THE EFFECT OF PERIPHERAL NERVE INJURY ON THE TRIGEMINAL GANGLION IN THE RAT

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PRECIS

Changes occur in the centrally located nerve cell bodies following injury to peripheral branches of the trigeminal nerve. However, these central effects of peripheral nerve injuries have not been well documented in the literature, particularly with regard to peripheral nerve cryoinjury.

The paucity of information prompted the present study which was initiated to:-

1. design an animal model for the comparison of the effects of peripheral mental nerve transection and peripheral mental nerve cryoinjury on the trigeminal ganglion.
2. perform qualitative and quantitative analyses of the morphological changes occuring within the trigeminal ganglion using this model.
3. draw conclusions as to the effects of these two types of nerve injuries on the trigeminal ganglion, and to attempt to relate these findings to the problem of pain mechanism within the trigeminal system.

Transection and cryoinjury were performed at the periphery on the mental branch of the mandibular division of the trigeminal nerve in male Sprague Dawley rats.

Light microscopic examination of the trigeminal ganglion ipsilateral to the side of the nerve injury was performed at 1 day, 3 days, 1 week, 2 weeks, 1 month, 3 months and 6 months.

The characteristic light microscopic features of the retrograde neuronal reaction and neuronal degeneration occurring within the trigeminal ganglion were scored and recorded for both types of peripheral nerve injuries.

Major changes occurred in the trigeminal ganglion following both types of peripheral nerve injuries. However, important differences were noted between the central effects of peripheral mental nerve transection and peripheral mental nerve cryoinjury.

The transection nerve injury produced a more intense retrograde neuronal reaction, delaying the onset of recovery and prolonging the recovery period. In addition, irreversible morphological changes indicative of cell death were observed in the transection series. The irreversible morphological changes were absent from the cryoinjury series. Consequently, in the transection series, trigeminal ganglion architecture was never completely restored to normal, whereas in the cryoinjury series, complete return to normal ganglion architecture occurred.

The central effects of peripheral nerve injury were more prevalent among the small nerve cells of the trigeminal ganglion.

It would appear, that the central effects following peripheral mental nerve cryoinjury are completely reversible and therefore more predictable in contrast to peripheral mental nerve transection.

DECLARATION

This thesis is submitted in partial fulfilment of the requirements for the Degree of Master of Dental Surgery, in the University of Adelaide. Candidature for the degree was satisfied by obtaining the Honours Degree of Bachelor of Science in Dentistry in 1981.

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any other University and, to the best of my knowledge, no material previously published or written by any other person, except where due reference is made in the text.

R.H.J. D'ROZARIO

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CHAPTER I

INTRODUCTION

The response of peripheral nerves to injury is an area of increasing interest in oral and maxillofacial surgery. The trigeminal nerve is the main sensory nerve innervating the oral and maxillofacial region. Impairment of function in branches of the trigeminal nerve subsequent to nerve injuries can create a range of problems such as involuntary egress of saliva and other fluids, lip biting and speech impediments. These unpleasant effects often result in patients presenting to the oral and maxillofacial surgeon for management of the nerve injury.

Conversely, certain types of elective nerve injuries, such as peripheral neurectomy (Quinn, 1965 ; Quinn and Weil, 1975) and peripheral nerve cryoinjury (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Goss, 1984 ; Nally, 1984) have been found to be effective treatment modalities in the management of chronic facial pain including trigeminal neuralgia.

To date, the main areas of interest in the literature have been the response of the nerve at the site of injury and distal to it (Choukas et al., 1974 ; Hribar, 1978 ; Girard, 1979), and the microsurgical correction of peripheral nerve injuries (Hausamen et al., 1974 ; Wessberg et al., 1982 ; Mozsary et al., 1982, 1983, 1984).

A review of the literature reveals that there is comparatively little available information on the central effects of peripheral nerve

injuries. In particular there have been no published studies on the central effects of peripheral nerve cryoinjury despite its clinical use as a treatment modality for trigeminal neuralgia.

The paucity of available information in this area prompted the present study, which was designed to demonstrate both qualitatively and quantitatively the morphological changes occurring in the trigeminal ganglion of the rat following two forms of nerve injury - peripheral neurectomy (peripheral nerve transection) and peripheral nerve cryoinjury.

In this study, the right mental nerve of the rat was neurectomised as it emerged from the mental foramen and the effects on the trigeminal ganglion over various post-neurectomy intervals were studied light microscopically. Similarly, the response of the trigeminal ganglion to cryoinjury of the rat mental nerve was recorded. The morphological responses of the trigeminal ganglion to these two types of peripheral nerve injuries were then compared over corresponding time intervals.

The fundamental importance of the morphological response of the trigeminal ganglion to peripheral nerve injury required a review of the basic anatomy of the rat trigeminal ganglion (Chapter II), the types of nerve injury and the changes occurring at the site of nerve injury (Chapter III), and the changes occurring central (within the trigeminal ganglion) to the site of nerve injury (Chapter IV). The biological principles of cryosurgery, cryosurgical apparatus, and the effect of cryosurgery on peripheral nerve structure and function are described in Chapter V. The experimental model utilized in this study is described in Chapter VI, with the results obtained presented in Chapter VIII.

Peripheral neurectomy temporarily relieves the pain of trigeminal neuralgia by interrupting the central flow of afferent impules (Quinn, 1965). However, irreversible changes occur at the site of injury in the form of neuroma formation and scarring and this may result in the recurrence of pain (Wall and Gutnick, 1974). In addition, irreversible changes occurring centrally within the trigeminal ganglion may also be responsible for the recurrence of pain (Gregg, 1971).

The reported ability of peripheral nerve cryoinjury to relieve the pain of trigeminal neuralgia in a non-invasive, reversible manner with minimum side effects (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Goss, 1984 ; Nally, 1984) is indeed an attractive concept. The mechanisms by which this therapeutic modality affects pain modulation requires elucidation to permit a better understanding of the clinical management of patients afflicted by this distressing disorder.

The findings of this study in relation to the clinical applications of peripheral neurectomy and peripheral nerve cryoinjury as treatment modalities in trigeminal neuralgia are discussed in Chapter VIII.

CHAPTER II

ANATOMY OF THE TRIGEMINAL GANGLION IN THE RAT

2.1 INTRODUCTION

The trigeminal (semilunar or gasserian) ganglion corresponds to a spinal ganglion ; that is, it belongs to the somatosensory nervous system. The histological architecture of the ganglion has been investigated by various authors in a number of vertebrates (Dixon, 1963 ; Kerr and Lysak, 1964 ; Moses, 1967 ; Pineda et al., 1967 ; Matsuura et al., 1969 ; Peach, 1972) and there is general agreement that it consists of two types of neurons surrounded by satellite or capsular cells. The nature of the nerve cells in the ganglion is important, because of their functional importance in the transmission of painful stimuli from orofacial tissues to the central nervous system.

2.2 GROSS ANATOMY

The trigeminal ganglion in the rat is formed by the union of the ophthalmic-maxillary division with the mandibular division of the trigeminal nerve, the latter lying in a lateral relationship to the former. The ganglion projects to the head and the face via the corresponding three divisions. A postero-lateral protuberance lies at the base of the mandibular division, posteriorly and lateral to the bifurcation between the ophthalmic-maxillary division and the mandibular division (Kerr and Lysak, 1964 ; Gregg and Dixon, 1973) (Figure 2.1).

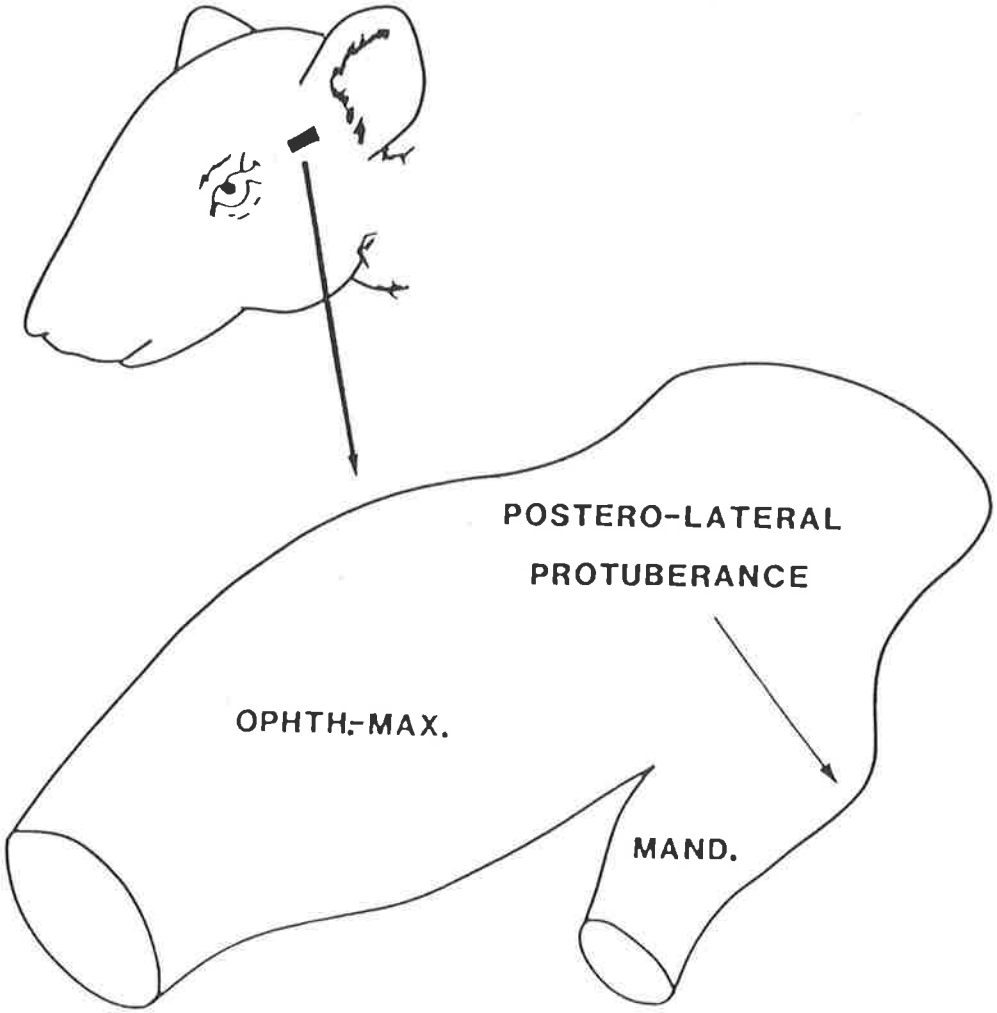


Figure 2.1: Three-dimensional relationship of the left trigeminal ganglion to the cranium.

The ganglion is located in a bony depression in the base of the skull, roofed by dura mater. The cavernous sinus and the basisphenoid bone lie on the medial aspect of the ganglion, while laterally a spur of petrous temporal bone separates the ganglion from the internal carotid artery and the mandibular division of the trigeminal nerve (Figure 2.2).

2.3 MICROSCOPIC ANATOMY

In the trigeminal ganglion of the rat, nerve cell bodies are concentrated predominantly at the periphery with only isolated columns of cell bodies occurring in the broad central zone which is occupied mainly by nerve fibres (Dixon, 1963 ; Peach, 1972).

At the light microscopic level, one type, the "dark" cell stains more intensely and tends to be smaller than the second type or "light" cell. At the electron microscopic level corresponding differences have been observed in the trigeminal ganglion (Dixon, 1963 ; Kerr and Lysak, 1964 ; Matsuura et al., 1969 ; Peach, 1972).

Despite speculation amongst authors, the functional significance of the two cell types remains obscure. An artifactual origin for the differences between light and dark cells is indicated by the reported absence of light and dark cells in aldehyde perfused trigeminal ganglia of the cat and monkey (Pineda et al., 1967). Here the matrix density of the cytoplasm was found to be relatively uniform from cell to cell, therefore providing no basis for classification of cells into separate types. However, the use of perfusion techniques and handling procedures designed to minimize the possibility of artifact production indicates that it is unlikely that light and dark cells of the trigeminal ganglion

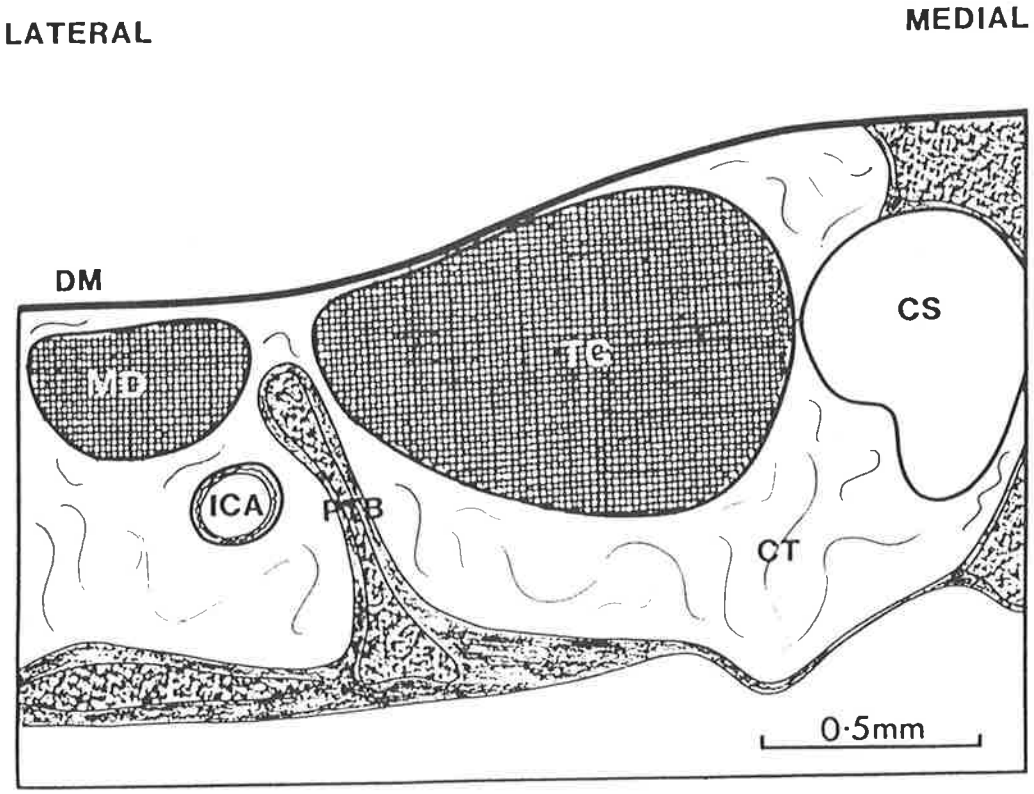


Figure 2.2: Schematic diagram of a coronal section through base of the skull. (After Mazza and Dixon, 1972)

Key:
DM = dura mater
TG = trigeminal ganglion
MD = mandibular division trigeminal nerve
ICA = internal carotid artery
PTB = petrous temporal bone
CS = cavernous sinus

of the rats are artifacts, and suggests that their appearance reflects some real differences existing between the neurons in the living state (Peach, 1972).

Furthermore, it has been suggested that the differing appearances of the trigeminal ganglion cells may indicate that they subserve different sensory functions. The possibility that the small dark cells are associated with autonomic function has been proposed (Dixon, 1963).

The nuclei of the nerve cells are enveloped by a double nuclear membrane and usually occupy a central position. One or more well defined nucleoli are contained within a pale particulate nucleoplasm (Matsuura et al., 1969).

The nerve cells contain granular Nissl substance which is scattered throughout the cytoplasm, except in the region of the axon hillock (Dixon, 1963). Nissl bodies are considered to be the structural basis of protein synthesis in the nerve cells, and a prominent feature of trigeminal neurons.

There is variation in the size and degree of staining of the neuronal cell bodies. Larger more numerous light neurons and smaller, less numerous dark neurons can be seen. It is now generally accepted that the relative differences in density between light cells and dark cells are due mainly to the concentration and distribution of cytoplasmic organelles. Both cell types are found to contain the usual sub-cellular components; for example, mitochondria, lysosomes, Golgi apparatus and numerous smooth and coated vesicles. However, the main structural differences between light and dark cells occurrs in the

distribution of neurofilaments, rough endoplasmic reticulum and ribosomes. In the light cell the ribosomes and rough endoplasmic reticulum are mainly clumped together, an arrangement typical of Nissl granules. These cells contain many neurofilaments and some neurotubules, however, mitochondrial profiles are less numerous (Dixon, 1963 ; Moses, 1967 ; Matsuura et al., 1969 ; Peach, 1972).

In nerve cells of both types, the majority of the mitochondria are concentrated in the perinuclear zone (Dixon, 1963 ; Matsuura et al., 1969). The mitochondria are circular or ovoid in shape and show well defined cristae. Mitochondria in the dark cells have an overall electron-dense internal structure compared to those in pale cells (Dixon, 1963).

Each nerve cell body is surrounded by a covering of satellite or capsular cell cytoplasm. These cells may be connected to one another by fine protoplasmic projections (Dixon, 1963). The nuclei of satellite cells are smaller than, and differ in density from those of the nerve cells, due to a finer granularity of the satellite cell nucleoplasm. Matsuura et al. (1969) observed that the nuclei of satellite cells were enclosed by a double nuclear membrane, and a number of clumps of chromatin particles were concentrated on the peripheral zones of the nucleus. The satellite cell plasma membrane immediately adjacent to the neuron may be infolded, thus giving an interdigitation effect between the nerve cell and its satellite cells (Dixon, 1963). This may be associated with the transfer of nutritional materials between the nerve cell and the intercellular spaces via the medium of the surrounding satellite cell cytoplasm. Small vesicles have been observed in the cytoplasm of satellite cells, and they have been implicated in this

transport mechanism (Peach, 1972). The cytoplasm of capsular cells contains similar organelles to the nerve cells. The number of mitochondria, however, in the nerve cell of the trigeminal ganglion in the rat is much greater than in the capsular cells (Matsuura et al., 1969).

Blood vessels are found in close proximity to the nerve cell bodies lying in the intercellular substance which contains varying amounts of collagen fibres. It has also been reported (Dixon, 1963 ; Matsuura et al., 1969) that in the intervals between adjacent nerve cells, clusters of small nuclei can be seen belonging to fibroblasts or Schwann cells associated with nerve fibres.

2.4 HISTOCHEMICAL FINDINGS

Light and dark cells have been demonstrated in the trigeminal ganglion in the rat by histochemical techniques, particularly those for acid phosphatase (Matsuura et al., 1969). The higher level of acid phosphatase activity observed in dark cells does not correlate with the numbers of lysosomal-like structures found at the fine structural level. Therefore it would appear that a non-lysosomal localization is involved. This may be partly in the tubular and vesicular elements associated with the Golgi apparatus (Peach, 1972).

Matsuura et al. (1969) proposed that besides the light and dark nerve cells present in the trigeminal ganglion, cells of a transitional type were also observable. They stated that it would be reasonable to assume that there was a transformation of the nerve cell types in various biological states from dark cell to clear cell. Futhermore,

these authors proposed that two types of capsular cells were distinguishable ; that is, clear capsular cells and dark capsular cells, and that the capsular cells enclosing one nerve cell body usually consisted of the cells of both types.

2.5 SOMATOTOPIC ORGANIZATION OF THE TRIGEMINAL GANGLION

Somatotopic organization of the trigeminal ganglion has been identified for many species, including the cat (Allen, 1924 ; Kerr and Lysak, 1964 ; Darian-Smith et al., 1965 ; Beaudreau and Jerge, 1968), rabbit (Strassburg, 1967), monkey (Carmel and Stein, 1969 ; Lende and Poulos, 1970) and rat (Mazza and Dixon, 1972 ; Gregg and Dixon, 1973). The internal organization of the ganglion and its sensory root are of interest, because it helps to explain how degenerative and irritative lesions of the ganglion may cause pain and paraesthesia that are referred to very specific peripheral orofacial tissues, for example, the establishment of precise orofacial trigger zones in trigeminal neuralgia.

Gregg and Dixon (1973) demonstrated with the technique of chromatolysis, that mandibular nerve branches project to the postero-lateral positions of the ganglion, with the ophthalmic projections anteromedially and the maxillary fields in an intermediate position. Furthermore, within this divisional arrangement there is a detailed suborganization of the individual branches for peripheral structures (Mazza and Dixon, 1972) (Figure 2.3).

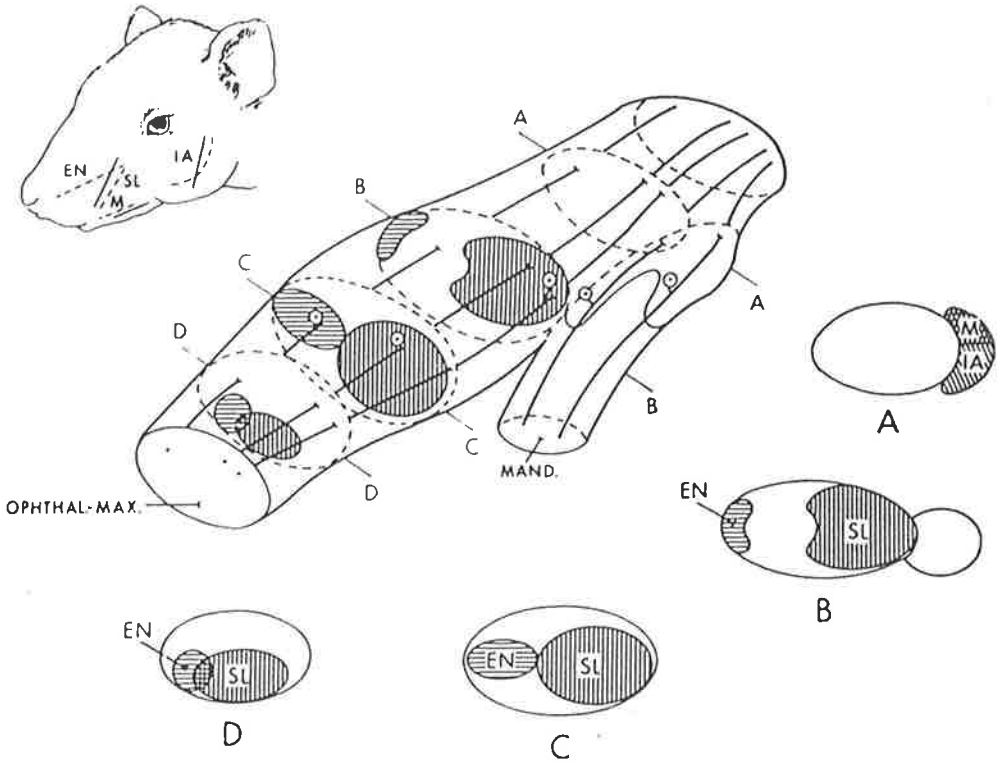


Figure 2.3: Three-dimensional representation of cell groups in the left trigeminal ganglion. Four planes of cross-section are shown A,B,C,D. (After Mazza and Dixon, 1972).

Key:
EN = external nasal nerve - terminal branch of nasociliary nerve, which is a branch of V_1.
SL = superior labial nerve - terminal branch of infra-orbital nerve, which is a branch of V_2.
IA = inferior alveolar nerve - branch of V_3
M = mental nerve - branch of V_3

Gregg and Dixon's studies (1973) determined quantitatively the somatotopic organization of the ganglion for the trigeminal nerve branches to the orofacial region. This was achieved by preparation of histograms based on percentages of chromatolytic cells for all ganglion regions and all trigeminal nerve branch lesions. A rather precise parallel arrangement of chromatolytic neurons was observed in the horizontal plane. Cell groups associated with more lateral orofacial structures supplied by the auriculotemporal, superior labial, inferior alveolar, mandibular incisor and molar branches of the trigeminal nerve, were found to be located laterally in the ganglion. Cell bodies related to more medial orofacial tissues supplied by the lingual, maxillary incisor, greater palatine and external nasal branches of the trigeminal nerve, were represented in the more medial regions of the ganglion (Figure 2.4).

The same authors found that the dorsoventral organization was more complex, although generally the nerve cells whose peripheral process supplied intra- and peri-oral structures were located in the more ventral portions of the ganglion.

In the antero-posterior plane, there appeared to be a concentration of cell bodies associated with intra-oral structures at the bifurcation of the ganglion. Extending anteriorly from the bifurcation were locations of cells associated with the more superficial maxillary tissues of the incisor, superior labial and external nasal regions. Posteriorly from the bifurcation, more superficial mandibular tissues such as the auriculotemporal, mental, lingual and incisor nerves were represented.

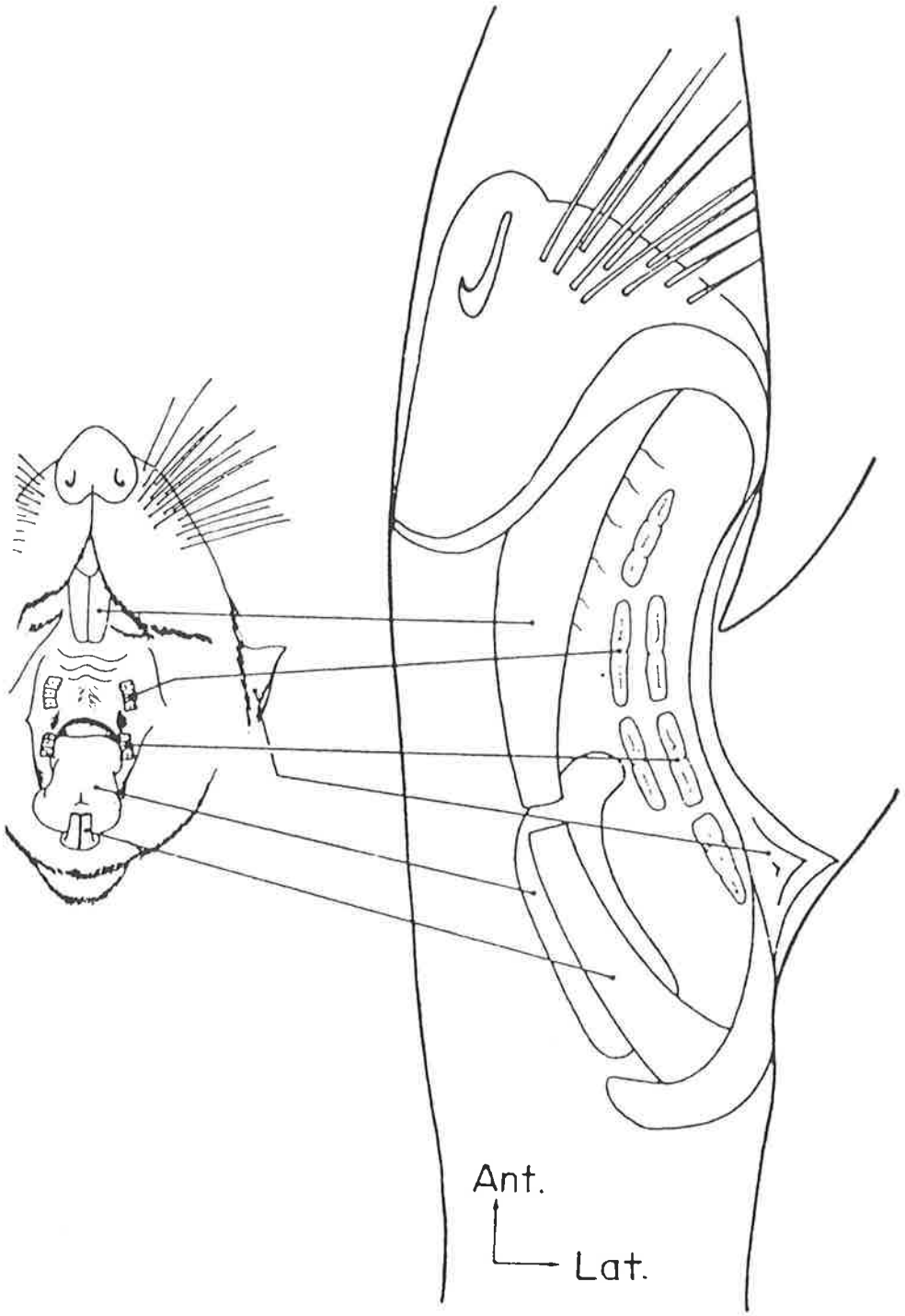


Figure 2.4: A representation of orofacial structures within the right trigeminal ganglion viewed from its superior aspect based on the percentage and distribution of chromatolytic cells. (After Gregg and Dixon, 1973).

CHAPTER III

PERIPHERAL NERVE FIBRE RESPONSE TO INJURY

3.1 INTRODUCTION

Injury to nerve fibres produces certain effects which depend on the nature, site and severity of the injury. Following injury to a peripheral nerve both physiological and anatomical changes occur.

The reactions of the nerve following injury proceed in two phases. The first is Wallerian degeneration which involves the disintegration of the axon and the myelin sheath. This is a feature below (distal to) the site of injury. However, the nerve fibre proximal to the injury site also undergoes changes which may, or may not, involve the degeneration of the whole neuron. Degenerative changes result in the separation of the end organ from its corresponding neuron.

The second phase of the reaction involves the restoration of continuity between the end organ and the neuron by regeneration of the intact part of the axon. Regeneration is critically influenced by the condition of the endoneurium. With an intact endoneurium, regeneration is complete and without complication. The growing axon is confined to the original endoneurial tube and is directed distally to the end organ it innervated initially. Severance of the entire nerve fibre complicates regeneration. The axon may be obstructed by scar tissue formed at the site of injury and may terminate somewhere other than at the original end organ. These phenomena will be discussed in further detail later in this review.

It is not always possible to make a clear distinction between these two phases described above, since almost from the outset the degenerative response is preparation for the regenerative response.

3.2 CLASSIFICATION OF PERIPHERAL NERVE INJURIES

The principal reason for proposing a classification of peripheral nerve injuries is so that a standardized term may be used to refer to a particular nerve injury and convey some idea of prognosis. However, peripheral nerve injuries cannot in all instances be placed into well defined categories. Many factors associated with injuries in general, and in particular with nerve injuries, will affect both diagnosis and prognosis. For example:-

1. In some instances it is very difficult, or even impossible, to diagnose the type of nerve injury purely on clinical grounds.

2. The extent of nerve injury affects the rate and extent of recovery and can make the diagnosis and prognosis difficult.

3. The proximity of the nerve injury to the nucleus affects both diagnosis and prognosis.

4. Infections delay or may even prevent regeneration.

5. Multiple injuries of the same or different types to the same nerve complicate diagnosis and prognosis.

At present in the literature there exists three classifications of peripheral nerve injuries. Seddon (1943) described three types of nerve injury:-

1. Neurapraxia - a term used to describe paralysis in the absence of peripheral degeneration.

2. Axontmesis - damage occurs to the nerve fibres leading to peripheral degeneration. The internal connective tissue architecture of the nerve is fairly well preserved.

3. Neurotmesis - a term used to describe a nerve in which there has been complete severence of all essential structures, such as the axon and its myelin sheath and the connective tissue elements.

Sunderland (1951), proposed a more comprehensive classification:-

1. First degree injury - this refers to blockage of nerve conduction at the site of injury without loss of continuity of the essential neural structures (equivalent to Seddon's neurapraxia). The blockage of conduction is only transient and the axon undergoes functional recovery.

2. Second degree injury - this is where there is damage to the axon with subsequent Wallerian degeneration. The connective tissue components, Schwann cells and basement membrane remain intact (equivalent to Seddon's axontmesis). Regeneration then occurs at approximately 1-2 mm/day and is usually effective

unless the injury is in very close proximity to the central nervous system.

3. Third degree injury - in addition to axonal disintegration and Wallerian degeneration, the internal structure of the fasciculus is disorganised so that endoneurial tube continuity is lost. However, the integrity of the perineurium is maintained (equivalent to Seddon's axontmesis). Regeneration is less complete than in second degree injury, with cross-shunting of nerve fibres being a possibility.

4. Fourth degree injury - this refers to a situation where the entire fasciculus is involved, all bundles being breached and disorganized. The nerve remains macroscopically intact due to maintenance of the integrity of the epineurium. However, as the perineurium and endoneurium are damaged, there may be axon loss and scarring (equivalent to Seddon's neurotmesis). Regeneration is poorly orientated and ineffective.

5. Fifth degree injury - this is where there is complete loss of continuity of the nerve trunk (equivalent to Seddon's neurotmesis). Regeneration is very poorly orientated, very ineffective and in many cases requires surgical intervention.

More recently, Rood (1983) has extended Seddon's classification by proposing six types of nerve injury:-

1. Neurapraxia grade 1 - functional conduction block caused by ischaemia and/or oedema with no anatomical change.

2. Neurapraxia grade 2 - demyelination which is a minimal structural change with no axon loss.

3. Axontmesis grade 1 - axon degeneration (Wallerian degeneration).

4. Axontmesis grade 2 - axon degeneration with endoneurial disruption.

5. Neurotmesis grade 1 - destruction of the axons with perineurial disruption.

6. Neurotmesis grade 2 - complete nerve division.

Rood's classification is essentially an extension of Seddon's earlier classification which accounts for grades of functional and anatomical disruption under the main headings of neurapraxia, axontmesis and neurotmesis. These two classifications have found wide usage in clinical practice, however, the variety of clinical patterns of sensory loss cannot always be categorised into the three main groups proposed by these classifications.

Sunderland's more comprehensive classification does not appear to have been as widely accepted by clinicians. However, it is the method of classification adopted by most researchers studying histological changes in peripheral nerves following injury, and as such, it is used in the present study.

A summary of the three peripheral nerve injury classifications is presented in composite table form (Table 3.1).

Seddon (1943)	Sunderland (1951)	Rood (1983)
NEURAPRAXIA	FIRST DEGREE INJURY	NEURAPRAXIA GRADE 1
paralysis in the absence of peripheral nerve degeneration.	conduction along axon is interrupted with no loss of continuity of essential neural structures.	functional conduction block (Ischaemia and oedema).
		NEURAPRAXIA GRADE 2
		demyelination (no axon loss).
AXONTMESIS	SECOND DEGREE INJURY	AXONTMESIS GRADE 1
damage to the nerve fibres leading to peripheral degeneration. Internal neural architecture is fairly well preserved.	axon fails to survive below level of injury and for a short distance proximally.	axon degeneration (Wallerian degeneration)
	THIRD DEGREE INJURY	AXONTMESIS GRADE 2
	axonal disintegration plus internal structure fasciculi disorganised.	axon degeneration plus endoneurial disruption.
NEUROTMESIS	FOURTH DEGREE INJURY	NEUROTMESIS GRADE 1
complete severance of all essential structures.	entire fasciculus involved Perineurium and endoneurium damaged.	perineurial disruption (closed nerve injury)
	FIFTH DEGREE INJURY	NEUROTMESIS GRADE 2
	loss of continuity of the nerve trunk.	nerve division (epineurium disrupted).

Table 3.1 Classifications of peripheral nerve injuries.

3.3 DEGENERATION OF PERIPHERAL NERVES

Nerve fibres degenerate at different rates, the rate depending on factors such as:-

1. The type of experimental animal used.
2. The age of the experimental animal.
3. The size of the nerve fibre.
4. The degree of myelination of the fibre.
5. Temperature.

(Sunderland, 1978)

The degenerative aspects that are important are the retrograde changes occurring in the fibre central (proximal) to the site of injury, and the responses occurring in the nerve fibre at, and distal to the injury site.

3.3.1 The retrograde fibre reaction

The changes that occur in the nerve segment proximal to the injury are directed centrally and the extent varies with the severity of the injury. In injuries that do not rupture the endoneurial tube the retrograde degeneration is usually confined to the adjacent few millimetres of the fibre ; in severe injuries the effects may extend proximally for several centimetres.

The pattern of the retrograde reaction is the same as that occurring below (distal to) the level of the injury, however, the

Schwann cell proliferation is not as great in the proximal nerve segment (Logan et al., 1953).

Lubinska (1961) reported that the retrograde effects were usually confined to the immediate neighbourhood of the lesion with crush injuries. She further reported, that myelin degeneration is dependent upon the preservation of the nucleated region of the Schwann cell. Physiologically, the proximal nerve segment exhibits a reduced conduction velocity when an electrical stimulus is applied to it.

3.3.2 Changes occurring in the nerve fibre at and distal to the site of injury

1. Degeneration of the axon and myelin

Within the first 24 hours of the injury axon changes are exhibited and are manifested as initial swelling, followed by fragmentation of the neurofilaments and separation of the axon from the myelin sheath. The axon has a varicose appearance and 48-72 hours after the injury it breaks into twisted fragments which are dispersed along the fibre. It is generally agreed that the degenerative changes take place simultaneously along the length of the fibre, though they are somewhat slower at the nodes of Ranvier (Sugar, 1938 ; Weddell and Glees, 1941).

Changes in the myelin sheath become obvious 28-96 hours after the injury, and by this time axon disintegration is well advanced. Initially there is folding, lamellar splitting and fracturing of the myelin sheath. The myelin fragments into droplets which form around the axon debris. The above physical changes are completed by about the 8th day

when chemical degeneration begins and complex lipids are broken down into simpler fats. (Johnson et al., 1950). There is some evidence suggesting that breakdown of myelin proteins may precede these lipid changes (Adams et al., 1972). The interior of the fibre becomes occupied by an amorphous collection of myelin and axon debris which is removed by phagocytosis. Myelin degeneration is generally completed by the end of the 14th day (Weddell and Glees, 1941 ; Guth, 1956).

The ability of the fibre to conduct action potentials ceases with the loss of axonal continuity. Conduction failure is complete at about 5 days after the lesion is produced (Landau, 1953).

2. Schwann cell changes

Schwann cell changes follow the changes in the axon and myelin, and are usually evident 24 hours after the injury. The Schwann cell reaction is considered essentially hyperactive because it is associated with an increase in the protein-synthesizing apparatus of the cell manifested by enlarged nuclei, increased amount of cytoplasm and more cytoplasmic granules. These processes occupy the next 48 hours when Schwann cell proliferation commences. The factors controlling the rate of Schwann cell proliferation are unknown, but large myelinated fibres show more cell proliferation than smaller fibres (Thomas, 1948).

Sunderland (1978) noted that the height of Schwann cell activity appears to coincide with the period when Wallerian degeneration and the removal of debris are occurring most rapidly. On the basis of this observation he proposed that the initiator of Schwann cell proliferation

may be a chemical factor released when the myelin and axon degenerate, or by physical factors introduced when they collapse.

In the mouse, maximal Schwann cell proliferation occurs approximately 3 days following injury (Bradley and Asbury, 1970). Schwann cell activity decreases from about the 2nd week, when the cells form strands along the endoneurium which is now empty but for the remnants of myelin and axon. Schwann cell proliferation is seen to decline when the cell columns from the proximal and distal segments contact each other (Jurecka et al., 1975). On this basis it has been proposed that the duration of proliferation depends on the size of the defect resulting from the injury.

Wallerian degenerative changes of unmyelinated axons are similar to those of myelinated axons, but are slower. Furthermore, the strands of Schwann cells along the endoneurial tube seen in established degeneration of myelinated axons are not present in unmyelinated axons (Thomas and King, 1974).

The functional significance of Schwann cells in the degenerative process is not clearly defined. It has been proposed that they may provide enzymes that destroy the myelin or act as macrophages (Lee, 1963).

3. The macrophages

The origin of phagocytes in degenerating peripheral nerves is not clearly understood. The majority of workers, including Lee (1963) and Williams and Hall (1971) maintain a Schwann cell origin for phagocytes.

Ollsson and Sjostrand (1969), Bernier et al. (1973) and Mei Liu (1974) on the other hand proposed that macrophages are of haematogenous origin. Stenwig (1972), suggested a dual source for phagocytes.

Macrophages enter the endoneurium at about the 3rd day after injury and add to the nuclear increase. Macrophage activity then increases in intensity and the degeneration debris is removed as a result of this process. The removal of debris continues into the 14th day, and is usually finished by the 4th week after injury. On completion of their activity the macrophages leave and the Schwann cells are left as the only occupants of the endoneurial tube.

4. The endoneurium

The endoneurial sheath undergoes minimal and insignificant change. The endoneurium continues to provide the supporting framework of the endoneurial tubes which are maintained as distinct structural entities. The early onset of regeneration stops endoneurial shrinkage well in advance to it reaching a level that would grossly affect regeneration (Sunderland, 1978).

3.3.3 Changes occurring in the nerve fibre where there is loss of continuity of the nerve fibre

If the nerve fibre is completely sectioned, additional changes are superimposed on the pattern of Wallerian degeneration outlined above. These are concerned with the reaction occurring at the ends of the severed nerve fibre, the retrograde changes in the nerve fibre, and the effects of prolonged denervation of the distal segment of the endoneurial tube when it fails to receive a regenerating axon.

1. Reaction at the ends of the severed nerve fibre

When the endoneurial tube is completely severed, the proliferating Schwann cells and regenerating axon are not confined to the tube. Fibroblasts and Schwann cells proliferate at the ends of the severed tube, and depending on the distance between the ends of the tube, may or may not meet. The net result of this reaction is scar tissue which is composed of a haphazard arrangement of Schwann cells and fibroblasts. Collagen is deposited in the gap between the severed ends and its extent influences axon regeneration (Sunderland, 1978). The Schwann cell activity at the nerve ends is at a peak when proliferation within the endoneurial tube is at a peak.

2. Retrograde changes in the nerve fibre

The fibres in the proximal segment of a sectioned nerve show a progressive reduction in diameter if contact is not re-established with the periphery. This reduction is manifested in axon diameter and myelin thickness (Cragg and Thomas, 1961).

The retrograde diameter reduction has been attributed to the outflow of axoplasm that occurs during regeneration, to the failure of the regenerating axon to establish appropriate connections with the periphery and to disuse (Sunderland, 1978). Furthermore, there is an associated progressive reduction in conducting velocity (Cragg and Thomas, 1961, 1964).

3. The effects of prolonged denervation on the distal segment of the endoneurial tube

The regenerating axon emerges from the severed end of the proximal endoneurial tube, and must cross the gap of connective tissue between the nerve ends in order to reach the distal segment of endoneurial tube. As a result of this "obstacle encounter", reinnervation of the distal section may be delayed or it may remain permanently denervated.

Endoneurial collagenisation occurs in the denervated distal segment of the severed nerve. The new endoneurial collagen is deposited at the external surface of the basement membrane of the Schwann cell, giving the sheath a thickened appearance (Thomas, 1964).

With the phagocytic removal of the axon and myelin debris the endoneurial tube shrinks. The denervated endoneurial tubes are initially swollen, but by the 12th day the lumen becomes smaller. The reduction increases progressively with the duration of denervation, and is proportional to the fibre diameter. This reduction occurs rapidly over the first 3 months, at the conclusion of which there is little further change (Sunderland and Bradley, 1950a,b).

This progressive decrease in the calibre of the endoneurial tube with increasing periods of denervation is mainly due to shrinkage, and not to thickening of the tube wall at the expense of the lumen. The shrinkage is thought to be caused by loss of intracellular pressure from the degenerating axon and myelin and their removal. Tension is thereby reduced in the endoneurial wall which collapes. This change is general throughout the fasciculus and therefore tension is relieved in the

endoneurium and perineurium (Sunderland, 1978). In contrast, the epineurial tissues do not react in the same way and undergo little change.

Despite narrowing of the endoneurial tubes, they will still receive regenerating axons and guide them to the periphery (Sunderland, 1978). The endoneurial collagenisation hinders the expansion of the endoneurial tube on reinnervation and so prevents the restoration of normal fibre diameter.

3.4 REGENERATION OF PERIPHERAL NERVES

The essential feature of regeneration in the nerve fibre is the axon outgrowth to replace the fibre segment that perishes as a result of the injury. The time taken for regeneration to be completed is divisible into the following periods (Sunderland, 1978).

1. The initial delay. This is the time taken for the neuron to recover from the insult, for the axon growth to commence, and for the axon to reach the site of injury. It depends on the severity of the injury and the proximity of the injury to the cell body.

2. The scar delay. This is the time taken for the axon to cross the injured area. It represents the time taken for the axon to enter the distal segment of the endoneurial tube.

3. The period of axon growth distal to the site of the injury. This is the time taken for the axon to reach its peripheral terminations.

4. The period of functional recovery. This is the time required to complete the changes in the axonal pathway which determine the normal conduction properties of the individual fibres.

Regeneration may be delayed or prevented at any stage during, or after, the restoration of axonal continuity. This results in wide variations in the interval between injury and recovery, thereby making accurate predictions of regeneration times almost impossible for a given individual.

The condition of the endoneurial tube is critical in determining the course and results of regeneration as will be discussed shortly. However, irrespective of the integrity of the endoneurial tube, regeneration progresses as far as the injured zone in essentially the same way, differences relating only to time. From this point onwards, the course of regeneration differs significantly for the two forms of injury and will be considered separately.

3.4.1 Recovery of the neuron and the onset of regeneration

This involves the correction of disorganized physiochemical proceses that are not directly visible, the reversal of depressed protein synthesis and associated chromatolysis, and the reformation of the characteristic pattern of Nissl substance.

The recovery is coincident with regenerative activities at the axon tip. Later the regenerating axon makes demands on the protein synthesizing machinery of the nucleus (Bodian and Dziewiatkowski, 1950). Severity of the injury and the resulting retrograde effects determine

the time required for the neuron to recover sufficiently in order to allow regeneration to begin.

3.4.2 Axon growth as far as the injured zone

Due to the retrograde fibre reaction the axon tip is located some distance proximal to the injury site, and is therefore confined in the early stages of advance to the endoneurial tube. It is here that the first signs of axon branching may occur, although this is not usually a feature until the injured area is reached (Shawe, 1955).

The progress of the axon tip along the endoneurial tube is the result of the combination of three factors:-

(a) The central changes in the cell that propel the axon tip distally (Young, 1945).

(b) The axon tip, which, as it moves distally, leads to elongation of the axoplasm (Sunderland, 1978).

(c) The peripheral resistance offered by the tissue through which the axon must pass to progress distally. This must be overcome by the intrinsic growth forces (Sunderland, 1978).

3.4.3 Regeneration at and below the injured zone with an undamaged endoneurium

It is at the site of injury that axon advancement may be transiently slowed down and axon branching may occur. Shawe (1955) found

that with crush injuries each motor axon sprouted three branches. He also proposed that large axons formed more sprouts than small ones.

However, axon branching is not a feature of regeneration within intact endoneurial tubes. If it does occur, it is short lived and only one process survives - the one that has moved furthest peripherally down the endoneurial tube, the other branches being progressively resorbed (Denny-Brown and Brenner, 1944).

The progress of the axon tip down the endoneurial tube is determined by the resultant action of the three forces that have been discussed previously. Because the axon is confined to the endoneurial tube it is provided with ideal conditions for growth as the Schwann cells are optimally aligned to minimize resistance to the advancing axon (Sunderland, 1978). Axon advancement at the site of injury is not significantly obstructed by the debris of degeneration. However, Lubinska (1952) concluded that degeneration must reach a certain level before regenerating axons will enter and grow along denervated endoneurial tubes.

3.4.4 Regeneration at and below the site of injury with a severed endoneurial tube

The regenerating process is complicated with the complete transverse sectioning of nerves. When a nerve is sectioned, scar tissue forms at the site joining the ends of the severed nerve. The regenerating axon on leaving the proximal segment, enters and must pass through this scar which is never as suitable a medium for growth of the axon as the endoneurial tube. The scar tissue usually acts to obstruct, delay and misdirect the passage of the axon distally (Sunderland, 1978).

Resistance provided for the axon by the scar tissue results in the formation of multiple sprouts (Weddell, 1942). This branching is more extensive and variable than that occurring with a crush injury where the endoneurium remains intact (Shawe, 1955). As these axon branches enter the scar tissue they pass along the lines of least resistance. Several branches may reach, enter and descend along an endoneurial tube. Some of these axons may acquire a myelin sheath (Davenport et al., 1937). However, usually only one axon dominates the growth pattern, the others failing to survive (Weddell, 1942 ; Weiss and Taylor, 1944). Alternatively, it has been reported that one single regenerating axon may branch and reinnervate more than one endoneurial tube (Sunderland, 1978).

Choice of which endoneurial tube is entered by the axon branches is not clearly understood. Evidence has failed to demonstrate any selectivity of regenerating neurons and their corresponding end organs. Furthermore, it has been demonstrated that the original axons of a muscle have no advantage over any other motor axons in the reinnervation of that muscle (Bernstein and Guth, 1961). It has also been reported that axons of non-myelinated fibres grow into and along the endoneurial tubes of myelinated fibres in preference to their original pathway (Evans and Murray, 1954).

The resultant effect of disorganized growth occurring in the scar tissue is to reduce the fibre population available for reinnervation and to distort the innervation pattern. Both of these factors adversely affect the restoration of function.

Once the regenerating axon has entered the endoneurial tube of the distal segment and if reasonable conditions prevail, regeneration is similar to when the endoneurium is intact. This is the case irrespective of the period of denervation (Sunderland, 1978). However, regeneration in the distal segment is slower than if there was no loss of continuity of the endoneurial tube.

Myelination proceeds centrifugally along the fibre, proceeding just behind the advance of the axon tip. Once the axon traverses the scar and passes into the distal segment myelination keeps pace with axon growth (Davenport et al., 1939). There is a general lack of agreement as to when myelination begins. Commencement of myelination varies with the type of injury, the diameter of the axon, and the experimental animal; the range being from 6-21 days after injury.

The Schwann cells are re-aligned in such a manner that there is one Schwann cell to each myelin segment, thereby restoring the normal arrangement (Hiscoe, 1947). The axon diameter progressively enlarges after the commencement of myelination until the original dimensions are obtained. Finally, axonal continuity with the periphery, end organ connection and functional relationships are re-established.

CHAPTER IV

CENTRAL RESPONSE OF THE TRIGEMINAL GANGLION TO PERIPHERAL NERVE INJURY

4.1 INTRODUCTION

When a peripheral nerve is injured or sectioned, predictable morphological and physiological changes occur in all parts of the cell including : proximal to the site of injury, at, and distal to the site of injury (Chapter III), and in the centrally located cell body. In this section of the review, the central response to peripheral nerve injury will be discussed.

Peripherally directed nerve fibres of mammals are generally accepted as being able to survive an axon injury.The re-establishment of the original pattern of peripheral innervation depends largely on the type of injury, a second degree injury offering optimal conditions for successful regeneration, whereas a fifth degree injury offers only limited opportunities for successful regeneration (Sunderland, 1951).

However, a number of investigators have reported that a significant percentage of spinal ganglion cells degenerate and die following spinal nerve transection (Ranson, 1909 ; Cavanaugh, 1951). A similar result has also been reported in trigeminal ganglion cells by Galich et al. (1976) and Aldskogius and Arvidsson (1978).

It has also been shown that following lesions of the peripheral branches of the trigeminal nerve in kittens and rats, abundant nerve

fibre degeneration occurs in the trigeminal brain stem tract (Grant and Arvidsson, 1975). In the adult cat also, trigeminal tract degeneration was demonstrated following tooth pulpectomies (Westrum et al., 1976 ; Gobel and Binck, 1977). This phenomenon which seems to occur in all primary sensory neurons is referred to as transganglionic sensory degeneration (Grant and Arvidsson, 1975) (Figure 4.1). It appears that the two phenomena of ganglion cell degeneration and death, and transganglionic sensory degeneration, are related (Grant and Arvidsson, 1975 ; Aldskogius and Arvidsson, 1978).

4.2 MORPHOLOGICAL CHANGES IN THE TRIGEMINAL GANGLION FOLLOWING PERIPHERAL NERVE INJURY

The structural changes occuring in sensory ganglion cells following peripheral nerve lesions have been reported in light and electron microscopic studies (Lieberman, 1971). It is evident from the literature that the precise nature of the central changes induced by peripheral nerve injury is not fully known. Furthermore, little is known about the time course of these changes in transected neurons. The time course varies considerably with alterations in the experimental conditions.

A review of the characteristic structural changes occurring in peripherally transected sensory ganglion neurons follows:-

4.2.1 Cytoplasmic changes

Early cellular swelling is observed readily within the first week. The cells appear swollen and take on a granular appearance. It has been proposed that this is due mainly to an increase in the uptake of water and an increased amount of intracellular organic material (Gersh and

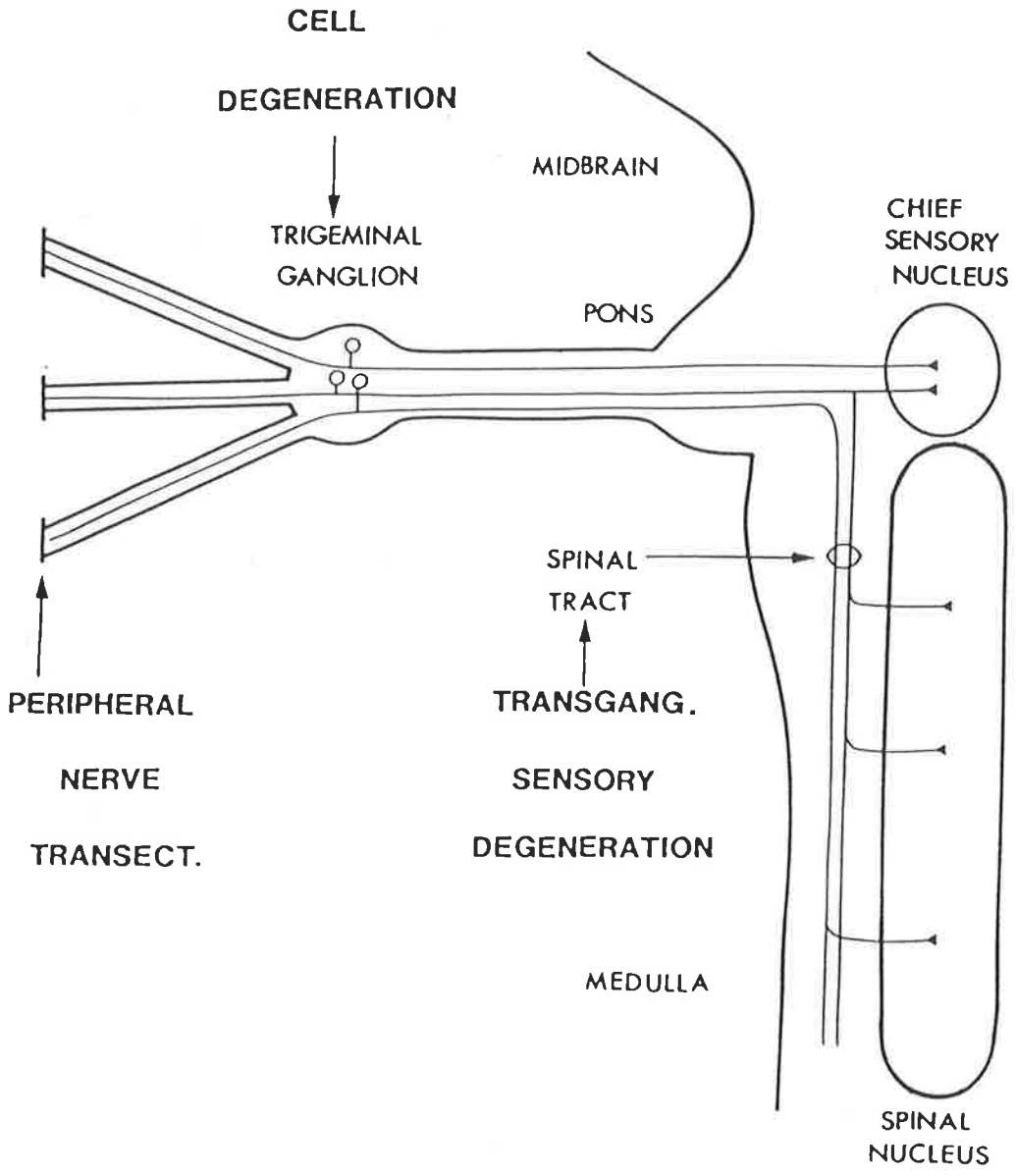


Figure 4.1: Schematic diagram showing the concept of transganglionic sensory degeneration.

Bodian, 1943). Subsequently, after about two weeks, the cell undergoes shrinkage and atrophy with a resultant decrease in cell body volume.

The disintegration of prominent basophilic staining Nissl granules was first described by Nissl (1892) in his original studies on rabbit facial neurons. Nissl granules are essentially clusters of ribosomes, and the decrease in the amount of Nissl granules and its dispersal through the cell in finer particles is referred to as chromatolysis.

Chromatolysis is an optical expression of a physico-chemical disturbance resulting in the loss of ribonucleoprotein (Sunderland, 1978). Chromatolysis occurs within the first 24 hours following axon section and is well advanced by the seventh day. A maximum is usually reached about 18 days after injury. It is generally agreed that at the peak of chromatolysis the central region of the cell is devoid of Nissl granules and contains very fine basophilic granules, with the residual Nissl substance largely confined to the periphery.

Following this, if recovery is to take place, there is levelling off of ribonucleoprotein loss. The regressive changes are now reversed, and the ribonucleoprotein loss is replaced. This occurs between the 18th and 30th day. Eventually there is restoration of cell size and shape, along with the original pattern of Nissl substance. The recovery period can vary from three to six months depending on the severity of the retrograde changes.

It is emphasized that the time course of chromatolysis, the severity of chromatolysis, the onset of regeneration and the recovery period, all vary considerbly with the experimental conditions.

Dilatation and reduction of granular endoplasmic reticulum with the disaggregation of polysomes and a decrease in ribosomal number have been reported in both spinal sensory neurons (Mackey et al., 1964) and motor neurons (Kirkpatrick, 1968). Similar changes have been observed in the trigeminal ganglion following transection of the infra-orbital nerve (Aldskogius and Arvidsson, 1978).

Finally, the appearance of many vacuoles and lysosomal-like structures within the cytoplasm is a consistently observed structural change following peripheral transection of sensory ganglion cells (Mackey et al., 1964 ; Aldskogius and Arvidsson, 1978).

4.2.2 Nuclear changes

Peripheral displacement of the nucleus from its usual central position has been reported by many investigators. Nuclear eccentricity is established after the early chromatolytic changes are apparent (Nissl, 1892). At later stages of the retrograde neuronal reaction the nucleus regains its central position at about the same time that the normal Nissl pattern is reconstituted providing there has been successful peripheral nerve regeneration (Gersh and Bodian, 1943).

The cause and significance of nuclear displacement is not understood. Among suggested primary causes are cellular water uptake (Barr and Hamilton, 1948) and changes in the axoplasmic flow characteristics after transection (Lieberman, 1971).

Nuclear indentations and folding of the nuclear membrane has been described (Mackey et al., 1964 ; Aldskogius and Arvidsson, 1978). It has

also been suggested that an increased nucleocytoplasmic interface is related to enhanced neuronal metabolism in regenerating cells. Nucleolar vacuolation and "paling" of the nucleoli have also been described following peripheral nerve transection (Aldskogius and Arvidsson, 1978).

The above changes, constitute a generalized or non-specific retrograde neuronal reaction to cellular injury. In addition Aldskogius and Arvidsson (1978) observed many cells exhibiting changes interpreted as reflecting stages in a process terminating with cell death. They stated that well preserved normal and chromatolytic neurons were observed adjacent to the degenerating and dying neurons, and on that basis they considered that the degenerative changes were non-artifactual.

The cytologic criteria for neuronal degeneration and death are at present far from unanimous. Furthermore, the changes associated with degeneration and death of transected neurons are extremely diverse and their significance is obscure. One of the major problems is that the reversible retrograde neuronal reaction and the morphological manifestations of cell death represent a continuum, with one stage merging imperceptibly with the next. It is therefore impossible to delineate accurately the point beyond which changes are irreversible.

For recognition of cell death we depend on indicators of loss of metabolic processes or loss of vital structures, manifested by the nuclear changes of pyknosis, karyorrhexis and karyolysis. By the time these changes are recognized, the cell has already been irreversibly injured or dead for some time (Robbins, 1979). Aldskogius and Arvidsson

(1978) stated that almost complete vacuolation and disintegration of cell organelles are likely manifestations of overt cell death.

Changes have also been observed in satellite cells which have been interpreted as reflecting nerve cell degeneration (Leech, 1967 ; Aldskogius and Arvidsson, 1978). Clusters of satellite cells surrounding degenerating neurons and occasionally associated with intracellular and/or extracellular debris indicate their phagocytic activity.

Although the structural changes resulting from transection of peripheral nerve fibre processes which are considered as signs of the retrograde neuronal reaction may be reversible (Engh and Schofield, 1972), it is likely that in some cases, where the cellular injury has been severe, they represent changes preceding cell death (Aldskogius and Arvidsson, 1978) (Figure 4.2).

4.3 THE SIGNIFICANCE OF THE CENTRAL RESPONSE TO PERIPHERAL NERVE INJURY

Despite some agreement among various authors concerning the major central morphological changes to peripheral nerve injury, interpretation of their significance, that is, degenerative or regenerative, remains controversial.

Originally Nissl (1892) interpreted chromatolysis as primary cellular irritation resulting from axon injury. This process is reversible providing peripheral axon regeneration is successful. Subsequently, these same changes were described as retrograde degeneration by Gersh and Bodian (1943), who were able to demonstrate decreased concentrations of ribonucleoprotein in chromatolytic neurons.

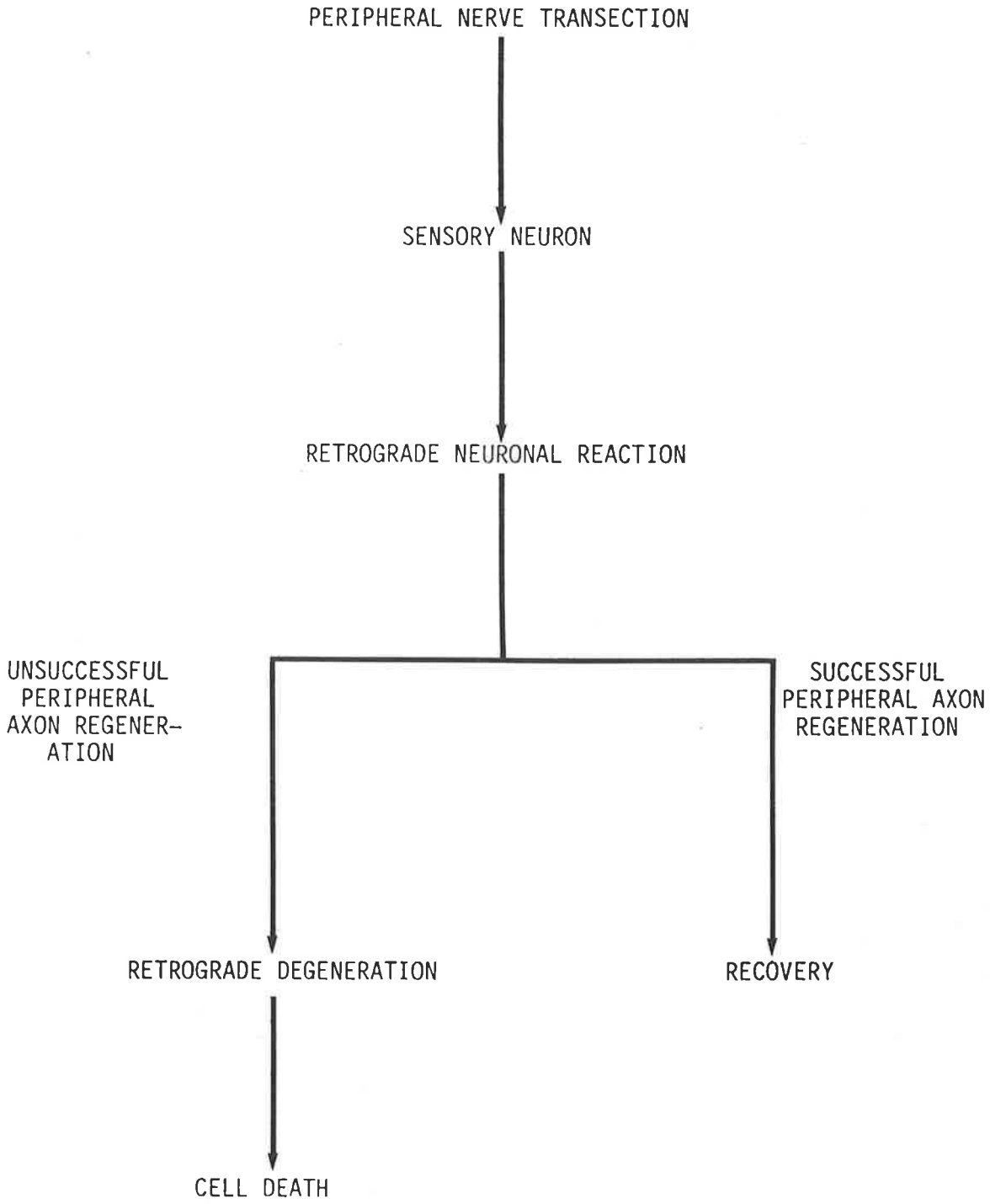


Figure 4.2: Possible sequelae of sensory ganglion neurons to peripheral nerve transection.

Other investigators disagreed with the degenerative concept and proposed that chromatolytic neurons were hyperactive and regenerative (Brattgard et al., 1957). These investigators put forward the argument that chromatolysis represented a redistribution rather than a reduction in ribonucleoprotein, and were able to show increased cellular ribonucleic acid in the ganglionic neurons two weeks after peripheral nerve injury.

Droz and Leblond (1963) demonstrated axon flow of centrally synthesized axonal cytoplasm by using autoradiographic techniques. This work provided the stimulus for other investigators to apply similar techniques to the study of the effects of nerve injuries on cell body regenerative activity (Watson, 1965 ; Scott et al., 1968). These later studies produced variable results with respect to the relative amount of labelled amino acids precursor incorporated into injured neurons and the rate of distal axon transport. The conflicting results may be rationalized to some degree by the differences in the experimental models used. Some of the variables in the experimental models included : the animal age, the animal species, the type of injury, the type of labelled precursor, the time interval from injury to sacrifice, and the time interval from systemic administration of labelled precursor to sacrifice. All of these variables could conceivably affect the end result.

The section of a peripheral nerve results in amputation of axonal volume of the component axons, the exact amount of axonal volume amputated depends on the level of amputation. Successful peripheral axon regeneration requires central cell body replacement of this loss in volume and, therefore, eventually an increase in the rate of axonal

cytoplasm production. Advocates of the regenerative interpretation of chromatolysis consider the process to be an adaptive cell body change necessary for an immediate increase in the synthetic activity of the cell (Brattgard et al., 1957).

More recently the regenerative interpretation of chromatolysis has been questioned (Engh and Schofield, 1972). Electron microscopic and histochemical studies of experimentally induced chromatolysis seem incompatible with the regenerative interpretation. Ultrastructural alterations in the cell body, as discussed above, include a decrease in ribosomal number, a disaggregation of polysomes, and an increased number of vacuoles and lysosmal-like structures. In conditions other than chromatolysis, for example, starvation and radiation, these same changes are usually considered as manifestations of a non-specific response to cellular injury and are associated with decreased R.N.A. and protein synthesis. It seems unlikely that the same changes following peripheral axon injury represent increased cell body synthetic activity.

Nissl's original concept of chromatolysis being a manifestation of primary cellular irritation seems to be correct, and a restoration of a normal cytoplasmic appearance with an organized ribosomal pattern appears to be necessary for a return to normal cell body protein synthesis (Engh et al., 1971).

Cavanaugh (1951) reported that a return to normal cytoplasmic appearance did not occur in dorsal root ganglion cells subjected to peripheral nerve transection unless there was successful peripheral axon regeneration.

Engh et al. (1971) using autoradiography found that peripheral axon injuries are initially associated with the decreased cell body synthetic activity, and the decreased synthetic activity continued until successful peripheral axon regeneration occurred. If successful peripheral axon regeneration were not allowed to occur, evidence of decreased protein synthesis persisted. However, with reversible nerve injuries, autoradiography demonstrated increased cell body synthetic activity. These authors believed that the initial decrease in cell body synthetic activity of neurons subjected to peripheral axon injury represents a generalized transient response by these cells to a non-specific cellular injury. This interpretation finds support from the aforementioned ultrastructural changes observed in chromatolysis.

Therefore, it seems that following peripheral nerve transection, the regressive changes are reversed if successful peripheral regeneration occurs, and an increase in cell body synthetic activity results. Finally, there is complete restoration of the size, shape and appearance of the cell. The delay before onset of the regenerative phase and the time taken for a neuron to recover completely depends on the severity of the injury. With mild injuries that do not rupture the endoneurial sheath (for example, first or second degree injury) the delay is short and the recovery is rapid and complete (Engh and Schofield, 1972).

4.4 TRIGEMINAL GANGLION CELL DEATH

Previous investigations have demonstrated that substantial cell loss occurs in spinal ganglia of rats following peripheral nerve transection (Ranson, 1909 ; Cavanaugh, 1951). Significant cell loss also

occurs in the trigeminal ganglion following infra-orbital nerve section (Galich et al., 1976 ; Aldskogius and Arvidsson, 1978) and in the trigeminal mesencephalic nucleus after mandibular nerve transection (Imamoto, 1972). The disappearance of sensory ganglion cells appears to be associated with degeneration of the ganglion cell processes.

The estimates of the number of cells perishing after nerve transection vary. This variation can be accounted for, at least in part, by the variation in experimental models used by different groups of investigators. Table 4.1 summarizes the range of cell loss obtained by various investigators, and the differences in their experimental models.

It can be seen that Ranson (1909) used young rats, where it is generally accepted that nerve cell death is more widespread after an axonal injury than in adult rats. Cavanaugh (1951), used adult rats, in which the intercostal nerves were transected and capped. The more pronounced trauma on the nerve fibres in addition to the prevention of axonal regeneration to the periphery imposed by this procedure may have contributed to the extensive cell loss. Furthermore, in both Ranson's and Cavanaugh's investigations, the nerve fibre transections were carried out more proximally relative to the cell bodies than in Aldskogius and Arvidsson's material. It is generally well accepted that the retrograde neuronal reaction and, therefore, presumably also the cell loss is more extensive the more proximal the site of the axonal injury to the parent cell body. Sunderland (1978) has related this to the amount of cytoplasm amputated from the cell.

It has been proposed that the different types of ganglion cells react differently to peripheral nerve section. Ranson (1909) and

EXPERIMENTAL PARAMETERS	INVESTIGATORS			
	Ranson (1909)	Cavanaugh (1951)	Galich et al. (1976)	Aldskogius & Arvidsson (1978)
Cell loss (%)	50	60	6-40*	17-29
Experimental Animal	Rat	Rat	Rat	Rat
Age of Animal	Young	Adult	Adult	Adult
Ganglia	Spinal	Spinal	Infra-orbital	Infra-orbital
Type of Peripheral Nerve Injury	Transection	Transection + Capping	Transection	Transection
Site of Peripheral Nerve Injury Relative to Ganglia	Proximal	Proximal	Distal	Distal

Table 4.1: Sensory ganglion cell loss following peripheral nerve transection.

* the 40% cell loss corresponded to the longer post-operative survival time of 70-140 days by these investigators.

Cavanaugh (1951), report that cell loss in spinal ganglia following peripheral nerve transection mainly affects small neurons. Confirmatory results were obtained by Aldskogius and Arvidsson (1978) in trigeminal ganglia following infra-orbital nerve section. These authors found that the majority of degenerating neurons in their studies were small. However, it may be possible that large neurons shrink extensively during degeneration, or they may degenerate quicker than small ganglion cells and, therefore, be harder to observe in a state of degeneration.

Conversely Galich et al. (1976) found that a substantial reduction of large neurons occurs in rat trigeminal ganglia following infra-orbital nerve section. However, this observation may reflect a general shift in the cell size spectrum towards smaller values by atrophy of the remaining cells rather than a selective loss of large neurons.

In conclusion, it must be pointed out that the possibility of differential cell loss with regard to size in the rat trigeminal ganglion is still open to question and further studies are required to verify this issue. However, the majority of available evidence suggests that the greatest effect is upon the small neurons. Pain impulses are mainly transmitted by thin unmyelinated (C group) fibres, which are presumably connected to correspondingly small cell bodies (Martinez and Freide, 1970). Therefore, it seems reasonable to assume that cell loss affecting this type of cell within the trigeminal ganglion would lead to structural and functional reorganization in the periphery or in the central nervous system which bears on the topic of pain mechanisms in the trigeminal system.

4.5 THE EFFECTS OF CELL LOSS WITHIN THE TRIGEMINAL GANGLION

Previous reports have demonstrated significant cell loss occurring in the trigeminal ganglion following peripheral nerve transection (Galich et al., 1976 ; Aldskogius and Arvidsson, 1978).

The observation of degeneration in the trigeminal tract at the same time as trigeminal ganglion cell degeneration, indicates that the death of the trigeminal ganglion cells and degeneration of trigeminal primary afferents in the brainstem (transganglionic sensory degeneration) are associated phenomena. It is thought that the nerve fibre degeneration in the trigeminal tract following peripheral nerve transection is a consequence of degeneration and death of trigeminal ganglion cells (Grant and Arvidsson, 1975 ; Aldskogius and Arvidsson, 1978).

Further investigations are required in this area to provide more information to elucidate this relationship.

CHAPTER V

PRINCIPLES OF CRYOSURGERY AND THE EFFECTS OF CRYOSURGERY ON PERIPHERAL NERVE STRUCTURE AND FUNCTION

5.1 INTRODUCTION

Cryosurgery is that branch of cryobiology and surgery which deals with the therapeutic application of extremely low temperatures (below 0°C) for the purpose of destroying tissues in selected target sites. The freezing process induces coagulation necrosis and is confined to the tissues within the region of the ice-ball, this localized effect of cryosurgery on the tissues has been termed a cryolesion (Neel, 1980).

The clinical application of cryosurgery is by no means new. As early as 1851, James Arnott described the cryosurgical treatment of cancer. However, it was not until a reliable cryosurgical system was devised using liquid nitrogen, that cryosurgery emerged as a potential clinical tool (Cooper, 1963).

It is widely known that freezing can also be used to preserve cells. Therefore, it is important to understand the factors that control these two opposite aspects of cryobiology. By varying the rate and method of cooling, and altering the local environment of the frozen tissues, the operator can manipulate the processes of either preservation or destruction according to the desired effect. In this review, we are concerned with the principles underlying the destruction of tissues by freezing, and the effect of freezing on nerve structure and function.

5.2 BIOLOGICAL MECHANISMS OF CRYONECROSIS

It is accepted that no single factor is responsible for the lethal effect of freezing on tissues, with cell death being induced by a combination of distinct cryobiological mechanisms (Meryman, 1956 ; Frazer and Gill, 1967). The relative contribution of each mechanism varies according to the apparatus used, the physical nature of the tissue, the distance from the cryoprobe, and the rate and degree of cooling and thawing (Leopard, 1975).

5.2.1 The direct effects of freezing

1. Cellular disruption

Where the rate of freezing is rapid (greater than 5°C per second), particularly in the vicinity of the probe tip where the cooling rate approaches -70°C per second (Whittaker, 1972), ice crystals form in both the extracellular and intracellular fluid. These ice crystals produce physical disruption of cell membranes and are lethal.

2. Cell dehydration and electrolyte disturbance

Within the outer zones of the ice-ball where the temperature is about -10°C (Whittaker, 1972), the cooling rate is much slower, and extracellular ice formation occurs. When crystals form extracellularly, water is removed from these spaces as ice and the remaining extracellular fluid becomes hypertonic, causing intracellular water to pass from the cells. The cells thus become dehydrated and undergo physical shrinkage. Additionally, the concentrations of electrolytes

both inside and outside the cell membranes increase to levels which become toxic to cell function (Leopard, 1975).

3. Inhibition of enzymes

Because each cellular enzyme system operates over a narrow temperature range, sudden cooling acts as an enzyme inhibitor. This modifies cellular metabolism, and may render the cell more vulnerable to metabolic disturbances (Leopard, 1975).

4. Denaturation of lipoprotein complexes

Water plays an important role in the spatial relationship of many cellular macromolecules and its removal could be a significant factor in denaturing proteins. Lipoprotein complexes in both cell membranes and mitochondria are denatured during freezing, with consequent damage to cell metabolism (Jolly, 1976).

5. The effects of thawing

When the freezing process stops and the tissues are allowed to rewarm, further damaging effects may take place. If the intracellular water was supercooled, then on thawing, ice crystals form about -21°C to -2°C, with resultant damage from crystallization. Again, because of the raised concentrations of intracellular electrolytes, and also due to the increase in the permeability of cell membranes during thawing (Meryman, 1956), extracellular water is taken into the cells, which then vacuolate, swell and rupture. Slow warming will prolong the duration of these damaging effects.

Thus it appears that maximum cell damage is achieved by combining a rapid freeze with a slow thaw.

5.2.2 The indirect effects of freezing

1. Vascular effects

It has been demonstrated that intracellular damage occurs within one minute of thawing (Whittaker, 1972). However, despite the complete vascular stasis evident during freezing, normal blood flow resumes after 10 minutes. Consequently, it appears that vascular changes are not the primary cause of cell death, rather they account for the delayed indirect effects of freezing including stasis and microthrombus formation, leading to ischaemic necrosis (Gill et al., 1970).

2. Immunological effects

It has been suggested that tissue damaged by freezing may act as an antigen and provoke an antibody response. The possible mechanisms involved in this immune response are :-

(i) the antigenic substances normally present within cells are liberated when those cells are killed by freezing, or

(ii) freezing may produce alterations in molecular structures which render normal cell components antigenic.

(Leopard, 1975).

The above hypotheses must be investigated in the future in order to clarify this issue.

Recently, Fazio et al. (1984) have produced evidence to support the hypothesis that cryosurgery can stimulate specific delayed hypersensitivity towards tumours as well as non-specific immunology parameters.

However, at present it cannot be claimed that cryosurgery is a valid form of immunotherapy in patients with tumours. Thus the significance of cryo-immunization remains hypothetical.

5.3 CRYOSURGICAL APPARATUS

Currently, there are two principal systems used in cryosurgery, the "open" and "closed" systems.

5.3.1. Open systems

These involve the direct application of carbon dioxide snow (-79°C) or liquid nitrogen (-196°C) applied on cotton pellets or as an open spray.

Open systems are more applicable to thickened proliferative and invasive lesions where control of the depth of destruction assumes secondary importance, and in the treatment of bone conditions.

5.3.2. Closed systems

Closed systems afford a greater degree of control, however, the instrumentation is more complex and the depth of freezing less profound compared with open systems. There are three main types of closed system.

1. Evaporative

This depends on the controlled evaporation of liquid nitrogen. This technique provides a minimum temperature approximating -195°C.

2. Joule-Thomson

With this principle the rapid expansion of a compressed gas (usually nitrous oxide) through a narrow orifice, results in the extraction of heat from the local environment causing a profound fall in temperature. These instruments produce a temperature at the probe tip in the vicinity of -70°C, and are used for the treatment of a wide variety of superficial lesions.

Cryosurgical units utilizing this principle are flexible to use and have heating coils built into the probes that defrost the tip, freeing it from the tissues when the supply of nitrous oxide has been cut off. The probes may be sterilized by autoclaving, and the probe tips are self-sterilizing by virtue of the low temperatures obtained.

3. Thermo-electric

This system is inefficient and cumbersome. Operating by the Peltier effect, the apparatus uses a D.C. current and water cooling.

5.4 FACTORS AFFECTING THE BIOLOGICAL RESPONSE OF TISSUES TO CRYOSURGERY

There are a number of factors that affect the response of individual cells to freezing.

5.4.1 The rate of freezing and thawing

The rate at which freezing and thawing takes place appears to be of much greater importance than the minimum temperature reached within the tissue (Poswillo, 1971).

The rate of freezing determines the way in which the frozen cell will undergo necrosis (Section 5.2.1), either by shrinkage from cellular dehydration, or by intracellular crystal formation. The latter mechanism is more lethal (Neel et al., 1971). It is widely accepted that slow thawing is more harmful than rapid thawing. Therefore, the cycle of a rapid freeze followed by a slow thaw produces the maximum lethal effect.

5.4.2 The effect of repeated freeze-thaw cycles

With repeated freezing the volume of tissue affected by freezing increased until a maximum is achieved after 5 to 7 applications (Gill et al., 1968). Repeated freeze-thaw cycles seem to enhance the thermal conductivity of the tissues and thus allow the formation of a larger ice-ball. Furthermore, the repeated cycles result in the tissues being frozen at successively more rapid rates so that each zone is subjected to more than one mechanism of cryonecrosis (Gill et al., 1968).

5.4.3 The effect of local heat sources

When a single source of freezing is applied to living tissue, the cryolesion is surrounded by a very effective heat source in the form of the local vascular supply. Digital compression of afferent vessels, or the injection of a chemical vasoconstrictor into the locality reduces

the heat source, enhances freezing, and slows thawing of the frozen tissues (Leopard, 1975 ; Anker, 1984).

5.5 THE APPLICATION OF CRYOSURGERY TO SELECTIVE TISSUE DESTRUCTION

Effective cryosurgery requires controlled tissue destruction. It is generally accepted that most cells are killed by freezing to -20°C or below, however, a few individual cells may survive conditions which are lethal to their neighbours.

When a cryoprobe is applied to tissue the resultant ice-ball reaches equilibrium after about a minute. Furthermore, a tissue temperature gradient is set up in the vicinity of the cryoprobe and ice-ball (Fraser and Gill, 1967). In the zone adjacent to the cryoprobe the tissue is cooled rapidly and intracellular ice-crystal formation predominates. Beyond this zone is an area in which freezing is slower, so that intracellular ice-crystal formation is less. However, in this zone the rate of freezing may be such that the toxic concentration of electrolytes in the intercellular tissues has little time to act before the cell is completely frozen and further damage is thus inhibited. It is here that some cells may escape the lethal effects of freezing. In the third zone, on the periphery of the ice-ball, the cooling rate is slow. Ice crystal formation is unlikely but the damage by toxic electrolytes is highest. In this zone maximum cell death can be achieved by the freeze-thaw cycle if thawing can be slowed by control of local heat sources (Section 5.4.3).

Cells in all three zones of the cryolesion are affected by the process of ischaemic infarction. This effect is promoted by the local reduction of blood supply to the frozen area (Section 5.4.3).

5.6 CLINICAL EVENTS FOLLOWING CRYOSURGERY TO NORMAL TISSUE

Following the application of a cryoprobe to normal tissue an ice-ball forms. When the ice-ball thaws out and within the first hour there is tissue swelling and hyperaemia (Neel et al., 1971). Gross accumulation of extracellular fluid may lead to vesicle formation. At three days ulceration of the epithelial surface occurs (Poswillo, 1973) and the area becomes covered by a greyish yellow slough in the case of mucosa, or a thick eschar on skin.

Healing is by secondary intention, and re-epithelialization is completed by 2 to 3 weeks, with minimal scar formation and wound contraction (Gill et al., 1970).

Throughout the healing period the cryolesion provokes little inflammatory reaction in the surrounding tissues.

Pain, secondary infection and bleeding are rare occurrences during the healing period.

5.7 THE EFFECTS OF CRYOSURGERY ON PERIPHERAL NERVE STRUCTURE & FUNCTION

The effects of freezing on peripheral nerves have been noted for several hundred years. The analgesic effect of freezing was recorded by Hippocrates (Davison, 1959), and in 1832, during Napoleon's defeat in Russia it was reported by the Surgeon General, that soldiers who were subjected to freezing in the snow could have their limbs amputated relatively painlessly.

More recently experimental work has shown that a complete but reversible functional loss follows the freezing of peripheral nerves, with good recovery occurring over a period of a few weeks (Carter et al., 1972 ; Beazley et al., 1974 ; Barnard, 1980).

The histological changes produced following cryoinjury to a peripheral nerve are similar to a Sunderland second degree type of nerve injury (Carter et al., 1972 ; Beazley et al., 1974). In Sunderland's classification, a second degree lesion consists of Wallerian degeneration with axonal disintegration and fragmentation of myelin sheaths without breaching the endoneurium and other connective tissue neural sheaths. Thus the residual fibrous skeleton provides an accurate pathway for the regenerating axons, thereby, promoting the return of normal function (Chapter III).

The ability of peripheral nerves to regenerate following cryoinjury is not a new finding. Bielschowsky and Valentine (1922) used freezing to interrupt nerve function. They noted the absence of scarring and neuroma formation at the freeze site, as well as the excellent regeneration.

Carter et al. (1972) studied the effect of cryoinjury on the median-ulnar nerve in the rat. The nerves were frozen to a minimum temperature of -100° for 1 minute. There was evidence of cellular damage after 24 hours, involving all components of the nerve. Both sheath and axon were devitalized, followed by Wallerian and retrograde degeneration. Although devitalized, the connective tissue sheaths maintained their integrity as sheaths with little evidence of an inflammatory reaction. Revitalization of the connective tissue sheaths

and axon sprouting were seen at 12 days after cryoinjury. Normal nerve structure and function were restored by 30 days.

Barnard (1980) evaluated the histological changes occurring in the infra-orbital nerves of rats following a cryolesion of around -40°C. Two freeze-thaw cycles of 1 minute duration were used. There was complete degeneration of the myelin sheaths across the nerve bundle at 7 days. Previous studies had shown that a selective destruction of large and medium sized myelinated fibres would result from mild freezing or cooling of peripheral nerves, with small unmyelinated fibres being spared (Denny-Brown et al., 1945 ; Douglas and Malcolm, 1955). However, this selective response is unlikely after exposure to the lower temperatures used in cryosurgery where a pan-necrosis occurs to include the small unmyelinated fibres. At 14 days in Barnard's study, degenerating myelin was still evident, but small-diameter regenerating nerves were noted. By six weeks post-injury, the myelin sheaths appeared clearly defined, with an appearance similar to that of a normal control. Therefore, Barnard was able to demonstrate that cryosurgery provides a method of producing a complete degeneration but without disrupting the fibrous architecture of the peripheral nerve.

It appears that the combination of a minimal degree of inflammation and subsequent scarring which occurs with a cryolesion (Poswillo, 1971), and the maintenance of the integrity of the neural connective tissue sheaths, facilitate early axonal regeneration which is consistent with the functional recovery following cryosurgery.

The above experimental findings are supported by the clinical findings that return of sensation occurs in the distribution of

peripheral nerves which become incidently incorporated in the cryolesion. Bradley and Fisher (1975) used cryosurgery in the treatment of keratocysts in the mandible. They reported return of function of the inferior alveolar nerve over 3 to 6 months. The use of cryosurgery as a palliative treatment modality in oncology has received some attention (Poswillo, 1971 ; Beazley et al., 1974 ; Neel, 1980). The possibility of destruction of tumour adjacent to neural tissue with subsequent accurate functional return of the nerve is an important concept. This is of particular interest in the case of the trigeminal and facial nerves where accuracy of reinnervation is highly desirable.

Both experimental and clinical findings suggest that peripheral nerve cryoinjury may be used to produce an extended, but reversible block in peripheral nerves. This concept of cryoblockade, has been developed in the management of chronic facial pain (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Goss, 1984 ; Nally, 1984) and post-thoracotomy pain (Nelson et al., 1974 ; Katz et al., 1980).

Previously, the reversible blocking of peripheral nerves was achieved using the injection of local anaesthetic solutions. However, even the long acting agents are generally not effective for more than 12 hours (Watt et al., 1970). Therefore, the injections needed to be repeated frequently to obtain a sufficiently long pain-free period, but this is impractical in certain situations. To increase the duration of analgesia, neurolytic agents were employed. There is, however, significant morbidity associated with the use of these agents on peripheral nerves, as incomplete destruction of the nerve may cause neuritis. Furthermore, intraneural scarring may be responsible for secondary neuralgia (Nathan et al., 1965). Following surgical section,

recovery cannot be predicted and subsequent neuroma formation and scarring may result in the recurrence of pain (Wall and Gutnick, 1974). Cryoblockade of peripheral nerves appears to have distinct advantages over the above mentioned modalities for nerve block, in that a reliable, prolonged, but reversible nerve block results without pain from neuritis or scarring. This branch of cryosurgery has been termed cryoanalgesia (Lloyd et al., 1976 ; Barnard et al., 1981).

There are at least two factors that affect the time taken for structural regeneration and subsequent functional recovery of peripheral nerves subjected to a cryolesion. The first, and probably the most important, is the distance between the cryolesion and the peripheral innervation of the nerve. The greater the distance the longer the period required for regeneration (Beazley et al., 1974). The second factor is the duration of the cryolesion. There is a linear relationship between the time below 0°C and the time required for regeneration (Gaster et al., 1971 ; Beazley et al., 1974).

There appears to be no definite relationship between the minimum temperature of the cryolesion and the time required for regeneration (Gaster et al., 1971 ; Beazley et al., 1974).

In summary, cryoinjury to peripheral nerves produces a complete functional loss associated with a Sunderland second degree type of nerve injury. This accounts for the subsequent complete return of normal structure and function over a period of a few weeks after freezing. The time required for structural regeneration and functional recovery is directly related to the distance between the cryolesion and the peripheral innervation, and the time period that the cryolesion is below 0°C.

CHAPTER VI

MATERIAL AND METHOD

6.1 INTRODUCTION

The experimental method adopted in this study and its chronological sequence is summarized in Figure 6.1.

6.2 CHOICE OF ANIMAL

The experimental animal chosen for this study was the Sprague Dawley strain of rat. Healthy young (approximately 3 months old) male rats, weighing approximately 300 grams at the beginning of the experiment were used. The animals were housed under standardized conditions and fed on a stock diet (Appendix 1).

6.3 SURGICAL ANAESTHESIA

Surgical anaesthesia was induced by chloral hydrate. A dose of 300 mg/kg of body weight of the rat was administered as an intra-peritoneal injection (Lehman and Knoeffel, 1938). The average weight of each rat was 300 gms, therefore 90 mg of chloral hydrate was required to anaesthetize each rat. Fresh solutions of chloral hydrate for injection were prepared as required.

The animal was held securely by an assistant, while the intra-peritoneal injection was administered.

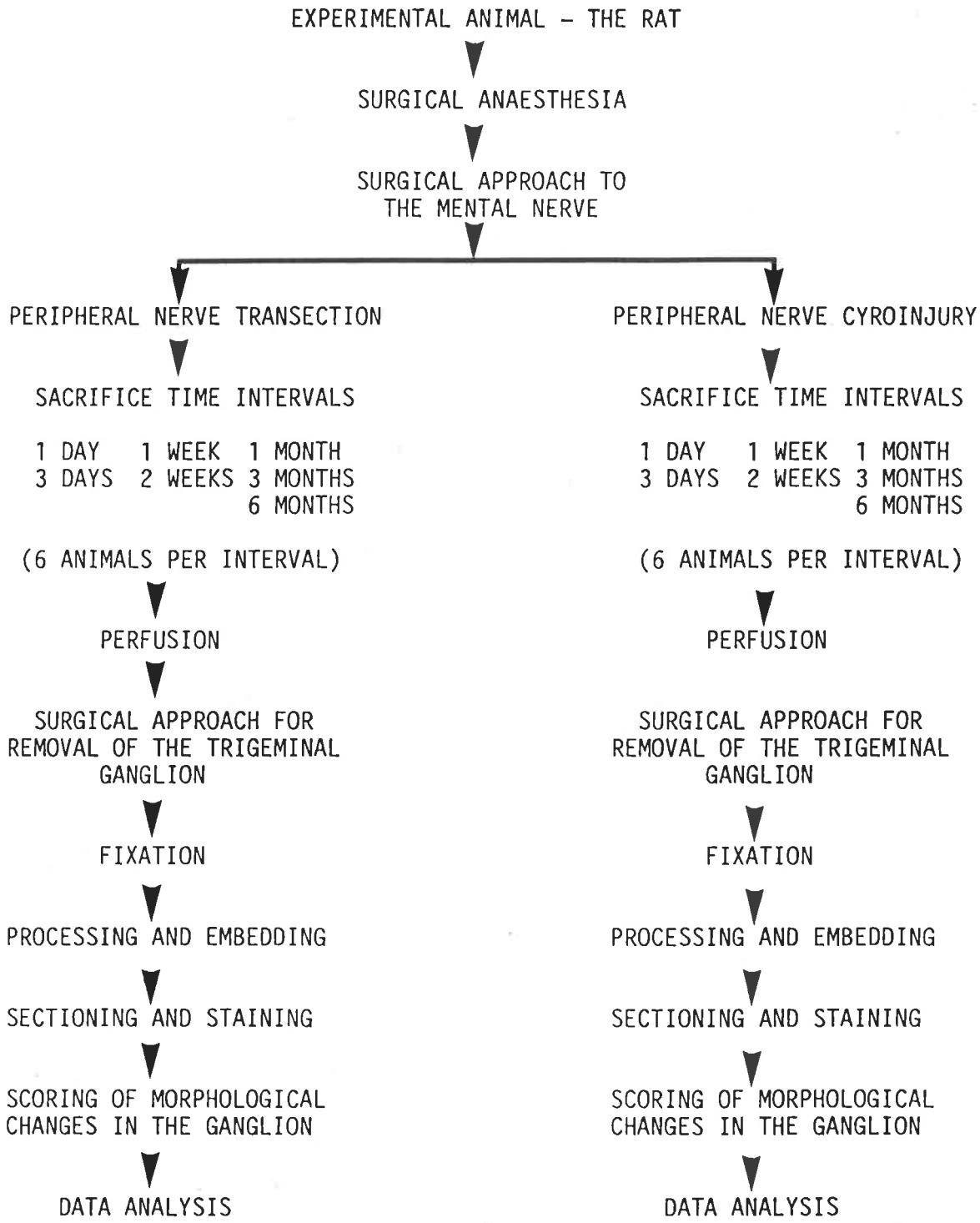


Figure 6.1: The experimental method.

N.B. A further 6 animals were not subjected to peripheral nerve injury, and were used as controls.

Surgical anaesthesia was consistently obtained within 10 minutes following administration of the anaesthetic agent.

6.4 SURGICAL INSTRUMENTATION

Surgical instruments as described in Appendix 2 were used in this study.

6.5 SURGICAL APPROACH

The surgical approach used to gain access to the mental nerve was a submandibular approach described by Hribar (1978).

Once anaesthetized, the rat was placed in a supine position on the operating table and stabilized with elastic bands placed on the limbs and one around the maxilla (Figure 6.2).

The mandible was liberally disinfected with 70% isopropyl alcohol. Using an aseptic technique a skin incision was made along the lower border of the right body of the mandible. The skin was then elevated and retracted with skin hooks to allow incision of the periosteum, being careful to avoid the facial artery. The periosteum was then elevated from the underlying bone. This readily exposed the right mental nerve as it emerged from the mental foramen (Figure 6.3).

The nerve was then injured directly as it emerged from the mental foramen (Section 6.6).

Finally, the skin was sutured with multiple interrupted 3/0 black silk suture (Figure 6.4).

6.6 TYPES OF NERVE INJURY

Two types of peripheral nerve injury were investigated in this study.

1. Clean complete transverse sectioning of the mental nerve with a scalpel directly as it emerged from the foramen (Figure 6.5).

2. A cryolesion was produced in the mental nerve using a Spembly cryosurgical unit fed by a CS size nitrous oxide cylinder capable of a minimum pressure of 600 lb/in^2 and a maximum pressure of 700 lb/in^2 (Figure 6.6).

The probe tip measured 3 mm in diameter, and the minimum probe tip temperature measured by a Wescor digital thermocouple thermometer type TH-65 (Figure 6.7), was $-40^{o}C \pm 2.5^{o}C$.

Once the mental nerve was isolated (Section 6.5) it was frozen for 90 seconds, being timed from the establishment of equilibrium in the ice-ball as assessed by the recording of a steady temperature in the order of $-40^{o}C$ (Figure 6.8). This was followed by a thawing period of 5 minutes, then a further 90 second freeze was performed. During the thaw the temperature rose to above $0^{o}C$.

6.7 POST-OPERATIVE CARE

The animal was positioned on its side with its head down after the operation to prevent the possibility of respiratory obstruction by allowing drainage of salivary secretions, and for forward positioning of the tongue.

Direct supervision was carried out until fully mobile, which for most animals took approximately one and a half hours post-induction. The animals were then returned to their cages.

No more than four rats were housed in each cage. Polypropylene cages with stainless steel tops were used. The dimensions of the cages were 45 cm x 28 cm x 22 cm. The storage area was maintained at a controlled temperature of 65-70°F.

6.8 SACRIFICE TIME INTERVALS

The time intervals of sacrifice chosen for this study are presented in Table 6.1. Six rats were included in each time interval and each was sacrificed at 1 day, 3 days, 1 week, 2 weeks, 1 month, 3 months and 6 months. In addition, six rats served as controls.

At the appropriate intervals the rats were re-anaesthetized (Section 6.3) for perfusion and sacrifice.

6.9 THE PERFUSION TECHNIQUE

All the animals used in this study were perfused. The apparatus for the "once-through" procedure used in this study is shown in a schematic diagram (Figure 6.9).

POST-INJURY INTERVAL	TRANSECTION	CRYOINJURY
1 day	6 rats	6 rats
3 days	6 rats	6 rats
1 week	6 rats	6 rats
2 weeks	6 rats	6 rats
1 month	6 rats	6 rats
3 months	6 rats	6 rats
6 months	6 rats	6 rats
	42 rats	42 rats
CONTROL	6 rats	
TOTAL	90 rats	

Table 6.1: Sacrifice times.

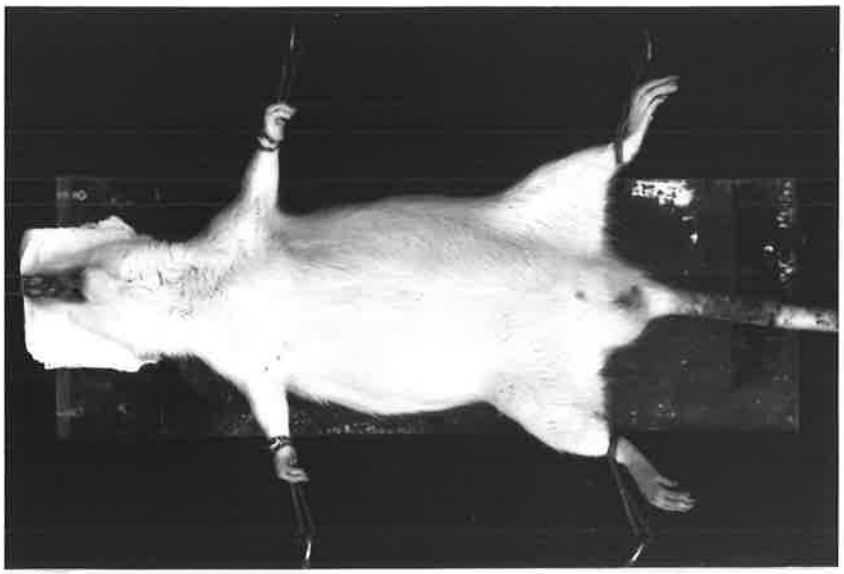


Figure 6.2: Anaesthetized rat in the operating position.

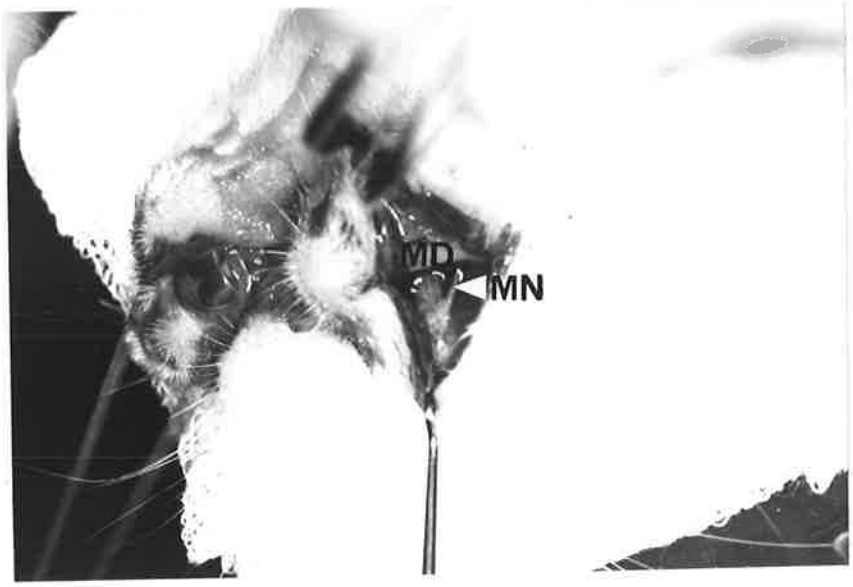


Figure 6.3: The exposed mental nerve.
Mandible (MD) and mental nerve (MN).

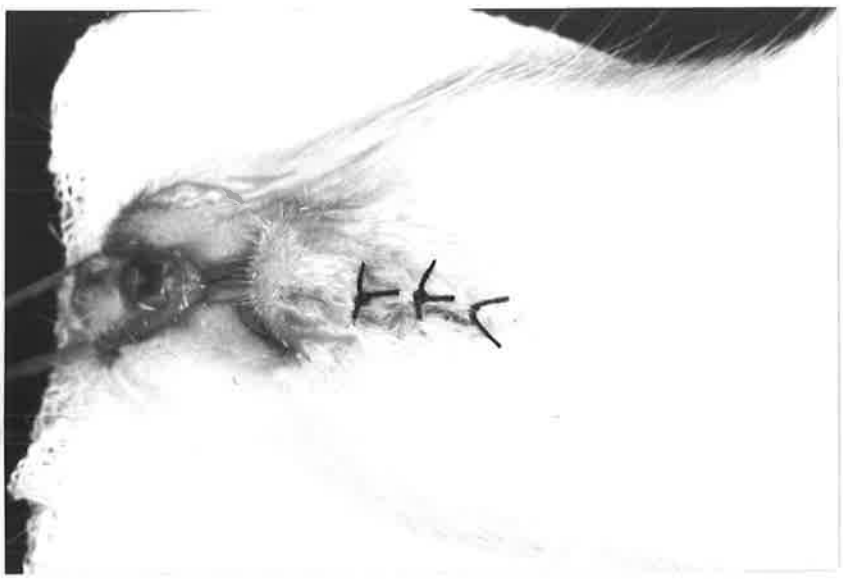


Figure 6.4: Immediately post-operative after final suturing of the skin.

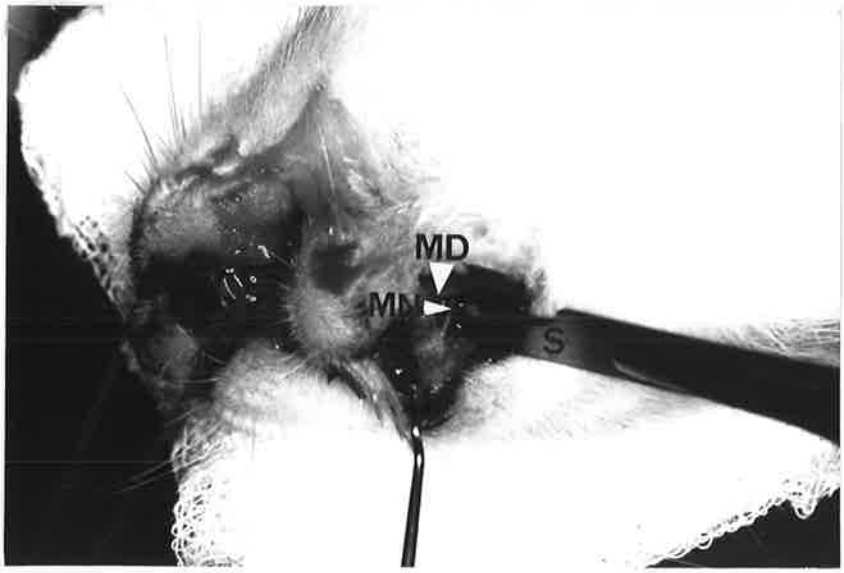


Figure 6.5: Exposed mental nerve immediately prior to sectioning. Mandible (MD), mental nerve (MN) and scalpel (S).

Figure 6.6: The Spembly cryosurgical unit.

Figure 6.7: The Wescor thermocouple thermometer.

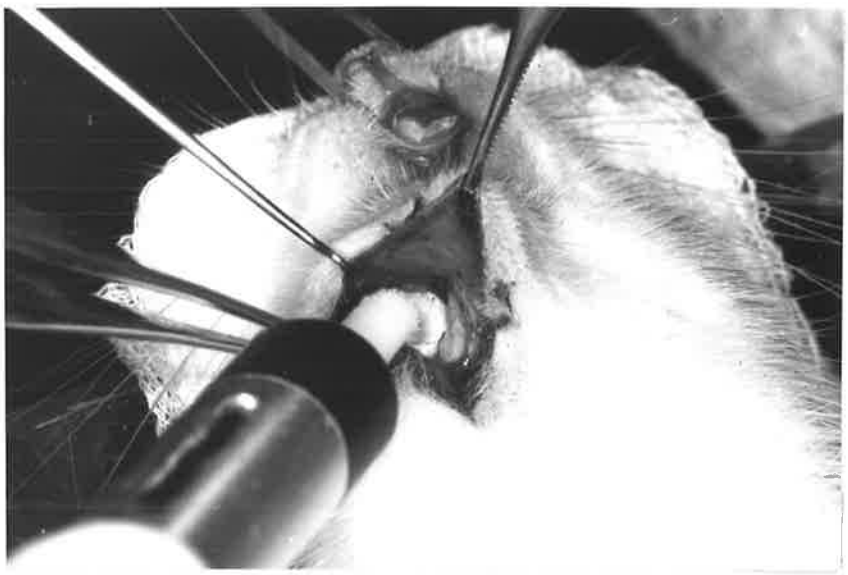


Figure 6.8: Cryolesion of the mental nerve.

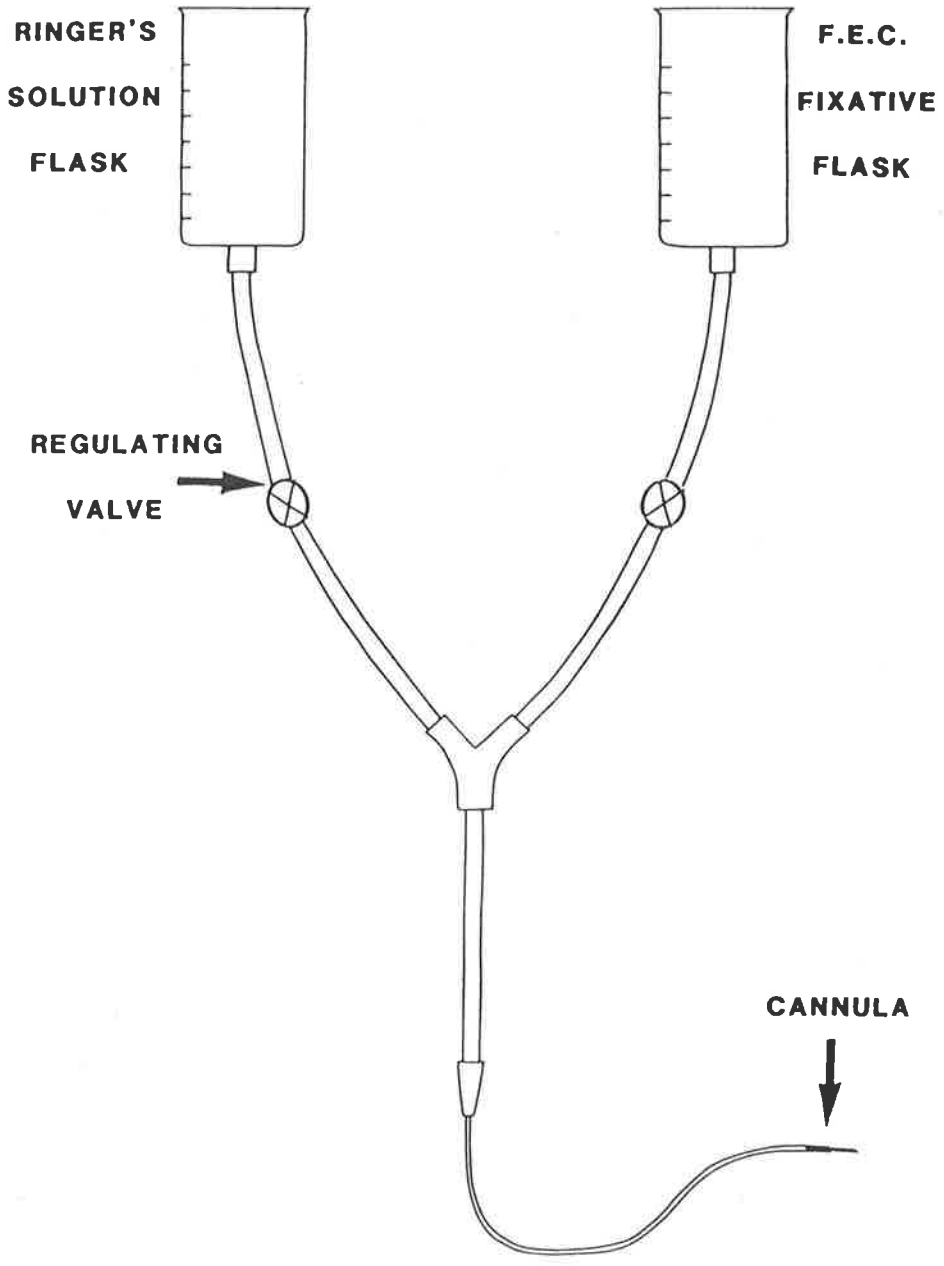


Figure 6.9: Schematic diagram of the perfusion apparatus.

The anaesthetized animal was placed on its back. Next a longitudinal mid-ventral skin incision was made from the sternal notch to the abdomen (Figure 6.10).

Access to the thoracic cavity was obtained by incising through the thoracic musculature and the rib cage and reflecting these structures laterally. At this stage the animal's heart was exposed, and was found to be beating in all the animals operated on in this series. The cannula of the perfusion apparatus was inserted into the left ventricle (Figure 6.11), and perfusion with Ringer's solution was commenced by opening the valve on the channel of the Ringer's solution flask. Almost immediately the superior vena cava was cut transversely to allow the Ringer's solution to escape after it has passed through the head. As the blood was displaced from the head; the eyes, ears and oral mucous membrane became progressively paler.

At this stage, the flow of Ringer's solution was stopped, and the flow of F.E.C. fixative was commenced by adjustng the regulating valves. Subsequent to the perfusion of 100 ml of fixative, the trigeminal ganglion was immediately removed.

6.10 SURGICAL APPROACH FOR REMOVAL OF THE TRIGEMINAL GANGLION

Following perfusion, a dorsal mid-sagittal skin incision was made from the nose, between the pinnae and then continued backwards to the level of the forelimbs (Figure 6.12).

The skin was then dissected off the skull and reflected laterally on both sides. At this stage the brain could be seen through the roof of

the skull. The calvarium and the cervical vertebrae were split in the mid-sagittal plane and reflected laterally to expose the brain and brainstem. (Figure 6.13).

Next the brain and brainstem were carefully dissected out from the cranial base and spinal canal using a combination of sharp and blunt dissection. This exposed the two underlying trigeminal ganglia roofed by dura mater (Figure 6.14).

The right trigeminal ganglion was carefully dissected out with approximately 3 millimetres of each of the principal divisions of the trigeminal nerve attached. During dissection each of the divisions were gently stabilized by passing a skin hook under the nerve divisions. Each division was then sectioned approximately 3 millimetres from the ganglion.

Following removal, the ganglion was placed on a flat piece of cardboard with each of the nerve divisions placed relative to labelled corners of the piece of cardboard. This was done to aid orientation of the specimen during subsequent embedding.

The specimen was then immediately immersed in fixative for 2 weeks. F.E.C. neural fixative (Hribar, 1977) was used exclusively in these experiments (Appendix 3).

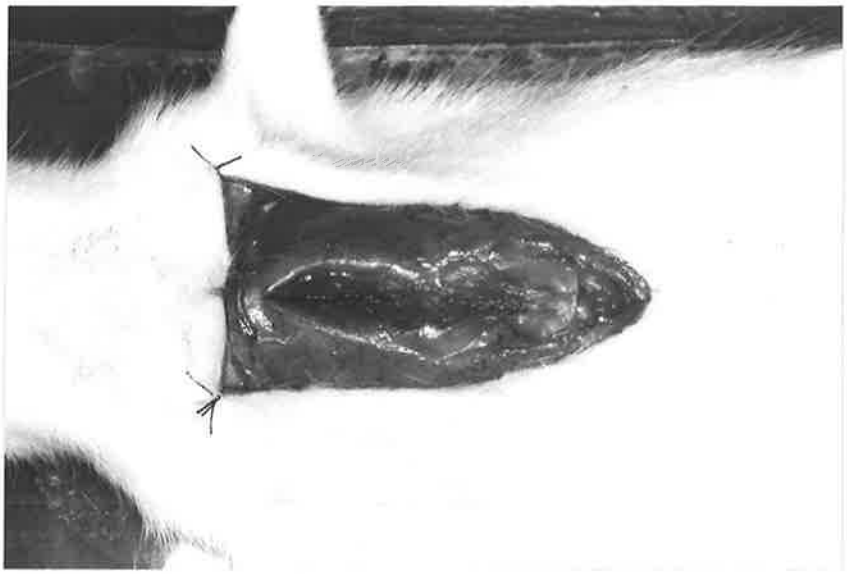


Figure 6.10: The mid-ventral skin incision.

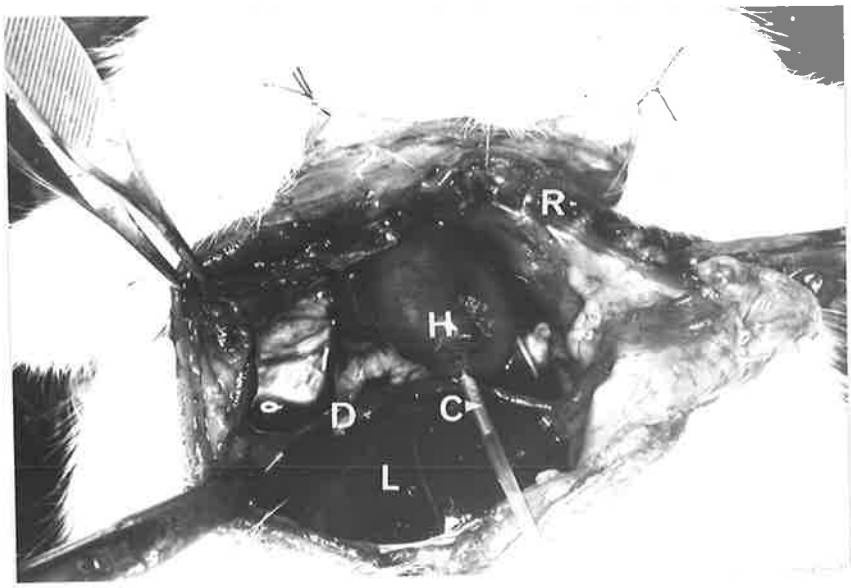


Figure 6.11: Insertion of cannula (C) into the left ventricle of the heart (H). Rib cage (R), liver (L) and diaphragm (D).

Figure 6.12: The dorsal mid-sagittal skin incision.

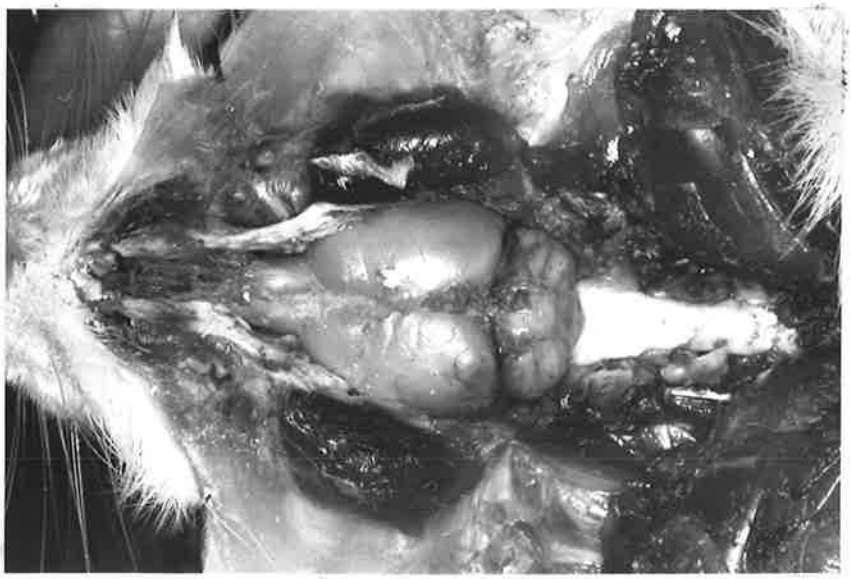


Figure 6.13: The brain and brainstem exposed.

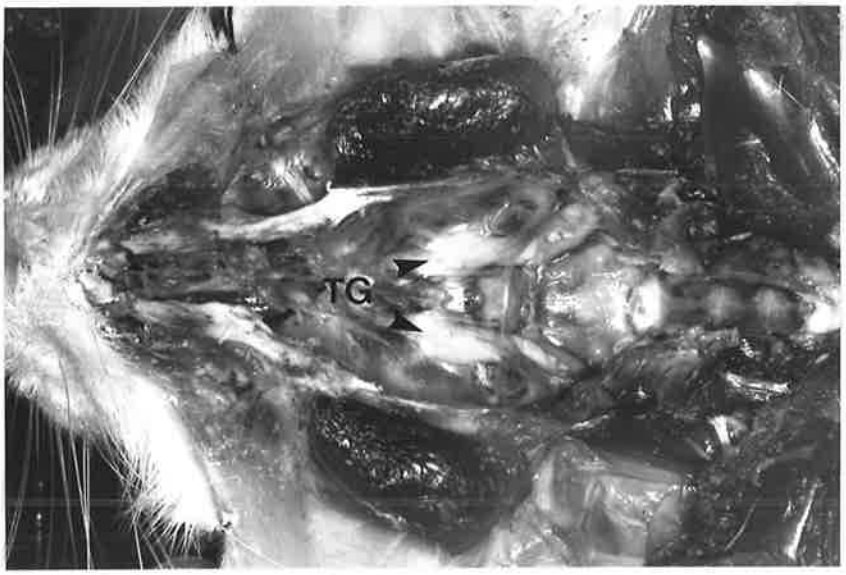


Figure 6.14: Exposure of the trigeminal ganglia (TG).

6.11 HISTOLOGICAL TECHNIQUE

Double paraffin embedding (Appendix 4) of the specimens was done such that in longitudinal sections, the ganglion and parts of the divisions of the trigeminal nerve situated close to the ganglion could be visualized at the same level (Figure 6.15). The ganglion was held down to the base of the wax dish, dorsal surface of the ganglion uppermost, while molten wax was poured in. Positioning in this manner aided orientation for subsequent longitudinal sectioning of the specimens.

Each paraffin block contained four trigeminal ganglia from the same post-operative period. The remaining two ganglia from the same post-operative interval were blocked together in another paraffin block.

Each block was sectioned with serial longitudinal sections 6 microns thick being obtained from a rotary microtome and then mounted on numbered glass slides. Every tenth slide was stained with standard haematoxylin and eosin technique (Appendix 5) to determine depth, orientation and details of the total histological picture. Sections that did not contain any nerve tissue pertinent to the investigation were discarded. Every ninth retained slide was stained with cresyl violet (Appendix 5) to demonstrate the cytoplasmic Nissl granules in the cell body of the neurons. The remaining slides were then used as a reserve if any particular stain failed to give satisfactory results.

Light microscopic histological examination was carried out on the sectioned trigeminal ganglion ipsilateral to the site of peripheral

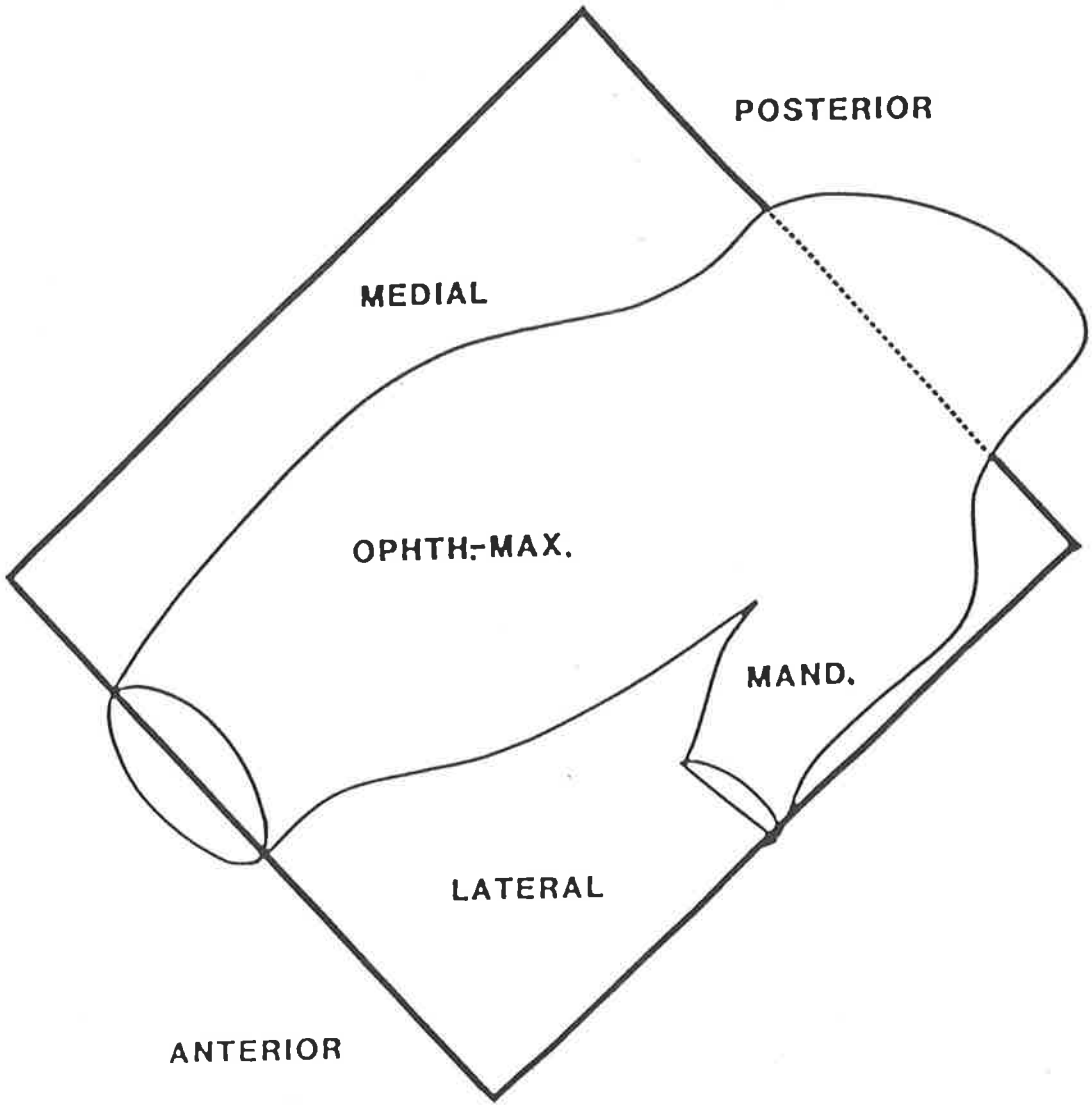


Figure 6.15: The lonitudinal plane of sectioning of the trigeminal ganglion.

nerve injury. An Olympus BH light microscope was used at powers Plan 4, 10/0.25, 20/0.40, and 40/0.65.

Sections examined were from the dorsal aspect of the region from the posterolateral protuberance across the base of the mandibular division. This area is where the mental nerve cell bodies are concentrated (Mazza and Dixon, 1972) (Figure 6.16).

6.12 HISTOLOGICAL ASSESSMENT

Histological assessment was made on the morphological reactions of the trigeminal ganglion neurons following injury to the mental nerve for the post-operative time intervals stated above (Section 6.8).

6.12.1 Histological features under investigation

The characteristic features of the retrograde neuronal reaction to cellular injury and neuronal degeneration were determined, scored and recorded:-

1. Chromatolysis - Nissl granules dispersed in finer particles towards the cell periphery, with the remainder of the cytoplasm being relatively clear of granular material (Figure 6.18).

2. Peripheral displacement of the nucleus (Figure 6.19).

3. Cytoplasmic fragmentation (Figure 6.20).

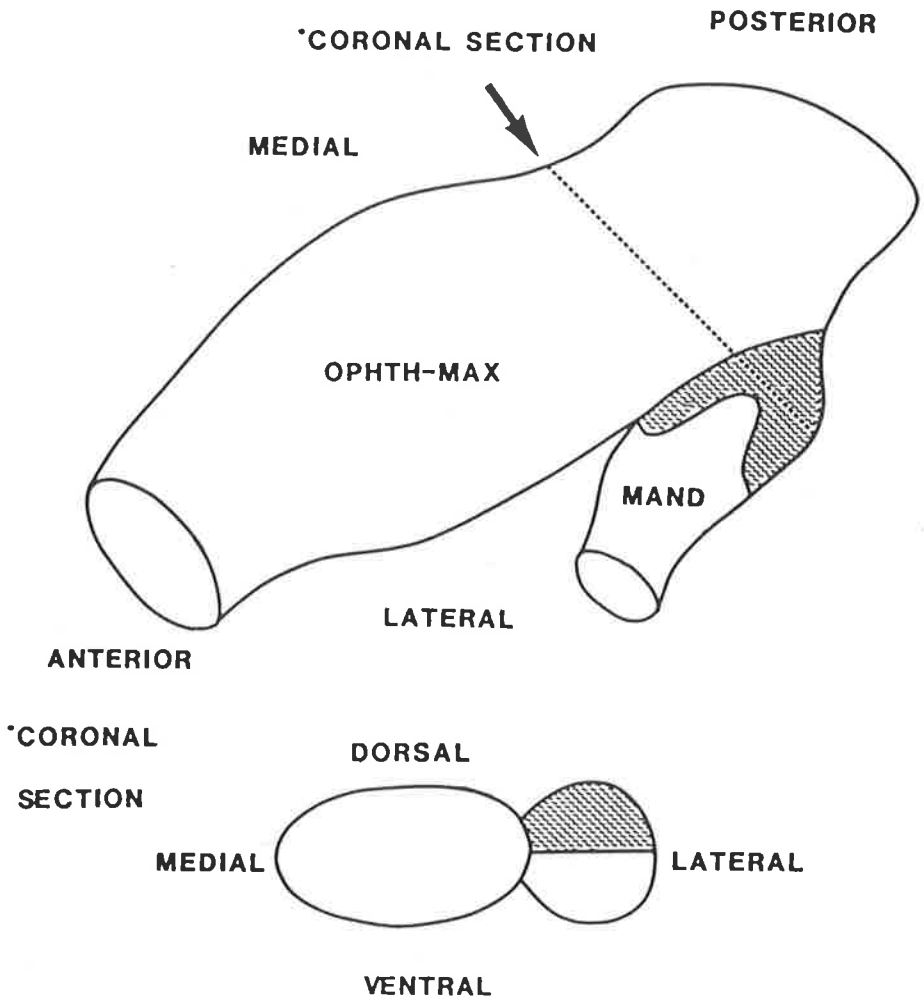


Figure 6.16: The left trigeminal ganglion in the rat demonstrating the position of the mental nerve cell group.

4. Nuclear fragmentation (Figure 6.21).

5. Pericellular shrinkage or atrophy (Figure 6.22).

6. Satellite (capsular) cell clusters associated with degenerating neurons (Figure 6.23).

6.12.2 Method of scoring

Each of the six histological features were scored for both the large cell population and the small cell population within the ganglia, according to the proforma in Appendix 6. Only those cells contained within the same histological plane as indicated by the presence of the nucleolus, nucleus and cytoplasm were counted. The remaining cells were designated as "indeterminate". Cell counts were performed under light microscopy at magnification 20/0.40 to cover as large an area as possible but allowing identification of cell morphology. Cell counts were performed using an Olympus optical eyepiece graticule (260 microns x 260 microns).

The histological features studied were scored according to the following scale:-

0 - no change demonstrable

1 - change demonstrable

The above method was used for all the histological features evaluated except for the pericellular shrinkage feature. Here the following scale was used:-

1 - moderate pericellular shrinkage - cell occupies approximately 50% of the pericellular space.

2 - severe pericellular shrinkage - cell occupies less than 50% of the pericellular space.

(Figure 6.22)

Absolute cell counts were expressed as a percentage of the total number of cells in that particular cell group.

Where doubt existed concerning the score for a particular feature, the lesser score was assigned.

Standard photomicrographs, previously selected for each feature (Section 6.12.1), were available for comparison with the slides being evaluated. Normal large and small cells are illustrated in Figure 6.17.

6.12.3 Reliability of scoring

The validity of the scoring method used was assessed by a double determination procedure. 20% of the total number of ganglia were randomly selected and then re-examined and recorded approximately two months after the initial assessment as per the original method. The scores obtained on the two separate occasions were then compared.

6.13 DATA ANALYSIS

Data obtained from the histological scoring proformas (Appendix 6) was grouped according to the percentage of cells showing a particular histological feature at a particular time interval.

Mean percentages were calculated for each group. As a measure of variability of the scores obtained the standard errors of the means were also calculated.

The standard error of the mean (s.e. $\bar{x}$) is defined as the standard deviation (s) of a distribution of means for an infinite set of samples of the same size drawn randomly from the same population (Maxwell, 1970).

$$\text{i.e. s.e. } \bar{x} = \frac{s}{\sqrt{n}}$$

where s = standard deviation

n = sample size

The standard deviation (s) is a measure of the dispersion of scores about their mean and is used to compare samples with one another (Maxwell, 1970).

$$\text{i.e. } s = \sqrt{\frac{(\bar{x} - x)^2}{n - 1}}$$

where x = score

$\bar{x}$ = mean

n = sample size

The data obtained from these statistical methods was tabulated and illustrated graphically and will be presented in Chapter VII. For clarity, the standard error of the means were omitted from the graphic data but were included in the tabulated data.

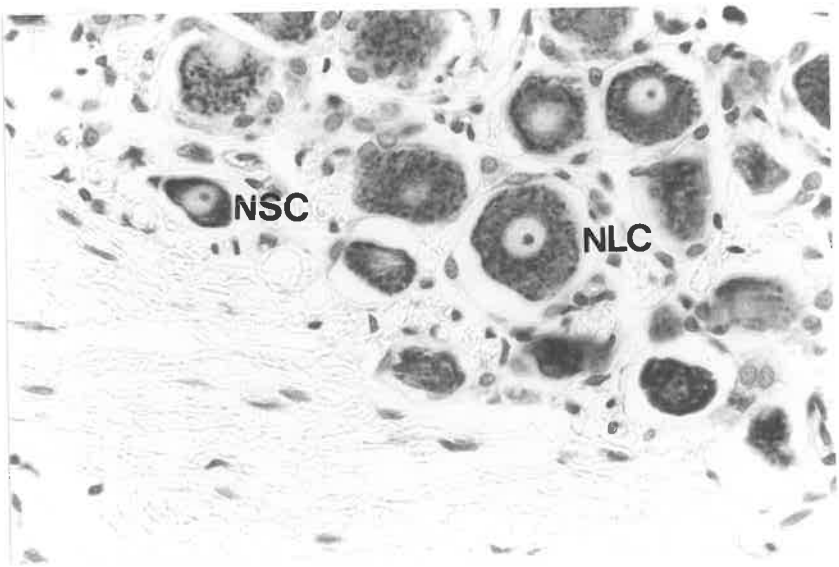


Figure 6.17: Normal trigeminal gangliom cells. Normal large cell (NLC). Normal small cell (NSC). Cresyl violet, x400.

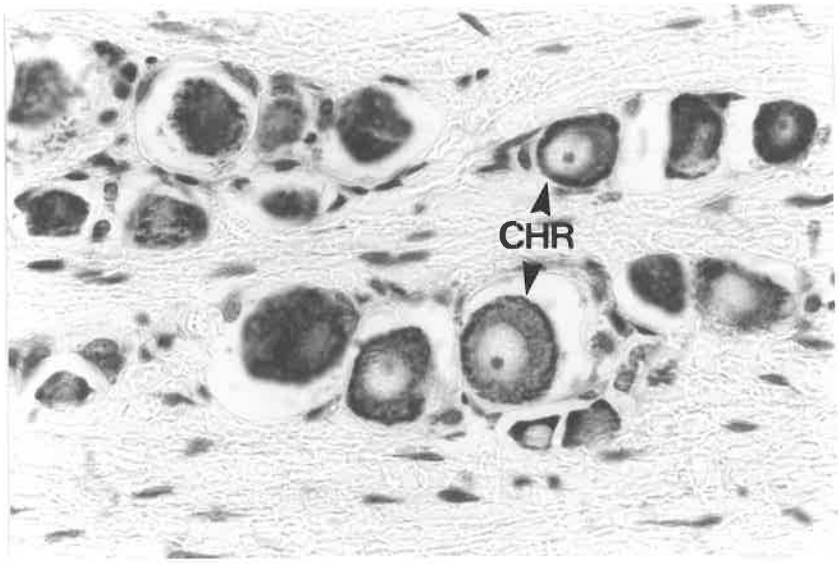


Figure 6.18: Chromatolysis (CHR). Cresyl violet, x500.

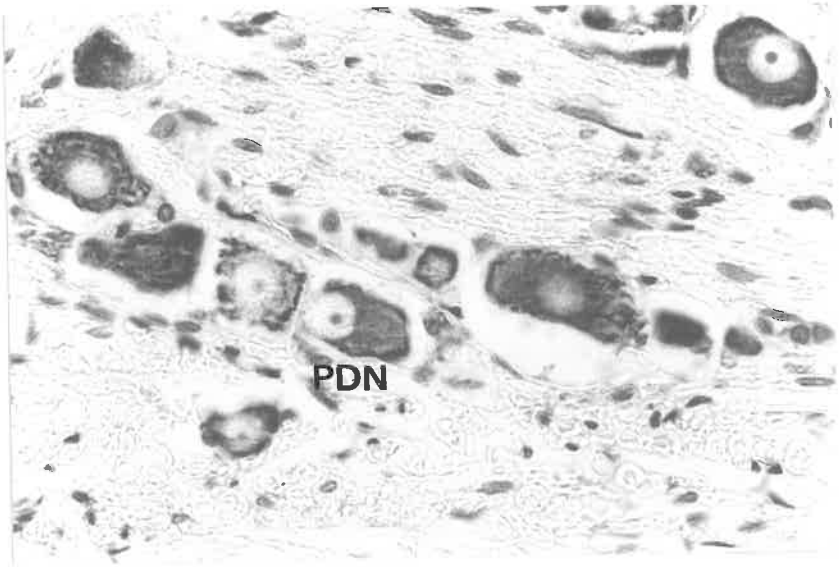


Figure 6.19: Peripheral displacement of the nucleus (PDN). Cresyl violet, x500.

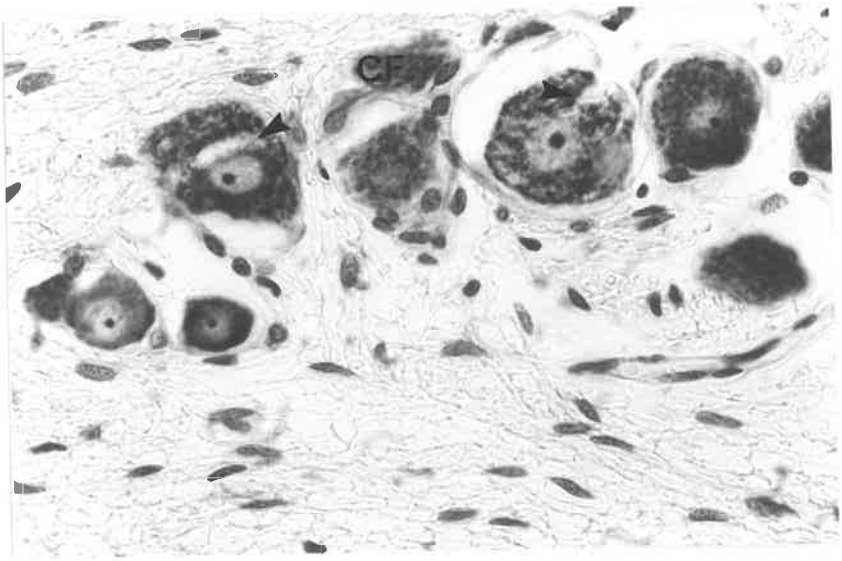


Figure 6.20: Cytoplasmic fragmentation (CF). Cresyl violet, x500.

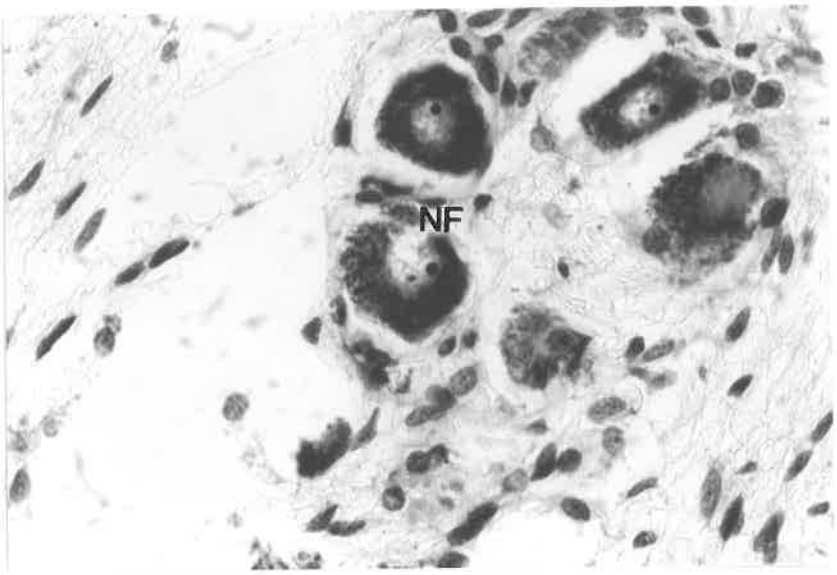


Figure 6.21: Nuclear fragmentation (NF). Cresyl violet, x500.

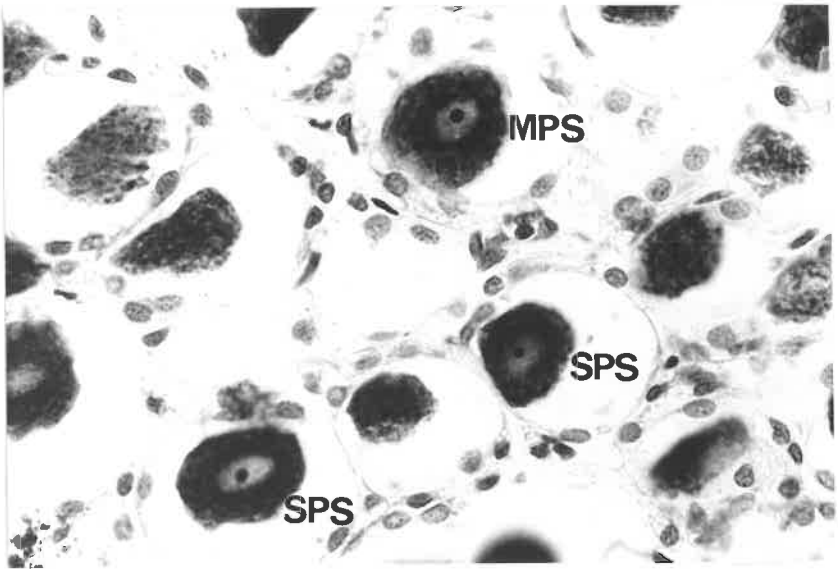


Figure 6.22: Pericellular shrinkage or atrophy.
Moderate pericellular shrinkage (MPS).
Severe pericellular shrinkage (SPS). Cresyl violet, x500.

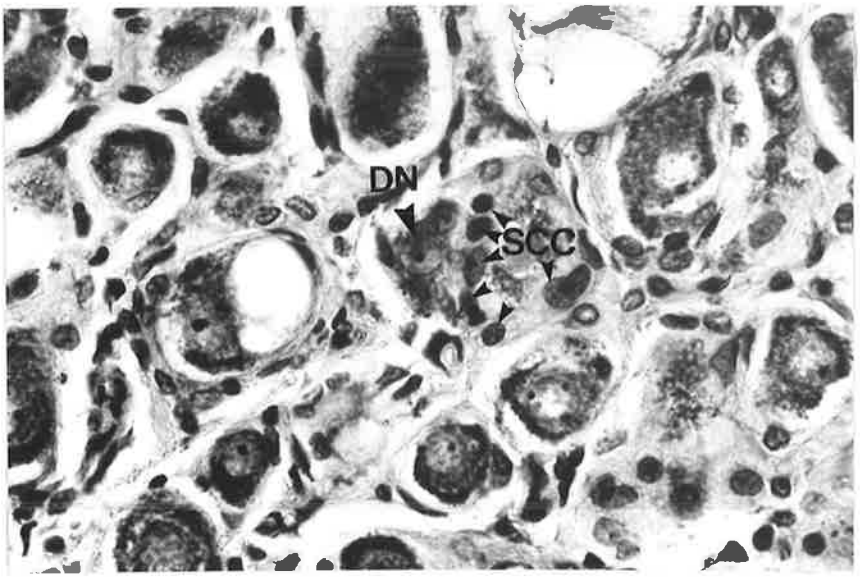


Figure 6.23: Satellite cell cluster (SCC) associated with degenerating neuron (DN). Cresyl violet, x500.

CHAPTER VII

RESULTS

7.1 CONTROL - NORMAL TRIGEMINAL GANGLION

Macroscopically, the trigeminal ganglion was composed of an ophthalmic-maxillary division and a mandibular division in a lateral relationship. A postero-lateral protuberance was situated at the base of the mandibular division, posterior and lateral to the bifurcation between the two divisions (Figure 7.1).

On light microscopic examination, nerve cell bodies were situated mainly at the bifurcation, in the postero-lateral protuberance, and in columns occurring within the ophthalmic-maxillary division (Figure 7.2).

The mental nerve cell bodies were situated in the postero-lateral protuberance (Mazza and Dixon, 1972) (Figure 7.3).

There appeared to be essentially two cell types:-

1. small cells with a darker staining, more homogenous cytoplasm.
2. more numerous large cells with a pale, granular cytoplasm.

(Figure 7.4)

Large and small cells were not topographically distributed, but were found to be randomly arranged in all areas of the ganglion.

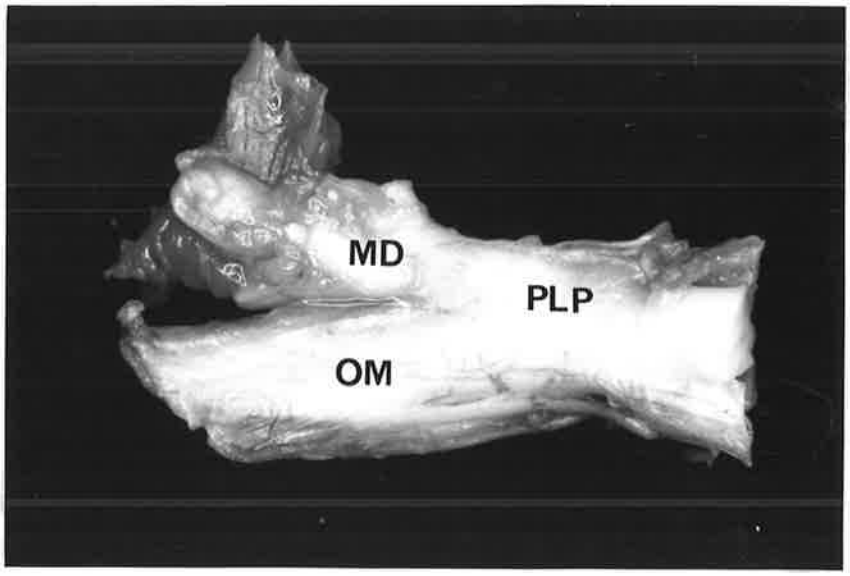


Figure 7.1: Macroscopic dorsal view of the right trigeminal ganglion. Ophthalmic-maxillary division (OM). Mandibular division (MD). Postero-lateral protuberance (PLP).

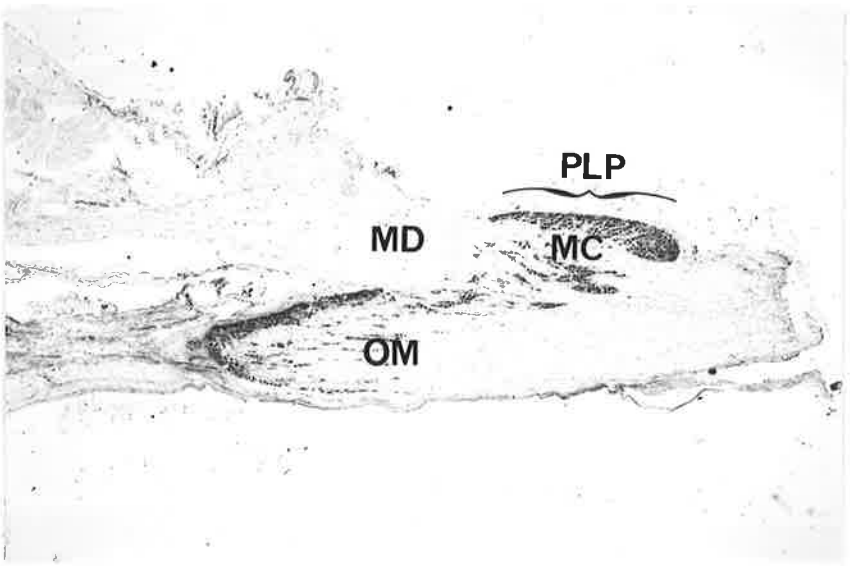


Figure 7.2: The trigeminal ganglion. Ophthalmic-maxillary division (OM). Mandibular division (MD). Postero-lateral protuberance (PLP). Mental nerve cell bodies (MC). Cresyl violet, x40.

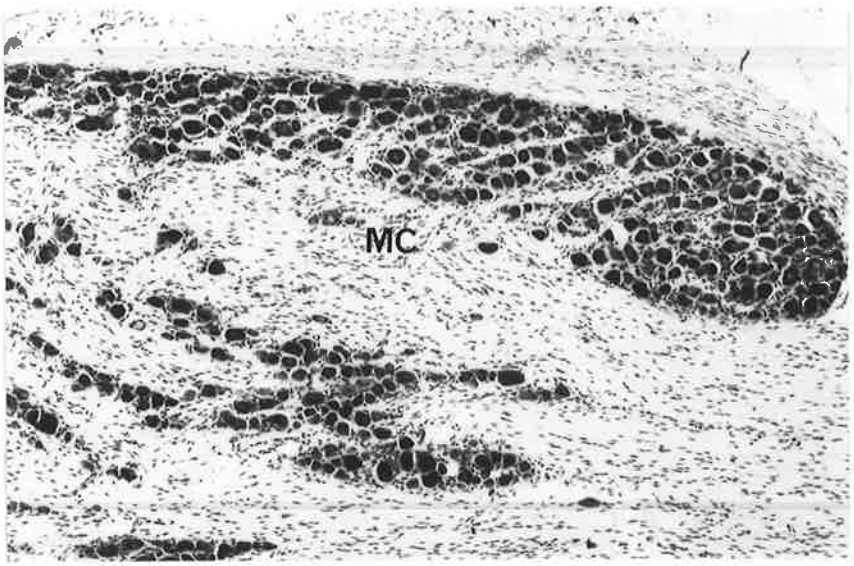


Figure 7.3: The mental nerve cell bodies (MC). Cresyl violet, x100.

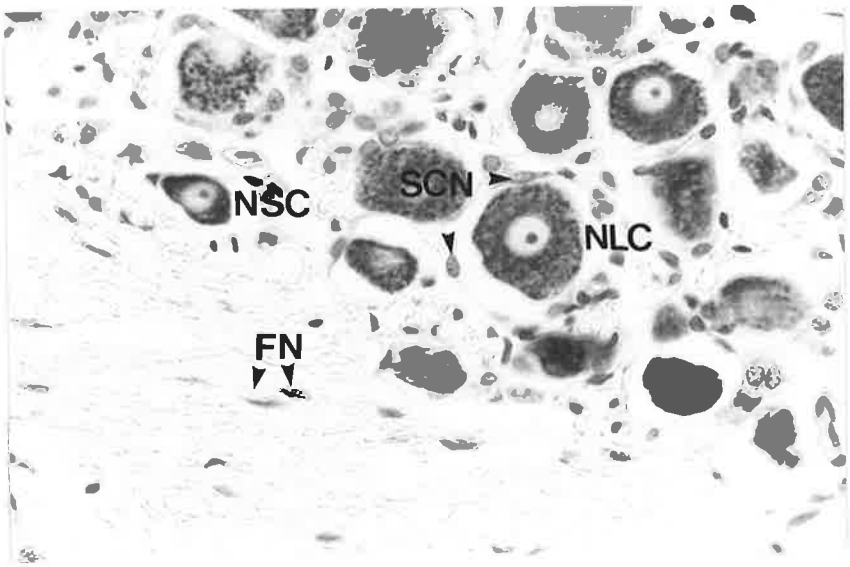


Figure 7.4: Normal trigeminal ganglion cells. Normal large cell (NLC). Normal small cell (NSC). Satellite cell nucleus (SCN). Fibroblast nucleus (FN). Cresyl violet, x400.

The nuclei of both types of nerve cells were typically large, centrally placed, contained one or two intensely staining nucleoli and a pale particulate nucleoplasm. Granular Nissl substance was scattered uniformly throughout the cytoplasm.

Smaller rounded or ovoid nuclei of satellite cells surrounded the nerve cell bodies. In the interneuronal spaces, and in the intervals between adjacent groups of nerve cells, collagen fibres and randomly arranged small elongated nuclei of fibroblasts were found.

7.2 RELIABILITY OF SCORING

The results of a double determination, in which 20% of the total number of ganglia were randomly selected and re-scored approximately two months after the initial examination, are given in Table 7.1. These figures indicate the difference between the first and second counts expressed as a mean percentage. Thus, a small figure represents a greater reliability of scoring, and a large figure represents a lesser reliability of scoring.

The least reliability of scoring was recorded with respect to cell shrinkage, with the maximum reliability of scoring being recorded with respect to peripheral displacement of the nucleus. Generally, a somewhat larger discrepency in scoring was obtained for the scoring of the histological features in the small nerve cells compared with the large nerve cells.

HISTOLOGICAL FEATURE	NERVE CELL TYPE	
	LARGE	SMALL
Chromatolysis	7.0%	6.9%
Peripheral Displacement of Nucleus	1.2%	3.2%
Cytoplasmic Fragmentation	4.8%	7.2%
Nuclear Fragmentation	2.5%	5.0%
Moderate Pericellular Shrinkage	7.1%	10.3%
Severe Pericellular Shrinkage	10.5%	9.7%
Satellite Cell Clusters	4.4%	4.5%

Table 7.1: Reliability of scoring - double determination

7.3 TRANSECTION OF THE MENTAL NERVE

1 DAY

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at one day post-operatively are presented in Table 7.2 and Figures 7.5 and 7.20.

One day post-operatively, most of the nerve cells resembled those of the normal control specimens. These cells typically presented large, centrally positioned nuclei, and a uniformly granular cytoplasm due to the presence of Nissl substance throughout the cytoplasm.

Some nerve cells had undergone chromatolysis having lost the uniform granular pattern of Nissl substance with its peripheral accumulation. The rest of the cytoplasm was relatively clear of Nissl substance and appeared somewhat agranular.

A few nerve cells were also seen to be displaying an eccentrically placed nucleus.

A larger proportion of small nerve cells were seen reacting to peripheral mental nerve transection compared to the large nerve cells.

No evidence of nerve cell degeneration or death was found, and no pericellular shrinkage was observed.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	4.7 (0.40)	3.2 (0.35)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	14.6 (1.3)	7.9 (0.83)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	8.4 (0.72)	5.0 (0.47)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.2: 1 day - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

3 DAYS

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at three days post-operatively are presented in Table 7.3 and Figures 7.6 and 7.21.

Three days post-operatively, there was an increase in the percentage of nerve cells showing both chromatolysis (Figure 7.27) and peripheral displacement of the nucleus (Figure 7.28). Chromatolysis, however, showed the more marked increase.

The above changes were more prevalent amongst the small nerve cell population compared to the large nerve cell population.

No evidence of nerve cell degeneration or pericellular shrinkage was observed.

1 WEEK

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at one week post-operatively are presented in Table 7.4 and Figures 7.7 and 7.22.

One week post-operatively, the percentage of nerve cells exhibiting chromatolysis and peripheral displacement of the nucleus, had again shown a marked increase compared with the previous post-operative interval (Figures 7.27 and 7.28).

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	12.2 (1.3)	10.5 (0.95)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	44.1 (2.6)	24.6 (1.2)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	23.5 (0.70)	15.6 (1.1)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.3: 3 days - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	22.6 (1.6)	27.3 (1.6))	6.4 (1.4)	0 (0)	mod: 23.0 (1.7) sev: 4.2 (0.61)	0 (0)
Small	55.6 (1.8)	58.6 (2.2)	5.6 (0.93)	0 (0)	mod: 24.0 (1.6) sev: 6.6 (0.90)	0 (0)
Combined	35.2 (2.1)	39.1 (2.7)	5.9 (0.42)	0 (0)	mod: 23.3 (1.6) sev: 5.1 (0.62)	0 (0)

Table 7.4: 1 week – peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

It was clearly evident that the greater increase was with respect to the peripheral displacement of the nucleus. The percentage of nerve cells exhibiting chromatolysis and peripheral displacement of the nucleus was of the same order.

Chromatolysis and peripheral displacement of the nucleus were more prevalent amongst the small nerve cells compared to the large nerve cells.

A certain percentage of nerve cells were seen to be exhibiting pericellular shrinkage, with moderate shrinkage being more common than severe shrinkage. Large and small nerve cells appeared to be similarly affected in both cell shrinkage categories.

A small percentage of nerve cells with cytoplasmic fragmentation was observed. These cells were considered to be undergoing degeneration. Approximately equal percentages of large and small cells exhibited this feature.

2 WEEKS

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at two weeks post-operatively are presented in Table 7.5 and Figures 7.8 and 7.23.

Two weeks after peripheral mental nerve transection, the extent of chromatolysis and peripheral displacement of the nucleus had reached a peak (Figures 7.27 and 7.28) respectively). The increase in percentage of nerve cells exhibiting the above two histological features was of the

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	26.7 (1.6)	34.0 (1.1)	19.1 (1.3)	5.3 (1.9)	mod: 23.8 (1.3) sev: 12.2 (0.79)	9.2 (0.90)
Small	64.3 (2.5)	65.6 (1.4)	19.7 (1.7)	12.1 (0.6)	mod: 33.3 (1.1) sev: 12.7 (1.8)	11.4 (1.5)
Combined	41.6 (1.5)	46.6 (1.3)	19.3 (1.2)	8.0 (0.80)	mod: 27.5 (1.2) sev: 12.3 (0.97)	10.2 (1.1)

Table 7.5: 2 weeks - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

same order. Some nerve cells were seen displaying pronounced chromatolysis, and appeared achromatic (Figure 7.12).

As was the case for previous post-operative time intervals, the percentage of small nerve cells affected by chromatolysis and peripheral displacement of the nucleus were greater than those for large nerve cells.

Pericellular shrinkage was more prevalent than in the previous post-operative time interval (Figure 7.31). Moderate cell shrinkage was more commonly observed than severe cell shrinkage. However, unlike the one week stage, small nerve cells were more widely affected than large nerve cells in the moderate cell shrinkage category. In the severe cell shrinkage category about equal proportions of large and small nerve cells were affected.

There was an increase in the percentage of nerve cells showing cytoplasmic fragmentation, with equal percentages of large and small nerve cells showing this feature (Figure 7.29).

A small percentage of nerve cells undergoing nuclear fragmentation was also observed, with small cells being more widely affected than large cells. Cells showing this feature together with those showing cytoplasmic fragmentation were considered to be in a state of degeneration that would ultimately lead to cell death.

A further indicator of nerve cell degeneration, satellite cell clusters consisting of 5-6 satellite cells, were seen surrounding degenerating nerve cells. Small nerve cells were affected with a

slightly larger frequency compared to large nerve cells. Satellite cell clusters were also seen in association with histological structures which may have been either indeterminate nerve cells (cells with the nucleus and nucleolus outside the plane of section), or nerve cell remnants. It was difficult to differentiate between these two structures.

1 MONTH

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at one month post-operatively are presented in Table 7.6 and Figures 7.9 and 7.24.

One month post-operatively, a marked fall in the percentage of chromatolytic nerve cells was observed, to a level below that seen at one day post-operatively (Figure 7.27). A less marked decrease in the percentage of nerve cells with peripheral displacement of the nucleus was observed (Figure 7.28). The result was that peripheral displacement of the nucleus was more prevalent than chromatolysis.

Small nerve cells displayed chromatolysis and peripheral displacement of the nucleus with greater frequency than large nerve cells.

The most striking feature at this post-operative interval was the incidence of pericellular shrinkage or atrophy, with both moderate and severe cell shrinkage being observed with frequency. Small nerve cells were noted to be more widely affected than their large cell

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	3.7 (0.89)	10.7 (1.6)	14.6 (0.93)	3.3 (1.3)	mod: 35.0 (3.0) sev: 19.0 (1.5)	11.1 (2.1)
Small	13.6 (1.4)	26.2 (1.2)	10.0 (1.9)	3.7 (0.60)	mod: 44.5 (2.8) sev: 28.4 (3.3)	12.4 (2.7)
Combined	7.7 (0.90)	17.0 (0.96)	11.3 (1.5)	3.5 (0.67)	mod: 38.8 (2.9) sev: 22.8 (1.9)	11.7 (2.1)

Table 7.6: 1 month - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

counterparts. The vast majority of nerve cells exhibiting severe cell shrinkage appeared to be more intensely staining or hyperchromatic.

A decline in the percentage of nerve cells exhibiting the features of cytoplasmic and nuclear fragmentation was observed (Figures 7.29 and 7.30 respectively). The frequency of nuclear fragmentation was about equal in the large and small cell population. However, cytoplasmic fragmentation was more frequent amongst the large cells.

The frequency with which satellite cell clusters were observed in association with degenerating large and small nerve cells remained about the same. However, satellite cell clusters in relation to indeterminate cell remnants were more prevalent (Figure 7.32).

3 MONTHS

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at three months post-operatively are presented in Table 7.7 and Figures 7.10 and 7.25.

Three months post-operatively, very few nerve cells appeared chromatolytic. Occasionally an eccentric nucleus would be observed, with this feature being marginally more frequently observed in the small cells.

Signs of nerve cell degeneration, namely cytoplasmic and nuclear fragmentation were almost entirely absent.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	0.93 (0.47)	1.4 (0.81)	0.97 (0.97)	0 (0.97)	mod: 32.4 (1.5) sev: 17.6 (0.60)	0 (0)
Small	1.5 (1.5)	3.5 (0.68)	0.73 (0.73)	0.73 (0.73)	mod: 41.8 (3.5) sev: 29.0 (0)	0.73 (0.73)
Combined	1.1 (0.73)	2.2 (0.71)	0.87 (0.57)	0.27 (0.27)	mod: 36.1 (1.2) sev: 22.1 (0)	0.27 (0.27)

Table 7.7: 3 months - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

Overall there was not a marked change in the incidence of pericellular shrinkage. However, it was again noticeable that small cells were affected with greater frequency. These shrunken, atrophic cells retained their hyperchromatic staining characteristics.

Satellite cell clusters in association with degenerating distinguishable large or small nerve cells were almost totally non-existent. However, there was an increase in frequency of satellite cell clusters in association with either cell remnants or satellite cell clusters without any obvious relationship to nerve cells in areas which appeared to be scarred.

6 MONTHS

The histological results of peripheral mental nerve transection on trigeminal ganglion nerve cells at six months post-operatively are presented in Table 7.8 and Figures 7.11 and 7.26.

At six months post-operatively, chromatolytic cells and cells showing a peripherally displaced nucleus were almost totally absent.

Furthermore, signs of nerve cell degeneration were very rare if not totally absent.

There appeared to be a slight recovery in the incidence of pericellular shrinkage, with the small cells again being more frequently affected (Figure 7.31).

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	0.43 (0)	1.3 (0)	1.3 (0)	0 (0)	mod: 28.3 (0) sev: 15.0 (0)	0 (0)
Small	0 (0)	2.1 (0)	2.1 (0)	0 (0)	mod: 38.8 (0) sev: 22.5 (0)	0 (0)
Combined	0.27 (0)	1.6 (0)	1.6 (0)	0 (0)	mod: 32.4 (0) sev: 17.9 (0)	0 (0)

Table 7.8: 6 months - peripheral mental nerve transection. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

A small increase in incidence of satellite cell clusters in association with either cell remnants or no obvious relation to nerve cells was noted (Figure 7.32).

The histological results of peripheral mental nerve transection have been summarized in Table 7.9 for comparison purposes.

7.4 CRYOINJURY OF THE MENTAL NERVE

1 DAY

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at one day post-operatively are presented in Table 7.10 and Figures 7.13 and 7.20.

One day post-operatively, the majority of the nerve cells appeared normal. These cells had centrally positioned nuclei and uniform distribution of Nissl substance in the cytoplasm.

A few nerve cells were undergoing chromatolysis, and occasionally a nerve cell with an eccentric nucleus would be seen.

Small nerve cells appeared to be affected with marginally greater frequency compared to the large nerve cells.

No pericellular shrinkage was observed. Signs of nerve cell degeneration were also absent.

POST-INJURY INTERVAL	NERVE CELL TYPE	HISTOLOGICAL FEATURE					
		CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
1 DAY	Large	4.7	3.2	0	0	mod: 0 sev: 0	0
	Small	14.6	7.9	0	0	mod: 0 sev: 0	0
	Combined	8.4	5.0	0	0	mod: 0	0
3 DAYS	Large	12.2	10.5	0	0	mod: 0 sev: 0	0
	Small	44.1	24.6	0	0	mod: 0 sev: 0	0
	Combined	23.5	15.6	0	0	mod: 0	0
1 WEEK	Large	22.6	27.3	6.4	0	mod: 23.0 sev: 4.2	0
	Small	55.6	58.6	5.6	0	mod: 24.0 sev: 6.6	0
	Combined	35.2	39.1	5.9	0	mod: 23.3	0

	Large	26.7	34.0	19.1	5.3	mod: 23.8 sev: 12.2	9.2
2 WEEKS	Small	64.3	65.6	19.7	12.1	mod: 33.3 sev: 12.7	11.4
	Combined	41.6	46.6	19.3	8.0	mod: 27.5	10.2
	Large	3.7	10.7	14.6	3.3	mod: 35.0 sev: 19.0	11.1
1 MONTH	Small	13.6	26.2	10.0	3.7	mod: 44.5 sev: 28.4	12.4
	Combined	7.7	17.0	11.3	3.5	mod: 38.8	11.7
	Large	0.93	1.4	0.97	0	mod: 32.4 sev: 17.6	0
3 MONTHS	Small	1.5	3.5	0.73	0.73	mod: 41.8 sev: 29.0	0.73
	Combined	1.1	2.2	0.87	0.27	mod: 36.1	0.27
	Large	0.43	1.3	1.3	0	mod: 28.3 sev: 15.0	0
6 MONTHS	Small	0	2.1	2.1	0	mod: 38.8 sev: 22.5	0
	Combined	0.27	1.6	1.6	0	mod: 32.4	0

Table 7.9 : Histological results of peripheral mental nerve transection. Figures represent the percentage of nerve cells displaying the histological feature.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	3.2 (0.95)	2.2 (0.38)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	6.7 (1.4)	3.7 (0.26)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	4.5 (0.98)	2.8 (0.18)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.10: 1 day - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

3 DAYS

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at three days post-operatively are presented in Table 7.11 and Figures 7.14 and 7.21.

Three days post-operatively, there was a large increase in the percentage of nerve cells exhibiting chromatolysis and peripheral displacement of the nucelus (Figures 7.33 and 7.34 respectively). The more marked increase was with respect to chromatolysis.

The differential response to peripheral cryoinjury between the two cell populations was very evident, with small nerve cells being more widely affected.

No pericellular shrinkage or signs of nerve cell degeneration were observed.

1 WEEK

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at one week post-operatively are presented in Table 7.12 and Figures 7.15 and 7.22.

One week post-operatively, the percentage of nerve cells exhibiting chromatolysis and peripheral displacement of the nucleus had reached a peak (Figures 7.33 and 7.34 respectively). The increase in percentage of chromatolysis and peripheral displacement of the nucleus was

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	27.2 (2.1)	21.7 (1.5)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	44.9 (1.1)	37.1 (2.0)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	33.6 (1.5)	27.1 (1.7)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.11: 3 days - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	36.7 (0.81)	32.8 (1.3)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	55.9 (1.9)	43.2 (1.9)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	43.6 (0.59)	36.5 (1.5)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.12: 1 week - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

approximately of the same order. Some chromatolytic cells appeared almost achromatic with gross dissolution of Nissl substance.

The percentage of small nerve cells responding to peripheral cryoinjury was greater than that for the large cells.

There was no evidence of pericellular shrinkage or nerve cell degeneration.

2 WEEKS

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at two weeks post-operatively are presented in Table 7.13 and Figures 7.16 and 7.23.

Two weeks post-operatively, a marked decline in the percentage of nerve cells with chromatolysis and eccentric nuclei was observed (Figures 7.33 and 7.34 respectively). The more marked decline occurred with respect to chromatolysis. It was evident that recovery from peripheral cryoinjury had occurred.

The incidence of chromatolysis and peripheral displacement of the nucleus was approximately the same. Small nerve cells were more commonly affected than the large cells.

As was the case for the previous post-operative times, no pericellular shrinkage or nerve cell degeneration was noted.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	16.1 (1.4)	13.8 (0.65)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	20.5 (0.8)	19.0 (1.7)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	17.7 (1.1)	15.7 (0.91)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.13: 2 weeks - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

1 MONTH

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at one month post-operatively are presented in Table 7.14 and Figures 7.17 and 7.24.

One month post-operatively, a further substantial decline in chromatolytic nerve cells and nerve cells with peripherally displaced nuclei was observed (Figure 7.33 and 7.34 respectively). Overall, the level of chromatolysis and peripherally displaced nuclei observed amongst the nerve cells was of the same order.

With regard to both chromatolysis and peripheral displacement of the nucleus, the small nerve cells were again more commonly affected than the large nerve cells.

No signs of pericellular shrinkage or nerve cell degeneration were noted.

3 MONTHS

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at three months post-operatively are presented in Table 7.15 and Figures 7.18 and 7.25.

Three months post-operatively, only the occasional nerve cell appeared chromatolytic or had an eccentric nucleus. The vast majority of both small and large nerve cells resembled those from the normal control

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	3.2 (0.64)	3.5 (0.73)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	9.8 (0.64)	7.2 (1.1)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	5.6 (0.45)	4.9 (0.57)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.14: 1 month - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	2.2 (0.31)	1.6 (0.83)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	2.6 (0.50)	3.1 (0.96)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	2.3 (0.34)	2.2 (0.79)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.15: 3 months – peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

specimens. The frequency with which chromatolysis and peripheral displacement of the nucleus were observed was of the same order.

The differential response of the small nerve versus the large nerve cells that was evident during previous post-operative time intervals was absent.

No pericellular shrinkage or signs of nerve cell degeneration were noted.

6 MONTHS

The histological results of peripheral mental nerve cryoinjury on trigeminal ganglion nerve cells at six months post-operatively are presented in Table 7.16 and Figures 7.19 and 7.26.

Six months post-operatively, nerve cells of the trigeminal ganglion resembled those of the normal control specimens.

No chromatolytic nerve cells, or nerve cells with an eccentric nucleus were noted. Additionally, as had been the case for previous post-operative intervals in the peripheral cryoinjury series, pericellular shrinkage and signs of nerve cell degeneration were also absent.

The histological results of peripheral mental nerve cryoinjury have been summarized in Table 7.17 for comparison purposes.

NERVE CELL TYPE	HISTOLOGICAL FEATURE					
	CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
Large	0 (0)	0 (0)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Small	0 (0)	0 (0)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)
Combined	0 (0)	0 (0)	0 (0)	0 (0)	mod: 0 (0) sev: 0 (0)	0 (0)

Table 7.16: 6 months - peripheral mental nerve cryoinjury. Figures in parentheses represent the standard error of the mean. All figures quoted are percentages.

POST-INJURY INTERVAL	NERVE CELL TYPE	HISTOLOGICAL FEATURE					
		CHROMATOLYSIS	PERIPHERAL DISPLACEMENT NUCLEUS	CYTOPLASMIC FRAGMENTATION	NUCLEAR FRAGMENTATION	PERICELLULAR SHRINKAGE	SATELLITE CELL CLUSTERS
	Large	3.2	2.2	0	0	mod: 0 sev: 0	0
1 DAY	Small	6.7	3.7	0	0	mod: 0 sev: 0	0
	Combined	4.5	2.8	0	0	mod: 0 sev: 0	0
	Large	27.2	21.7	0	0	mod: 0 sev: 0	0
3 DAYS	Small	44.9	37.1	0	0	mod: 0 sev: 0	0
	Combined	33.6	27.1	0	0	mod: 0 sev: 0	0
	Large	36.7	32.8	0	0	mod: 0 sev: 0	0
1 WEEK	Small	55.9	43.2	0	0	mod: 0 sev: 0	0
	Combined	43.6	36.5	0	0	mod: 0 sev: 0	0

2 WEEKS	Large	16.1	13.8	0	0	mod: 0 sev: 0	0
	Small	20.5	19.0	0	0	mod: 0 sev: 0	0
	Combined	17.7	15.7	0	0	mod: 0 sev: 0	0
1 MONTH	Large	3.2	3.5	0	0	mod: 0 sev: 0	0
	Small	9.8	7.2	0	0	mod: 0 sev: 0	0
	Combined	5.6	4.9	0	0	mod: 0 sev: 0	0
3 MONTHS	Large	2.2	1.6	0	0	mod: 0 sev: 0	0
	Small	2.6	3.1	0	0	mod: 0 sev: 0	0
	Combined	2.3	2.2	0	0	mod: 0 sev: 0	0
6 MONTHS	Large	0	0	0	0	mod: 0 sev: 0	0
	Small	0	0	0	0	mod: 0 sev: 0	0
	Combined	0	0	0	0	mod: 0 sev: 0	0

Table 7.17 : Histological results of peripheral mental nerve cyroinjury. Figures represent the percentage of nerve cells displaying the histological feature.

7.5 COMPARISON BETWEEN TRANSECTION AND CRYOINJURY OF THE MENTAL NERVE

For chromatolysis and peripheral displacement of the nucelus, the overall pattern of response of the trigeminal ganglion nerve cells to the two forms of peripheral nerve injuries studied were similar. By comparing Figure 7.27 and Figure 7.28 with Figure 7.33 and Figure 7.34 a general trend of a rapid rise to a peak incidence followed by a more gradual recovery was noted.

However, there were important differences noted between the transection and cryoinjury series. First, the overall frequency levels of chromatolysis and peripheral displacement of the nucleus were higher following mental nerve transection. This was particularly evident amongst the small nerve cell population. Secondly, the peak incidence of chromatolysis and peripheral displacement of the nucleus occurred concurrently, at two weeks for mental nerve transection and one week for cryoinjury. Thirdly, recovery from the above changes, although essentially complete for both transection and cryoinjury, was more rapid following cryoinjury.

A further point of comparison between the transection and cryoinjury series was the absence of signs of nerve cell degeneration in the cryoinjury series. The return to normal morphology of the trigeminal ganglion was almost complete at three months, and absolute at six months. In the transection series, cytoplasmic and nuclear fragmentation were observed, particularly at the two week interval (Figures 7.29 and 7.30 respectively). Although the level of frequency of these histological features decreased with time to almost nil, the morphology of the trigeminal ganglion was never restored to normal. This was due to

the persistence of residual pericellular shrinkage and atrophy amongst the cell population (Figure 7.31), and the presence of satellite cell clusters in association with either cell remnants, or in no obvious relation to nerve cells seen in the long post-operative time intervals (Figure 7.32).

It was evident that mental nerve transection resulted in irreversible changes within the trigeminal ganglion associated with nerve cell degeneration preceding cell death and cell loss. Apart from the previously discussed morphological signs observed histologically, further support for cell loss occurring within the ganglion was obtained by comparing the mean nerve cell counts from the control specimens with the mean nerve cell counts from the six month specimens (Table 7.18).

Conversely, cryoinjury to the mental nerve resulted in reversible changes within the trigeminal ganglion, with no appreciable cell loss as evidenced by the absence of nerve cell degeneration and death, and the similarity between the mean nerve cell counts of the control specimens and the six month specimens.

	MEAN NERVE CELL COUNT	MEAN NERVE CELL DIFFERENCE
Control	511	-
Transection 6 Month Specimens	371	140
Cryoinjury 6 Month Specimens	483	28

Table 7.18: Mean nerve cell counts - Transection vs. Cryoinjury.

Figure 7.5: 1 day - peripheral mental nerve transection.

The majority of the nerve cells appeared normal (NC). Occasionally a chromatolytic (CHR) cell or a cell with peripheral displacement of the nucleus (PDN) would be encountered. Cresyl violet, x320.

Figure 7.6: 3 days - peripheral mental nerve transection.

There was an increase in incidence of chromatolysis (CHR) and peripheral displacement of the nucleus (PDN). Cresyl violet, x320.

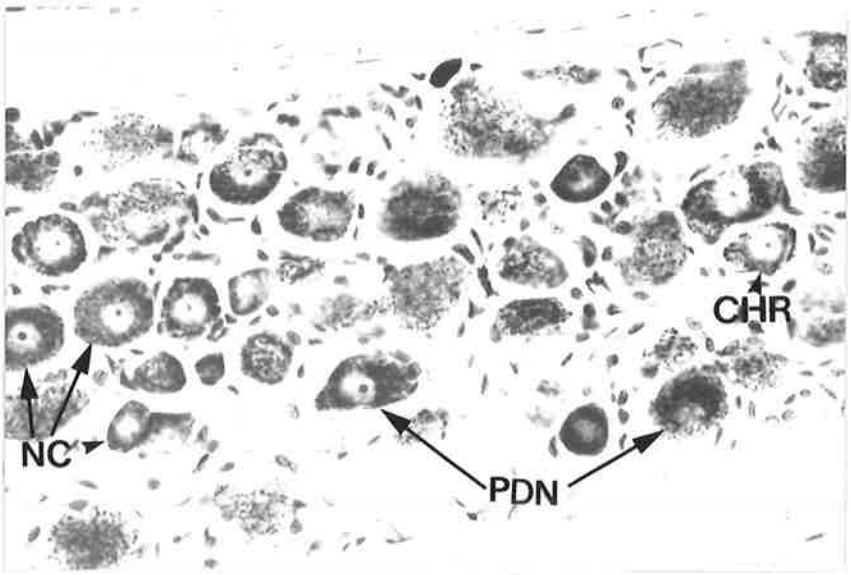


Figure 7.5: 1 day - peripheral mental nerve transection.

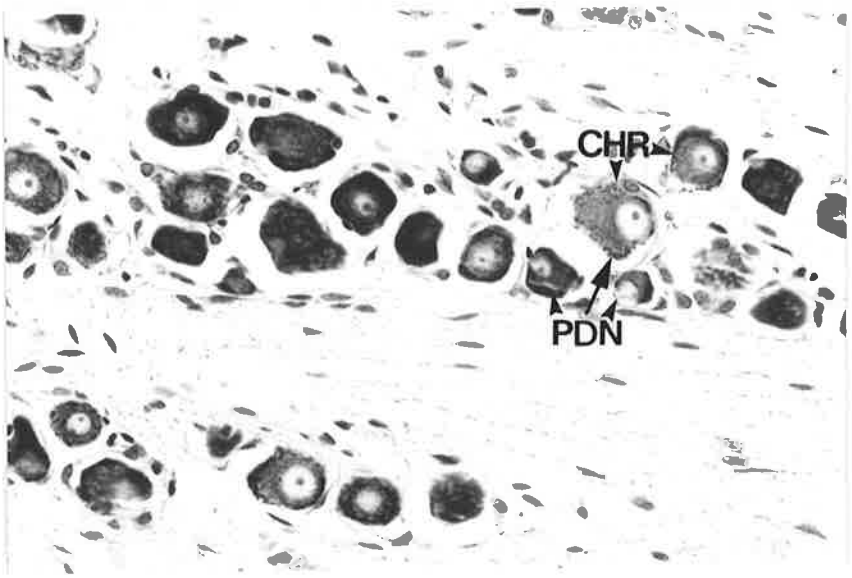


Figure 7.6: 3 days - peripheral mental nerve transection.

Figure 7.7: 1 week - peripheral mental nerve transection.

In addition to chromatolysis (CHR) and peripheral displacement of the nucleus (PDN), some cells were showing both moderate pericellular shrinkage (MPS) and severe pericellular shrinkage (SPS). A few cells were undergoing cytoplasmic fragmentation (CF). Cresyl violet, x320.

Figure 7.8: 2 weeks - peripheral mental nerve transection.

Chromatolysis (CHR) and peripheral displacement of the nucleus (PDN) were widespread. There was an increase in the incidence of cytoplasmic fragmentation (CF), nuclear fragmentation (NF), and pericellular shrinkage (MPS, SPS). Satellite cell clusters (SCC) in association with degenerating neurons were also evident. Cresyl violet, x320.

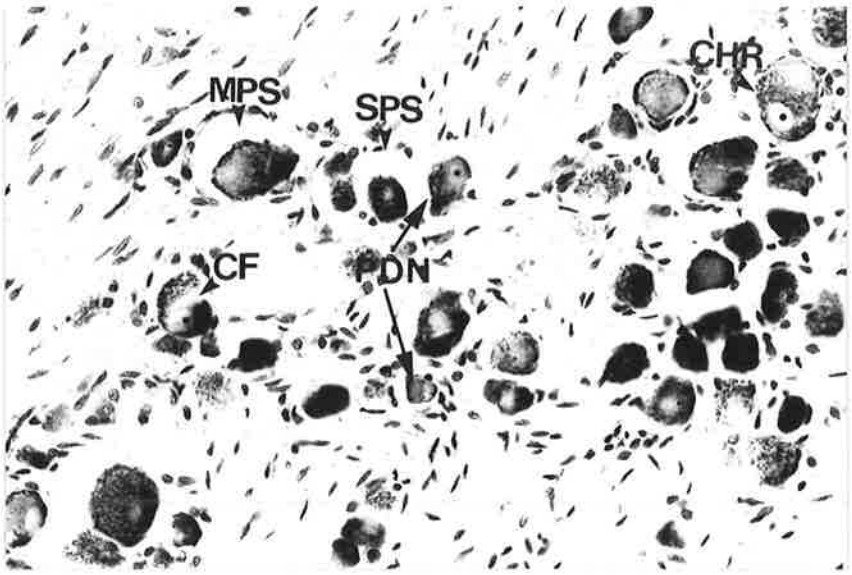


Figure 7.7: 1 week - peripheral mental nerve transection.

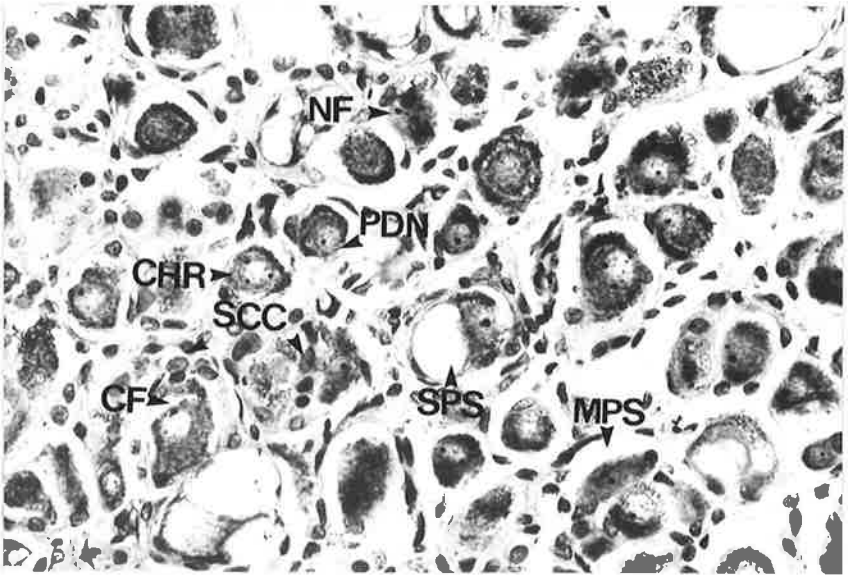


Figure 7.8: 2 weeks - peripheral mental nerve transection.

Figure 7.9: 1 month - peripheral mental nerve transection.

Moderate pericellular shrinkage (MPS) and severe pericellular shrinkage (SPS) were widespread, with the affected cells taking on a hyperchromatic appearance. Satellite cell clusters (SCC) in association with cell remnants were also evident. Cresyl violet, x320.

Figure 7.10: 3 months - peripheral mental nerve transection.

Pericellular shrinkage (MPS, SPS) was again a noticeable feature. Satellite cell clusters (SCC) in no obvious relationship to nerve cells were noted. Cresyl violet, x320.

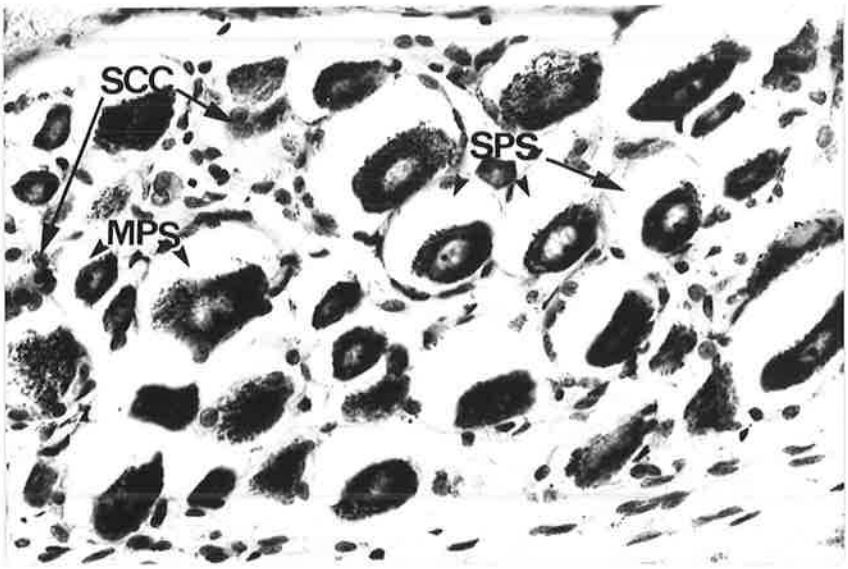


Figure 7.9: 1 month - peripheral mental nerve transection.

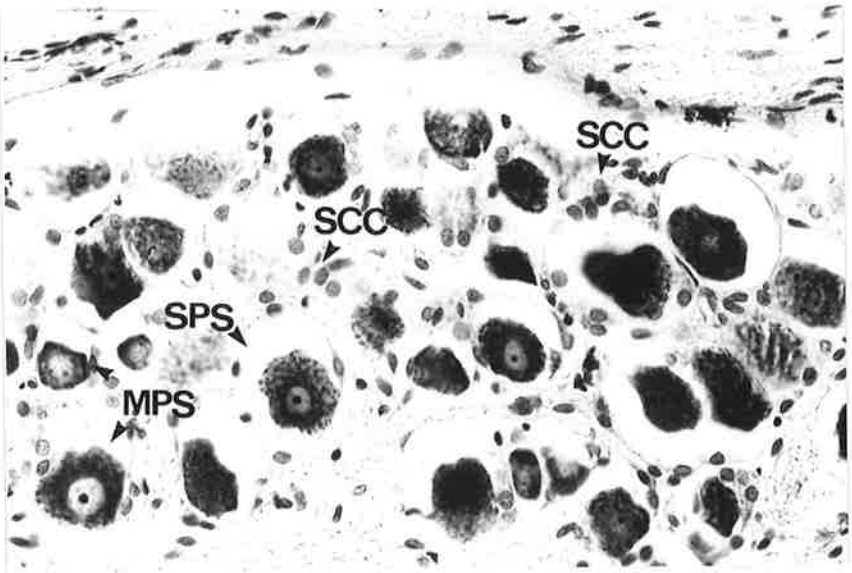


Figure 7.10: 3 months - peripheral mental nerve transection.

Figure 7.11: 6 months - peripheral mental nerve transection

The majority of the nerve cells were normal. However, residual pericellular shrinkage (MPS, SPS) was evident. Satellite cell clusters (SCC) in no obvious relationship to nerve cells were a noticeable feature. Cresyl violet, x320.

Figure 7.12: Severe chromatolysis - achromatic nerve cells (ACH). Cresyl violet, x500.

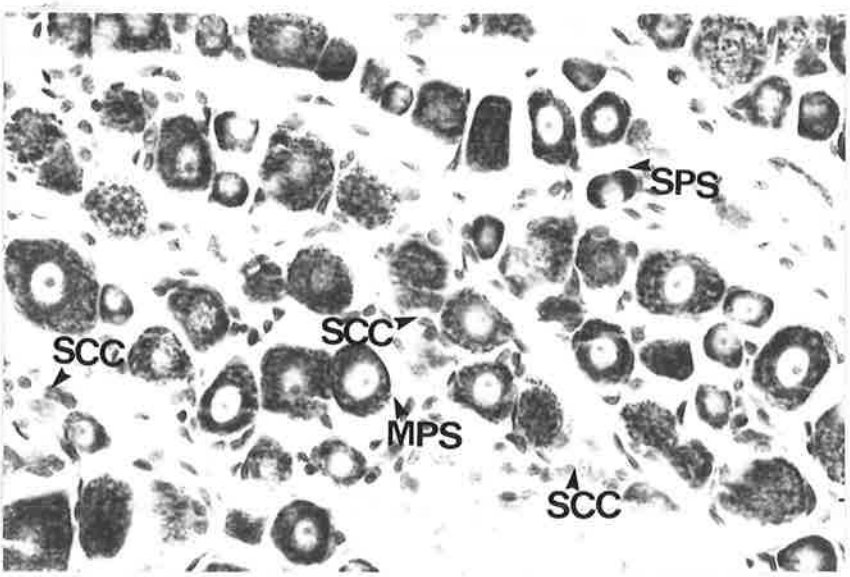


Figure 7.11: 6 months - peripheral mental nerve transection

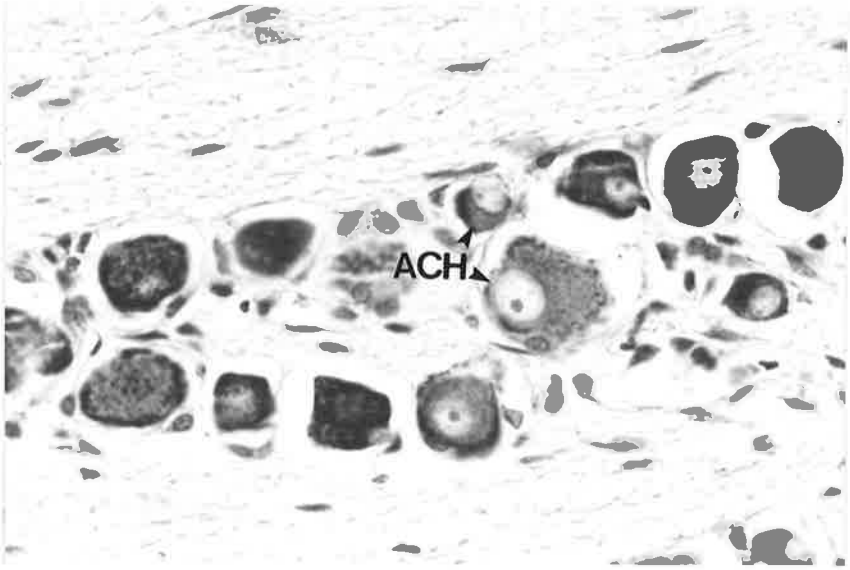


Figure 7.12: Severe chromatolysis - achromatic nerve cells (ACH).

Figure 7.13: 1 day - peripheral mental nerve cryoinjury.

The majority of the nerve cells appeared normal (NC). A few nerve cells were showing chromatolytic changes (CHR). Cresyl violet, x320.

Figure 7.14: 3 days - peripheral mental nerve cryoinjury.

There was an increase in incidence of chromatolysis (CHR) and peripheral displacement of the nucleus (PDN). Cresyl violet, x320.

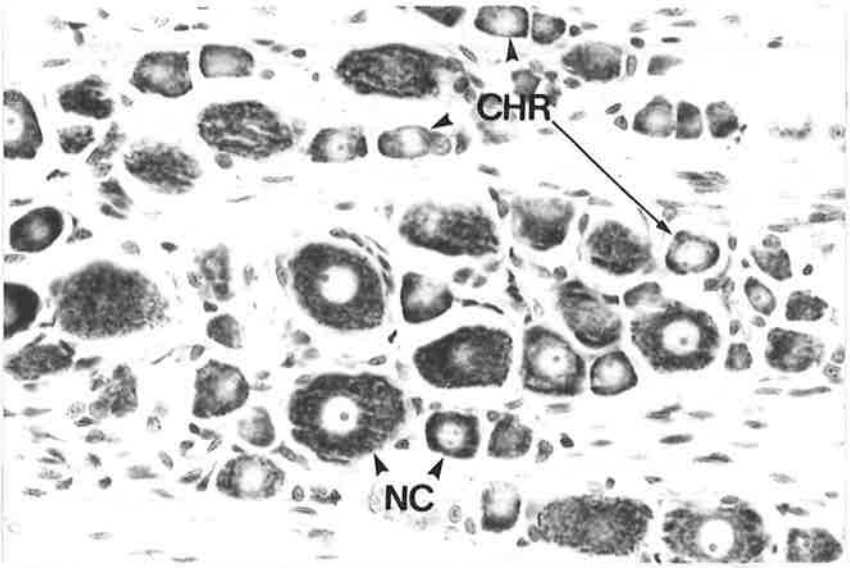


Figure 7.13: 1 day - peripheral mental nerve cryoinjury.

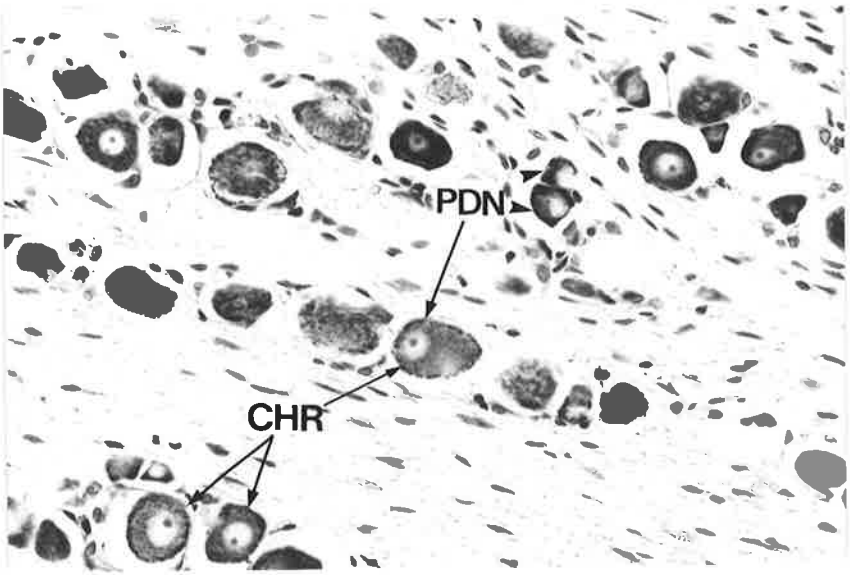


Figure 7.14: 3 days - peripheral mental nerve cryoinjury.

Figure 7.15: 1 week - peripheral mental nerve cryoinjury.

Chromatolysis (CHR) and peripheral displacement of the nucleus (PDN) were widespread. Cresyl violet, x320.

Figure 7.16: 2 weeks - peripheral mental nerve cryoinjury.

The majority of the nerve cells appeared normal (NC). Chromatolysis (CHR) and peripheral displacement of the nucleus (PDN) were observed with a reduced frequency. Cresyl violet, x320.

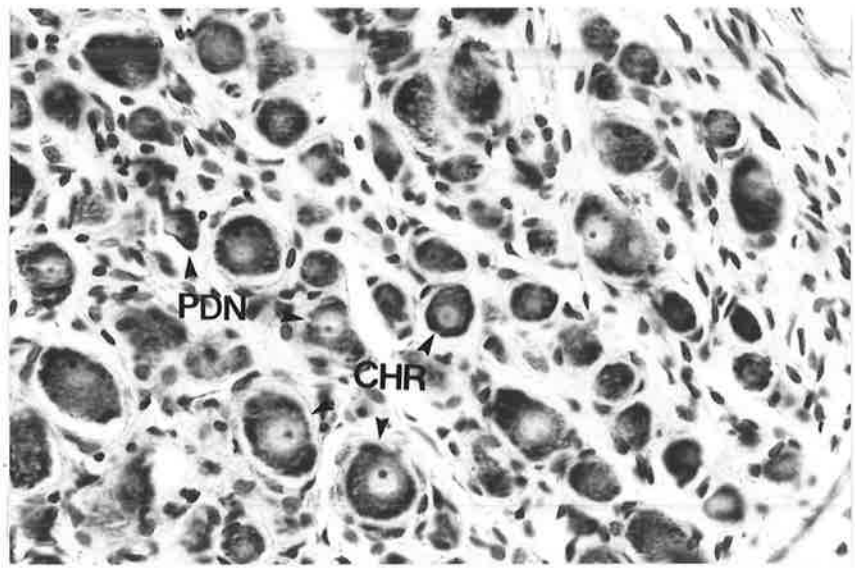


Figure 7.15: 1 week - peripheral mental nerve cryoinjury.

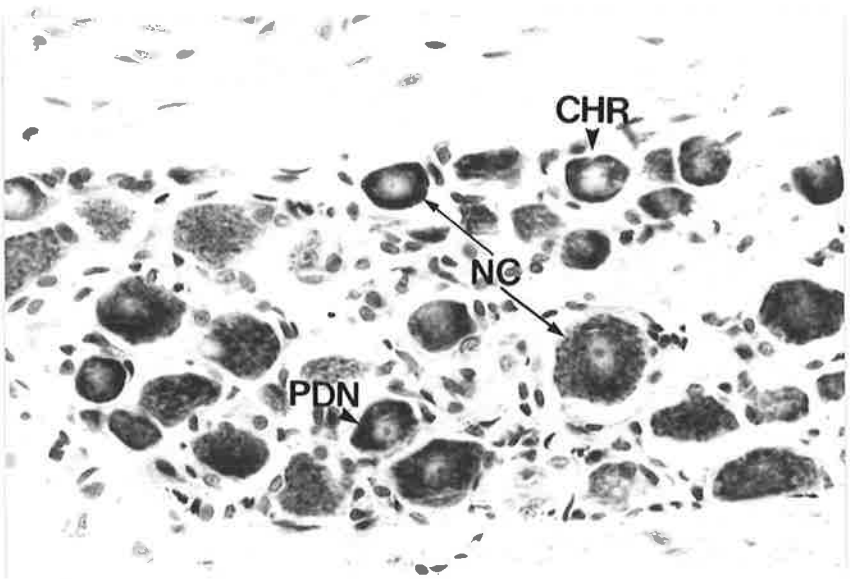


Figure 7.16: 2 weeks - peripheral mental nerve cryoinjury.

Figure 7.17: 1 month - peripheral mental nerve cryoinjury.

The majority of the nerve cells appeared normal, with chromatolysis (CHR) and peripheral displacement of the nucleus (PDN) being an occasional feature. Cresyl violet, x320.

Figure 7.18: 3 months - peripheral mental nerve cryoinjury.

The majority of the nerve cells appeared normal (NC). Very few cells exhibited either chromatolysis (CHR) or peripheral displacement of the nucleus (PDN). Cresyl violet, x320.

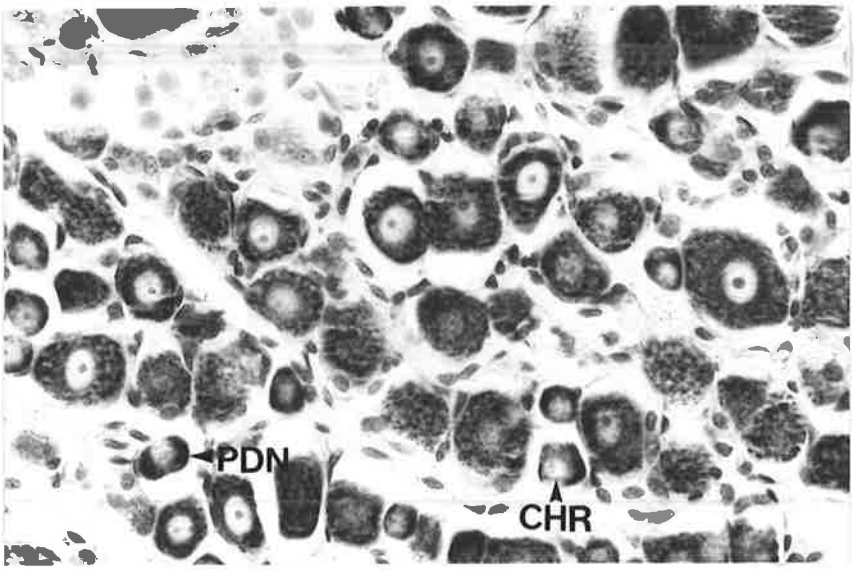


Figure 7.17: 1 month - peripheral mental nerve cryoinjury.

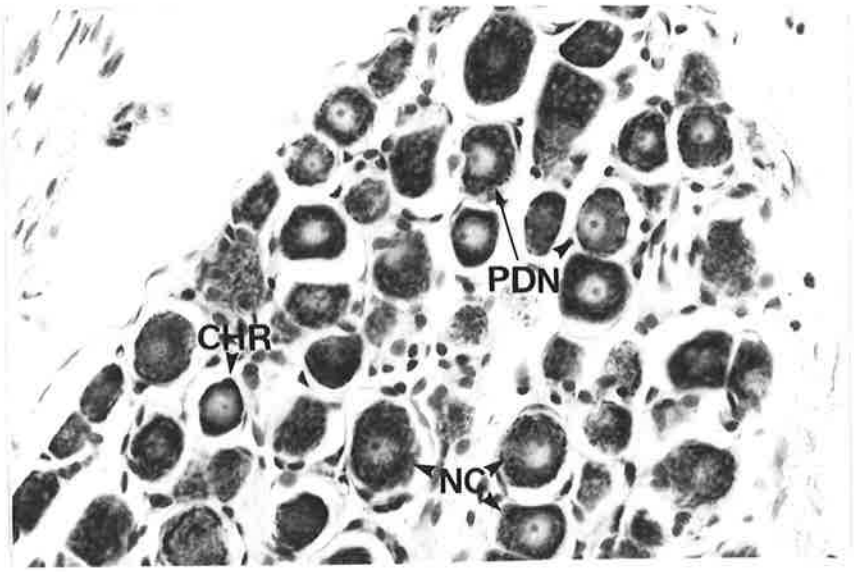


Figure 7.18: 3 months - peripheral mental nerve cryoinjury.

Figure 7.19: 6 months - peripheral mental nerve cryoinjury.

The nerve cells of the trigeminal ganglion (NC) resembled those of the normal control specimens. Cresyl violet, x320.

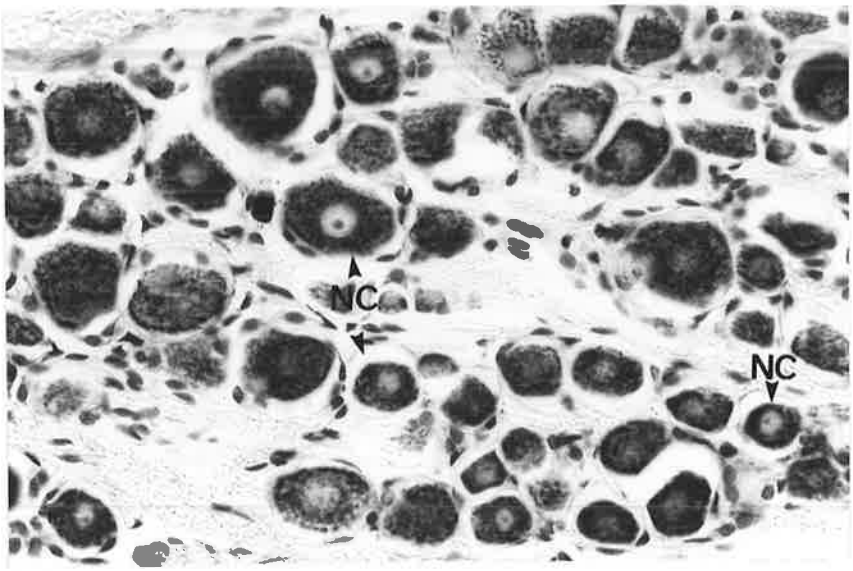


Figure 7.19: 6 months – peripheral mental nerve cryoinjury.

KEY FOR BAR GRAPHS

CHR = Chromatolysis.

PDN = Peripheral displacement of the nucleus.

CF = Cytoplasmic fragmentation.

NF = Nuclear fragmentation.

MPS = Moderate pericellular shrinkage.

SPS = Severe pericellular shrinkage.

SCC = Satellite cell clusters.

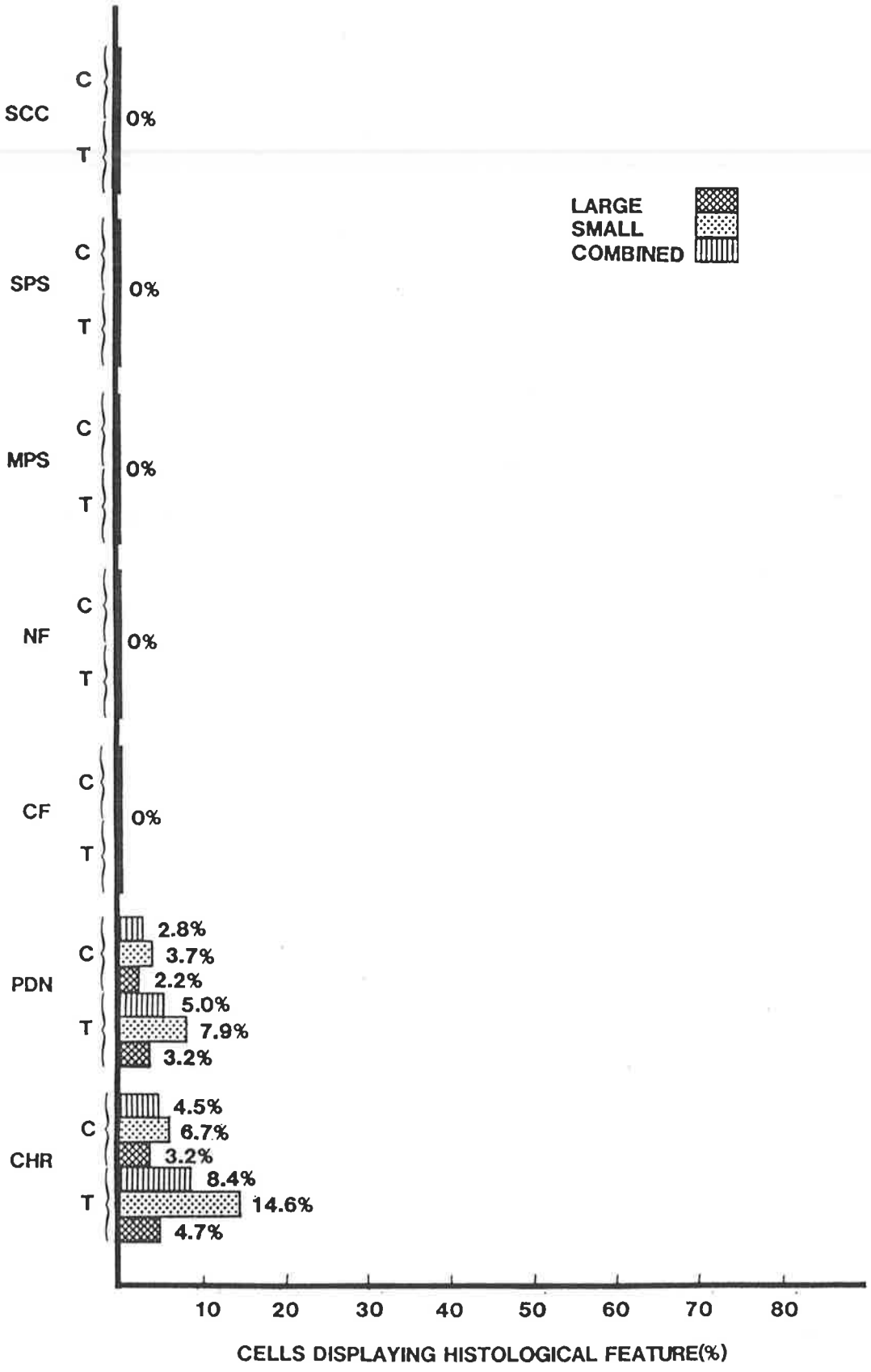


Figure 7.20: Results of peripheral mental nerve injury at 1 day post-operatively.

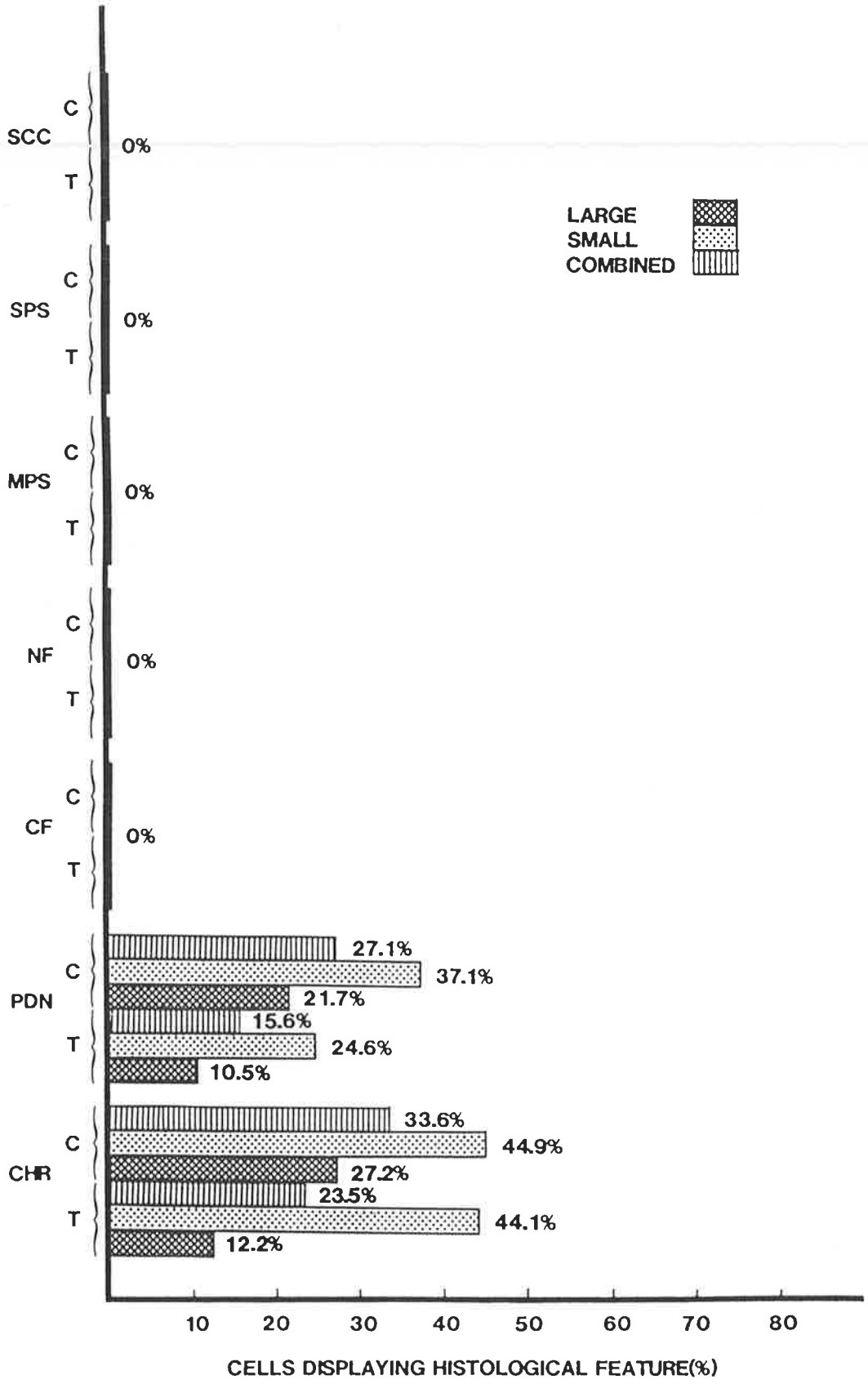


Figure 7.21: Results of peripheral mental nerve injury at 3 days post-operatively.

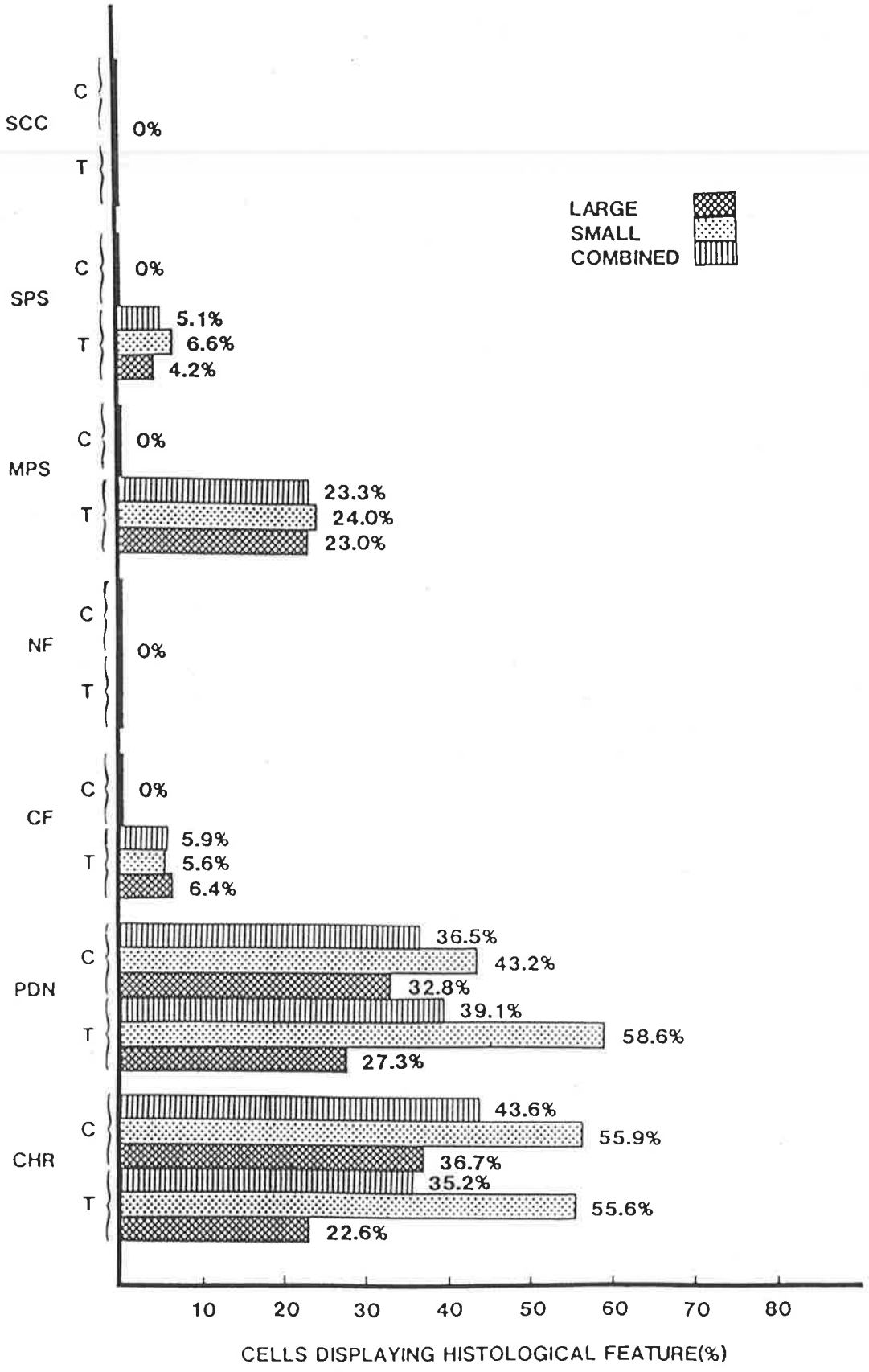


Figure 7.22: Results of peripheral mental nerve injury at 1 week post-operatively.

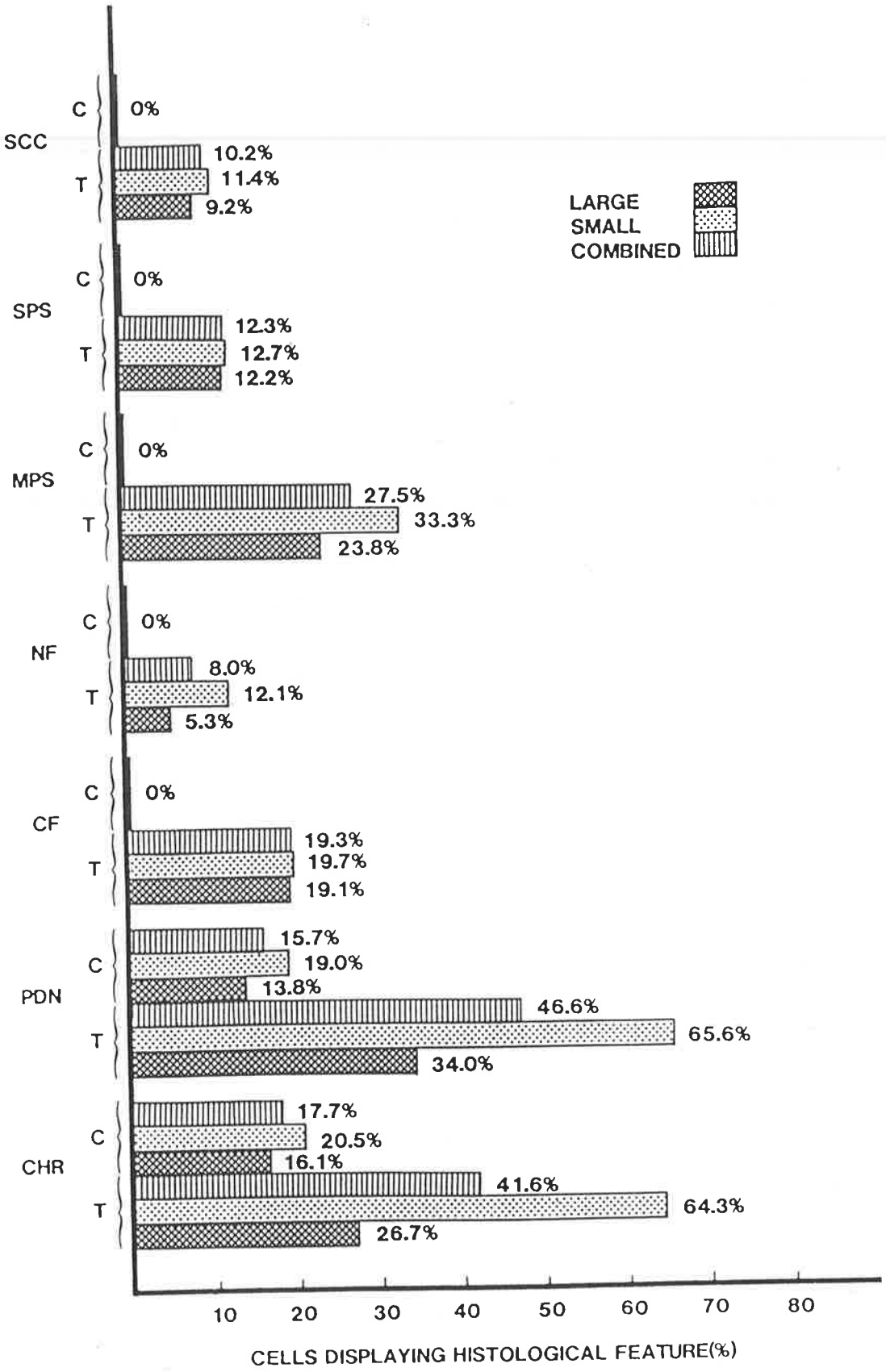


Figure 7.23: Results of peripheral mental nerve injury at 2 weeks post-operatively.

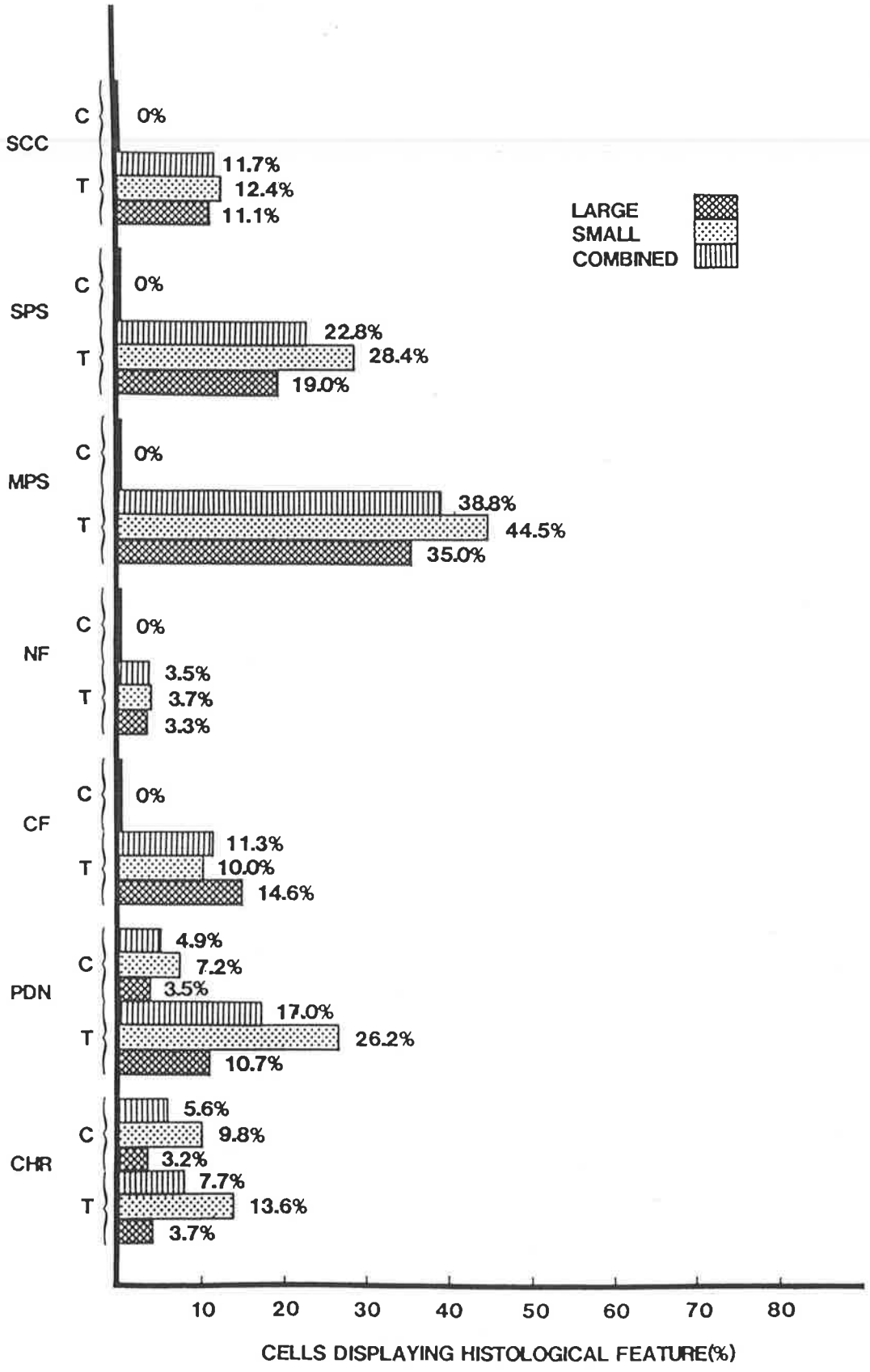


Figure 7.24: Results of peripheral mental nerve injury at 1 month post-operatively.

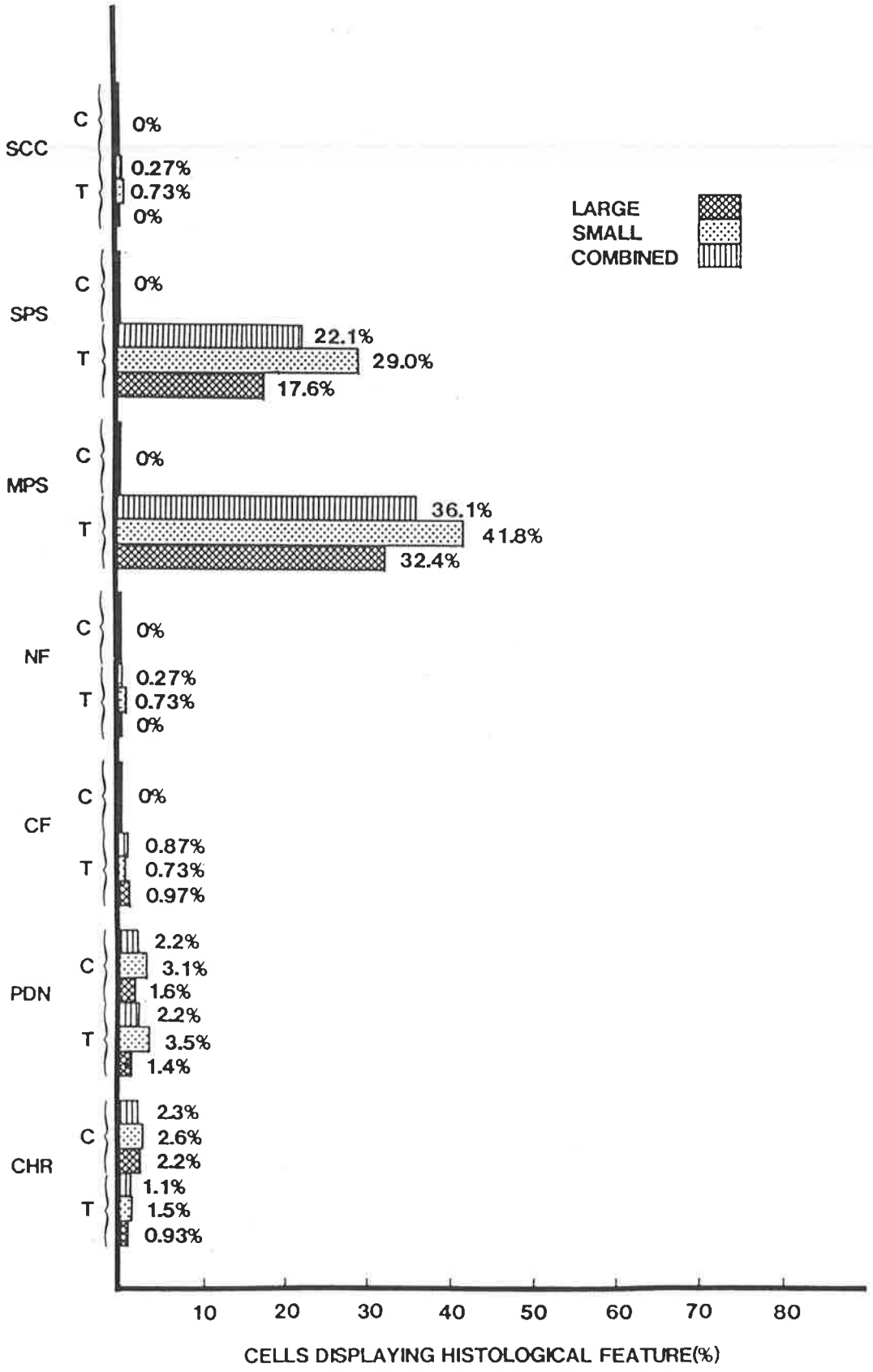


Figure 7.25: Results of peripheral mental nerve injury at 3 months post-operatively.

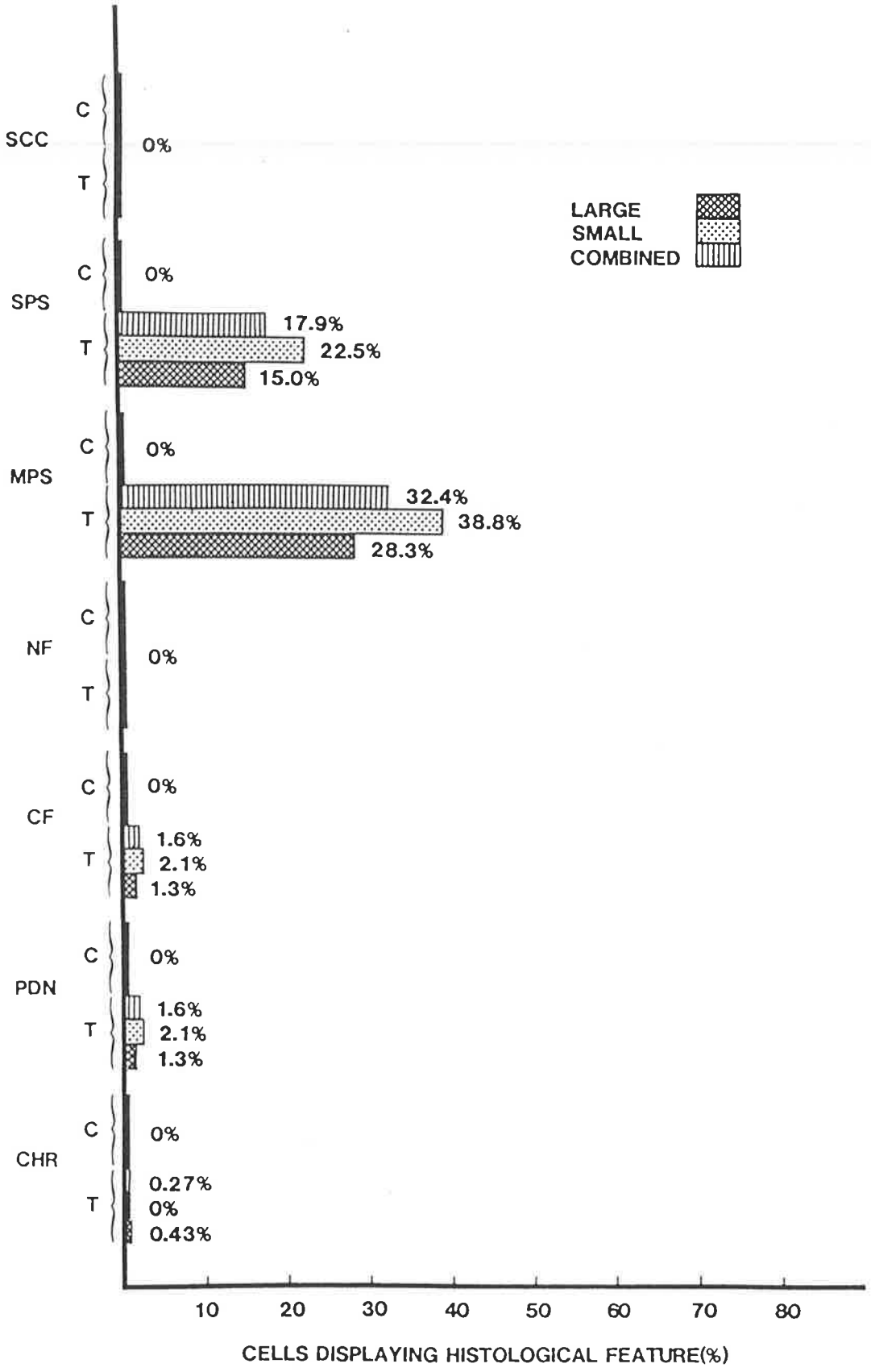


Figure 7.26: Results of peripheral mental nerve injury at 6months post-operatively.

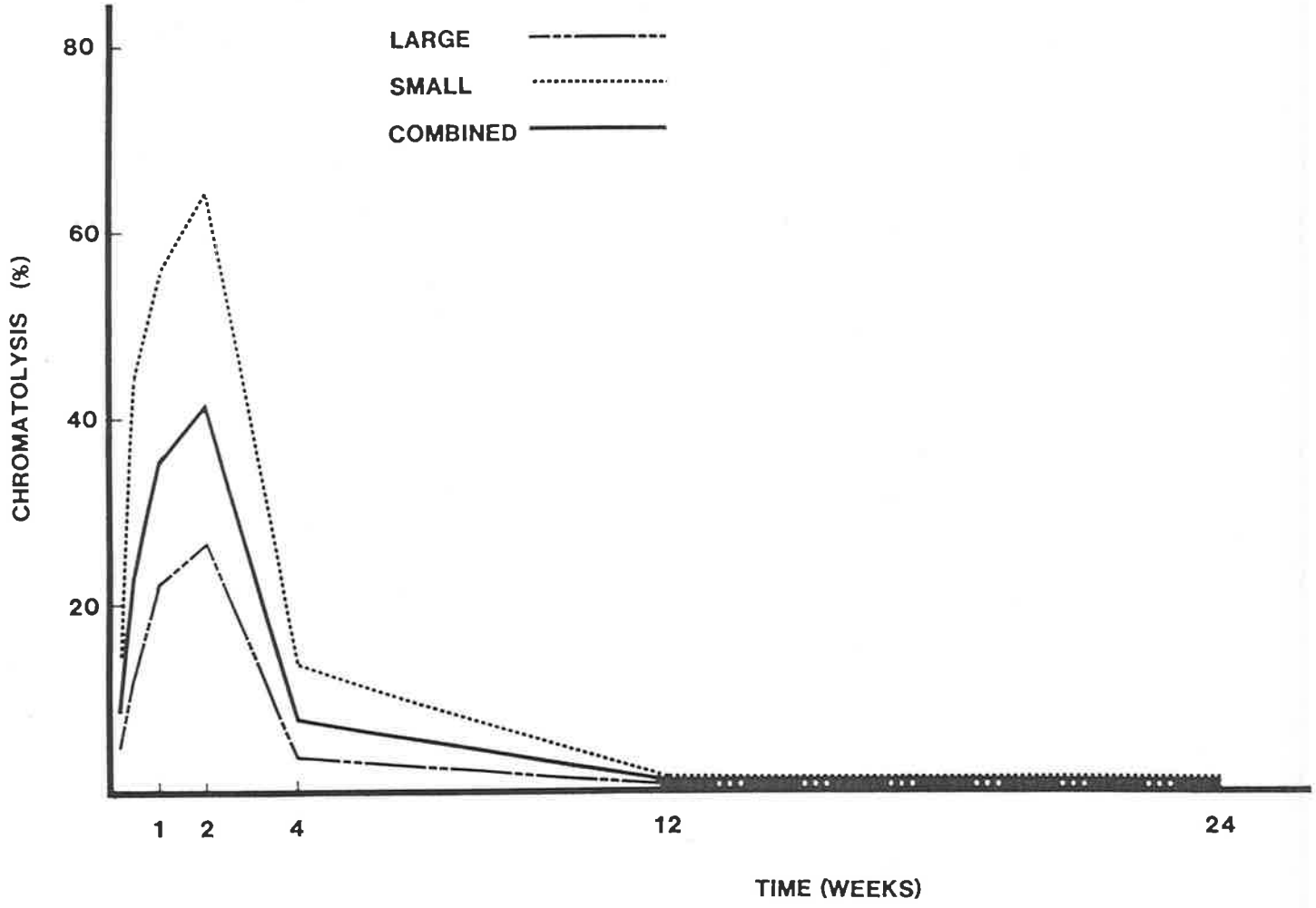


Figure 7.27: Chromatolysis following peripheral mental nerve transection.

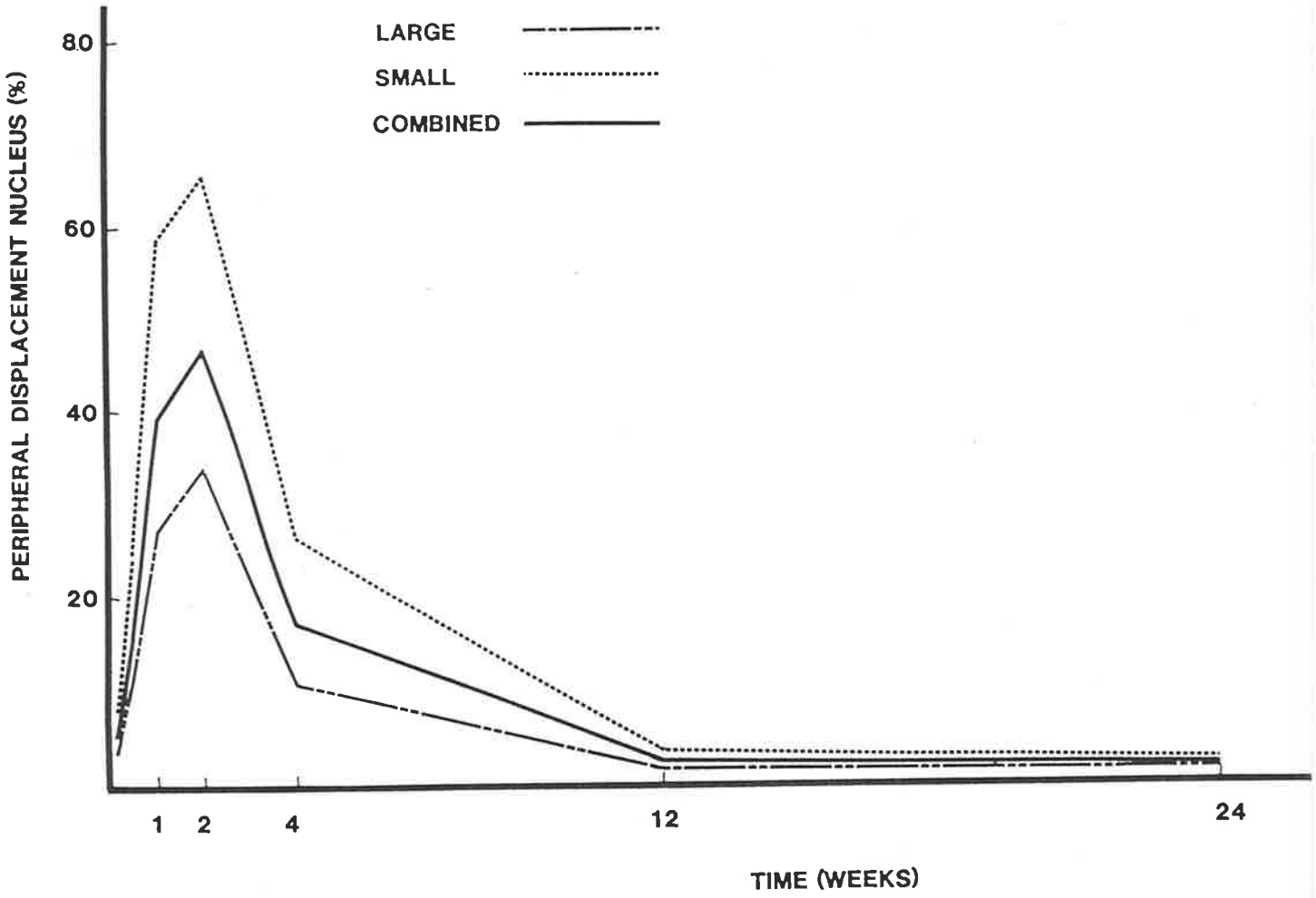


Figure 7.28: Peripheral displacement of the nucleus following peripheral mental nerve transection.

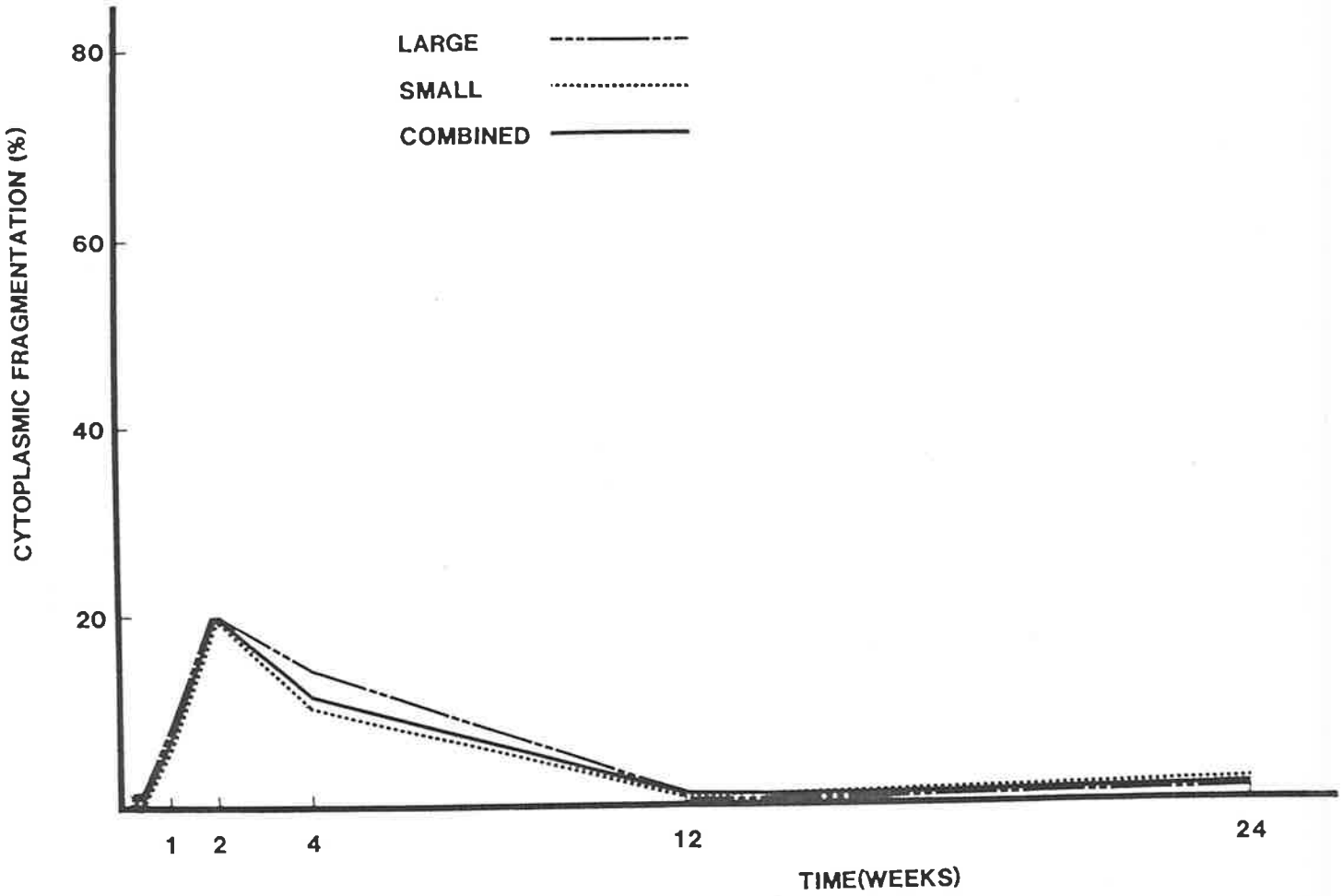


Figure 7.29: Cytoplasmic fragmentation following peripheral mental nerve transection.

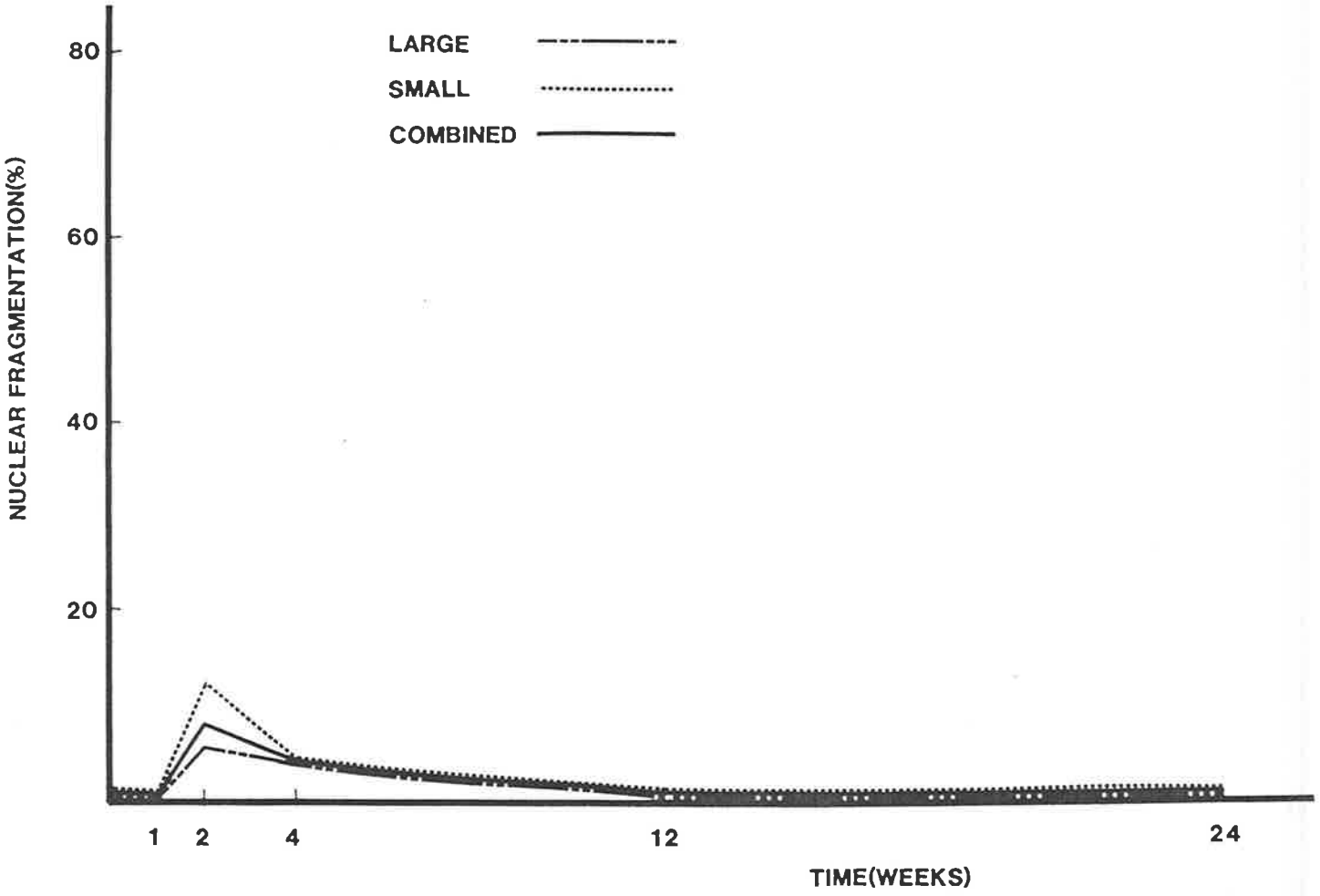


Figure 7.30: Nuclear fragmentation following peripheral mental nerve transection.

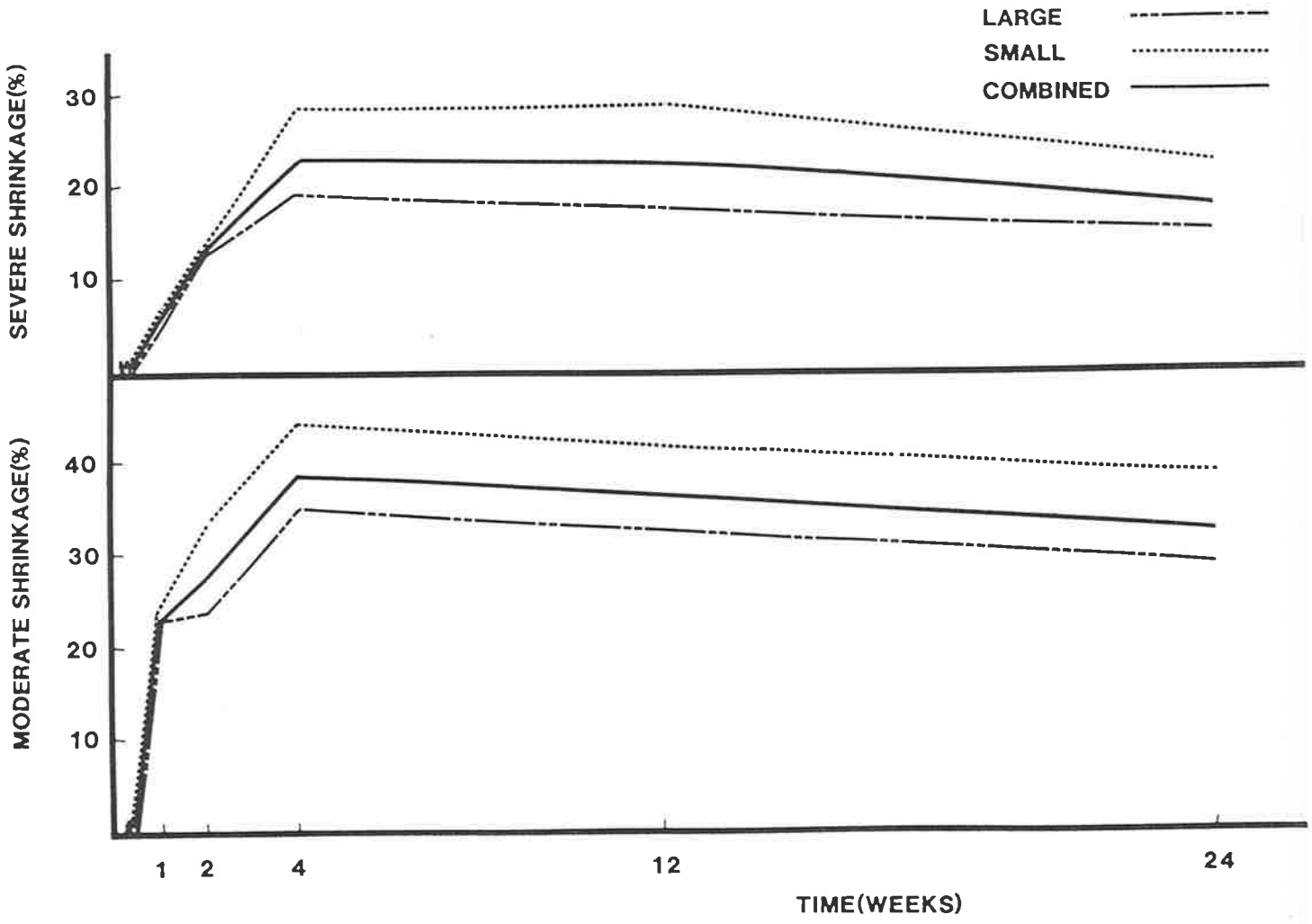


Figure 7.31: Pericellular shrinkage following peripheral mental nerve transection.

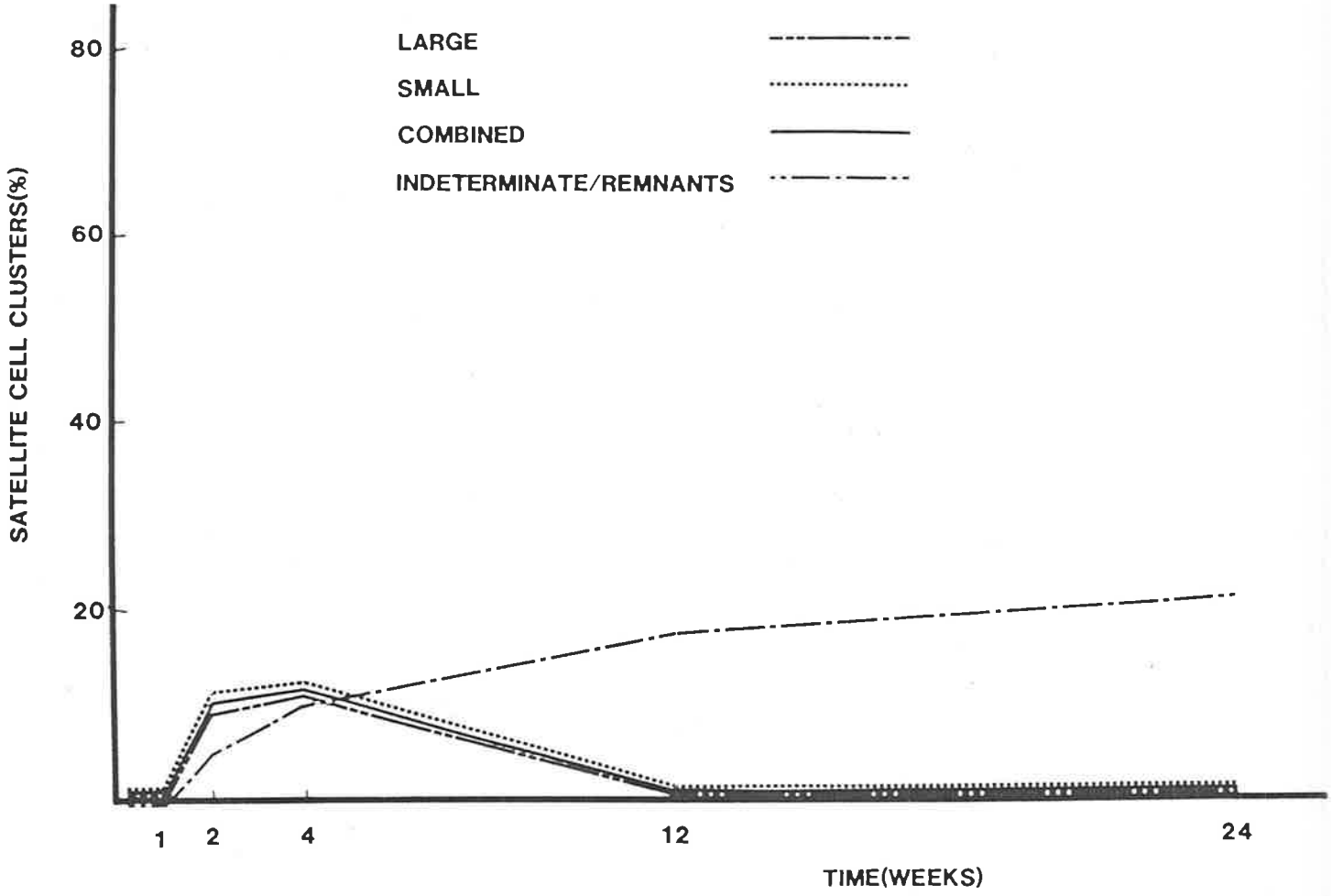


Figure 7.32: Satellite cell clusters following peripheral mental nerve transection.

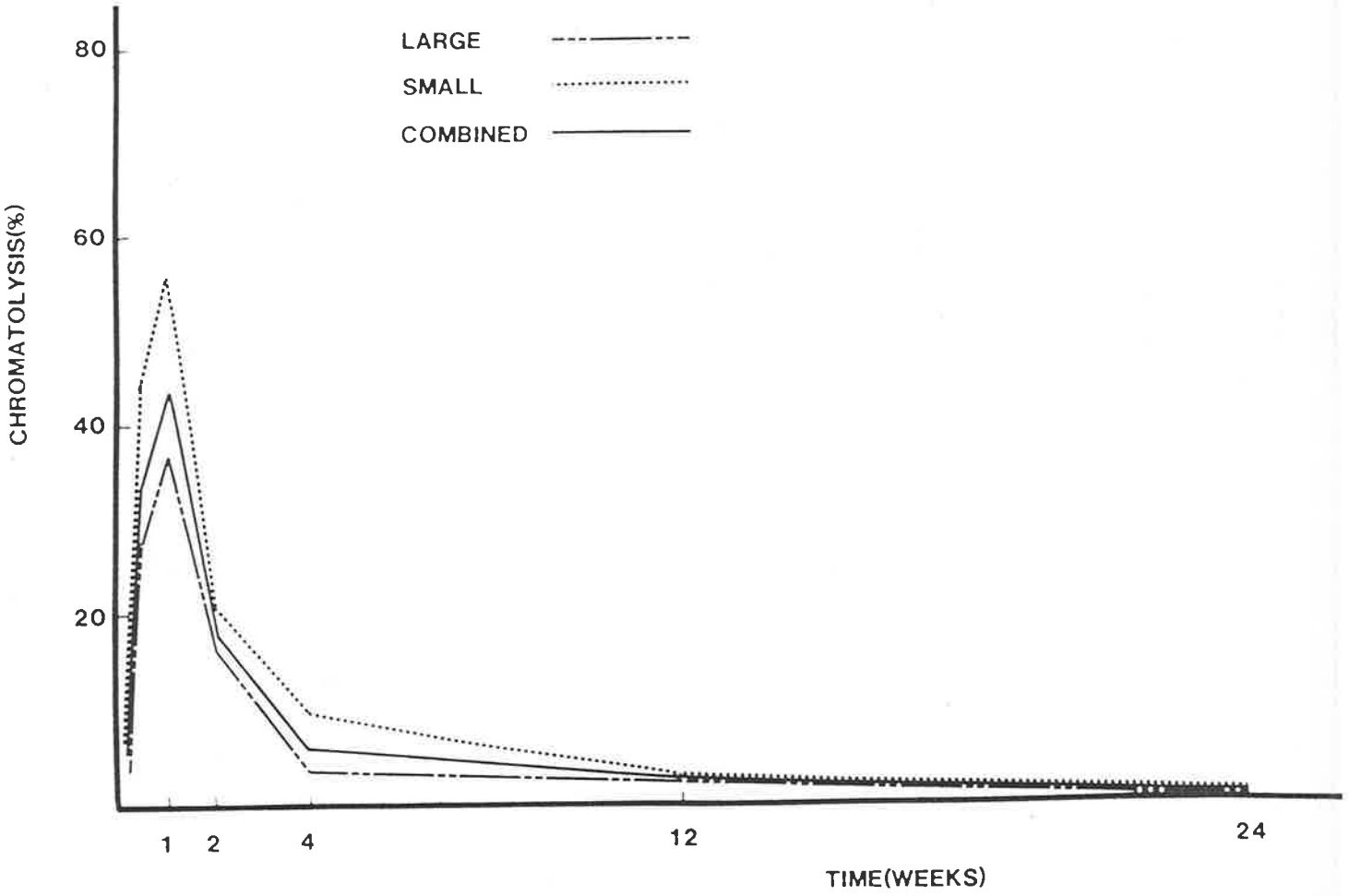


Figure 7.33: Chromatolysis following peripheral mental nerve cryoinjury.

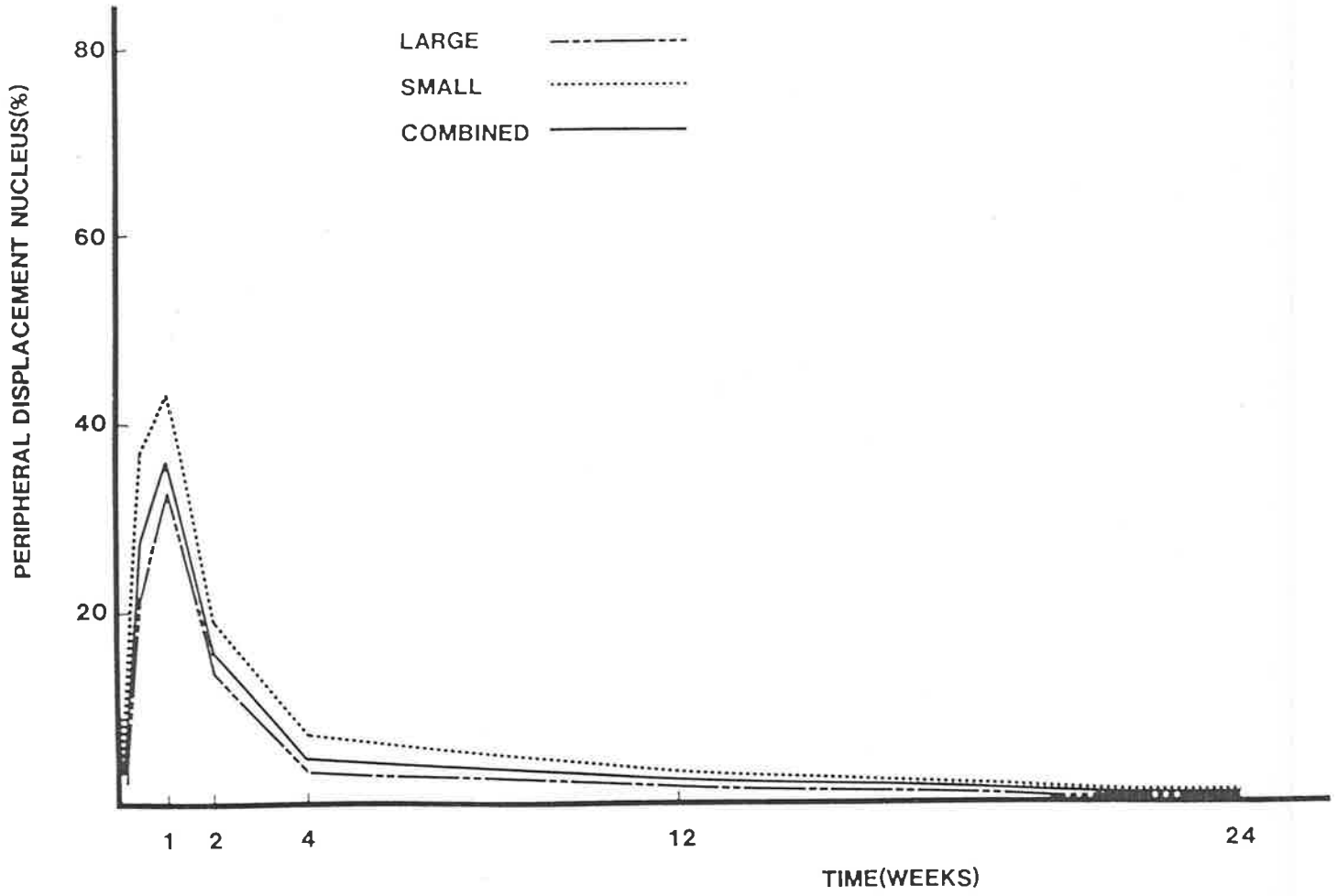


Figure 7.34: Peripheral displacement of the nucleus following peripheral mental nerve cryoinjury.

CHAPTER VIII

DISCUSSION

This study illustrated that major changes occurred within the trigeminal ganglion following peripheral injury to the mental branch of the mandibular division of the trigeminal nerve. The initial responses to the two types of nerve injuries investigated were similar, varying only in extent. However, with mental nerve transection both reversible and irreversible changes occurred within the ganglion, whereas with cryoinjury to the mental nerve there was complete return to normal ganglion architecture.

These findings will be discussed under the following headings:-

1. Experimental design.
2. Histological results.
3. Clinical significance of the results to trigeminal neuralgia and pain mechanisms within the trigeminal system.
4. Further avenues of research.

8.1 EXPERIMENTAL DESIGN

The experimental model used in this study was designed as much as possible to build on the findings of previous researchers in closely related fields.

8.1.1 Animal model

The experimental animal used in this study was the male Sprague Dawley strain of white rat. The choice was made on the basis of this animal to fulfil a number of important criteria. There is a wealth of previously published data in related areas of research where the rat was chosen as the experimental animal (Ranson, 1909 ; Cavanaugh, 1951 ; Gregg, 1971 ; Galich et al., 1976 ; Aldskogius and Arvidsson, 1978). Secondly, the rat has a mental nerve of sufficient size to be operated on. Thirdly, the rat's trigeminal ganglion is relatively easy to remove using the trans-cranial approach (Chapter VI), and it is of sufficient size to submit to light microscopic histological evaluation. Fourthly, the rat's ability to withstand general anaesthesia and surgical stress with low mortality. Finally, rats are readily available for experimental purposes because they are cheap to buy, are easy to handle and to house.

The age of the experimental animal is important. It is thought that neurons undergo a more rapid and complete degeneration after nerve injury in the young than in the adult (Lieberman, 1971). Consequently, young (approximately 3 months old) rats were chosen for this study to ensure that these changes were well demonstrated. Thus these animals proved to be a good model for the production of peripheral nerve injuries and the subsequent study of their effects within the trigeminal ganglion.

The ability of the rat to withstand general anaesthesia and surgical stress with low mortality was an important consideration. In the present study, the general anaesthetic used was chloral hydrate administered intra-peritoneally, with no resultant mortality.

The surgical approach used to expose the mental nerve (Hribar, 1978) afforded rapid and easy access to the mental nerve without mortality and minimum morbidity.

At the end of the experimental time interval the rat's trigeminal ganglion was relatively easy to remove without excessive manipulation of the ganglion and the major nerve branches emanating from it. This reduced the chance of experimental error due to the production of artifacts.

8.1.2 Injury production

Peripheral nerve transections for the purpose of studying changes in sensory ganglia have been produced in a variety of experimental animals, such as rats (Gregg, 1971 ; Grant and Arvidsson, 1975 ; Aldskogius and Arvidsson, 1978), Rhesus monkeys (Carmel and Stein, 1969) and rabbits (Strassburg, 1967). In this study, clean complete transection of the mental nerve as it emerged from the mental foramen proved to be relatively reproducable.

Cryoinjuries to peripheral nerves have also been produced in various experimental animals, including Rhesus monkeys and dogs (Beazley et al., 1974), rats (Carter et al., 1972 ; Barnard, 1980) and hamsters (Whittaker, 1974). In human subjects, peripheral sensory nerves have been subjected to cryoinjuries for the management of chronic facial pain (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Goss, 1984 ; Nally, 1984) and post-thoracotomy pain (Nelson et al., 1974 ; Katz et al., 1980). These studies were designed to evaluate the effect of cryoinjuries on peripheral nerve structure and function. The principal

method of producing peripheral nerve cryoinjuries utilized the cryoprobe operating on the Joule-Thomson principle with compressed nitrous oxide as the refrigerant gas. The flexibility and control of this method were the main factors influencing this choice (Chapter V). The present study also made use of this method of producing the cryoinjury.

Compared with transection of the mental nerve, the cryoinjury was more difficult to reproduce accurately due to the introduction of more variables such as the probe tip temperature, and the time interval over which the temperature was maintained.

The importance of accurately reproducing the type of injury cannot be over-emphasized because the injury was studied in continuum with different animals of the same species over varying periods of time. Because the animals were inbred and subjected to standardized environmental conditions, their responses should have been similar to the same injuries over the same period of time.

8.1.3 Timing of sacrifice

The sacrifice time intervals were chosen to observe:-

1. The classic acute chromatolytic response and peripheral displacement of the nucleus originally described by Nissl (1892) and later by Lieberman (1971).

2. The progression of the above morphological changes to reach a maximum between two to three weeks (Lieberman, 1971).

3. The morphological changes of neuronal degeneration and death described of by Aldskogius and Arvidsson (1978) occurring between one week to one month.

4. The subsequent return to normal appearing neurons observed by Strassburg (1968) and Aldskogius and Arvidsson (1978).

8.1.4 Perfusion of animals

Perfusion of animals prior to sacrifice is a technique that has been widely practised by investigators studying normal trigeminal ganglia (Peach, 1972), and changes in trigeminal ganglia following nerve injury (Carmel and Stein, 1969 ; Grant and Arvidsson, 1975 ; Aldskogius and Arvidsson, 1978).

All animals in the present study were perfused prior to sacrifice. This was done on the basis of perfusion to provide two important advantages. Firstly, extended operating time for exposure and removal of the ganglion without degenerative changes occurring within the nervous system. It is widely accepted that the neuron is one of the most susceptible cells to ischaemia (Barr, 1974 ; Robbins, 1979). Secondly, the bloodless surgical field makes for rapid and easy removal of the trigeminal ganglion.

8.1.5 Fixation of Specimens

The F.E.C. neural fixative pH 7.2 gave excellent consistent results, was relatively cheap and easy to prepare and therefore was

strongly recommended for fixation. Because of toxicity and expense, osmium tetroxide was not investigated for a neural fixative.

In the present study, a combination of perfusion and extended fixation times (2 weeks) was used in order to minimize degenerative artifacts (Peach, 1972).

8.1.6 Assessment of morphological changes in the trigeminal ganglion

There have been no published studies on the quantitative assessment of morphological changes in sensory ganglia in response to peripheral nerve injury, other than those designed to estimate cell loss following peripheral nerve injury (Aldskogius and Arvidsson, 1978).

In the present study, the characteristic features of the retrograde neuronal reaction and retrograde neuronal degeneration that could be studied at the light microscopic level, were determined from a review of the literature (Chapter IV).

These histological features were scored for both large and small cells independently. All the histological features were scored on the basis of the presence or absence of the feature displayed by the nerve cells. Due to the striking variation in the degree of cell shrinkage observed, this parameter was graded as nil, moderate and severe.

In accordance with other investigators (Dixon, 1963 ; Peach, 1972), large nerve cells were found to be more numerous than small nerve cells within the rat trigeminal ganglion. For this reason, absolute counts of cells displaying a particular histological feature were expressed as a

percentage, allowing a more accurate evaluation of the incidence of the histological feature within the two nerve cell populations.

8.1.7 Errors

Two types of errors may have occurred in this study, systematic and accidental. Systematic errors may have occurred as a result of limitations in the technology employed. Great attention to detail was given to the accurate reproduction of the peripheral nerve injuries, so that the responses of different animals could be compared over the various post-injury time intervals. In order to minimize the degree of degenerative artifacts, all animals were perfused prior to the removal of the trigeminal ganglia and the ganglia were then subjected to long fixation times. In addition, all specimens were processed and stained together in a standardized manner in an attempt to reduce processing artifacts.

Accidental errors may have resulted from observer limitations in the scoring and recording of data. In an attempt to maximize observer consistency, a series of standard photomicrographs of the histological changes under investigation were used during the scoring procedure. However, ultimately a subjective decision had to be made by the observer and discrepancies may have occurred in borderline cases. Observer variation in the interpretation of histological changes is a recognized phenomenon.

Further measures taken to minimize accidental errors in the scoring and recording of data included limiting the length of each scoring session to one hour thus minimizing observer fatigue. Scoring was

facilitated by the use of an eye piece graticule which made the cell counts more accurate.

The magnitude of the accidental errors was evaluated using a double determination procedure which involved the re-scoring of a randomly selected sample of 20% of the total number of ganglia. The results indicated an acceptable level of replicability for the ganglia scored on the two separate occasions, with the poorest correlation being obtained for cell shrinkage (Table 7.1). This may have been due to the variation in degree of cell shrinkage observed and the correspondingly more complex grading index used to score this morphologic feature. For instance, a cell may have been scored as exhibiting moderate cell shrinkage on the first occasion and severe cell shrinkage on the second occasion, or vice-versa. This is particularly likely to have occurred in borderline cases.

Conversely, the strongest correlation was obtained for peripheral displacement of the nucleus. Whether a neuron had a centric or an eccentric nucleus was relatively easy to score.

The generally poorer correlation obtained for the scoring of the morphological features in the small cell population may have been related to the features being more difficult to score accurately in the small cells on the basis of cell size.

8.2 HISTOLOGICAL RESULTS

The results from the present study are of interest from the point of view that they confirm and extend our knowledge about the effects of

peripheral nerve injuries on the sensory neurons comprising the trigeminal ganglion. In addition, they help to explain, at least in part, the problem of pain mechanisms within the trigeminal system.

8.2.1 The normal trigeminal ganglion of the rat

Previous investigators have described the macroscopic anatomy of the normal rat trigeminal ganglion (Mazza and Dixon, 1972) and its histology (Dixon, 1963 ; Peach, 1972 ; Mazza and Dixon, 1972). The observations of the present study are in general agreement with their findings.

There has been some evidence for an artifactual origin for the differences between the large pale cells and the small dark cells, by the reported absence of pale and dark cells in aldehyde perfused cats and monkeys (Pineda et al., 1967). Here the cytoplasm was of relatively uniform density, thus classification of cells into separate cell types was not indicated.

In the present study, following perfusion fixation and extended immersion fixation (2 weeks) two distinct cell types were observed. They were found adjacent to one another, their appearance did not suggest local tissue damage and there was no evidence of shrinkage. All these factors suggest a non-artifactual origin for the large pale cells and the small dark cells of the rat trigeminal ganglion. Further support for distinct cell types within the rat trigeminal ganglion has been provided by Matsuura et al. (1969) using histochemical techniques and comparing the acid phosphatase activity between the cell types.

8.2.2 The morphological response of the trigeminal ganglion to peripheral nerve injury

Previous investigators have described the response of spinal ganglia (Ranson, 1909 ; Cavanaugh, 1951), and the trigeminal ganglion (Gregg, 1971 ; Galich et al., 1976 ; Aldskogius and Arvidsson, 1978) in the rat to peripheral nerve transection. In general, the morphological responses of the trigeminal ganglion nerve cells to mental nerve transection observed in the present study were confirmatory.

To date there have been no published studies concerning the morphological responses of trigeminal ganglion nerve cells to peripheral nerve cryoinjury. This study demonstrates that there were important differences between the effects of mental nerve transection and cryoinjury to the mental nerve, on the cells of the trigeminal ganglion. These findings will be discussed below.

Both qualitative and quantitative light microscopic data were assessed in this study. The quantitative histological assessment was achieved by performing absolute cell counts and converting these to percentages as per the proforma in Appendix 6. This served to quantitate the characteristic light microscopic features of the trigeminal ganglion response to mental nerve injury.

Following transection and cryoinjury to the mental nerve the initial retrograde neuronal reaction consisting of chromatolysis and peripheral displacement of the nucelus, was observed at one day post-operatively. Thereafter, there was a rapid increase in the incidence of nerve cells displaying the retrograde neuronal reaction until a peak incidence was reached, followed by a gradual but definite recovery.

However, the retrograde neuronal reaction in the transection series was generally more intense, and took longer for the onset and duration of recovery (Figures 7.27, 7.28, 7.33 and 7.34).

In addition to the morphological changes which constitute part of a general retrograde neuronal reaction which may be reversible (Lieberman, 1971), nerve cells with irreversible changes interpreted as reflecting stages in a process terminating with nerve cell death were seen in the transection series. These morphological changes of cytoplasmic fragmentation, nuclear fragmentation and satellite cell clusters were not found in the ganglia from the control series or the cryoinjury series. The presence of normal or chromatolytic nerve cells adjacent to degenerating nerve cells, and the use of perfusion fixation in conjunction with extended immersion fixation times, negates the suggestion that these degenerative nerve cell changes were artifacts.

Controversy still surrounds the cytologic criteria for nerve cell degeneration and death, principally because the changes associated with degeneration and death of traumatized or injured nerve cells seem to be diverse. For a nerve cell body to be classified as dead, there must be an indication of loss of metabolic functions or loss of vital structures manifested by nuclear changes. Robbins (1979) states that by the time nuclear changes are recognized the nerve cell has already been irreversibly injured or dead for some time.

In the present study, morphological signs of nerve cell degeneration preceding cell death were observed in the transection series betwen one week and three months post-operatively, with the peak incidence generally occurring at two weeks post-operatively (Figures

7.29, 7.30 and 7.32). These findings are in general agreement with those of Leiberman (1971), Gregg (1971) and Aldskogius and Arvidsson (1978).

In accordance with the findings of Aldskogius and Arvidsson (1978), satellite cell clusters with phagocytic activity were observed in close association with degenerating nerve cells in the transection series. These satellite cell changes occurred in association with both small and large nerve cells during the same period of time that cytoplasmic and nuclear fragmentation were observed (Figure 7.32). Although the incidence of this morphological feature had decreased to almost zero in relation to small and large nerve cells, in the longer time periods, satellite cell clusters were also observed in relation to indeterminate cell remnants or in no obvious relation to ganglion cells in areas of scarring. These satellite cell changes which have been interpreted as indicators of nerve cell degeneration and death followed by phagocytosis (Leech, 1967 ; Aldskogius and Arvidsson, 1978) were absent in the controls and the cryoinjury series of the present study.

On the basis of the results obtained from this study it can be stated that the morphological response of the trigeminal ganglion to peripheral cryoinjury was completely reversible, with the vast majority of nerve cells resembling the normal control specimens at four weeks post-operatively (Figures 7.33 and 7.34).

Conversely, in the transection series, although the majority of the nerve cells exhibited structural changes which were interpreted as being reversible, there were also nerve cells seen displaying irreversible degenerative signs heralding imminent cell death and cell loss (Figure 8.1). In the transection series it was evident that the morphology of

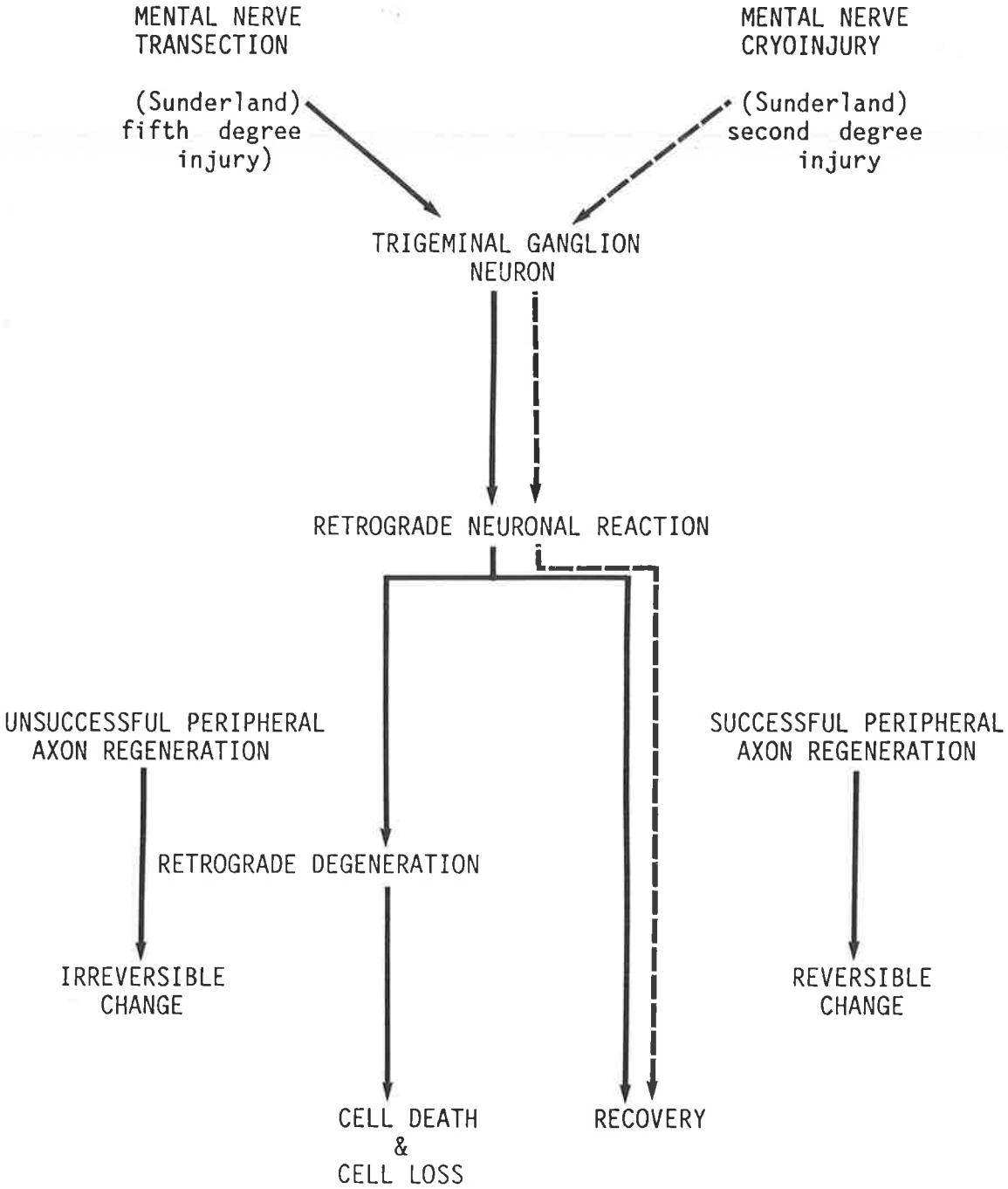


Figure 8.1 : Possible sequelae of trigeminal ganglion neurons to mental nerve transection and peripheral mental nerve cryoinjury.

the trigeminal ganglion was never completely restored to normal due to nerve cell loss, the presence of satellite cell clusters and the residual nerve cell shrinkage and atrophy.

Significant cell loss has been reported to occur in the trigeminal ganglion following transection of the infra-orbital nerve in the rat (Galich et al., 1976 ; Aldskogius and Arvidsson, 1978). In the present study it was evident that mental nerve transection resulted in nerve cells showing irreversible degenerative signs which would ultimately lead to cell death and cell loss. In contrast, no appreciable cell loss was detected in the cryoinjury series. Aldskogius and Arvidsson (1978) reported cell loss figures in the order of 17 - 29% following infra-orbital nerve transection. However, in the present study no attempt was made to accurately quantitate the degree of cell loss after mental nerve transection for the following reasons. Firstly, although the majority of the nerve cell bodies contained within the sections studied corresponded to the mental nerve, some would have belonged to the inferior alveolar nerve, the lingual nerve and the auriculotemporal nerve (Mazza and Dixon, 1972 ; Gregg and Dixon, 1973). There have been no published studies in the literature on the percentage of nerve cell bodies in the rat trigeminal ganglion corresponding to various peripheral branches of the mandibular nerve. This makes the accurate evaluation of cell loss difficult.

Secondly, the figure obtained would not be of much value in comparison with other studies because the amount of cell loss occurring in sensory ganglia following peripheral nerve transection varies considerably with the experimental model used. Lieberman (1971) in his excellent review of the axon reaction described three experimental

parameters that directly affect the degree of cell loss occurring within ganglia. These parameters being the severity of injury, the site of injury and the age of the animal.

The severity of the injury affects the degree of cell loss. The greater the injury to the peripheral nerve the more intense the degeneration and cell loss.

The age of the animal is also important because nerve cell death subsequent to nerve injury is more extensive in young rats than adult rats.

The intensity of the reaction also varies inversely with the distance of the injury from the nerve cell bodies. Thus cell loss is greater after lesions close to the parent nerve cell body than after injury more peripherally. Sunderland (1978) has related this to the amount of cytoplasm amputated from the cell.

The differences in the morphological response of the trigeminal ganglion to the two types of peripheral nerve injuries investigated in this study can be rationalized, at least in part, on the basis of the severity of the injuries. Carter et al. (1972) and Beazley et al. (1974) have demonstrated that a second degree nerve injury, according to Sunderland's classification, results from a cryolesion. This type of nerve injury refers to the occurrence of Wallerian degeneration in the axon fibre and myelin sheath but with minimal disruption to the perineurium, the epineurium and the endoneurium (Sunderland, 1951). In contrast, transection of a nerve results in a more advanced fifth degree

nerve injury with complete disruption of the neural connective tissue sheaths (Sunderland, 1951).

Lieberman (1971) proposed that the greater the injury to the peripheral nerve fibres the more intense the morphological alterations occurring within the ganglion. This concept was confirmed in the present study where not only was the retrograde neuronal reaction more extensive, but also irreverisble degenerative changes within nerve cells terminating with cell death and cell loss were observed in the more severe fifth degree injury of transection.

Barr and Hamilton (1948) found that the delay before the onset of recovery, and the duration for complete recovery from the retrograde neuronal reaction depends on the severity of the nerve injury. In the present study, the delay before the onset of recovery, and the time taken for recovery from the retrograde neuronal reaction, was greater in the case of the more severe nerve injury of transection.

The crucial factor to consider in the severity of nerve injuries is the preservation of the connective tissue architecture, in particular the endoneurial tubes (Carter et al., 1972). Following a cryoinjury to a peripheral nerve, axonal regeneration is facilitated by the preservation of the neural connective tissue sheaths. Transection of a peripheral nerve, however, results in disorganization of the neural connective tissue architecture consistent with a Sunderland fifth degree injury. This was found to adversely affect axonal regeneration because of the inevitable intraneural scarring and fibrosis which serves to divert and delay axonal regeneration (Hribar, 1978).

It has been stated by Cavanaugh (1951) and Engh et al. (1971) that the reversibility of the morphological responses of ganglia are directly related to the occurrence of successful peripheral axon regeneration. The results of the present study support this view, where reversible morphological changes were seen in the trigeminal ganglion following a Sunderland second degree injury (cryoinjury) to the mental nerve which facilitates peripheral axon regeneration. Conversely, both irreverisble and reversible changes were seen following a Sunderland fifth degree injury (transection) to the mental nerve where peripheral axon regeneration is less well directed (Figure 8.1).

8.2.3 Differential nerve cell response

The concept that the different nerve cell types may react differently to peripheral nerve injury is an important one. Ranson (1909) and Cavanaugh (1951) stated that it was amongst the small dark nerve cells of spinal ganglia that retrograde neuronal degeneration was more widespread following peripheral nerve lesions. In a concurring report Aldskogius and Arvidsson (1978) found that following infra-orbital nerve transection, the majority of trigeminal ganglion neurons exhibiting signs of degeneration were small.

Similarly, in the present study, the majority of the trigeminal ganglion nerve cells showing signs of retrograde neuronal reaction and neuronal degeneration were of the small dark variety. Although the overall pattern of the response was similar with respect to time, it was clearly evident that at each post-injury time interval the histological changes under investigation were generally more widespread within the small nerve cell population.

Aldskogius and Arvidsson (1978) stated that it was possible that large neurons degenerated more quickly than small neurons and therefore were harder to observe in a state of degeneration. The results of the present study does not support this proposal, because large and small nerve cells were found to be degenerating at similar rates (Figures 7.29, 7.30 and 7.32).

Conversely, Gregg (1974) and Galich et al. (1976) found that there was a marked tendency towards the large pale nerve cells of the trigeminal ganglion showing the effects of peripheral mandibular nerve transection. The report by Galich et al. (1976) was brief and it was not possible to assess their experimental method to determine their histological criteria and method of scoring. Gregg's report was based on a qualitative light microscopic study where it may well have been that the degenerative changes appeared more pronounced in the large cells because, based on their size their evaluation was easier.

It must be emphasized that at present, the question of a differential cell response with regard to cell size in the trigeminal ganglion is far from settled. Further research is required in this area to elucidate this important issue because it bears on the problem of pain mechanisms within the trigeminal ganglion.

8.2.4 Significance of results with respect to trigeminal neuralgia and pain mechanisms within the trigeminal system

Barnard (1980) suggested that all fibre types are damaged following cryoinjury, in contrast to earlier studies which pointed towards a selective destruction of large myelinated fibres sparing small nerve fibres (Denny-Brown et al., 1945 ; Whittaker, 1974). This latter effect

is disadvantageous because it would serve to intensify the pain by reducing large nerve fibre activity and result in an "open-gate" situation (Melzack and Wall, 1965).

The "gate-control" theory of Melzack and Wall appears to be particularly relevant to the modulation of pain in the trigeminal system and may be used to explain certain central mechanisms that may occur subsequent to peripheral nerve injury. Melzack and Wall made four basic proposals that were integral to their theory. Firstly, the substantia gelatinosa (S.G.) of the spinal cord functions as a gate - control system that modulates the afferent impulses before they influence the central transmission (T) cells in the dorsal horn. It is known that there is substantia gelatinosa in the nucleus caudalis of the spinal tract of the trigeminal nerve, and that the first synapses of the pain afferent system are located here. Secondly, there is a central control trigger which influences the modulating properties of the gate - control system. Thirdly, the T cells activate neural mechanisms which comprise the action system responsible for perception and response. Finally, pain phenomena are determined by interactions among the above three systems (Figure 8.2).

From a study of Figure 8.2, it can be seen that large and small nerve fibres project to the substantia gelatinosa and the T cells. The T cells are activated by large and small nerve fibres, and in turn project centrally to the action system giving rise to perception and response. Furthermore, the inhibitory effect exerted by the SG on the afferent fibre terminals to the T cells is increased by activity in the large fibres and decreased by activity in the small fibres. Thus pain perception can be affected in the synapse of the primary sensory neuron.

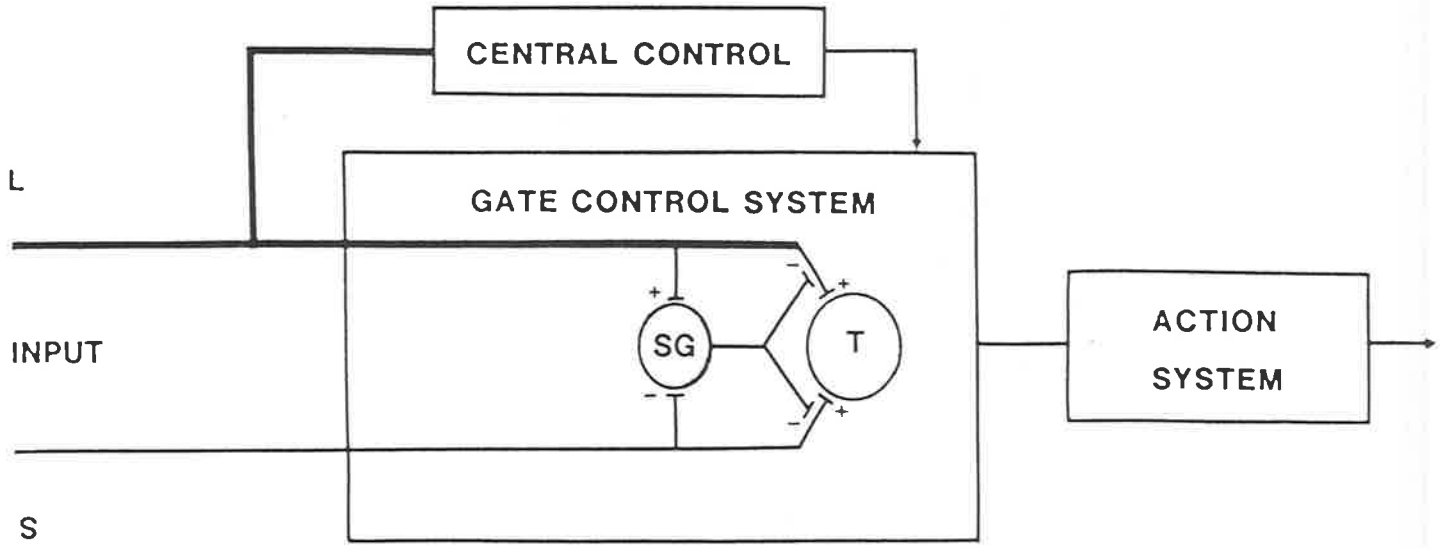


Figure 8.2: Schematic diagram of the gate-control theory (After Melzack and Wall, 1965).

Key:
L = Large - diameter fibres
S = Small - diameter fibres
S.G. = Substantia gelatinosa
T = Central transmission cells
\+ = Stimulation
\- = Inhibition

In the present study, small nerve cells which are connected to small nerve fibres (Martinez and Freide, 1970) appeared to be more widely affected in both the peripheral nerve transection and peripheral nerve cryoinjury series. Using the concept of the gate - control theory, one could postulate that the decreased activity in the small nerve fibres would result in an increased inhibitory effect of the S.G. on the afferent fibre terminals thereby "closing the gate" and reducing pain perception. Furthermore, a situation is produced where small nerve cells which are known to be the principal conductors of painful impulses, have a reduced influence on T cells.

The clinical observation that the pain of trigeminal neuralgia recurs following peripheral neurectomy (= peripheral nerve transection) (Quinn, 1965 ; Quinn and Weil, 1975) and peripheral cryoblockade (= peripheral nerve cryoinjury) (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Goss, 1984 ; Nally, 1984), albeit after an extended time ranging from months to years, is possibly related to the reversibility of the changes observed within the majority of the nerve cells in this study. With regard to the peripheral transection series, the presence of some permanent pathological changes in the trigeminal ganglion subsequent to cell degeneration and death, may also be a cause for the recurrence of a neuralgia-like pain (Gregg, 1971).

The reported ability of sensory function to return more rapidly than pain following peripheral nerve cryoinjury in trigeminal neuralgia (Lloyd et al., 1976 ; Barnard et al., 1978, 1981 ; Barnard, 1980 ; Goss, 1984 ; Nally, 1984), may be explained on the basis of the differential nerve cell response, and pain mechanisms within the trigeminal system. The results of this study show that the effects of peripheral nerve

cryoinjury are more widely manifested amongst the small nerve cells of the trigeminal ganglion, and affect the larger nerve cells to a lesser extent. Thus there are fewer small nerve cells which can conduct painful impulses centrally. Conversely, the larger nerve cells which are known to subserve the other sensory modalities are structurally and functionally less affected by peripheral nerve cryoinjury.

It is clear that the mechanisms by which peripheral nerve transection and peripheral nerve cryoinjury affect pain modulation within the trigeminal system are still open to question. Many of the concepts are speculative and further research in this field is required.

8.3 FURTHER AVENUES OF RESEARCH

This study was limited to light microscopic investigation of the morphological changes occurring in the trigeminal ganglion after peripheral nerve transection and peripheral nerve cryoinjury. The use of more detailed techniques such as electron microscopy (Aldskogius and Arvidsson, 1978), histochemistry (Brattgard et al., 1957), and autoradiography (Droz and Leblond, 1963) may reveal important anatomical and biochemical changes at the cellular level which could not be observed with the light microscope. These changes may demonstrate with more accuracy the central response to peripheral mental nerve transection and peripheral mental nerve cryoinjury.

CHAPTER IX

CONCLUSION

The central effects of peripheral mental nerve transection and peripheral mental nerve cryoinjury in the rat were compared. The present study has shown:-

General Findings

1. The Sprague Dawley strain of rats provided a satisfactory animal model for the investigation of light microscopic morphological changes occurring centrally within the trigeminal ganglion following peripheral nerve injuries.

2. Major morphological changes occurred in the trigeminal ganglion following both types of peripheral nerve injury to the mental branch of the mandibular division of the trigeminal nerve.

3. The scoring of light microscopic histological features showed a high level of reproducibility using a double determination procedure.

Specific Histological Findings

1. The trigeminal ganglion in the rat was composed of two distinct cell types: small darkly staining cells, and more numerous large pale cells. This finding is consistent with the majority of previously published reports.

2. The morphological responses of trigeminal ganglion nerve cells to peripheral mental nerve transection in this study are in general agreement with the literature.

3. There have been no previous reports concerning the morphological responses of trigeminal ganglion nerve cells to peripheral nerve cryoinjury. This study demonstrates that important differences exist between the effects of peripheral mental nerve transection and peripheral mental nerve cryoinjury on the cells of the trigeminal ganglion.

Comparison between Nerve Injuries

1. The pattern of the initial retrograde neuronal reaction consisting of chromatolysis and peripheral displacement of the nucleus was similar for both transection and cryoinjury. However, the retrograde neuronal reaction in the transection series was generally more intense, the onset of recovery delayed, and the recovery period prolonged.

2. In the transection series, irreversible morphological changes precedent to cell death were also observed. These changes were observed between one week and three months post-operatively. This finding is in agreement with the literature. These irreversible changes were not found in the trigeminal ganglia from the cryoinjury series.

3. In the transection series, both reversible and irreversible changes occurred within the trigeminal ganglion. Consequently,

the morphology of the trigeminal ganglion was never completely restored to normal. This finding is consistent with the literature. Conversely, in the cryoinjury series only reversible changes occurred with complete return to normal ganglion architecture.

4. The effects of both types of nerve injuries were more prevalent amongst the small nerve cell population. This is in agreement with the predominant view of the literature. However, contrary reports have been published in the literature and, as such, the issue of differential cell response remains controversial and further research is required in this area.

Thus, it would appear that the central effects following peripheral nerve cryoinjury are more consistent. This may well account for the more consistent clinical results obtained using this therapeutic modality in the management of trigeminal neuralgia.

The findings of the present study confirm and extend our knowledge about the central effects of peripheral nerve injuries. However, it is clear that the mechanisms by which peripheral nerve transection and peripheral nerve cryoinjury affect pain modulation within the trigeminal system remain largely speculative, and further research in this field is required.

APPENDIX

1. DIET

All animal used in this investigation were fed on a stock diet with the following composition:

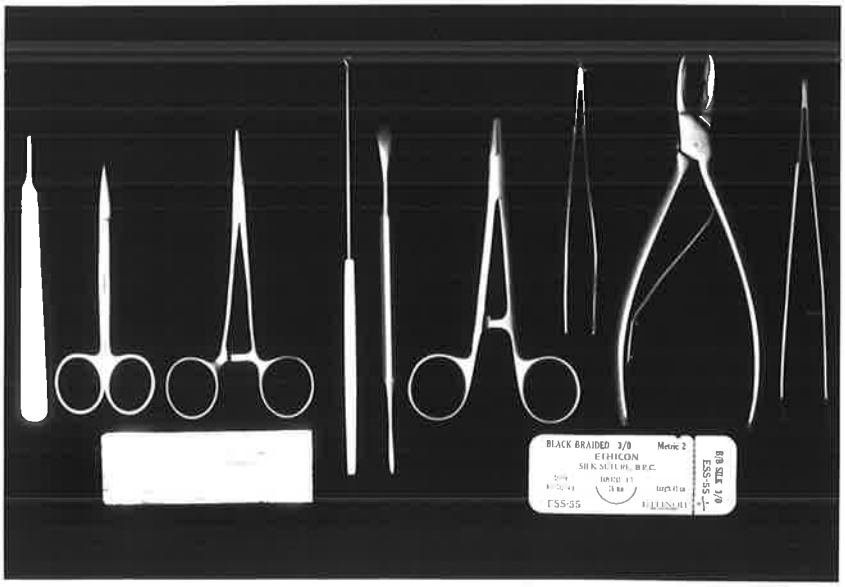
Diet:-

Ground wheat	40.0%
Ground barley	18.0%
Bran and pollard	12.0%
Meat and bone meal	9.6%
Extracts of soya meal	6.2%
Fish meal	6.2%
Milk powder	3.0%
Brewer's yeast	1.0%
Salt	1.0%
Molasses	3.0%

Vitamin supplement/kg of feed:-

Vit A_3	3928 I.U.
Vit D_2	928 I.U.
Vit B_6	1.5 mg
Vit B_{12}	0.2 mg
Vit B	3.4 mc gms
Vit E	1.2 mg
Vit K	0.5 mg
Pantothenic acid	0.5 mg
Choline chloride	25.0 mg

2. SURGICAL INSTRUMENTS



(i) Scalpel handle and No 15 scalpel blade

(ii) Sharp pointed, straight 4 inch scissors

(iii) Straight mosquito artery forceps

(iv) Gilles skin hook

(v) No. 7 wax spatula

(vi) Kilner needle holder

(vii) 3/0 black silk suture (Ethicon)

(viii) Adson's toothed tissue forceps

(ix) Side cutting bone rongeur

(x) College forceps

3. TISSUE FIXATIVE

F.E.C. fixative (HRIBAR 1977)

37-40% formalin, neutral	80 mls
50% ethyl alcohol	720 mls
chloral hydrate	38 gms

Each specimen was placed in a sealed container with 50 mls of the fixative for two weeks at room temperature.

4. PROCESSING AND EMBEDDING PROCEDURE

All tissue specimens passed through the following reagents:

at 37°C	(i)	Alcohol	70%	1 hour
	(ii)	Alcohol	80%	1 hour
	(iii)	Alcohol	90%	1 hour
	(iv)	Alcohol	95%	1 hour
	(v)	Absolute Alcohol		1 hour
	(vi)	Absolute Alcohol		1 hour
	(vii)	Absolute Alcohol		1 hour
	(viii)	Methyl Salicylate and Absolute Alcohol (50:50)		2 days
	(ix)	Methyl Salicylate and Celloidin 0.5%		2 days
	(x)	Celloidin 1%		2 days
at 60°C	(xi)	Wax and Methyl Salicylate (1:2)		1 hour
	(xii)	Wax and Methyl Salicylate (1:1)		1 hour
	(xiii)	Wax and Methyl Salicylate (2:1)		1 hour
at 56°C	(xiv)	Wax		1 hour
	(xv)	Wax		1 hour
	(xvi)	Wax		overnight

All specimens were then placed in a vacuum for one hour to eliminate air bubbles, and were subsequently blocked in wax at 56°C.

Sections were cut in a rotary microtome set at 6 microns and then mounted on numbered glass slides.

5. STAINING PROCEDURES

Haematoxylin and Eosin Stain

Method:

(i) Xylol 5 minutes
(ii) Xylol 5 minutes
(iii) Absolute alcohol 2 minutes
(iv) Absolute alcohol 2 minutes
(v) Rinse in tap water 5 minutes
(vi) Haematoxylin 5 minutes
(vii) Rinse in running tap water 5 minutes
(viii) Differentiate in 0.5 to 1.0% hydrochloric acid in 70% alcohol for 30 seconds
(ix) Rinse in running tap water for 10 minutes
(x) Eosin 45 seconds
(xi) Absolute alcohol 1 minute
(xii) Absolute alcohol 1 minute
(xiii) Absolute alcohol and xylol mix 2 minutes
(xiv) Xylol 2 minutes
(xv) Xylol 2 minutes
(xvi) Mount in Depex

Cresyl Violet Stain

Method:

(i) Rinse in distilled water 10 minues
(ii) Pre-heat *Cresyl fast violet stain to 60°C
(iii) Cresyl fast violet stain at 60°C 5 minutes
(iv) Rinse in 95% alcohol
(v) Differentiate in 95% alcohol - controlling staining under the microscope
(vi) Dehydrate in absolute alcohol 2 minutes
(vii) Xylol 2 minutes
(viii) Xylol 2 minutes
(ix) Mount in Depex

* Cresyl fast violet solution

0.5% aqueous cresyl fast violet 100 mls
glacial acetic acid 4 drops

6. HISTOLOGICAL PROFORMA WITH SPECIFIC EXAMPLE

POST-OPERATIVE INTERVAL: 2 WEEKS — SECTION NUMBER: 51
ANIMAL/GANGLION NUMBER: 1 — INJURY: TRANSECTION

CELL NUMBER
LARGE: 72
SMALL: 52
INDETERMINATE: 278
TOTAL: 402

HISTOLOGICAL FEATURES	LARGE CELLS ABSOLUTE	LARGE CELLS %	SMALL CELLS ABSOLUTE	SMALL CELLS %	COMBINED ABSOLUTE	COMBINED %	COMMENTS
Peripheral* displacement of nucleus	26	36.1%	36	69.2%	62	50.0%	
Chromatolysis	17	23.6%	34	65.4%	51	41.1%	
Cytoplasmic fragmentation	14	19.4%	8	15.4%	22	17.4%	
Nuclear fragmentation	2	2.8%	6	11.5%	8	6.5%	
Pericellular shrinkage/ atrophy — mod:	15	21.0%	17	32.7%	32	25.8%	
Pericellular shrinkage/ atrophy — sev:	9	12.5%	4	7.7%	13	10.4%	
Satellite cell clusters around degenerating neurons	6	8.3%	5	9.6%	11	8.9%	

* The calculation of the percentage of the large, small and combined cell population showing peripheral displacement of the nucleus is shown below:-

LARGE CELLS	SMALL CELLS	COMBINED
26/72 x 100/1 = 36.1%	36/52 x 100/1 = 69.2%	62/124 x 100/1 = 50.0%

7. PHOTOGRAPHIC FORMAT

The histological sections were photographed with the Leitz Orthoplan microscope and the Leitz Vario-Orthomat camera system.

BIBLIOGRAPHY

ADAMS, C.W.M., CSEJEEY, J., HALLPIKE, J.F. and BAYLISS, O.B. 1972.
Changes in the myellin proteins of the peripheral nerve undergoing Wallerian degeneration. Electrophoretic and microdensitometric observations.
J. Neurochem. 19 : 2043-2048.

ALDSKOGIUS, H. and ARVIDSSON, J. 1978.
Nerve cell degeneration and death in the trigeminal ganglion of the adult rat following peripheral nerve transection.
J. Neurocytol. 7 : 229-250.

ALLEN, W.F. 1924.
Localization in the ganglion semilunare in the cat.
J. Comp. Neurol. 38 : 1-25.

ANKER, A.H. 1984.
Healing of cryosurgical wounds.
Thesis submitted in part fulfilment for the Degree of Master of Dental Srugery.
University of Adelaide.

BARNARD, D. 1980.
The effects of extreme cold on sensory nerves.
Ann. R. Coll. Surg. Eng. 62 : 180-187.

BARNARD, J.D.W., LLOYD, J.W. and GLYNN, C.J. 1978.
Cryosurgery in the management of intractable facial pain.
Br. J. Oral. Surg. 16 : 135-142.

BARNARD, J.D.W., LLOYD, J.W. and EVANS, J. 1981.
Croyanalgesia in the management of chronic facial pain.
J. Max-fac. Surg. 9 : 101-102.

BARR, M.L. 1974.
The human nervous system : an anatomical viewpoint.
Second edition.
Harper Row : Maryland.

BARR, M.L. and HAMILTON, J.D. 1948.
A quantitative study of certain morphological changes in spinal motor neurons during axon reaction.
J. Comp. Neurol. 89 : 93-122.

BEAZLEY, R.M., BAGLEY, D.H. and KETCHAM, A.G. 1974.
The effect of cryosurgery on peripheral nerves.
J. Surg. Res. 16 : 231-234.

BEAUDREAU, D.E. and JERGE, C.R. 1968.
Somatotopic representation in the gasserian ganglion of tactile peripheral fields in the cat.
Archs. oral. Biol. 13 : 247-256.

BERNIER, A., TORVIK, A. and STENWIG, A.E. 1973.
Origin of macrophages in traumatic lesions and Wallerian degeneration in peripheral nerves.
Acta. Neuropath. 25 : 228-236.

BERNSTEIN, J.J. and GUTH, L. 1961.
Non selectivity in establishment of neuromuscular connections following nerve regeneration in the rat.
Expl. Neurol. 4 : 262-275.

BIELSCHOWSKY, M. and VAENTINE, B. 1922.
Die histologischen veranderungen in durchforen nervenstrecken.
J.F. Psychol. Neurol. 29 : 133-144.

BODIAN, D. and DZIEWIATKOWSKI, D. 1950.
The disposition of radioactive phosphorus in normal, as compared with regenerating and degenerating nervous tissue.
J. Cell. Comp. Physiol. 35 : 155-168.

BRADELY, W.G. and ASBURY, A.K. 1970.
Duration of synthesis phase in neurilemma cells in mouse sciatic nerve during degeneration.
Exp. Neurol. 26 : 275-282.

BRADELY, P.F. and FISHER, A.D. 1975.
The cryosurgery of bone. An experimental and clinical assessment.
Br. J. Oral. Surg. 13 : 111-127.

BRATTGARD, S.O., EDSTROM, J.E. and HYDEN, H. 1957.
The chemical changes in regenerating neurons.
J. Neurochem. 1 : 316-325.

CARMEL, P.W. and STEIN, B.M. 1969.
Cell changes in sensory ganglia following proximal and distal nerve section in the monkey.
J. Comp. Neurol. 135 : 145-166.

CARTER, D.C., LEE, P.W.R., GILL, W. and JOHNSON, R.J. 1972.
The effect of cryosurgery on peripheral nerve function.
Ann. R. Coll. Surg. Edin. 17 : 25-31.

CAVANAUGH, M.W. 1951.
Quantitative effects of the peripheral innervation area on nerves and spinal ganglion cells.
J. Comp. Neurol. 94 : 181-219.

CHOUKAS, N.C., TOTO, P.D. and NOLAN, R.E. 1974.
A histologic study of the regeneration of the inferior alveolar nerve.
J. Oral. Surg. 32 : 347-352.

COOPER, I.S. 1963.
Cryogenic surgery : a new method of destruction or extirpation of benign or malignant tissues.
N. Eng. J. Med. 268 : 743-749.

CRAGG, B.G. and THOMAS, P.K. 1961.
Changes in conduction velocity and fibre size proximal to peripheral nerve lesions.
J. Physiol. 157 : 315-327.

CRAGG, B.G. and THOMAS, P.K. 1964.
The conduction velocity of regenerated peripheral nerve fibres.
J. Physiol. 171 : 164-175.

DARIAN-SMITH, I., MUTTON, P. and PROCTOR, R. 1965.
Functional organization of tactile cutaneous afferents within the semilunar ganglion and trigeminal spinal tract of the cat.
J. Neurophysiol. 28 : 682-694.

DAVENPORT, H.A., CHOR, H. and DOLKART, R.E. 1937.
The ratio of myelinated to unmyelinated fibres in regenerated sciatic nerves of the macacus rhesus.
J. Comp. Neurol. 67 : 483-491.

DAVENPORT, H.A., CHOR, H. and CLEVELAND, D.A. 1939.
Fibre ratios in regenerating nerves : II. The status of regrowth of the sciatic and nerves to the gastrocnemius in macacus 3 months after section.
J. Comp. Neurol. 70 : 153-162.

DAVISON, M.H.A. 1959.
The evolution of anaesthesia.
Br. J. Anaes. 31 : 134-143.

DENNY-BROWN, D. and BRENNER, C. 1944.
The effect of percussion of nerve.
J. Neurol. Neurosurg. Psychiat. 7 : 76-95.

DENNY-BROWN, D., ADAMS, R.D., BRENNER, C. and DOHERTY, M.M. 1945.
The pathology of injury to nerve induced by cold.
J. Exp. Neuropath. Exp. Neurol. 4 : 305-312.

DIXON, A.D. 1963.
Fine structure of nerve cell bodies and satellite cells in the trigeminal ganglion.
J. Dent. Res. 42 : 990-999.

DOUGLAS, W.W. and MALCOLM, J.L. 1955.
The effect of localized cooling on conduction in cat nerves.
J. Physiol. 130 : 53-71.

DROZ, B. and LeBLOND, C.P. 1963.
Axonal migration of proteins in the central nervous system and peripheral nerves as shown by radioautography.
J. Comp. Neurol. 121 : 325-346.

ENGH, C.A. and SCHOFIELD, B.H. 1972.
A review of the central response to peripheral nerve injury and its significance in nerve regeneration.
J. Neurosurg. 37 : 195-203.

ENGH, C.A., SCHOFIELD, B.H. and DOTY, S.B. 1971.
Perikaryal synthetic function following reversible and irreversible peripheral axon injuries as shown by autoradiography.
J. Comp. Neurol. 142 : 465-480.

EVANS, D.H.L. and MURRAY, J.G. 1954.
Regeneration of non-medullated nerve fibres.
J. Anat. 88 : 465-480.

FAZIO, M., AIROLDI, M., NEGRI, L., MARCHESA, P. and GANDOLFO, S. 1984.
Specific immunological stimulation induced by cryosurgery in patients with squamous-cell carcinoma of the oral cavity.
J. Max-fac. Surg. 12 : 153-156.

FRASER, J. and GILL, W. 1967.
Observations on ultrafrozen tissue.
Br. J. Surg. 54 : 770-776.

GALICH, J.W., GREGG, J.M. and DUNCAN, G.H. 1976.
Quantitative study of trigeminal ganglion response to peripheral nerve trauma.
J. Dent. Res. 55. 190 (Abstract).

GASTER, R.N., DAVIDSON, T.M., RAND, R.W. and FONKALSRUD, E.W. 1971.
Comparison of nerve regeneration rates following controlled freezing or crushing.
Arch. Surg. 103 : 378-383.

GERSH, I. and BODIAN, D. 1943.
Some chemical mechanisms in chromatolysis.
J. Cell. Comp. Physiol. 21 : 253-279.

GILL, W., FRASER, J. and CARTER, D.C. 1968.
Repeated freeze-thaw cycles in cryosurgery.
Nature. 219 : 410-413.

GILL, W., FRASER, J., DaCOSTA, J. and BEAZLEY, R. 1970.
The cryosurgical lesion.
Amer. Surg. 36 : 437-445.

GIRARD, K.R. 1979.
Considerations in the management of damage to the mandibular nerve.
J. Am. Dent. Assoc. 98 : 65-71.

GOBEL, S. and BINCK, J.M. 1977.
Degenerative changes in primary trigeminal axons and in nucleus caudalis following pulp extirpations in the cat.
Brain Research. 132 : 347-354.

GOSS, A.N. 1984.
Peripheral cryoneurotomy in the treatment of trigeminal neuralgia.
Aust. Dent. J. 29 : 222-224.

GRANT, G. and ARVIDSSON, J. 1975.
Transganglionic degeneration in trigeminal primary sensory neurons.
Brain Research. 95 : 265-279.

GREGG, J.M. 1971.
Posttraumatic pain : experimental trigeminal neuropathy.
J. Oral. Surg. 29 : 260-267.

GREGG, J.M. and DIXON, A.D. 1973.
Somatotopic organization of the trigeminal ganglion in the rat.
Archs. Oral. Biol. 18 : 487-498.

GUTH, L. 1956.
Regeneration in the mammalian peripheral nervous system.
Physiol. Reviews. 36 : 441-478.

HAUSAMEN, J.E., SAMII, M. and SCHMIDSEDER, R. 1974.
Indication and technique for the reconstruction of nerve defects in head and neck.
J. Max-fac. Surg. 2 : 159-167.

HISCOE, H.B. 1947.
Distribution of nodes and incisures in normal and degenerated nerve fibres.
Anat. Rec. 99 : 447-475.

HRIBAR, D.L.A. 1977.
Comparison of F.E.C. with established neural fixatives.
International Association for Dental Research, Australian and New Zealand Division, 17th Annual Meeting, p. 11.

HRIBAR, D.L.A. 1978.
Response of a sectioned mental nerve.
Thesis submitted in part fulfilment for the Degree of Master of Dental Surgery.
University of Adelaide.

IMAMOTO, K. 1972.
Histochemical changes in the mesencephalic nucleus and motor nucleus following neurotomy of the third division of the trigeminal nerve.
Archivum histologicum japonicum. 34 : 19-33.

JOHNSON, A.C., McNABB, A.R. and ROSSITER, R.J. 1950.
Chemistry of Wallerian degeneration.
Archs. Neurol. Psychiat. 64 : 105-121.

JOLLY, M. 1976.
Cryosurgery.
Aust. Dent. J. 21 : 35-47.

JURECKA, W., AMMERER, H.P. and LASSMANN, H. 1975.
Regeneration of a transected peripheral nerve. An autoradiographic and electronmicroscopic study.
Acta. Neuropath. 32 : 299-312.

KATZ, J., NELSON, W., FOREST, R. and BRUCE, D.L. 1980.
Cryoanalgesia for post thoractomy pain.
Lancet. i : 512-513.

KERR, F.W.L. and LYSAK, W.R. 1964.
Somatotopic organization of trigeminal ganglion neurons.
Arch. Neurol. 11 : 593-602.

KIRKPATRICK, J.B. 1968.
Chromatolysis in the hypoglossal nucleus of the rat : an electron microscope analysis.
J. Comp. Neurol. 132 : 189-212.

LANDAU, W.M. 1953.
The duration of neuromuscular function after nerve section in man.
J. Neurosurg. 10 : 64-68.

LEE, J.C.Y. 1963.
Electron microscopy of Wallerian degeneration.
J. Comp. Neurol. 120 : 65-80.

LEECH, R.W. 1967.
Changes in satellite cells of rat dorsal root ganglia during central chromatolysis. An electron microscope study.
Neurology. 17 : 349-358.

LEHMAN, G. and KNOEFFEL, P.K. 1938.
Trichloroethanol, Tribromethanol, Chloral Hydrate and Bromal Hydrate.
J. Pharm. Exp. Ther. 63 : 453-465.

LENDE, R.A. and POULOS, D.A. 1970.
Functional localization in the trigeminal ganglion in the monkey.
J. Neurosurg. 32 : 336-343.

LEOPARD, P.J. 1975.
Cryosurgery, and its application to oral surgery.
Br. J. Oral. Surg. 13 : 128-152.

LIEBERMAN, A.R. 1971.
The axon reaction : a review of the principal features of perikaryal respones to axon injury.
Int. Rev. Neurobiol. 14 : 49-124.

LLOYD, J.W., BARNARD, J.D.W. and GLYNN, C.J. 1976.
Cryoanalgesia - a new approach to pain relief.
Lancet ii. 932-934.

LOGAN, J.E., ROSSITER, R.J. and BARR, M.L. 1953.
Cellular proliferation in the proximal segment of a sectioned or crushed peripheral nerve.
J. Anat. 87 : 419-422.

LUBINSKA, L. 1952.
The influence of the rate of the peripheral stump on the early stages of nerve regeneration.
Acta. Biol. Exp. 16 : 55-63.

LUBINSKA, L. 1961.
Demyelination and remyelination in the proximal parts of regenerating nerve fibres.
J. Comp. Neurol. 117 : 275-290.

MACKEY, E.A., SPIRO, D. and WIENER, J. 1964.
A study of chromatolysis in dorsal root ganglia at the cellular level.
J. Neuropath. Exp. Neurol. 23 : 508-526.

MARTINEZ, A.J. and FRIEDE, R.L. 1970.
Changes in nerve cell bodies during the myelination of their axons.
J. Comp. Neurol. 138 : 329-338.

MATSUURA, H., MORI, M. and KAWAKATSU, K. 1969.
A histochemical and electron-microscopic study of the trigeminal ganglion of the rat.
Archs. Oral. Biol. 14 : 1135-1146.

MAXWELL, A.E. 1970.
Basic statistics in behavioural research.
Penguin Books : Middlesex.

MAZZA, J.P. and DIXON, A.D. 1972.
A histological study of chromatolytic cell groups in the trigeminal ganglion of the rat.
Archs. Oral. Biol. 17 : 337-387.

MEI LIU, H. 1974.
Schwann cell properties II. The identity of phagocytes in the degenerating nerve.
Am. J. Pathol. 75 : 395-416.

MELZACK, R. and WALL, P.D. 1965.
Pain mechanisms : a new theory.
Science. 150 : 971-979.

MERYMAN, H.T. 1956.
Mechanics of freezing in living cells and tissues.
Science. 124 : 515-521.

MOSES, H.L. 1967.
Comparative fine structure of the trigeminal ganglia, including human autopsy studies.
J. Neurosurg. 26 : 112-126.

MOZSARY, P.G., MIDDLETON, R.A. and SZABO, Z. 1982.
Microsurgical treatment of traumatic neuroma of the inferior alveolar nerve.
J. Oral. Maxillofac. Surg. 40 : 446-448.

MOZSARY, P.G. and MIDDLETON, R.A. 1983.
Microsurgical reconstruction of the infraorbital nerves.
J. Oral Maxillofac. Surg. 41 : 697-700.

MOZSARY, P.G. and MIDDLETON, R.A. 1984.
Microsurgical reconstruction of the lingual nerve.
J. Oral and Maxillofac. Surg. 42 : 415-420.

NALLY, F.F. 1984.
A 22-year study of paroxysmal trigeminal neuralgia in 211 patients with a 3-year appraisal of the role of cryotherapy.
Oral Surg. 58 : 17-23.

NATHAN, P.W., SEARS, T.A. and SMITH, M.C. 1965.
Effect of phenol solutions on nerve roots of the cat : an electrophysiological and histological study.
J. Neuro. Sci. 2 : 7-15.

NEEL, H.B. 1980.
Cryosurgery for the treatment of cancer.
Laryngoscope. 90 : 1-48.

NEEL, H.B., KETCHAM, A.S. and HAMMOND, W.G. 1971.
Requisites for successful cryogenic surgery of cancer.
Arch. Surg. 102 : 45-48.

NELSON, K.M., VINCENT, R.G., BOURKE, R.S., SMITH, D.E., BLAKELEY, W.R., KAPLAN, R.J. and POLLAY, M. 1974.
Intraoperative intercostal nerve freezing to prevent post thoracotomy pain.
Ann. Thoracic. Surg. 18 : 280-284.

NISSL, F. 1892.
Uber die veranderungen der ganglienzeuen am facialis kern der kaninchen nach austreissung der nerven.
Allg. Z. Psychiat. 48 : 197-198.

OLSSON, Y. and SJOSTRAND, J. 1969.
Origin of macrophages in Wallerian degeneration of peripheral nerves demonstrated autoradiographically.
Exp. Neurol. 23 : 102-112.

PEACH, R. 1972.
Fine structural features of light and dark cells in the trigeminal ganglion of the rat.
J. Neurocytol. 1 : 151-160.

PINEDA, A., MAXWELL, D.S. and KRUGER, L. 1967.
The fine structure of neurons and satellite cells in the trigeminal ganglion of the cat and monkey.
Am. J. Anat. 121 : 461-488.

POSWILLO, D.E. 1971.
A comparative study of the effects of electrosurgery and cryosurgery in the management of benign lesions.
Br. J. oral. Surg. 9 : 1-7.

POSWILLO, D.E. 1973.
Cryosurgery in and around the oral cavity.
In, Recent advances in surgery.
Edit. Taylor, S.
Churchll-Livingston : London.

QUINN, J.H. 1965.
Repetitive peripheral neurectomies for neuralgias of the second and third divisions of the trigeminal nerve.
J. Oral. Surg. 23 : 600-608.

QUINN, J.H. and WEIL, T. 1975.
Trigeminal neuralgia : treatment by repetitive peripheral neurectomy.
Supplemental report.
J. Oral. Surg. 33 : 591-595.

RANSON, S.W. 1909.
Alterations in the spinal ganglion cells following neurotomy.
J. Comp. Neurol. 19 : 125-153.

ROBBINS, S.L. 1979.
Pathology.
Third edition.
W.B. Saunders Co. : Philadelphia.

ROOD, J.M. 1983.
Degrees of injury to the inferior alveolar nerve sustained during the removal of impacted mandibular third molars by the lingual split technique.
Br. J. Oral. Surg. 21 : 103-116.

SCOTT, D. Jr., GUTMAN, E. and HORSKY, P. 1968.
Regeneration in spinal neurons : proteosynthesis following nerve growth factor administration.
Science. 152 : 787-788.

SEDDON, H.J. 1943.
Three types of nerve injury.
Brain. 66 : 237-288.

SHAWE, G.D.H. 1955.
The number of branches formed by regenerating nerve fibres.
Br. J. Surg. 42 : 474-488.

STENWIG, A.E. 1972.
The origin of brain macrophages in traumatic lesions, Wallerian degeneration, and retrograde degeneration.
J. Neuropath. Exp. Neurol. 31 : 696-704.

STRASSBURG, M. 1967.
Morphologic reaction of the trigeminal ganglion after experimental surgery on the maxillodental region.
J. Oral. Surg. 25 : 107-114.

SUGAR, O. 1938.
The non-centrifugal degeneration of severed peripheral nerve.
J. Neurophysiol. 1 : 7-15.

SUNDERLAND, S. 1951.
A classification of peripheral nerve injuries producing loss of function.
Brain. 74 : 491-516.

SUNDERLAND, S. 1978.
Nerves and nerve injuries.
Second edition.
Churchill-Livingston : Edinburgh.

SUNDERLAND, S. and BRADLEY, K.C. 1950a.
Endoneurial tube shrinkage in the distal segment of a severed nerve.
J. Comp. Neurol. 93 : 411-420.

SUNDERLAND, S. adn BRADLEY, K.C. 1950b.
Denervation atrophy of the distal stump of a severed nerve.
J. Comp. Neurol. 93 : 401-410.

THOMAS, G.A. 1948.
Quantitative histology of Wallerian degeneration. II. Nuclear population in two nerves of different fibre spectrum.
J. Anat. 82 : 135-145.

THOMAS, P.K. 1964.
Changes in the endoneurial sheaths of peripheral myelinated nerve fibres during Wallerian degeneration.
J. Anat. 98 : 175-182.

THOMAS, P.K. and KING, R.H.M. 1974.
The degeneration of unmyelinated axons following nerve section ; an ultrastructural study.
J. Neurocytol. 3 : 497-512.

WALL, P.D. and GUTNICK, M. 1974.
Properties of afferent nerve impulse originating from a neuroma.
Nature. 248 : 740-743.

WATSON, W.E. 1965.
An autoradiographic study of the incorporation of nucleic-acid precursors by neurons and glia during nerve regeneration.
J. Physiol. 180 : 741-753.

WATT, M.J., AKHTAR, M. and ATKINSON, R.S. 1970.
Clinical comparison of bupivicaine and amethocaine. Onset and duration in extradural blockade.
Anaes. 25 : 24-29.

WEDDELL, G. 1942.
Axonal regeneration in cutaneous nerve plexuses.
J. Anat. 77 : 49-62.

WEDDELL, G. and GLEES, P. 1941.
The early stages in the degeneration of cutaneous nerve fibres.
J. Anat. 76 : 65-93.

WEISS, P. and TAYLOR, A.C. 1944.
Impairment of growth and myelination in regenerating nerve fibres subject to constriction.
Proc. Soc. Exp. Biol. Med. 55 : 77-80.

WESSBERG, G.A., WOLFORD, L.M. and EPKER, B.N. 1982.
Experiences with microsurgical reconstruction of the inferior alveolar nerve.
J. Oral. Maxillofac. Surg. 40 : 651-655.

WESTRUM, L.E., CANFIELD, R.C. and BLACK, R.G. 1976.
Transganglionic degeneration in the spinal trigeminal nucleus following removal of tooth pulps in adult cats.
Brain Research. 101 : 137-140.

WHITTAKER, D.K. 1972.
Cryosurgery of the oral mucosa : a study of the mechanisms of tissue damage.
Dent. Pract. 22 : 445-451.

WHITTAKER, D.K. 1974.
Degeneration and regeneration of nerves following cryosurgery.
Br. J. Exp. Path. 55 : 595-600.

WILLIAMS, P.L. and HALL, S.M. 1971.
Chronic Wallerian degeneration - an *in vivo* and ultrastructural study.
J. Anat. 109 : 487-503.

YOUNG, J.Z. 1945.
The history of the shape of a nerve fibre.
Essays on growth 2nd form, ed. Le Gros Clark, W., Medawar, P.B.
Oxford Clarendon Press 41-94.