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Cell counts in the trigeminal ganglion of the cat after inferior alveolar nerve injuries

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INTRODUCTION

This paper describes neuronal changes in the trigeminal ganglion following lesions of one of its peripheral branches. Previous studies have shown that damage to trigeminal nerve branches gives rise to central changes in both the ganglion (Strassburg, 1967; Aldskogius & Arvidsson, 1978) and the nucleus (Guttman & Medawar, 1942; Grant & Arvidsson, 1975; Gobel & Binck, 1977). In some circumstances these changes seem to lead to neuronal death. Aldskogius & Arvidsson (1978) found that infra-orbital nerve section in the rat caused a 14% reduction in the number of trigeminal ganglion cells on the operated side. The percentage loss of neurons supplying the infra-orbital nerve would presumably be much higher. Reductions in ganglion cell numbers have also been noted after injuries to other nerves (Aldskogius, Arvidsson & Grant, 1985). Losses of 35% of cells were found in thoracic sensory ganglia examined up to 180 days after sectioning intercostal nerves and sciatic nerve section resulted in comparable cell loss in lumbar dorsal root ganglia. This loss of cells is surprising in view of the results of Sugimoto & Gobel (1982) who found that after transecting and blocking regeneration of the superficial radial nerve, primary neurons of all sizes survived and their central arborisations maintained normal topography, synaptic vesicles and connections and they were capable of transporting horseradish peroxidase transganglionically. In addition, counts of the number of myelinated axons in the proximal stumps of transected nerves have shown that the whole original population of myelinated fibres persists (Horch & Lisney, 1981; Waite, 1984). Studies by ourselves (Holland & Robinson, 1987) and others (Fried & Erdelyi, 1982; Berger & Byers, 1983) have shown that, after sectioning the inferior alveolar nerve, reinnervation by regenerating fibres is extensive, suggesting that there is either little cell death in the trigeminal ganglion or extensive branching of fibres at the point of nerve section. In view of the controversy concerning the extent of the cell loss, we have counted the number of cells in the trigeminal ganglion following inferior alveolar nerve section using methods very similar to those used by Aldskogius & Arvidsson (1978). In an attempt to maximise the changes which may occur, in some of these experiments nerve regeneration was prevented.

MATERIALS AND METHODS

Twelve adult cats, age 8–16 months at the time of histological examination, were used in this investigation. At each stage of each experiment the animals were

anaesthetised with a mixture of alphaxalone and alphadolone acetate (Saffan, Glaxo Laboratories; induction: 18 mg/kg i.m, maintenance; 2 mg/kg i.v). In nine of the animals, using aseptic conditions, the left inferior alveolar nerve was exposed in the mandibular canal, just distal to the mandibular foramen, by displacing the masseter muscle posteriorly and removing bone from the ramus of the mandible. The nerve, which at this point consists of either one or two fascicles, was sectioned, care being taken not to disrupt the adjacent blood vessels. In 3 of these animals the cut nerve ends were reapposed and the wound closed. In the other 6, extensive steps were taken to prevent regeneration. As merely inserting the central stump inside a sealed tube is inadequate (Waite, 1984) the central stump of the nerve was soaked for approximately one minute with formaldehyde coloured with methylene blue on a pledget of cotton wool (Guttman & Medawar, 1942). The epineurial sheath was then drawn over the end of the nerve, ligated, and the stump was sealed inside a nylon tube (Wall & Gutnick, 1974). The animals were allowed to recover and 15 weeks later they were reanesthetised and perfused through the carotid arteries with a fixative mixture. Before the perfusion was begun, the cervical sympathetic trunk was sectioned bilaterally to remove any neurally mediated vasoconstriction. The aorta was clamped and the external jugular veins were cut to maximise the flow of fixative and allow for its efflux. The fixative mixture consisted initially of a prewash of 300 mosmol phosphate buffer containing 2·7% dextran T-40 (Pharmacia Fine Chemicals, Sweden), followed by a similar mixture to which 5% glutaraldehyde had been added. The three remaining unoperated animals were perfused in a similar manner to act as controls. The head was postfixed overnight in 5% buffered glutaraldehyde without dextran and the trigeminal ganglia were then removed, embedded in paraffin and serially sectioned at 5 μm. Sections were stained with cresyl violet to reveal the neuronal nucleoli as darkly staining bodies within a pale nucleus. The number of cells per ganglion was estimated using the method described by Konigsmark (1970) and used by Aldskogius & Arvidsson. (1978). Every third section was counted. All of the counts were made by the same individual who was unaware of which ganglia he was examining. The sections were also scanned for qualitative evidence for chromatolysis and neuronal degeneration.

The empirical correction factor recommended by Coggeshall, Chung, Greenwood & Hulseboch (1984) was determined by observing different nuclei in serial sections and determining the number of nucleolar profiles that were present in each nucleus. Three hundred and three nucleolar profiles were found in 243 nuclei giving a correction factor of 0·80 which calibrates for multiple nucleoli, split nucleoli, invisible fragments, nucleolar size changes and section thickness differences that may cause the nucleolar number to differ from the number of nuclei.

Comparisons between counts on the right and left sides of operated animals were made using a paired Student's t test and between counts in operated and control animals using an unpaired Student's t test.

RESULTS

Qualitative assessment

The operated and control sides could not be differentiated on qualitative grounds (Figs. 1, 2). We looked for chromatolysis and changes in the cytoplasm and could see none. We looked for nuclear enlargement and eccentricity and could find no difference between right and left. No degenerative material was found nor were phagocytic cells observed.

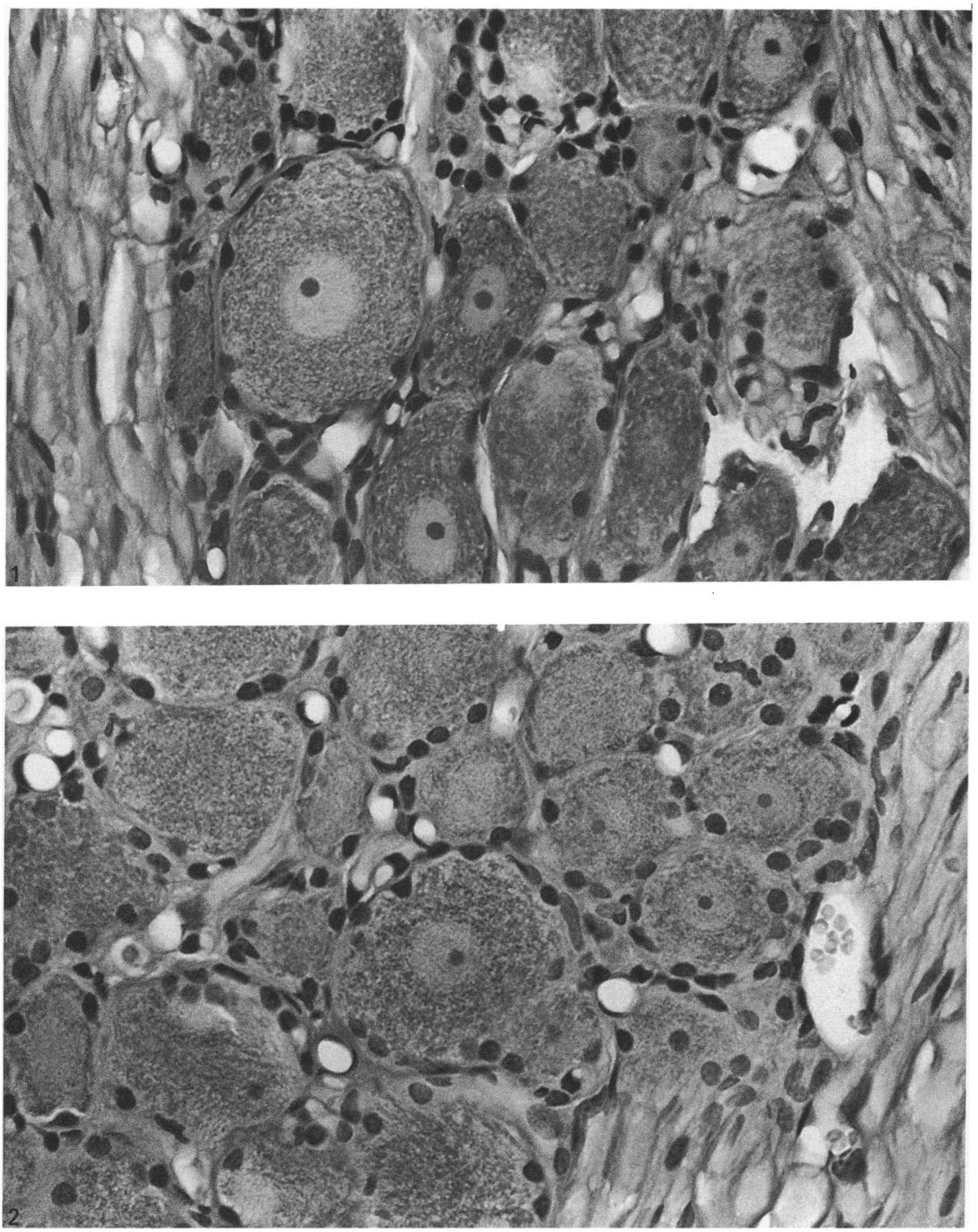

Fig. 1. Part of a trigeminal ganglion from the unoperated side of a cat in which the contralateral inferior alveolar nerve had been sectioned and regeneration prevented 15 weeks earlier. Cresyl violet. × 400.

Fig. 2. Part of the trigeminal ganglion from the operated side of the same animal as illustrated in Fig. 1. Cresyl violet. × 400.

Table 1. *Ganglion cell counts.*

Age at perfusion (months)	Right	Left	Difference between sides $\left(\frac{L-R}{R} \times 100\right)$
	Unoperated control group		
8	13577	15085	+11%
8	11618	12033	+4%
9	17908	15722	−12%
Mean	14367·7	14280·0	+1%
	IAN cut and reapposed		
11	17922	18609	+4%
11	14148	15518	+10%
11	16267	15864	−2%
Mean	16112·0	16664·0	+4%
	IAN cut and regeneration blocked		
16	14426	12851	−11%
15	15218	7160	−53%
14	17421	12870	−26%
11	23710	22167	−7%
11	19392	16584	−14%
11	20076	11615	−42%
Mean	18373·8	13874·5	−24%

IAN, inferior alveolar nerve

Quantitative assessment (*Table* 1)

The mean number of cells in the trigeminal ganglia of the control animals was 14324. The difference in cell count on each side was up to 12% but there was no significant difference between the mean counts on the right and left sides ($P > 0{\cdot}09$). After sectioning the left inferior alveolar nerve and reapposing the cut ends, there was no significant difference between the counts on each side ($P > 0{\cdot}4$) or from the counts in control animals (right, $P > 0{\cdot}10$; left, $P > 0{\cdot}05$). In contrast, after sectioning the left inferior alveolar nerve and preventing regeneration, there were always fewer cells on the operated side than on the unoperated side and this difference was significant ($P > 0{\cdot}002$). On average there were 24% fewer cells on the operated side but this difference ranged from 7% to 53%. Comparison between groups revealed, however, that after sectioning and blocking regeneration, there was no significant difference between the mean cell count on the operated side and that found in the control animals ($P > 0{\cdot}08$), but the mean cell count on the *unoperated* side was significantly higher than that of the control animals ($P < 0{\cdot}03$).

If the data from the control animals and the unoperated sides of the experimental animals are taken together, there is no significant correlation between the cell count and the age of the animal (Pearson's correlation coefficient, $r = 0{\cdot}23$, $P > 0{\cdot}42$).

DISCUSSION

No previous studies have determined cell numbers in the cat trigeminal ganglion. Surprisingly, however, our counts are lower than those obtained in the rat (Aldskogius

& Arvidsson, 1978). The explanation may lie in the resolution of the counting system. In an effort to maximise accuracy we used thinner sections than virtually all previous studies and counted a higher proportion of sections than most. The lower section thickness results in thinner and hence more lightly staining nucleolar fragments and, as Devor, Govrin-lippman, Frank & Raber (1985) have suggested, smaller fragments may be overlooked. As all counts were conducted under the same conditions by the same investigator using the same criteria, the underestimate, if such it is, is systematic and the counts remain comparable.

Our data show no significant difference in cell counts between operated and unoperated sides of animals in which the inferior alveolar nerve was sectioned and the cut ends reapposed, but a significant difference if regeneration of the cut nerve was prevented. Nevertheless counts on both sides of the former group were considerably greater than those of unoperated control animals. The failure to reach significance could be due to the very small number of animals examined. The severity of chromatolytic changes, and presumably therefore the extent of cellular degeneration, is affected by the maturity of the animal, the proximity of the lesion to the ganglion, the type of lesion and the type of neuron (Cammermeyer, 1963; Lieberman, 1974). Peripheral nerve section in neonates gives rise to extensive cell loss (Schmalbruch, 1984; Waite, 1984) and is followed by marked compensatory changes centrally as a result (Rhoades, Fiore, Math & Jacquin, 1983; Waite, 1984). In adults the potential for regeneration of the peripheral nerve is greater but there is less central plasticity (Waite, 1984). Variations in the proximity of the lesion to the ganglion (and hence the proportion of the axoplasm lost) may explain some difference in the extent of cell loss found in different experiments (Lieberman, 1974). The type of peripheral nerve injury may be important because of its influence on the number of fibres which will re-establish functional connections with the periphery (Cavanaugh, 1951), although this would appear to be of little importance in neonates (Chiaia, Hess & Rhoades, 1987). Despite the severe nerve injury used in some of the present experiments we have previously shown that some axons in the inferior alveolar nerve are still capable of regenerating to supply the teeth, skin and mucous membrane if the obstruction is removed (Robinson, 1984). Previous experiments have shown a differential response of different neuron types to nerve injury and this would result in a change in the size distribution of ganglion cells after the lesion. Cavanaugh (1951) found an apparent differential loss of small cells in thoracic sensory ganglia after sectioning intercostal nerves. Ranson (1906) reported a similar pattern whereas others (Aldskogius & Risling, 1981; Janig & McLachlan, 1984) support a selective loss of large cells. The differences may be due to variations in experimental protocol and this factor is under further investigation.

The analysis of our data suggests that the difference in ganglion cell counts after nerve section and regeneration blocking results from an increase in cells on the unoperated side rather than a decrease on the operated side. The small number of animals used in this study combined with the considerable inter-animal variation make it imperative that caution should be exercised in drawing conclusions. Nonetheless a closer look at data published by others also reveals an increase in cell counts contralateral to axotomy although this has not been commented upon by the authors. Aldskogius & Arvidsson (1978) found a mean of 25016 cells in the trigeminal ganglion of unoperated control rats; this was not significantly different from the cell counts obtained 60–70 days after removing a segment from the ipsilateral infra-orbital nerve (29763) but was significantly smaller than the cell counts from the trigeminal ganglia on the contralateral side (34705). Similar relationships are found in other studies

(Aldskogius & Risling, 1981; Ygge, Aldskogius & Grant, 1981; Ygge & Aldskogius, 1984; Devor *et al.* 1985; Arvidsson, Ygge & Grant, 1986). Devor *et al.* (1985) discussed the increase in contralateral cell counts from spinal ganglia in rats, after sciatic nerve section. They explained this result by showing that "the number of sensory neurons in mature rat DRGs is not constant but rather increases with the age of the animal. As a consequence, retrograde cell loss is reflected more in a decrease in the normal rate of accretion than in an actual decline in cell numbers". They based this suggestion on longitudinal studies on unoperated adult rats. Their calculations suggested that cell death does, in fact, occur but only at the rate of 8% of the originally axotomised neurons per 100 post-operative days. Our data was obtained from fully developed cats (rather than rats, which continue to grow throughout adult life) and we found no evidence of cell accretion in unoperated ganglia from animals aged between 8 and 16 months.

How else can we explain the increased cell counts contralateral to axotomy? Devor *et al.* (1985) commented that "we cannot rule out the possibility that neuronal accretion in adult DRGs reflects the later maturation of cells that were actually generated prenatally." We would elaborate on this hypothesis. Our counts depend upon the criteria by which a ganglion cell is recognised and included in the enumeration. The method used relies on the characteristic large dark nucleolus centrally placed in a pale nucleus. There may, however, be a normal component of the neuronal population, possibly quiescent neurons, that have smaller and paler nucleoli and these may be missed. Following an appropriate stimulus, such as a peripheral nerve injury, these cells may enlarge and their nuclei and nucleoli become more prominent, as occurs in chromatolysis. Such cells would then be included in the counts. The effect on the operated side could be to mask the cell loss but if a similar change took place on the unoperated side where there is no cell death it would result in increased counts. Contralateral effects following peripheral nerve injury have been reported and range from the sprouting of peripheral terminals (Robinson, 1981; Holland & Robinson, 1987; Rotshenker & Tal, 1985) to the accumulation of peptides (Hughes & Smith, 1988).

Recent experiments have suggested that neurotrophic proteins regulate neuronal survival during development (Hofer & Barde, 1988). They also appear to play a role in the response to peripheral nerve injuries, as nerve growth factor (NGF) appears to initiate the development of peripheral nerve sprouts (Owen, Logan & Robinson, 1989) and it has also been shown that NGF can counteract the neurophysiological and neurochemical effects of peripheral nerve section (Fitzgerald, Wall, Goedert & Emson, 1985; Otto, Unsicker & Grothe, 1987). The role of such neurotrophic agents in the cellular changes seen in the present study remains to be established.

SUMMARY

This investigation was designed to determine the change in cell numbers in the trigeminal ganglia following unilateral section of one of its peripheral branches.

In 9 young adult cats, under general anesthesia, the inferior alveolar nerve was transected. In 3 of the animals the cut ends were reapposed and in the other 6 regeneration was blocked. After 15 weeks the trigeminal ganglia were removed, and the number of neurons present estimated by counting nucleoli in every third section. The counts were compared with those obtained from 3 unoperated control animals.

The mean number of cells in the ganglia of control animals was 14324 with no statistically significant difference between sides. There was no significant difference

between counts from opposite sides of cats whose nerves were allowed to regenerate. In the animals in which regeneration was prevented the mean count on the operated side was 13874 and on the unoperated 18374. These differences were statistically significant and appeared to result from an increase in cell counts on the unoperated side rather than a reduction in the counts on the operated side. This may be explained by the presence of neurons with inconspicuous nucleoli in normal ganglia, which are stimulated to enlarge and become more prominent following peripheral nerve injury. This change could occur on both sides and would mask cell loss on the operated side and produce an apparent increase in the count on the unoperated side.

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