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British Journal of Oral and Maxillofacial Surgery 57 (2019) 341–344

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Effect of peripheral cryoneurotomy and nerve transection on the trigeminal ganglion in rats

R. D'Rozario [a,b], K. Ito [c], A.N. Goss [c,*]

[a] *Oral & Maxillofacial Surgery Unit, Royal Adelaide Hospital, The University of Adelaide, Adelaide, Australia*
[b] *33 Thames St., West Woolongong, NSW 2500, Australia*
[c] *Oral & Maxillofacial Surgery, The University of Adelaide, Adelaide, Australia*

Accepted 3 November 2018
Available online 2 April 2019

Abstract

In this experimental study, we did peripheral neurectomyand peripheral cryoneurotomy of the mental nerve in rats and histologically assessed their effects on the trigeminal ganglion at timed intervals for six months. There were marked irreversible changes in the neurectomy group whereas the cryoneurotomy group made a full recovery. These results help to explain the differing effects of these procedures on trigeminal neuralgia.

Keywords: Cryoneurotomy; nerve transection; gasserian ganglion; rats; experimental study

Introduction

Peripheral cryoneurotomy of branches of the trigeminal nerve has resulted in good pain control for several years in patients with intractable trigeminal neuralgia.[1–4] On the other hand, peripheral neurectomy results in a short-term benefit only, and is often followed by severe dysaesthetic pain.[5–7] The reasons for this are poorly understood although they probably relate to the severity of damage to the peripheral nerve. Cryoneurotomy does not directly damage the structural integrity of the nerve whereas neurectomy amputates part of it.

Most research relates to the effect of change at the site of injury, and in the peripheral nerve that is directly distal to it.[8–10] We know of some experimental evidence of the effects of nerve transaction centrally on the Gasserian ganglion,[11,12] but know of none on the effect of cryoneurotomy centrally.

In this experimental study we have compared the histological effects of transection and cryoneurotomy on the trigeminal ganglion in rats over time.

Material and methods

We divided 93 male Sprague Dawley rats one month old into three groups: transection (n = 42); cryoneurotomy (n = 42), and control (n = 6). Under general anaesthesia with chloral hydrate, we exposed the left mental nerve through a submandibular incision. The nerve was transected as it emerged from the mental foramen in 42, and in a further 42 the nerve was cryofrozen with a nitrous oxide cryoprobe for 90 seconds twice. The remaining six had a submandibular incision and

* Corresponding author at: Oral & Maxillofacial Surgery Unit, Faculty of Health Sciences, The University of Adelaide, Adelaide, South Australia 5005, Australia. Tel.: +61 8 8303 5103; Fax: +61 8 8303 4402.
E-mail address: alastair.goss@adelaide.edu.au (A.N. Goss).

https://doi.org/10.1016/j.bjoms.2018.11.023

Table 1
Histological features by time,feature, and procedure in transection and cryoneurotomy groups (n = 42 in each).

Time	Histological feature											
	Chromatoloysis		Peripheral cell displacement		Cytoplasmic fragmentation		Nuclear fragmentation		Pericellular shrinkage		Satellite cell clusters	
	Trans	Cryo	Trans	Cryo	Trans	Cryo	Trans	Cryo	Trans	Cryo	Trans	Cryo
Day 1	3.4	2	2	1.3	0	0	0	0	0	0	0	0
Day 3	10	14.3	6.7	11.3	0	0	0	0	0	0	0	0
1 week	14.6	18.5	16.4	15.5	2.5	0	0	0	12	0	0	0
2 weeks	17.6	7.6	19.7	6.7	8	0	3.4	0	16.8	0	4.2	0
1 month	3.4	2.5	7.1	2	4.6	0	1.7	0	25.6	0	5	0
3 months	.4	.8	.8	.8	.4	0	0	0	24.4	0	.4	0
6 months	.4	0	.8	0	.8	0	0	0	22	0	0	0

exposure of the mental nerve but no operation. The wounds were then closed.

Animals in groups of six were killed at one day, three days, one week, two weeks, one month, three months, and six months. At these times the experimental animals were anaesthetised, and the heart exposed by an incision from the sternal notch to the abdomen to open the thoracic cavity. A cannula was placed in the left ventricle and initially perfused with Ringer's solution. The superior vena cava was cut transversally and once blood flow was replaced by the solution, perfusion with 100 ml of FEC (faecal egg counts) fixative began.[13]

The skin over the head was then incised, and the skull and upper cervical vertebrae split in the midsagittal plane to expose the brain and brainstem. These were then dissected out to expose the trigeminal ganglia. The left trigeminal ganglion was carefully dissected out with 3 mm of all three divisions attached. These were then placed on a flat card maintaining the orientation. The specimen was then immediately immersed in FEC fixative for two weeks.

Each specimen was then double paraffin embedded. Serial sections 6 μm thick were obtained, and every tenth slide stained with haematoxylin and eosin. These were used for location and orientation of the specimens. Every ninth slide was stained with cresyl violet to show the cytoplasmic Nissl granules in the cell body of the neurons.

Each slide was histologically examined using a light microscope (Olympus BH) at 100 to 400 magnification. The mental nerve cells were found concentrated in the posterior lateral protuberance across the base of the mandibular division.

Specimens were examined for chromatolysis (dispersion of Nissl granules into finer particles toward the periphery of the cells, with the remainder of the cytoplasmas being relatively clear of granular material), peripheral displacement of the nucleus, cytoplasmic fragmentation, nuclear fragmentation, pericellular shrinkage, and clusters of satellite cells.

Each feature was scored from 0 to 2 (0 = no change, 1 = moderate change, and 2 = severe change). Scoring was against standard photographs that showed each feature and the percentage of cells affected.

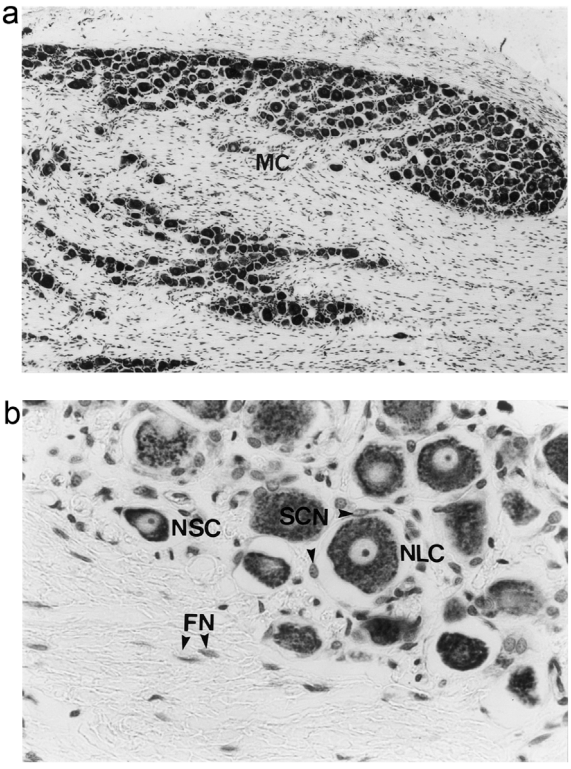

Fig. 1. (a). Normal trigeminal ganglion showing the mental nerve cell bodies (MC) (cresyl violet, original magnification x 100). (b). Normal trigeminal ganglion showing the mental nerve cells (NLC = normal large cell; NSC = normal small cell; SCN = satellite nucleus; FN = fibroblast nucleus) (cresyl violet, original magnification x 400).

A double determination from a randomly selected 20% sample was done two months after the initial score, and representative sections photographed.

Results

The key histological changes are shown in Figs. 1 and 2. The overall frequency of chromatolysis and peripheral nuclear displacement were highest in the transected group (Table 1). The peak of these changes was one week earlier in the cryoinjury group, which also recovered more rapidly. This group did not show degeneration of the nerve cells and by three

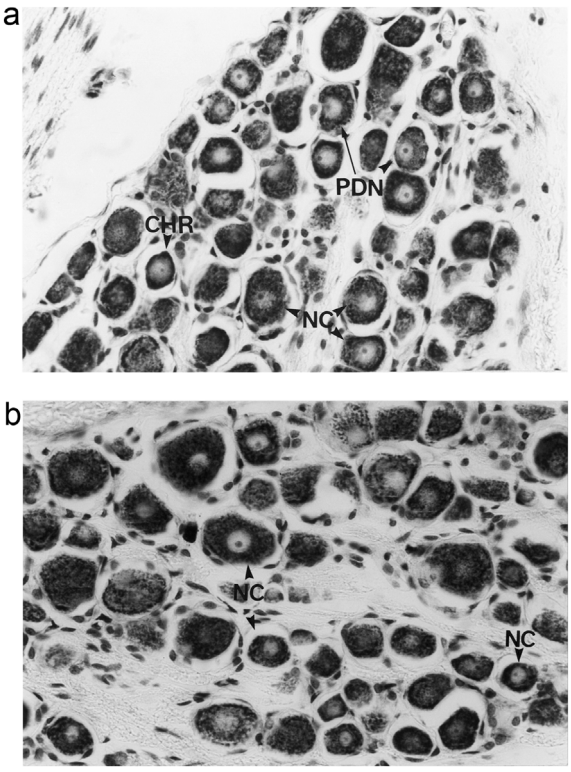

Fig. 2. (a). Trigeminal ganglion six months after transection showing pericellular shrinkage and loss of cells. (b). Trigeminal ganglion six months after cryoneurotomy. Cells similar to those of controls (cresyl violet, original magnification x 320).

Table 2
Mean nerve cell counts: transaction compared with cryoinjury. Group Mean nerve Mean nerve cell count cell difference.

Group	Mean nerve cell count	Mean nerve cell difference
Control	511	–
Transection	371	140
Cryoinjury	483	28

months, recovery was almost complete. In comparison, the transected group showed ongoing nuclear and cytoplasmic fragmentation, which was most prominent at two weeks, and the trigeminal ganglion never returned to normal, whereas in the cryoinjury group it did. Table 1 shows the histological changes by type over time between the two procedures. The double determinations showed a median (range) reliability of 5% (1.2% - 10.5%). Differences in small cells were greater than in large cells.

These irreversible changes in the ganglion in the transected group are further shown by the permanent reduction in cell count in the ganglion compared with the cryoinjury group (Table 2).

Discussion

This study showed important differences between the groups. Peripheral cryoneurotomy resulted in degenerative changes in the trigeminal ganglion that rapidly recovered. This change has been suspected but not shown experimentally, and it is noteworthy that the most extensive changes occurred in the small cell population. This may explain why sensation returns after cryoneurotomy earlier than pain, as the smaller nerve cells are associated more with its transmission.[1–3]

Transection of the mental nerve showed irreversible changes, and this finding is consistent with similar studies on sectioning of the infraorbital nerve (Galich JW, et al Quantitative study of the trigeminal ganglion response to peripheral nerve trauma. Paper presented at the IADR meeting in San Francisco, 1976).[12] One of the key differences between transection and cryoinjury is the extent of the injury. Transection results in full-thickness disruption of the peripheral nerve, which is consistent with a Sunderland fifth-degree injury, whereas with cryoinjury the structural integrity of the nerve is maintained. This is consistent with a Sunderland second-degree injury.[14]

This experiment was done with appropriate numbers of animals that were treated in a standard fashion to minimise experimental error. Young male animals were used, as they show the most extensive degenerative neural changes.[15] All animals had the brain infused with a specific nerve fixative followed by prolonged fixation to minimise any artifactual change.[13] The histological specimens were analysed in a standard fashion using both qualitative and quantitative methods.

In conclusion, this experimental study helps to explain why peripheral cryoneurotomy produces good pain control for trigeminal neuralgia without nerve damage and also why the symptoms eventually recur. Recurrence again responds to appropriate antineuralgic medications or further cryoneurotomy. Peripheral transection results in permanent changes in the ganglion, which helps to explain why there is an initial reduction in pain followed by irreversible dysaesthesia.[4]

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

Neither were applicable.

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