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A NOVEL TECHNIQUE FOR MULTIPLE INJECTIONS INTO THE MAMMALIAN OPTIC NERVE

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Introduction

As opposed to many areas of the CNS, the optic nerve may offer a relatively accessible number of CNS axons in a constrained space with access to both the cells of origin in the retina and their termination in the midbrain and thalamus. These properties make the optic nerve an approachable model in which CNS axons and their supporting cells can be studied, particularly in relation to central nervous system regeneration. Recently, research into the reasons for the general failure of central nervous system axonal regeneration has focused on the axonal growth inhibitory properties of oligodendrocyte membranes (Huber and Schwab 2000). The replacement of oligodendrocytes by the transplantation of Schwann cells (Vanevevercooren et al 1992) and more recently olfactory ensheathing cells (Raisman 2000: Ramon-Cueto 2000) into demyelinated or injured areas of CNS has shown some promise in the enhancement of axonal growth and/or functional recovery in CNS tracts. The intention of these manipulations is to alter the cellular composition of CNS tracts and provide an appropriate microenvironment for axonal growth. Because of the size of tissues involved

(usually spinal cord), it is difficult to construct a reasonably complete alteration of the cellular composition surrounding axons. The use of the optic nerve as a model of CNS tracts may offer a solution to some of these problems.

For the past several years, we have been interested in studying the potential various cells have of altering the cellular composition in a portion of the optic nerve that had previously been demyelinated. Currently, we are using olfactory ensheathing cells (Hallas, et.al. 2000) that normally surround the axons of neurons in the olfactory epithelium of the nasal cavities. Ensheathing cells accompany olfactory axons as they enter the olfactory bulb of the brain and, therefore surround growing axons in both central nervous system and peripheral nervous system environments. We have developed a technique in which various agents can be initially injected into an area of the mammalian (rat) optic nerve and after various periods of time the exact injection site can be re-located to re-inject various types of tissues or cultured cells. We have used this technique as part of an attempt to remyelinate portions of the rat optic nerve that had previously been demyelinted using lysolecthithin. The technique presented below outlines the technique which allowed us to inject the ensheathing cells into the demyelinated area of optic nerve (Hallas et al. 2000).

Materials and Methods

All surgical procedures were performed under barbiturate anesthesia using sterile techniques. Adult male Sprague-Dawley or Long -Evans Hooded rats (350g) were anesthetized using sodium pentobarbaritol (Nembutal) at a dose rate of 45mg/kg-body weight. Animals were placed into a Kopf stereotaxic using standard ear bars and kept warm using an underlying heating pad (fig. 1). The area of hair surrounding the orbit was wiped with alcohol, dried and carefully shaved avoiding the animal's whiskers. The skin was again wiped with alcohol and divided from the outer canthus toward the ear using a number 11-scalpel blade (fig. 2). The subcutaneous muscles (frontalis and ploiysma) were carefully retracted using fine spreaders. Care was taken to avoid severing the branches of the posterior facial, superficial temporal and intraorbital blood vessels. Any bleeding was controlled immediately with Gel foam (fig. 3). The branches of the facial and trigeminal nerves were also preserved. Severing of these nerves can contribute to corneal ulceration in the postoperation recovery period.

When hemostasis was achieved, the lateral conjuctiva was cut and the intraorbital lacrimal and Harderian glands and intraocular muscle were carefully retracted to expose the underlying optic nerve (fig. 3). Animals will have a better recovery if these glands and muscles are left intact rather than partially removed. A retraction suture (6-0) was placed through the connective tissue of the cut border of the conjuctiva and sclera, and the eyeball retracted forward using a hemostat, and gentle tension applied in an anterior direction. This tension moves the eyeball slightly anterior and out of the way of the injection site. Excessive tension may damage both the nerve and eye, both by direct injury and vascular compression. With the optic nerve exposed, a small hole in the dura covering the optic nerve was made by carefully grasping the dura with fine forceps and making an incision with a number 11 scalpel blade. This incision provides access to the underlying optic nerve and less compression of the nerve by the micropipette.

The injection needle to deliver various agents or cells into the optic nerve consisted of a small-bore glass micropipette pulled using a micropipette puller and

beveled (roughly 45 degrees) to yield a tip size of approximately 60 µ. This micropipette was attached to a positive displacement microsyringe and filled with the desired solution or combination of solutions (fig. 4). The filled microsyringe was placed in a delivery tower attached to the stereotaxic unit. The tip of the micropipette was then lowered toward the optic nerve and inserted into the small incision in the optic nerve dura (fig. 6). For the demonstration of this technique, the micropipette was filled with 0.1% methylene blue as a marker and injected into the optic nerve (fig. 7). In practice, a small amount of the dye is injected into the nerve with a demyelinating agent such as lysolecithin and serves as a marker for the injection site as a short-term indicator of the placement of the micropipette and the successful penetration of the agent into the nerve. Injections should be slow (under 0.5µ /min) to avoid injury to the nerve and allow diffusion. For rapidly diffusing agents such as lysolecithin, the methylene blue allows accurate relocation of the injection site later in the operation. Sterile carbon black or a 10-0 suture in adjacent connective tissue can also be placed on the dura to act as longer-term marker for the injection site.

During the above procedures care has to be taken to prevent the eye and optic nerve from drying out. We have found that bathing the area in either saline or lactated ringers solution will prevent drying of the eyeball or optic nerve. Using these techniques, we have made repeated injections of optic nerve after intervals of fifteen minutes to fourteen days from the initial injection. There have been no problems in locating the initial injection site using this technique although the connective tissue reactions at longer time intervals can make the placement of the micropipette into the nerve at precisely the same location somewhat difficult.

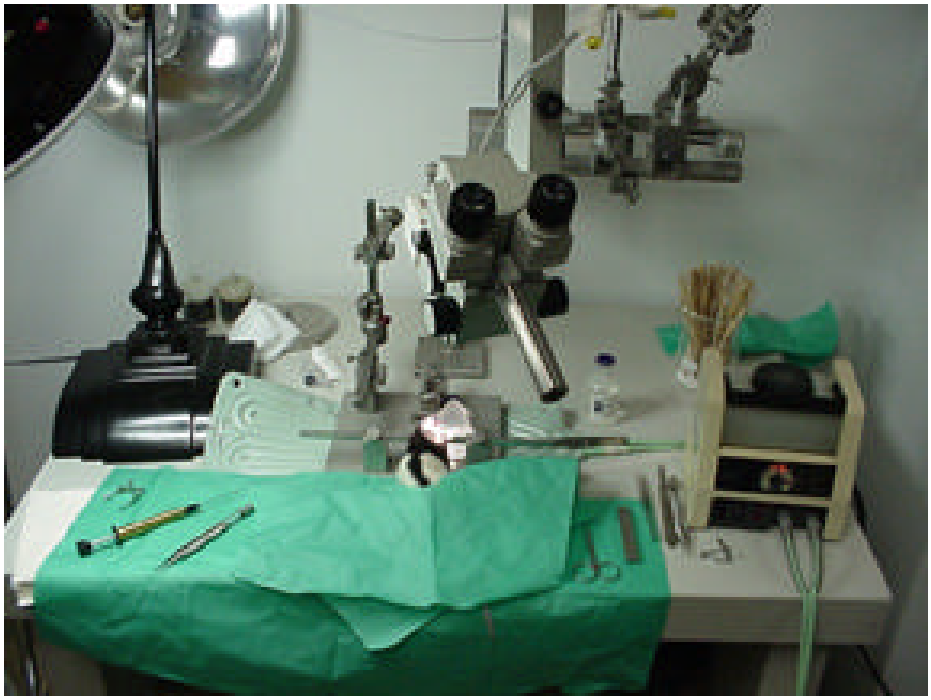

Figure 1. Demonstration of the surgical set-up described above using an animal killed with an overdose of anesthetic. The rat was restrained in a Kopf stereotaxic unit and during normal procedures kept warm by an underlying heating pad. The positive displacement syringe is attached to the carrier unit of the stereotaxic and can be lowered manually or with a microdriver.

Figure 2. Low power image of the shaved orbital area and the opening of the integument. The nose and ear bars of the stereotaxic unit hold the rat securely in place.

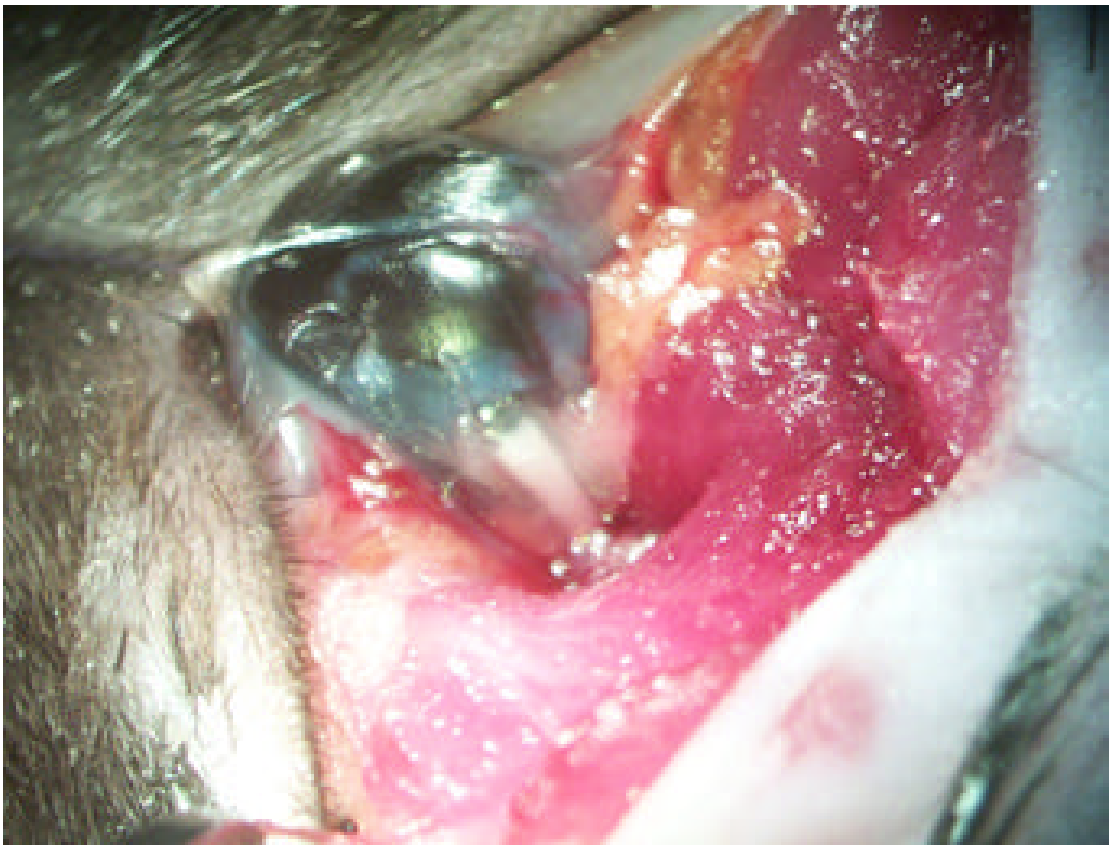

Figure 3. Higher manification image exposing the optic nerve. With the skin and underlying muscle retracted, the optic nerve is clearly visible. Care should be taken to avoid damaging the surrounding nerves and blood vessels.

Figure 4. Image of the positive displacement syringe with attached beveled micropipette. The micropipette is filled with methylene blue, which was used as a marker to locate the original injection site.

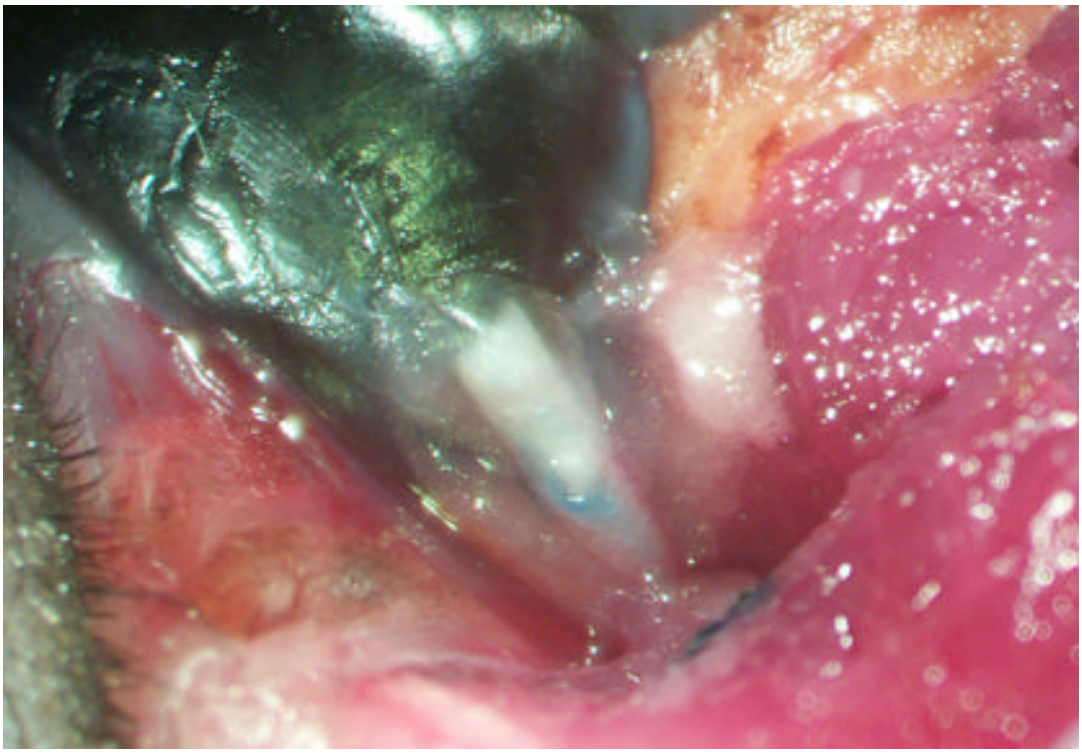

Figure 5. The exposed optic nerve with a small dural incision to allow the micropipette to enter. A small amount of dye was placed on the dura to show the incision site.

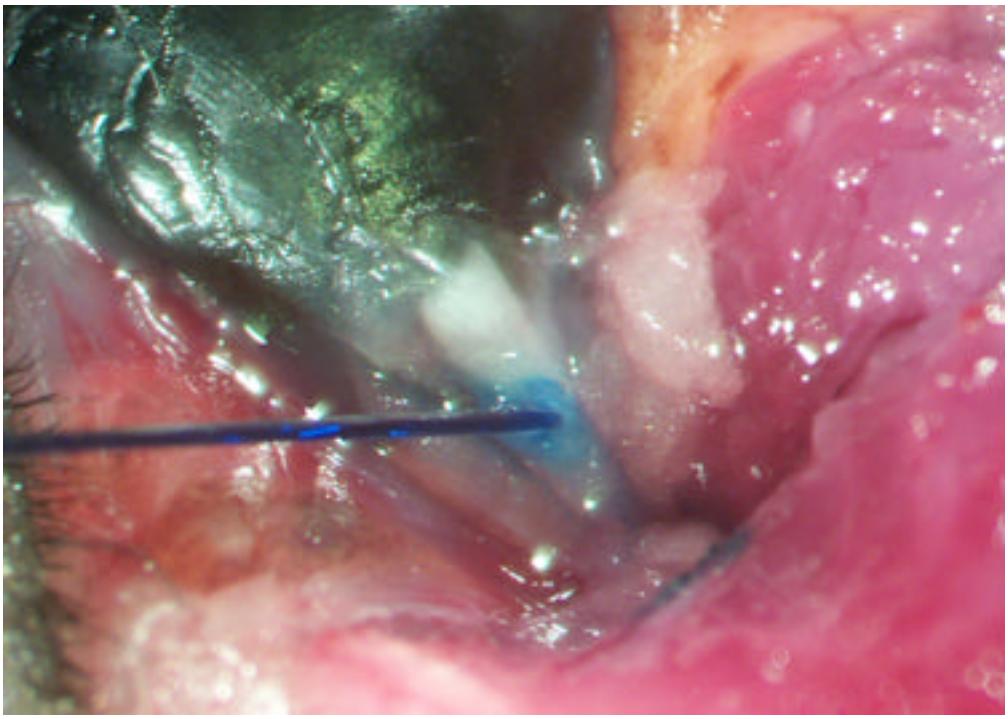

Figure 6. Introduction of the micropipette into the exposed optic nerve. The methylene blue in the injection media shows the infusion of fluid into the nerve.

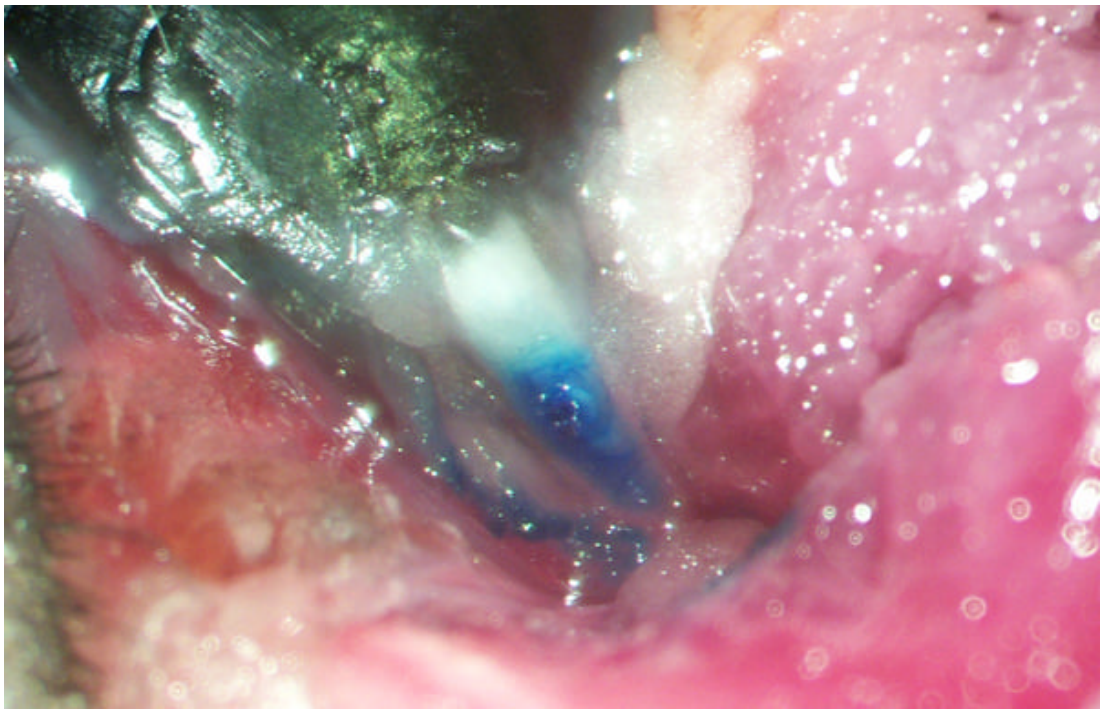

Figure 7. After the initial infusion, the injection site is clearly visible and can be used for short-term identification of the site. The muscles and overlying skin are sutured and the animal returned to a heated cage to recover from anesthesia.

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