The Visual System
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The goal of the session on the visual system, as described by Dr. R. Lund in his introductory remarks, was to integrate the biology of the visual system with transplantation studies. The presentations were selected as examples of different approaches to transplantation which would bring together problems from both spheres.

Dr. G. Bray, speaking in place of Dr. A. Aguayo on responses of injured retinal ganglion cells, considered the theme of injury-related phenomena. Dr. Bray introduced his talk by outlining experiments done in Dr. Aguayo's laboratory which formed the basis for the topic at hand. These experiments dealt with the results of axotomy in the optic nerve in rodents [1]. When transected, optic nerve axons do not regenerate, and the target (the superior colliculus, for over 90% of the fibers originating in the optic nerve in the rodent) remains denervated. In studies by Dr. Vidal-Sanz, it was shown that peripheral nerve, grafted to a totally transected optic nerve, survived and axons were shown to grow to the end of the graft [17]. Grafts could also be used to guide axons from the transected optic nerve back to the superior colliculus (SC). Grafts inserted in the SC survived and extended fibers which were shown to form well-differentiated synapses, and which activated neurons in the SC in response to light flashed on the retina. The reconnectivity potential of this model was limited by the fact that many neurons died as a result of retrograde degeneration following transection of the optic nerve. The experiments which followed were designed to explore the extent and pattern of death after axotomy in the hope of elucidating the mechanisms responsible.

In the first set of these experiments, by Vidal-Sanz and Villegas-Perez [18], lesions of the optic nerve were made at four distances from the eye, two close to the eye, intraorbitally, and two far from the eye, intracranially. The survival of the retinal ganglion cell (RGC) was determined and quantified two weeks to 20 months post-lesion. Two phases of cell loss were observed, an initial, acute phase which took place between 7 and 10 days following axotomy, and a second, protracted phase. The number of surviving RGCs varied with the distance of the axotomy from the eye. More distal lesions resulted in less cell death. It was speculated that RGCs may be temporarily sustained by trophic mechanisms originating within the optic nerve stump itself. Work by Lu *et al.* [13] was cited, in which lesions of the optic nerve were found to result in an 8-fold increase in amounts of NGF mRNA on the first day following transection. This finding suggests that the injured portion of the nerve is capable of producing trophic molecules, at least for a brief period, which may play a role in the transient survival of the RGCs.

The type of lesion may also affect cell survival. As Dr. Bray noted, a cut as opposed to a crush results in greater cell loss, possibly due to the production of molecules which are damaging to the cell. Thus, the cytological reaction at the site of injury may affect cell survival in either a positive or a negative way.

In discussing the second phase of cell death following axotomy, Dr. Bray described data from studies by Vidal-Sanz and David Carter with peripheral nerve grafts in the superior colliculus, which suggested that RGCs that have formed synapses by means of a graft may be sustained and do not die following transection [2,17].

In summary, the degree of cell loss occurring during the initial phase following axotomy appears to be related to the distance of the lesion from the

eye. The severity of the later, protracted phase does not appear to bear any relationship to the lesion site. The loss of neurotrophic support is probably not the sole factor in axotomy-induced neuronal death. Changes at the lesion site and along the course of the axotomized neuron may influence cell survival. If the molecular mechanisms associated with the two phases of cell death could be understood, it might be possible to interrupt the process leading to cell death and provide an environment which encourages reformation of connections by experimentally regenerated CNS axons.

Raymond Lund described a series of experiments dealing with the creation of functional connections to the host brain formed by retinal transplants /14/. When fetal retina was transplanted over the mid-brain of a neonatal host, connections with several visual areas in the host brain were formed /15/. The degree of afferentation of the transplant was enhanced if one eye of the host was removed at birth. The studies sought to determine whether: (i) the transplant could mediate light-activated responses in the host; (ii) the transplant was able to effectively analyze information it received; (iii) the host could use information derived from the transplant, and (iv) there was an interaction between the remaining host eye and the transplant.

In the first experiments described, rats were taught to press a bar for food /4/. They were then exposed to a conditioned suppression paradigm in which foot shock was preceded by a conditioned stimulus of a tone or light signal. A reduction in bar-pressing rate following the conditioned stimulus indicated an awareness that a shock was imminent. In one group of animals, the conditioned stimulus was a light signal. The host eye was covered, and the animal was trained with the transplant exposed. The animals demonstrated reduced bar pressing, indicating that the significance of the light stimulus had been learned through the transplant. When the transplant was covered and the host eye uncovered, information acquired via the transplant was transferred; i.e., the rate of bar pressing fell upon presentation of the light stimulus. If the paradigm was reversed, however, so that the host eye was trained first, and

the transplant tested later, no transfer of information occurred. When using transplant-derived light, the animal had to learn the task *de novo*. Different hypotheses were put forward to explain this phenomenon, such as the possibility of different pathways existing for host eye vs. the transplant, or the ability of the host eye to mediate rapid learning of the significance of visual stimuli, based on extensive past experience.

In a second series of experiments, rats were exposed to an open field in which some of the area was dark and some light /3/. With the transplant exposed and the normal eye covered, random behavior was seen. As much time was spent in the dark as in the light. When the intact eye was exposed, and the transplant covered, normal preference for the dark was observed. Following conditioned suppression, as described above, the animals were again exposed to the open field. If the animal had learned the light-shock association through the normal eye, it did not seek out the dark area with the implant exposed. The animals which had learned the association through the implant, however, did show a trend towards preferring the dark area. Dr. Lund suggested that the animals' behavior may indicate an "awareness" of the significance of the light. It was concluded that conditioning alerts the animal's CNS to pay attention to information coming from the implant. Dr. Lund stressed that, to show that transplant-derived sensory information is being used, it is not enough to demonstrate the presence of connections or physiological responses alone, but the animal must also be shown to be aware that the input to the transplant is significant.

In another series of studies by Klassen and Lund, pupilloconstriction was induced by shining light onto the transplant /12/. Brighter light produced greater constriction, indicating that the CNS was processing information accurately from the graft. The response to the light stimulus was predictable and robust. The nature of the host-transplant interaction was explored by testing the graft response while the optic nerve to the host eye was intact as compared to the response when the nerve was transected /16/. Interestingly, when the host optic nerve was cut, the transplant was more effective in producing pupilloconstriction. This

finding was not due to anatomical reorganization, as it occurred too rapidly for this to be a factor. Rapid receptor up-regulation or an increase in signal-to-noise ratio for transplant-derived information when the optic nerve was cut were proposed as possible explanations. These data suggest several important points, as noted by Dr. Lund: There is extensive overlap of visual projection fields of the transplant and host eye, as opposed to the projection fields of two normal eyes. This overlap leads to interactions, the nature and degree of which are related to the task, and which, in some cases, are dependent upon the "significance" attached to the transplant input. This work can be regarded as a useful model of how transplants can be used to reconstruct damaged circuitry, and the importance of defining the optimal conditions under which reconnection can be accomplished.

Douglas O. Frost presented work on the function of novel, surgically-induced visual circuits /9/. When the normal targets for retinal projections are surgically ablated, and the ascending afferent circuits to the somatosensory or auditory thalamic nuclei are also removed, retinal projections can be re-routed to form novel projections to these thalamic nuclei /11/. While these projections can innervate an inappropriate target, they retain their visual properties, forming themselves in a retinotopically organized manner. The synaptic complexes formed in the novel target area, on the other hand, do demonstrate the influence of their new milieu, in that their organization is typical of the novel environment rather than of the visual thalamic nucleus. The novel projections are formed by a subset of the same retinal ganglion cells that are found in normal retino-thalamic projections. Single unit recordings comparing normal animals with operated animals demonstrated that the spatial organization of the receptor fields was similar in both groups of animals, and that the same classes of neurons could be reliably distinguished according to stimulus characteristics in both groups /10/. The neurons in the somatosensory cortex of the operated animals responded in essentially the same way as did neurons in the visual cortex of normal animals to visual stimuli. Finally, preliminary data regarding the behavioral efficacy

of the novel connections were presented. Work currently in progress in collaboration with Dr. Larry Rosenblat indicated that operated hamsters were able to perform discriminations of vertically oriented versus horizontally oriented rows of squares nearly as well as normal hamsters, and that the ability to perform this task was lost if the cortex receiving the novel projections was ablated. These data indicate that novel circuitry can be functional at both neurophysiological and behavioral levels. As pointed out by Dr. Frost at the beginning of his presentation, once the problems of the survival of transplanted neurons and the conditions necessary for the induction of neuron regeneration are resolved, the potential for using surgically-created neuronal circuits to take over the function of damaged brain tissue may become a possibility.

The final speaker, Dr. M. del Cerro, presented work done in his laboratory on structural repair and visual recovery after retinal transplantation /7/. Using light-damaged phototoxic retinopathy as a model, del Cerro's group studied the feasibility of repair with fetal neural retina cells /8/. Following destruction of over 99% of the photoreceptor cells in the outer retina, a suspension of whole dissociated fetal neural retina cells was injected into the anterior chamber of the host eye. Electron microscopic examination of these grafts revealed clusters of photoreceptors growing in the host around which synaptic triads had developed. Using a behavioral test in which a startle reflex to an acoustic stimulus is partially suppressed by a light flash delivered prior to the sound, it was shown that the grafted rats were able to detect the light stimulus /6/. Blinded rats which were not grafted developed an exaggerated response to the acoustic stimulus. This facilitated response could be eliminated when these rats also received grafts. To test whether the results were cell-specific, both homogenized cells and immature cerebellar cells were implanted, with negative results. Enucleating the animals further demonstrated that the eye was, in fact, the route of information transmission to the CNS. Dr. del Cerro's work has shown that intraretinal grafts do grow, form connections and have functional effects. Studies using animals which are subject to age-related retinal degeneration were briefly discussed. In other

experiments, human retinoblastoma cells were used as grafts, in place of fetal cells, and were shown to form synapses within the host eye /5/. This work may provide a useful animal model for studying the feasibility of applying these methods in a clinical setting.

In summary, work presented in this session showed that grafts in the visual system can survive and grow. They are capable of making functional connections which can modify behavior. Because the circuitry of the visual pathways is well known, this system provides a very useful model for transplantation studies. The work presented shows promise for the development of interventions which can arrest the progress of processes leading to loss of function and may also provide us with the capability to repair damage that has already occurred. While the techniques that were described are not ready for clinical application, there is a possibility that they may ultimately be used for human conditions.

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