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Lack of synaptic reorganization in inner plexiform layer (IPL) of retina following ganglion cell degeneration[1]

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Summary. Ganglion cell degeneration in the rat retina following optic nerve lesion does not induce the formation of new synapses even after 1 year of postoperative recovery.

The retina is an attractive area for studies of synaptic plasticity due to its isolation and highly organized structure. However, while other investigators have reported retinal plasticity following either light deprivation or light damage[2-4], Chernenko and West[5] were unable to replicate these results when extensive controls were used. The purpose of this study was to determine whether the retinal inner plexiform layer (IPL) would exhibit synaptic reorganization more convincingly under the more extreme conditions which have demonstrated plasticity in other areas of the nervous system.

A variety of situations have been reported in which the central nervous system will undergo synaptic reorganization. In most of these studies it has been shown that the destruction of one input may cause another input to form new synapses onto the vacated postsynaptic sites[6-8]. However, a second model, reported by Ralston and Chow[9], suggests that there may also be situations in which the removal of postsynaptic membrane may induce the presynaptic processes to form synapses on membrane previously not involved in synaptic interaction.

In the present study it was desired to determine whether following optic nerve section and consequent degeneration of ganglion cells and their dendrites, the bipolar and amacrine cells would use amacrine processes to fill the postsynaptic sites vacated by ganglion cells. The model used here, then, is that of Ralston and Chow[9] as it considers whether a presynaptic process which has lost its postsynaptic element will seek out membrane which previously had not been involved in synaptic interaction. The amacrine cells were thought to be good candidates for such postsynaptic elements as a certain proportion of their processes already take part in postsynaptic relationships in a manner similar to ganglion cell dendrites. Amacrine and ganglion cell processes are both found to be postsynaptic to other amacrine processes, and amacrine and ganglion cell processes are found to be simultaneously postsynaptic to the same bipolar cell axons in synaptic complexes (called dyads)[10].

Method. 20 Sprague-Dawley rats averaging 39 days of age at the time of operation were used. In the interest of uniformity of treatment the rats were divided into 5 groups of 4 rats each so that each group, containing 3 lesioned and 1 control animal, could be processed at the same time. Radio frequency lesions were stereotaxically placed in the optic chiasms. Following 1 year of recovery, all rats were anesthetized with sodium pentobarbital and their eyes enucleated and fixed by immersion in osmium tetroxide and glutaraldehyde as described elsewhere[5]. All retinas were embedded in Epon and serially sectioned at 50 µm for accurate isolation of the desired area[11]. Areas 1 mm nasal to the optic disc were mounted on plastic stubs and sectioned at a silver-gold thickness for electron microscopy. These sections were mounted on formvar-coated 1×2 mm slot grids, stained with uranyl acetate and lead citrate, and examined in a Philips 300 electron microscope. Following enucleation, the brains were removed to verify optic nerve degeneration. From the 20 rats, 3 experimental and 3 control retinas (from corresponding groups) were selected for further study on the basis of complete optic nerve degeneration and good fixation. Retinal mosaics extending through the entire depth of the IPL were constructed for each retina at a print magnification of $\times 21{,}600$.

Results and discussion. The experimental mosaics covered a total of 5971 µm^2 and included 726 synapses while the control mosaics covered a total of 7592 µm^2 and included 687 synapses. All 3 experimental mosaics showed even on cursory inspection a loss of ganglion cell processes in the IPL (figures 1 and 2). This coincided with a 36% decrease in IPL thickness which is probably due entirely to the loss of ganglion cell dendrites. Because of these immediately apparent differences plus a loss of almost all ganglion cell axons and somata, it was not possible to score the mosaics without knowing the condition to which each mosaic belonged. However, we believe that the results were not biased by this as we had fully expected synaptic reorganization to occur, and, as will be seen, we found no evidence of this.

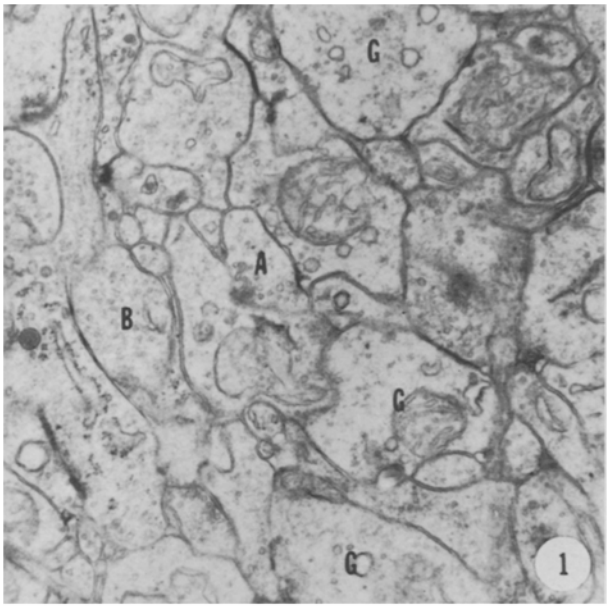
An effort was made to identify all processes (both pre- and postsynaptic) which participated in synapses. Each mosaic was gone over at least 3 times by 2 scorers, often simultaneously with much discussion. We have found this technique to give a minimum of drift to criteria used in synaptic

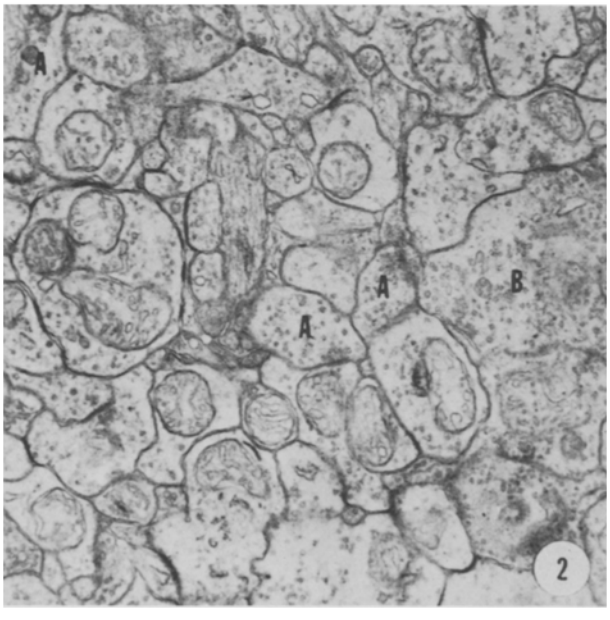
Columnar incidences of the various categories of synaptic relationships for the control and experimental groups

	Incidence (per 1 µm wide column)		Change (%)
	Control	Experimental	
Amacrine (total)	4.445	3.860	−13.2
A/A	0.899	0.886	− 1.4
A/B	0.699	0.665	− 4.9
A/G	0.671	0.292	−56.5
A/?	2.176	2.017	− 7.3
Bipolar (total)	0.457	0.432	− 5.5
B/A-or?	0.050	0.070	+40.0
B/(G-A) or (G-?)	0.057	0.018	−68.4
B/(A-A)	0.143	0.146	+ 2.1
B/(A-?) or (?-?)	0.207	0.198	− 4.3

identification. This is demonstrated by the similarity of incidences between experimental and control mosaics in those categories where no change is seen. The results are presented in the table. The mosaics were originally scored keeping track of differences in incidences for equal thirds through depth of the IPLs. Analyses of variance (2×3) were then performed for each synaptic type tabulated.

Because the experimental retinas decreased in thickness (retinal width was uniform), the gain or loss of synapses cannot be determined by a simple inspection of the incidence of a particular type of synapse per μm^2 of mosaic. In order to control for retinal compression, incidence in the table is reported for the average 1 µm wide strip extending through the entire depth of the IPL. Regardless of thickness changes, this *columnar* density will not change if there is no change in numbers of synapses, whereas, incidence/μm^2 would change and might lead to the erroneous conclusion that a change in synaptic incidence had occurred.





Figs. 1 and 2. Electron micrographs from a control retina (figure 1) and an experimental retina (figure 2). A few of the more readily recognizable processes have been identified (G, ganglion: A, amacrine; B, bipolar). The micrographs are from comparable areas of the IPL (adjacent to the amacrine cell somata) and are the same magnification ($\times 20{,}000$). The larger average size of the processes in the control retina is due to the lack of large ganglion cell processes in the experimental retina.

The table indicates that the total number of amacrine synapses decreased slightly (-13.2%). Further inspection indicates that this must be almost entirely due to the loss of the subset of amacrine synapses onto ganglion cell dendrites (A/G, -56.5%, $p < 0.05$). In addition, there was a decrease in bipolar synapses in which at least 1 of the 2 postsynaptic processes was a ganglion cell dendrite (B/(G-A) or B/(G-?), -68.4%, n.s.). These results are, of course, to be expected since most of the ganglion cells have degenerated.

The data in the table are consistent with the conclusion that no new synapses were formed. Neither the amacrine/bipolar (A/B, -4.9%) nor the amacrine/amacrine (A/A, -1.4%) synapses increased. Therefore, it is unlikely that the amacrine processes which were freed from postsynaptic ganglion cell dendrites made new presynaptic contacts with either bipolar cells or other amacrine cells. Also, there was no increase in B/(A-A) synapses as would be expected if B/(G-A) synapses were to have accepted amacrine processes in the place of lost ganglion cell processes. This observation, along with an accompanying increase in the bipolar synapses seen to occur on only 1 postsynaptic process (B/A-or-?, $+40.0\%$, n.s.), suggests that when a bipolar dyad loses a ganglion cell dendrite it becomes a 'monad'. It is doubtful, then that the bipolar cell synapses which have lost postsynaptic ganglion cell dendrites re-establish synaptic interaction with amacrine cells at the vacated synaptic sites.

In spite of a large percent decrease in the B/(G-A) or (G-?) and increase in the B/A-or-? synapses ('monads'), these differences were not statistically significant. The reason for this was obvious. Due to an unexpected difficulty in distinguishing ganglion cell dendrites from amacrine processes lacking synaptic specializations, and due to a small incidence of 'monads' (probably because B/(G-A) contacts make up a smaller proportion of bipolar synapses than is usually believed), the n for B/(G-A) and B/ A-or-? synapses was too small to give the statistical test much power. Nevertheless, when their direction of change is combined with the very stable values of the A/A, A/B, and B/(A-A) synapses, there is convincing evidence that synaptic reorganization did not occur. The synapses which would not be expected to change if no new synapses were formed show a satisfactory constancy, while the synapses which would be expected to change, change in the proper direction.

1 Acknowledgments. Supported by Canadian National Research Grant A-9755. We thank Barbara Vavasour for technical assistance, Carolyn Harley for critical reading of the manuscript, and Phil Hyam and the Memorial University School of Medicine for the use of the Philips 300 electron microscope.

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