coids in their postcranial anatomy and locomotor behavior. Similarities between this *Aegyptopithecus* ulna and that of the extant New World howler monkey, together with the absence of any particular similarity to ulnae of Miocene to Recent cercopithecoids, argue against such an interpretation. Rather, the evidence presented here suggests that early locomotor behavior of hominoids somewhat resembled that of extant New World monkeys. Such a similarity need not imply a particularly close phyletic relationship between these two groups.

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2. Although the ulna was not found in direct association with dental remains of *A. zeuxis*, assignment to that species appears highly probable for the following reasons. (i) On the basis of detailed morphological and metrical comparison (*3*) with mammalian ulnae from several orders, the fossil shows definite primate characteristics and is unlike the ulnae of rodents or carnivores. (ii) Among the known mammalian fauna from the Fayum [E. L. Simons and A. E. Wood, *Peabody Mus. Nat. Hist. Yale Univ. Bull. 28* (1968)] *Aegyptopithecus* is the most likely taxon to which this ulna could be assigned on the basis of the size of skull and dentition. There are no Fayum rodents or carnivores of a size correspondent to this ulna and all other Fayum primate species are disproportionately smaller. (iii) Limb bone fossils are much less common than teeth and jaws in the Fayum deposits and are known for only a few common animals. In consequence, it is improbable that this bone belongs to an animal not known from teeth or jaws (*3*). (iv) The bone is structurally that of an arboreal animal, but no group of arboreal nonprimates in this size range is known from any site throughout the whole Tertiary of Africa. (v) YPM 23940 resembles ulnae of Miocene apes and looks much like a robust version of the ulna of *Pliopithecus* from Czechoslovakia, which is, otherwise, the oldest well-preserved hominoid ulna.
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11 March 1975

Horizontal Cells in Cat Retina with Independent Dendritic Systems

Abstract. *Cat horizontal cells are retinal neurons with two functionally distinct parts; the cell body receives signals predominantly from cones, while the terminal arborization receives predominantly from rods. The long thin process connecting these parts neither generates impulses nor allows significant passive electrotonic conduction between them.*

One class of horizontal cells in the mammalian retina has an extraordinary appearance (*1*, *2*). A cell body with a radiating system of dendrites gives off a single, long, thin axon-like process that ends in an enormous terminal arborization (Fig. 1b) whose size far exceeds that of the dendritic field of the cell body (Fig. 1a). The terminals of this distant axonal arborization go only to rod spherules, while the dendrites at the cell body go only to cone pedicles (*2*). This pattern suggests that the cell is receiving signals from cones and conducting them to rods.

We have succeeded in recording from within the cell body and separately from within the terminal arborization of this type of horizontal cell in cat retina and have injected the structures in question with Procion Yellow (*3*). The results indicate that both ends of this cell receive signals from photoreceptors but that the long axon-like process plays no significant role in conducting these signals from one end of the cell body to the other.

Some horizontal cells injected with Procion bear a remarkable morphological similarity to axon-bearing, Golgi-impregnated cells (compare Fig. 2a with Fig. 1a). These horizontal cells have discrete round perikarya from which arise five main dendrites. The latter divide dichotomously, producing many overlapping wavy branches that in well-stained preparations can be seen to bear small clusters of terminals that are known to contact cone pedicles (*2*). The nuclei of these cells appear much brighter than the rest of the cell body and are thus readily discernible in preparations injected with Procion. Although the axon has not stained in our horizontal cells injected with Procion, we can distinguish the axon-bearing cell type by these morphological criteria and can thus be confident in attributing particular physiological responses to the correct structure.

The terminal arborizations of horizontal cells are quite different in appearance from cell bodies (compare Figs. 2b and 1b with Figs. 2a and 1a). The main axonal branches are, at their thickest, only about 5 μm in diameter, and we were surprised to be able to record from such small structures. A meshwork of ultrafine processes and bright dots can be seen in the Procion-injected terminal arborization of Fig. 2b, which is also particularly characteristic of Golgi-impregnated material and represents the fine branches and thousands of terminals that go to rod spherules (*2*). Nuclei have not been seen in these units.

Both cell bodies (*4*) and terminal arborizations respond to light of all wavelengths by hyperpolarizing shifts in membrane potential (S-potentials). The physiology of the terminal arborization is most reliably distinguished from the physiology of its cell body by the procedure shown in Fig. 1c. Responses were obtained to 658-nm (red) and 400-nm (blue) flashes which were adjusted to bleach equal amounts of rod pigment (*5*). The responses of the terminal arborization to these stimuli match at threshold and at low intensities, but those from the cell body do not match at any intensity. The fact that the low intensity responses of the terminal arborization have identical waveforms to these matched stimuli implies that the axon terminal is driven purely by rods at these intensities. The cell body, on the other hand, has a small rod input (*4*), but this is so insensitive that even at threshold the 658-nm light stimulates the cone input more strongly than the rod input (see Fig. 1d). Thus these traces are never superimposable.

At higher intensities the waveforms of the responses produced by the terminal arborization to rod-matched stimuli no longer match (Fig. 1c) because of a small cone input into this structure. Therefore, both terminal arborizations and cell bodies of these horizontal cells receive inputs from both rods and cones, but the proportion of these two inputs is quite different (*6*). In terminal arborizations about 80 $\pm$ 2 percent of the peak response was contributed by rods (three well-stained units), and the remainder by cones; in cell bodies, regardless of morphological characteristics, about 58 $\pm$ 14 percent of the peak response came from cones (five well-stained units), and the remainder from rods (means $\pm$ standard deviations).

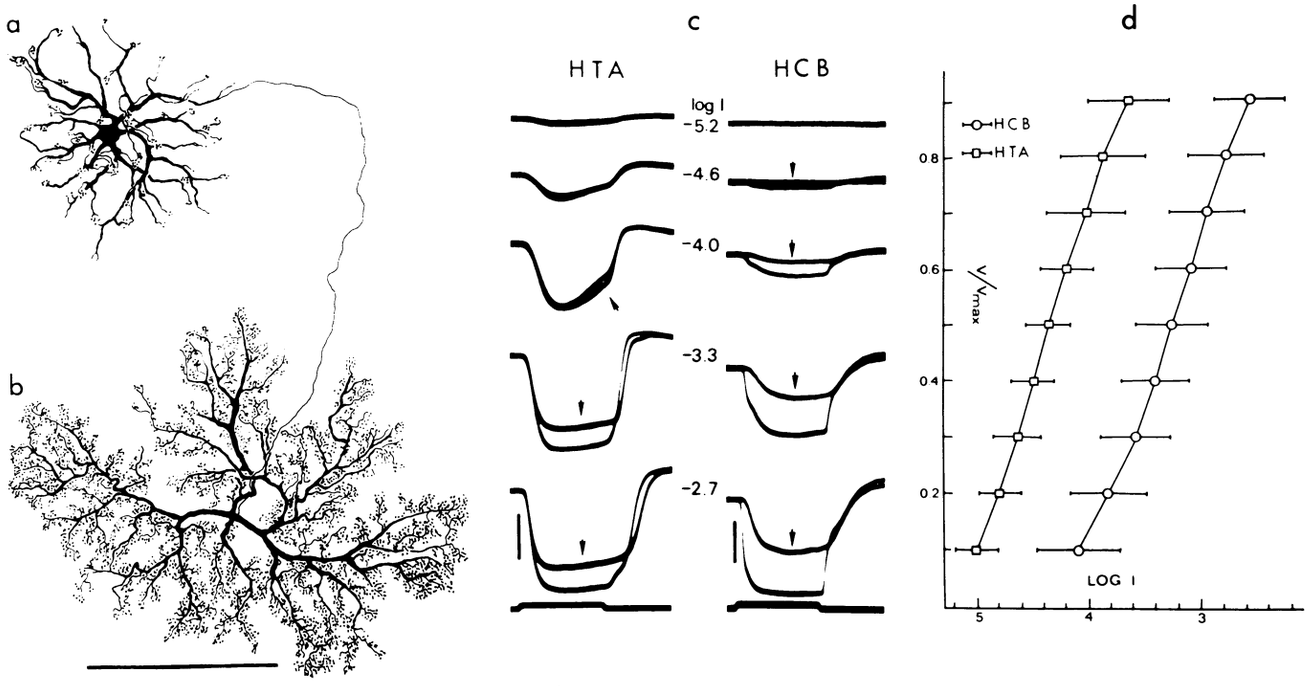

Fig. 1. (a and b) Golgi-impregnated cat horizontal cell of the axon-bearing variety. (a) Cell body; (b) terminal arborization. Calibration, 100 μm. (c) Responses of a horizontal terminal arborization (*HTA*) and a horizontal cell body (*HCB*) to stimuli matched for rods. One of each superimposed pair of traces was obtained with 400-nm light (arrows), and the other with 658-nm light. The energies of these two wavelengths were adjusted to stimulate rods equally. Where the traces are exactly superimposed, only rods contribute to the responses; where they differ some cone input is implied. Intensities refer to 400-nm light. Flash length, 520 msec; voltage calibration, 10 mv. The stimuli were large (about 1 cm) and diffuse. *HCB*, responses from cell of Fig. 2a; *HTA*, responses from a unit different from Fig. 2b (both stained). (d) Normalized, averaged intensity response curves for the rod inputs into five *HCB*'s and three *HTA*'s. Points are means ± standard deviations computed on the log I axis; 400-nm stimuli. Abbreviation: V/V_{max}, the fraction of the maximum rod response.

The rod limbs of the intensity response curves of both terminal arborizations and cell bodies can be seen in relative isolation from the cone limbs by using deep blue (400-nm) stimuli. If the rod component of the cell body response arrived from the terminal arborization by way of the long connecting process, the intensity response curves of the rod limbs of both terminal arborizations and cell bodies should be the same, except for possible voltage attenuation. To correct for this possible attenuation, these rod curves have been normalized to the peak rod response in each unit (*6*), and the results appear in Fig. 1d. The curves, however, are quite separate. It takes 1.1 ± 0.2 log units less light to half-saturate the rod input into terminal arborizations than it takes to half-saturate the corresponding rod input into cell bodies. Thus the rod signal that is seen in the cell body must not arrive from the terminal arborization by passive electrical conduction down the connecting process.

The way in which a rod input gets into cell bodies, or a cone input into terminal arborizations, is still unclear. Raviola and Gilula (*7*) have found numerous gap junctions, which appear to be the morphological counterparts of electrical synapses, between rods and cones. This could provide a means for some mixing of rod and cone signals at an early stage in the retina. There are also gap junctions between horizontal cell processes in cats, but so far we have found them only between dendrites and not between dendrites and terminal arborizations.

Certain horizontal cells of cat retina are, therefore, neurons that have a different physiology in their terminal arborizations than in their cell bodies. From an electrophysiological standpoint the "axon" of this cell serves more to isolate the cell body from its terminal arborization than to interconnect the two processes. These cells do not produce action potentials, and the small caliber (0.5 μm in diameter) and the great length (300 to 500 μm) of the "axon" most likely allow less than 1 percent passive electrical conduction of the signals between the two ends (*8*). Thus the axon puts electrotonic distance between the two parts of the cell while still maintaining a nutritive link.

One reason for limiting the conduction of signals from one end of this cell to the other may be to restrict interactions between the rod and cone systems of mammals, which have different dynamic ranges of function. Other instances of a separation of rod and cone channels in cat retina are found not only in the outer but also the inner plexiform layer where separate bipolars (*9*) and even amacrine (*10*) cells occur which appear to handle either exclusively or mostly one or the other system. It is in-

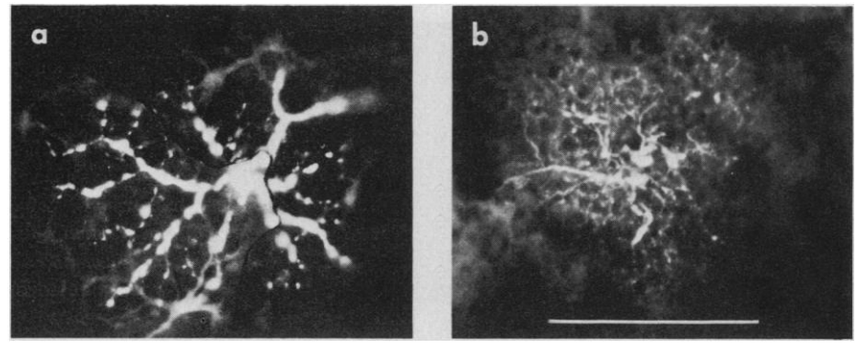

Fig. 2. Horizontal cells of the cat retina stained with Procion. (a) Cell body of the axon-bearing variety; (b) horizontal cell terminal arborization. Calibration, 100 μm.

triguing that in the teleost retinas entirely separate horizontal cells have evolved for rods and cones (*11*), whereas in mammals one cell of the axon-bearing variety appears to perform both functions. A possible advantage in having single neurons with electrically isolated regions is that the number of independent integrating units within the brain (*12*) can be increased without adding more cells. It would therefore not be surprising if similar arrangements were present elsewhere in the central nervous system.

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13. We thank B. B. Boycott for his assistance in helping us classify horizontal cells by light microscopy and for providing the Golgi-impregnated cell of Fig. 1. We thank E. V. Famiglietti and Wilfrid Rall for helpful discussion. Supported in part by National Eye Institute postdoctoral fellowship FO2 EY52967 and in part by a Max Kade postdoctoral research grant.
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Polyspermy Block of *Spisula* Eggs Is Prevented by Cytochalasin B

Abstract. *The eggs of the surf clam* Spisula solidissima *have a built-in mechanism that prevents polyspermy: the eggs show a 70 percent decrease in sperm receptivity 5 seconds after fertilization, and become completely resistant to sperm by 15 seconds. When the outer egg coat (vitelline layer) was removed, there was no change in fertilizability or the timing of the block to polyspermy. This suggests that the alteration occurs in or at the plasma membrane. Such changes in the egg surface were sensitive to low concentrations of cytochalasin B.*

A means for preventing more than one sperm nucleus from fusing with the egg nucleus is a prerequisite for preserving the diploid state of the genome in eukaryotic organisms. In eggs that do not contain yolks, this occurs by excluding all but one sperm and is referred to as the block to polyspermy. The mechanism of this block has been worked out for eggs of the sea urchin and the hamster. Earlier work on these eggs indicated that the fusion and exocytosis of the cortical granules, referred to as the "cortical reaction," was involved in the block to polyspermy (*1*). Later it was shown that macromolecules released from these granules at fertilization are the responsible factors (*2, 3*).

However, the cortical reaction may not constitute the sole block to polyspermy. In the sea urchin, numerous sperm attach to an egg within the first second after insemination and hundreds or even thousands of sperm are attached to the egg surface by the time the cortical reactions are initiated 25 seconds later (*4*). Many workers, therefore, have suggested that some earlier change in the egg results in the block to polyspermy, this change presumably occurring at the plasma membrane. Kinetic analyses of sea urchin fertilization indicate that two types of blocks occur. The first is rapid and only partially effective, and could correspond to a rapid change in the plasma membrane. The other block is later-acting and completely effective, and its time sequence corresponds to that of the cortical reactions (*5*). Whether the fast block occurs at all, or indeed whether it occurs in sea urchin eggs, is still a matter of debate (*4*).

There are eggs of other organisms that do not exhibit any sort of cortical reaction but must have a block to polyspermy. The best-studied examples of these are the pelecypod mollusk *Spisula solidissima* (*6, 7*) and the echiuroid worm *Urechis caupo* (*8*). These eggs are useful for studying blocks to polyspermy in the absence of cortical granule exocytosis. The experiments described here demonstrate a fast block to polyspermy in *Spisula* and may provide a model for the proposed fast incomplete block in eggs that do have cortical reactions, such as those of the sea urchin and hamster.

Ripe ovaries from *S. solidissima* females were minced with scissors, and the eggs were strained through several layers of cheesecloth into seawater. The eggs were immediately sedimented by a hand centrifuge, then suspended in filtered seawater; this washing procedure was repeated three to four times. Semen was obtained as the exudate from excised testes that had been stored at 4°C for 15 minutes; the exudate was filtered through cheesecloth and stored at 4°C until it was diluted just before use (*9*). All experiments were