar circuit to be considered is its divergence. As illustrated in Fig. 4, one rod projects to two rod bipolar cells, which project to 5AII amacrines, which in turn project to eight CBb₁s, which terminate on two beta ganglion cells. The over-
all pattern is progressive divergence through the first four stages, with a 
reconvergence at the end. This arrangement 

extends the number of independent copies of the quantal signal to eight by divergence and then finally brings them back together by reconvergence. This feature of the circuit resembles a signal averaging mechanism. Its effect would be to remove synaptic noise (due to spontaneous release of chemical transmitter) that would otherwise accumulate in the circuit, and this would be essential in order to retain the tiny quantal signal.

Concluding remarks
Certain definite conclusions emerge from what has been learned so far about the cat retina: (1) it is composed of 'identified types' of neuron, i.e., categories of neuron defined by invariant relationships between form and function; (2) the types are synaptically connected in invariant sequences that define 'identified pathways'; (3) the types are arranged in well defined, two-dimensional arrays, and (4) the arrays are connected in specific patterns of convergence and divergence that define 'identified circuits'.

A complete mechanistic account of the cat retina is not yet at hand. Only about one-third of the 60 morphological forms of neuron in it have been examined in any detail, and much remains to be learned about even the most well-studied ones. For example, the alpha and beta ganglion cells collect significant amacrine input, but which amacrine types are involved is unknown. Also, there must be switches that exchange one circuit for another, e.g., in the transition between light-adapted and dark-adapted states, and these are just beginning to be identified. It must be emphasized, therefore, that the relationships between form and function suggested here are based on incomplete knowledge. Circumpection is certainly warranted, especially in view of the errors into which the master, Cajal, was led by relying too much on inference. On the other hand, his inferences lent excitement and focus to his drive toward understanding the 'impeneetrable thicket of gray matter' and one hopes that the same may prove true for the present speculations. It is likely that continued probing by electron microscopy and microelectrodes, as well as by modern biochemical and molecular methods, will provide information of sufficient detail and resolution to achieve the kind of mechanistic account of this tissue that he envisioned.

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Selected references
20. Farnighetti, E. V. and Kolb, H. (1975) Brain Res. 84, 293–300

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