around the central matrix. The outer diameters of the circular profile of filaments seen surrounding the electron-lucent matrix in transverse sections were the same as in the longitudinal section, with the single exception of the smaller incomplete forms (Fig. 2, A). The outer diameters in this case were approximately 740 A. It is interesting to note that the cylinder structures were always enveloped by the cell processes.


Crossing axons in the third nerve nucleus.

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The research presented in this paper studied the pathway taken by the crossed fibers of the third nerve nucleus in an animal whose nucleus has been well mapped and found to correlate well with higher mammals and man. Autoradiography using tritiated amino acid labeled the cell bodies and axons of the left side of the oculomotor nucleus of the cat. Axons so labeled could be seen emerging from the ventral portion of the left nucleus through the median longitudinal fasciculus (mlf) to join the left oculomotor nerve. Labeled axons were also seen to emerge from the medial border of the caudal left nucleus, cross the midline, and pass through the right nucleus and the right mlf to join the right oculomotor nerve. These latter axons must be the crossed axons of the superior labial nucleus. The organization of the vertebrate retina has become increasingly well recognized in recent years. The present paper describes in detail for the first time the ultrastructural characteristics of cylinder-like structures (termed the cylinder organelle) present in the horizontal cell processes. Previous reports of this structure in the human retina have included only a partial description of its ultrastructural detail.

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The path of these crossed axons is through the caudal portion of the nucleus of the opposite side, the destruction of one lateral half of the oculomotor nucleus would result in a bilateral palsy of the crossed subnuclei. Bilateral palsy of the superior rectus and bilateral asymmetrical palsy of the levator palpebrae muscles would result.

Previous investigations in the cat and the monkey have established that the branch of the third nerve to the superior rectus consists entirely of crossed fibers, and that the branch to the levator palpebrae consists of both crossed and uncrossed fibers. This suggests that all the axons from the superior rectus and some from the levator palpebrae must cross the mesencephalic midline. The location of this crossing and the subsequent path taken by these crossed fibers has not been well studied though some suggestive information is available.

Stains for central nervous system myelin or for neuron fibers easily demonstrate an abundance of nerve fibers crossing the midline between the paired third nerve nuclei of the cat. Though present throughout the rostro-caudal extent of the nucleus, they are most prominent in the caudal one-third, suggesting that they are axons of the superior rectus, inferior oblique, and levator palpebrae subnuclei located there.

However, Cajal pointed out on the basis of Golgi studies of the third nerve nucleus that there are two other potential sources for crossing fibers in this nucleus, namely axons of afferents to the nucleus, e.g., from the vestibular nuclei, and dendrites of the cell bodies of the third nerve nucleus. Positive identification of these crossed fibers as the axons of the cell bodies of the opposite side could be made with a whole neuron marking technique such as autoradiography.

Radioactive amino acids, when injected in the vicinity of neurons, are taken up by the soma, incorporated into the protein of the cell, and ultimately distributed along the course of the entire cell thus labeling the cell body and the course of its axon. This new tracing device offers great promise for neuro-anatomic mapping since the amino acid is not taken up by axons passing through the region of the injection but only by cell bodies at the site of the injection. The cells that incorporate the radioactive amino acid identify themselves by labeling their cell bodies.

**Technique.** Five adult cats weighing from two to four kilograms were used. The cats were premedicated with 50 mg. of pentobarbital given intraperitoneally. Atropine sulphate, 0.1 mg. was given subcutaneously and the animals were deeply anesthetized with intramuscular ketamine hydrochloride. The head was placed in a stereotactic head holder and an occipital craniotomy was performed. A small amount of the tip of the bony tentorium was removed to allow the stereotactic placement of a glass micropipette into the left third cranial nerve nucleus. The micropipettes had previously been loaded with 1 to 2 μCi of desiccated tritiated proline or leucine. The dry amino acid was redissolved in a small amount of normal saline at the time of the experiment. The bolus of approximately 0.5 to 1.0 μlitters of solution was injected into the region of the left third nerve nucleus through the hydraulic pressure created by a Hamilton syringe connected to the micropipette with polyethylene tubing.

The scalp wound was sutured and the animals were killed after one week. The brains were fixed in 10 per cent formaldehyde buffered to pH 7.4, and the brain stem was processed for paraffin sectioning. Sections were cut at 10 micra and one section of every twenty was mounted. The mounted sections were processed down to the water stage and then dipped in 1/4 strength Kodak NTB-2 photographic emulsion. They were stored in dry, light-free boxes in a freezer at −13° C. for six weeks and developed in Dektol. The sections were lightly stained with toluidine blue.

**Results.** In three of the five cats the caudal portion of the third nerve nucleus was labeled. These three cats demonstrated radioisotopically labeled axons that originated in the left third nerve nucleus, crossed the midline, and entered the right third nerve after passing through the right third nerve nucleus. Since proline seemed to have a greater tendency to label small cells than leucine, and hence showed less contrast, the results were more striking with leucine. This differential uptake has been noted in other areas of the brain. With both amino acids, an abundance of radioactive fibers can be shown exiting from the ventral portion of the left nucleus destined for the muscles innervated by uncrossed axons. On the opposite side, exiting from the ventral portion of the right nucleus are radioactive fibers that have had their origin in cell bodies on the left side of the midline (Figs. 1 and 2).

The details of these crossing fibers is shown in Fig. 3. It should be noted that these radioactive axons cross the midline in the dorsoventral center of the nucleus and plunge into the mass of cell bodies of the opposite side before exiting through the medial longitudinal fasciculus to form the intramedullary portion of the third nerve. The level at which these crossing fibers are seen passing through the right third nerve nucleus would correspond to the location of the superior rectus, inferior oblique, and part of the levator palpebrae subnuclei.

Indirect evidence to support these findings was supplied by the two experiments which failed to inject the caudal third nerve nucleus. In one, only
Fig. 1. Autoradiograph and toluidine blue, ×50, the caudal third nerve nucleus in cross-section. The blackness overlying the large neuronal soma on the left side of the nucleus indicates they have incorporated the radioactive leucine. None of the neuronal soma in the right side of the nucleus are labeled. Axons which have incorporated radioactivity are seen streaming out of the left nucleus (arrow), passing through the right nucleus (arrow) and the right mlf to join the right third nerve. Uncrossed axons are seen exiting through the left mlf.

the rostral half of the third nerve nucleus was injected. No crossing fibers were seen and no radioactive fibers were seen in the contralateral third nerve. In the second, the periaqueductal grey matter and the left fourth nerve nucleus were injected but not the third nerve nucleus. Once again, no crossed fibers were seen in the third nerve nucleus or in the third nerve. Since these latter two experiments bracket the area of interest they indicate that the crossed fibers observed did not come from cells in the vicinity of the caudal half of the third nerve nucleus.

Finally, no cells of the interstitial nucleus, the superior colliculus or the vestibular nuclei were labeled in any of the experiments.

Discussion. Positive identification of the source of the abundant axons crossing in the caudal third nerve nucleus rests on a whole neuron marking technique. Since the autoradiographic injection can only label an axon by first reaching its cell body, the labeled axons exiting from the right ventral third nerve nucleus after crossing the midline must have originated from cells in the left third nucleus. No others cells with known projections to the third nerve nucleus were ever labeled.

The organization of the third nerve nucleus of the cat has been the most thoroughly studied. There are many articles in the French and German literature of the late nineteenth and early twentieth century postulating the existence of crossed axons in the third cranial nerve and its nucleus. With the exception of the articles by Perlia,4 Van Gehuchten,5 and Cajal,6 they fail to positively identify the course and location of these crossed fibers. Thus, although the proof of the origin of these crossed fibers from the superior rectus and levator palpebrae subnuclei could have been deduced from the fiber degeneration studies of Perlia4 and Van Gehuchten5 or from the Golgi stains of Cajal,6 these were done in the rabbit and mouse, respectively, and do not have as much pertinence to the human as does the cat.

The fact that the axons of the superior rectus subnucleus of one side cross the midline and pass through the superior rectus subnucleus of the other side implies that if one side of the third nerve nucleus is destroyed there will result a bilateral paralysis of superior rectus function. Since the axons of the levator palpebrae subnuclei are mixed-crossed and uncrossed, the lids would be expected to exhibit profound ipsilateral ptosis and partial contralateral ptosis.

Since the anatomy of the third nerve nucleus in man resembles that of the cat with respect to
Fig. 2. Same as Fig. 1 except that radioactive proline was used. Many small cells (probably glia) as well as the large cells are labeled in the left side of the third nerve nucleus. No large cells of the right nucleus are labeled. Labeled crossed axons are seen exiting from the right ventral portion of the nucleus (arrow) through the mlf.

Fig. 3. Detail of Fig. 1 x120. The radioactive change over the neuronal soma in the right side of the nucleus is no greater than the background in contrast to the neurons of the left side (arrow). Midline indicated by vertical lines.
the location of as yet unidentified crossed axons, the clinical neurologic findings following destruction of one lateral half of the third cranial nerve nucleus may be similar.

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Key words: oculomotor nucleus, superior rectus, levator palpebrae, autoradiography, axons.

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6. Ramon y Cajal, S.: Histologie du systeme nerveux of each eye cross at the optic chiasm. The Siamese cat would then seem to be a potentially good model for concomitant human squint, but a difficulty in the viability of this model for the human arises from the fact that in Siamese cats, the binocular vision necessary to maintain ocular alignment would presumably not be available, and squint would be predicted. Squint does in fact occur very commonly in Siamese cats, and would seem to be of the concomitant type described above. Mechanical adhesions in the periorbital adnexa do occur in some Siamese cats, but they are the exception and are not frequent enough to explain the incidence of squint in Siamese cats.

The Siamese cat would then seem to be a potentially good model for concomitant human squint, but a difficulty in the viability of this model for the human arises from the fact that