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Developing Chicken Trigeminal Ganglia Show Varying Patterns of Axon Outgrowth Following Ablation *in vivo* of The Maxillary and Mandibular Target Tissues

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Abstract.- Developing trigeminal neurons chemotropically grow towards maxillary and mandibular targets under *in vitro* conditions but *in vivo* data in support is lacking. The present study was designed to investigate *in vivo* if nerve outgrowth was target dependent. Targets of the trigeminal ganglion, maxillary and mandibular processes, were removed in chicken embryos by electrocauterization before their innervation by trigeminal neurons. Nerve patterns were analyzed immunohistochemically on tissue sections immediately after the operation and at 24, 48 and 80 hours post-operation. Trigeminal neurons extended axons in a normal pattern even when their peripheral target tissues were removed or absent. In those embryos where a complete removal of targets was achieved, major nerve divisions from the ganglion grew out but followed aberrant courses. When the maxillary and mandibular processes were removed maximally, the maxillary and mandibular divisions again formed along the proximal part of their normal course. When these processes were reduced but still visible, the major nerve trunks grew a considerable distance; but they failed to form terminal arbors and did not make contact with the target tissues even though an epithelial covering had regenerated. The peripheral target tissues appear to regulate fine-tuning the axons for branch formation or to make synapse when they are already in the vicinity of the target but initial outgrowth may be target-independent.

Key Words: Trigeminal ganglion, chemotropism, target ablation, nerve development, axon guidance.

INTRODUCTION

Developing axons reach their innervation targets with remarkable precision and accuracy. A target-directed outgrowth and migration of several axon populations is attributed to the target tissues shown to elicit axon outgrowth and guide growing axons by the release of chemotropic or chemorepulsive factors (Tessier-Lavigne and Goodman, 1996; Yoshikawa and Thomas, 2004; Yu and Bargmann, 2004). Target tissues of the mouse trigeminal ganglion, the maxillary and mandibular processes influence target-directed outgrowth of developing trigeminal neurites *in vitro* if co-cultured together in collagen gels. The epithelium of these tissues has been shown to be the source of a putative trigeminal neurotropic factor(s) in eliciting and orienting neurite outgrowth from the trigeminal ganglion (Lumsden and Davies, 1983, 1986) but evidence in support if the same mechanism operates *in vivo* for this group of cranial sensory neurons has been lacking. One possible way to address this question is to remove *in vivo* the epithelium of maxillary and mandibular target tissues. Because such an investigation in mammalian embryos is difficult to conduct, the role of trigeminal target tissues *in vivo* has been elusive. Presently, developing chick embryos were used as a model system to study the outgrowth and migration of trigeminal sensory axons *in ovo* to investigate if the nerves were capable of directed growth after removal of target tissues. Here, it is shown that ablation of target tissues in stage 19-20 (E3.0) chick embryos; the earliest stages of target field innervation, did not prevent axon outgrowth or the formation of major nerves but a misrouting of axons occurred near the ablated targets suggesting that initial outgrowth toward the targets occurred in a predetermined pattern.

MATERIALS AND METHODS

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Animal procedures were as approved by The Animals Scientific Procedures Act 1986, United Kingdom. Fertilized chicken eggs (n=250; mixed breed) were obtained from Hamish-Morrison and incubated at 37±1°C to embryonic day E3.0 (stages 19-20); earliest stages of trigeminal axon outgrowth (Hamburger and Hamilton, 1951). Time of placement of eggs for incubation was considered as zero hours. A few eggs were checked at random by windowing to make sure that embryonic growth had not already begum. Eggs in which embryonic growth was noted were discarded to avoid confusion regarding stages of embryonic development. Each time eggs were received, they were incubation in batches, each of 20 eggs, to obtain various developmental stages.

Electrocauterization

Eggs were taken out on day E 3 (72 hours) of incubation and windowed. The maxillary and mandibular processes of the first branchial arch on one side of the embryos were ablated by electrocautery. The voltage was kept moderate and current was applied for few seconds (1.81 V) to avoid immediate death of embryos or removal of the trigeminal ganglion. The ophthalmic target was inaccessible and indistinct at these stages and it was therefore not operated upon. The other lateral half of the embryos served as controls. Following operation, a group (n=19) of embryos was immediately processed to assess nerve outgrowth, the amount of damage and the presence of the trigeminal ganglion. The remaining embryos were allowed to recover for a period of further three days incubation (stages 27-28) at the conditions described above. Of these, 10 embryos were processed at 24 and 48 hours post-operatively. The major group (n=80) was processed 80 hours post-operation. A total of 250 embryos were used for the present study of which 118 + 2 survived the operation. The remaining embryos (n=132), which died immediately or during the recovery period were not analyzed.

Fixation, histology and immunolabelling

Fixation and histological procedures were according to the methods as described (Lee *et al.*, 1995). Serial transverse sections from the head region were collected onto gelatin coated slides and every 8th slide from each block of tissue was stained with Gill's hematoxylin and eosin to visualize the trigeminal ganglion and its target field. After de-paraffinization and hydration, sections were washed in Tris buffered saline (TBS) for 10min each and incubated overnight at 4°C under humidified conditions with a primary antibody, chick-specific monoclonal anti-β-tubulin isotype I + II or monoclonal anti-HNK-1/NCAM (Sigma Immunochemicals, UK) diluted 1:500 in diluting solution containing TBS, DMSO, and 0.5gm thimerosal, 0.02% Triton X-100 and 2% horse serum to block non-specific binding. Sections were washed and incubated overnight as above with an affinity purified biotinylated horse anti-mouse IgG (H+L), secondary antibody (Vectastain ABC Kit) diluted 1:300 in TBS. Sections were washed again and incubated in Vectastain ABC avidin-biotin complex (Vector Laboratories, UK) for 3h at 4°C, developed with 3, 3'-diaminobenzidine (DAB) for 15 min using a DAB substrate kit, washed, dehydrated, and mounted in DPX.

RESULTS

Immediate post-operative analysis (n=19 embryos), (E2; stages 19-20)

Embryos processed immediately after the operation showed that the trigeminal ganglia, the ophthalmic nerve and the central connections with the brainstem were preserved during the operative procedure. The trigeminal ganglia did not show any abnormality in their morphology, appearance or size indicating that the electrocauterization in a moderate and controlled way achieved removal of the maxillary and the mandibular processes causing minimal damage to other structures.

Control

Short thin nerve fibres in the ophthalmic territory represented the ophthalmic nerve (Fig. 1A, arrowhead, left side). The maxillary process was inconspicuous as compared with the mandibular process. The trigeminal ganglia were 50μm distant from these targets. A complete formation of the maxillo-mandibular trunk was not evident but immunoreactive fibres were present at 10-20μm

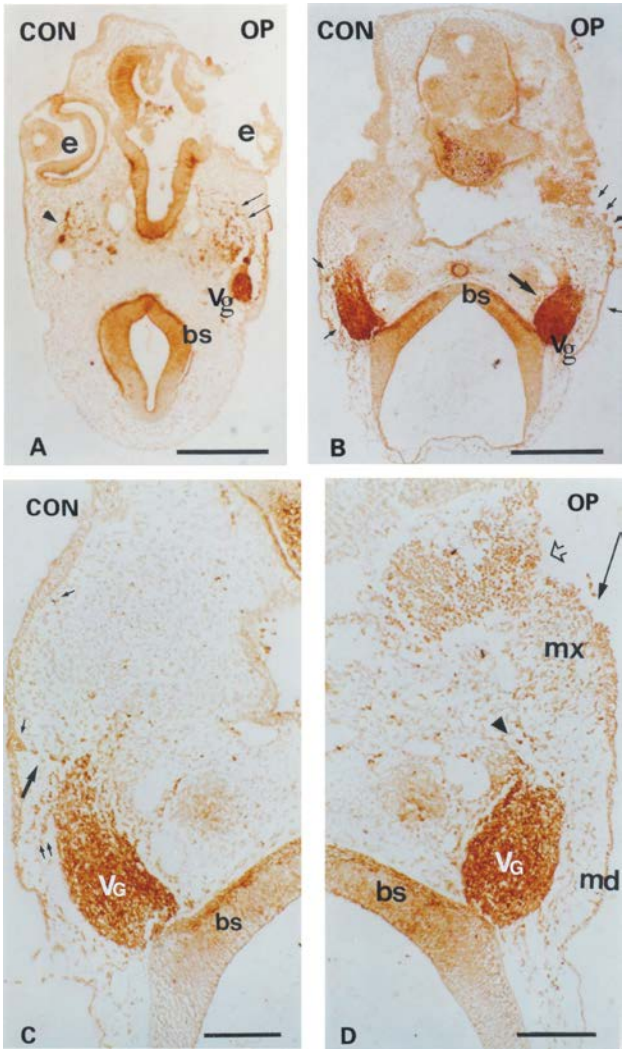

Fig. 1. Immediate post-operative sections of stage 19-20 chick embryos at the level of first branchial arch. Scale bars A-B = 200μm (x40), C-D =100μm (x100).
Abbreviations throughout figures: CON, control side; OP, operated side; e, eye, oph, ophthalmic nerve; Vg, trigeminal ganglion; bs, brainstem; mx, maxillary process; md, mandibular process; cg, ciliary ganglion; mV, motor root of trigeminal; ION, infraorbital nerve, G; Geniculate ganglion.

distance and a few contacted the epithelium of maxillary and mandibular processes (Fig. 1B and C, small and large arrows on left side).

Operated

The trigeminal ganglion and the ophthalmic nerve were normal (Fig. 1A, right side, arrows). Ophthalmic axons were dispersed and intermixed with the coagulated blood cells only in those embryos where the operative procedure removed maximum amount of tissue and resulted in a reduction or obliteration of the eye (Fig. 1B, three small arrows on the right side). Maxillary processes were more successfully removed than the mandibular processes. No detectable axons were found (n=11) near the maxillary and mandibular processes where a lesion was incurred (Fig. 1B and D, right side, thick arrow and arrowhead). In other operated embryos (n=6), a few short nerve fibres were visible near the trigeminal ganglia but they did not approach the maxillary or mandibular processes. Connections with brainstem were normal (Fig. 1B, and D, right side).

Axon outgrowth 24 hours post-operation (n=5), (E3; stages 22-23)

Control

The ophthalmic nerve showed a trajectory towards the fronto-nasal process and was present as a 300-400μm thick nerve bundle (Fig. 2A, left side, arrow). The maxillo-mandibular lobe was prominent and the maxillary and mandibular nerve fibres were evident (Fig. 2B and C, left side, arrows). Axons were found in contact with the epithelia of the maxillary and mandibular processes. The nerve fibres appeared as thick clusters of axons and gave off side branches. Normally developed neurons were fasciculated near the ganglion but non-fasciculated near the target tissues (Fig. 2E and G, left side, arrows).

Operated

The ophthalmic nerve extending towards the fronto-nasal process was normal and comparable to that of control nerve (Fig. 2B, right side, arrow). Eyes were abnormally developed, reduced or absent indicating greater damage than normal (Fig. 2D, right side). The trigeminal ganglia and their central connections developed normally (Fig. 2F and H, right side, arrowheads) with a nerve pattern similar to the control side (Fig. 2E and G, left side).

An epithelium covered the cut surface of the removed or reduced maxillary and mandibular

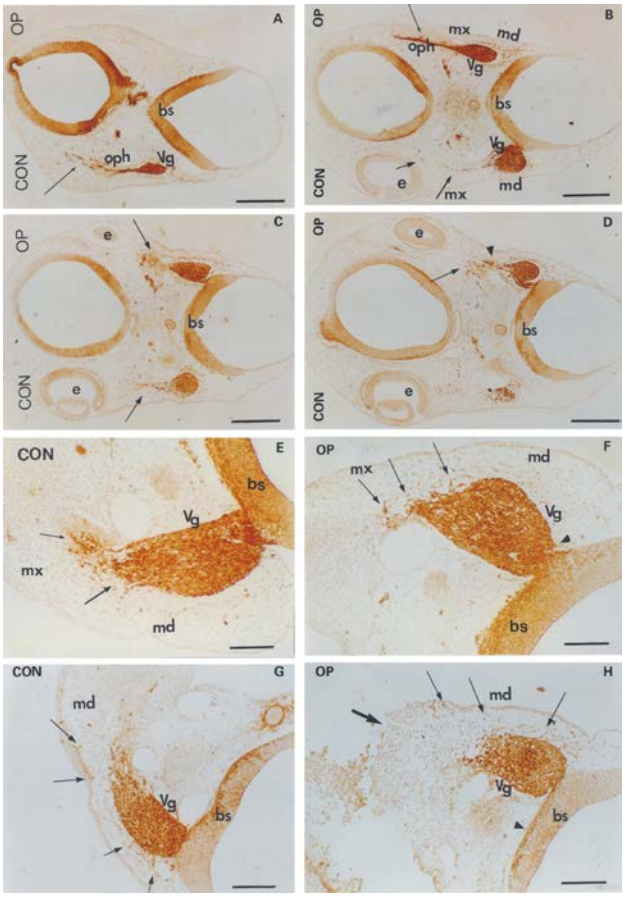

Fig .2 Sections of chick embryos through the first branchial arch region 24 hours post-operation. Scale bars A-B = 200μm (x40), Scale bars E-H = 100μm (x100).
For abbreviations see Figure 1.

processes in 50% embryos. The maxillo-mandibular trunk was short and its maxillary and mandibular nerve fibres were visible but indistinct and did not show branch formation. A reduction but not complete absence of axons was found in the remnants of maxillary and mandibular processes. A few 30-50μm long nerve fibres were apparent in the mesenchyme of the first branchial arch and a few were found very close to the epithelium (Fig. 2D right side, arrowhead). Axons appeared halted a short distance from the maxillary and mandibular process epithelia (Fig. 2F right side, long arrows) and were present as scattered fibres near the trigeminal ganglion and in the maxillary and mandibular territory (Fig. 2H, right side, long arrows).

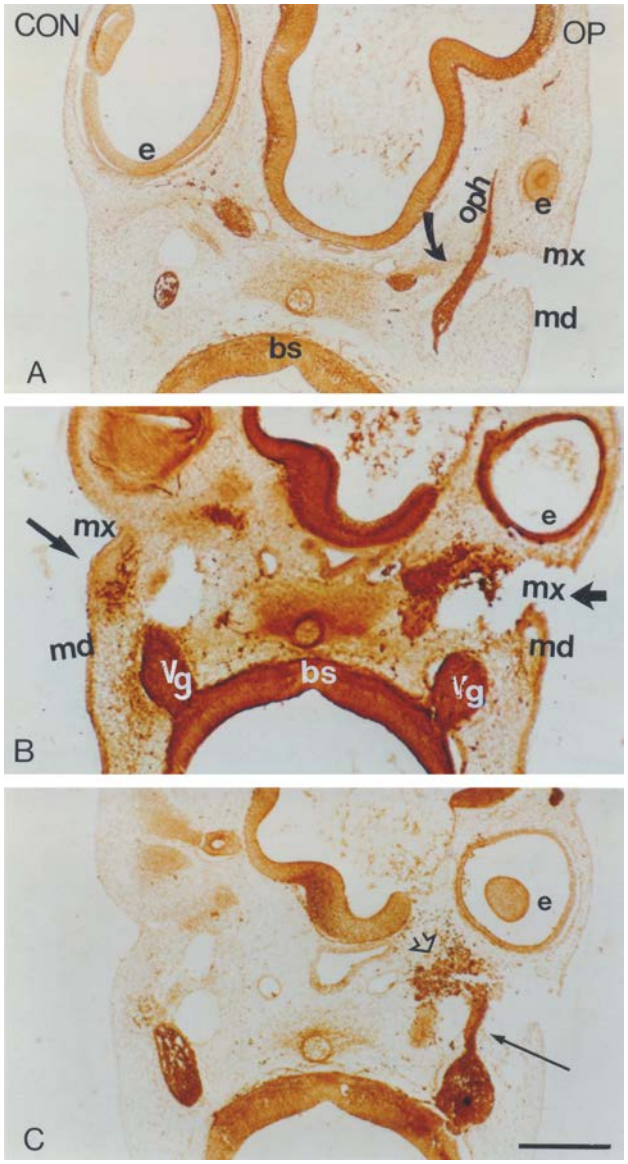

Fig. 3 Sections of an embryo 36 hours post-operation. Scale bars A-C= 200μm (x40).
For abbreviations see Figure 1.

In an attempt to completely remove the maxillary and mandibular processes, two (n=2) embryos did not survive beyond 36 hours post-operation, thus they were fixed and analyzed. The maxillo-mandibular nerve trunk, instead of projecting laterally towards the peripheral targets, took an abnormal frontal trajectory towards the eye. It consisted of thick fasciculated nerve bundles,

which did not form nerve branches (Fig. 3C, right side, arrow) compared to the non-fasciculated axons on the control side (Fig. 3B, left side, arrow). Blood coagulation (Fig. 3C, right side, clear arrowhead) and a complete loss of first branchial arch indicated excessive damage and the presence or absence of nerve fibres was not possible to be assessed (Fig. 3B, right side, arrowhead). Despite this degree of damage, the trigeminal ganglion, the main maxillo-mandibular nerve trunk, the ophthalmic nerve and the central trigeminal tract developed normally. A reduction in size of the eye was however noticeable (Fig. 3A-B, right side).

Axon outgrowth 48 hours post-operation (n=5), (E5; stages 25)

Control

By 48 hours the ophthalmic nerve was located between the optic surface and telencephalic vesicles as it headed toward the fronto-nasal process. It was 600μm long and fasciculated (Fig. 4A). The bifurcation of the maxillo-mandibular nerve trunk into maxillary and mandibular nerves was evident (Fig. 4C) axons of which were highly fasciculated near the trigeminal ganglia. Axons of the infraorbital branch of the maxillary nerve were located below the eye (Fig. 4B). Axons formed short thick clusters and several contacted with the epithelium of maxillary and mandibular processes (Fig. 4D, arrows). Axons growing laterally at the level of the second branchial arch were also found (Fig. 4C, long thin arrow).

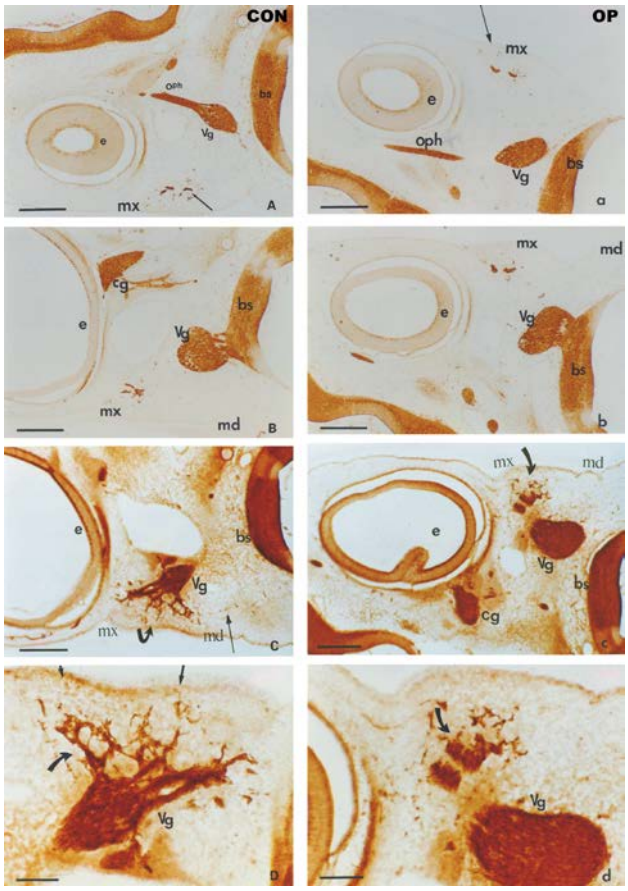

Fig. 4 Sections of chick embryo through the first branchial arch region 48 hours post-operation. Scale bars A-C = 200μm (x40), Scale bars C-D = 100μm (x100).
Scale bars a-c = 200μm (x40) Scale bar d = 100μm (x100).
For abbreviations see Figure 1.

Operated

The ophthalmic nerve developed normally and was about 500-600μm long. It was present in-between the outer border of the eye and the telencephalic vesicles (Fig. 4A) and was fasciculated similar to control nerve. The trigeminal ganglia and the central trigeminal tracts developed normally (Fig. 4B, compare with control 4B).

A reduction in the extent of axons was noticeable at the site of the first branchial arch where the operation was performed. The nerve fibres showed an arrested pattern of outgrowth and branch formation in four of the five embryos. Infraorbital nerve fibres were present as small clusters below the eye (Fig.4A, long arrow). In later serial sections the maxillary nerve was evident as composed of thick clustered axons near the trigeminal ganglia (Fig. 4C, curved arrow). Although the axons were present a short distance (about 50-100μm) from the epithelial border as they entered the branchial arch mesenchyme, they did not make contact with the epithelium of these arches, remained clustered to form maxillary or mandibular nerve branches (Fig. 4D, curved arrow) like those of control axons (Fig. 4D, arrows).

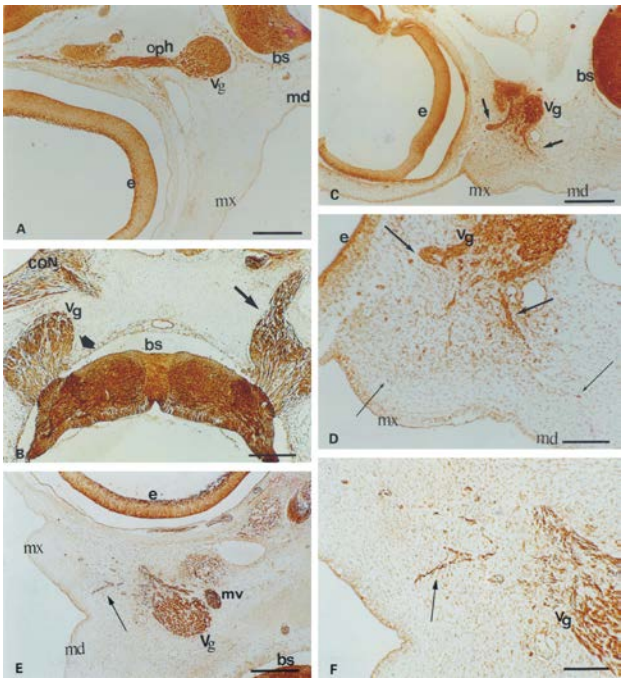

Fig. 5 Control side sections of chick embryo through the first branchial arch region 80 hours post-operation. Scale bars A-C, E = 200μm (x40). Scale bars D, F = 100μm (x100). For abbreviations see Figure 1.

Axon Outgrowth 80 hours post-operation (n=80), (E6-7; stages 27-28)

Control

By this stage the trigeminal ganglion and its three peripheral target fields were well developed. Highly fasciculated ophthalmic nerve followed a rostral trajectory towards the fronto-nasal process and was ~ 750-1800μm long (Fig. 5A). The central trigeminal tract consisted of thick nerve fibres (Fig. 5B, arrowhead on left). The maxillary and mandibular processes were fully developed and large. The maxillo-mandibular trunk was thick and bifurcated into distinct maxillary and mandibular nerves (Fig. 5C, arrows). These nerve fibres remained highly fasciculated near the trigeminal ganglia (Fig. 5D, long arrows) but branched as they invaded the mesenchyme of the maxillary and mandibular processes and were found close to the maxillary and mandibular epithelia (Fig. 5E and F, long thin arrows).

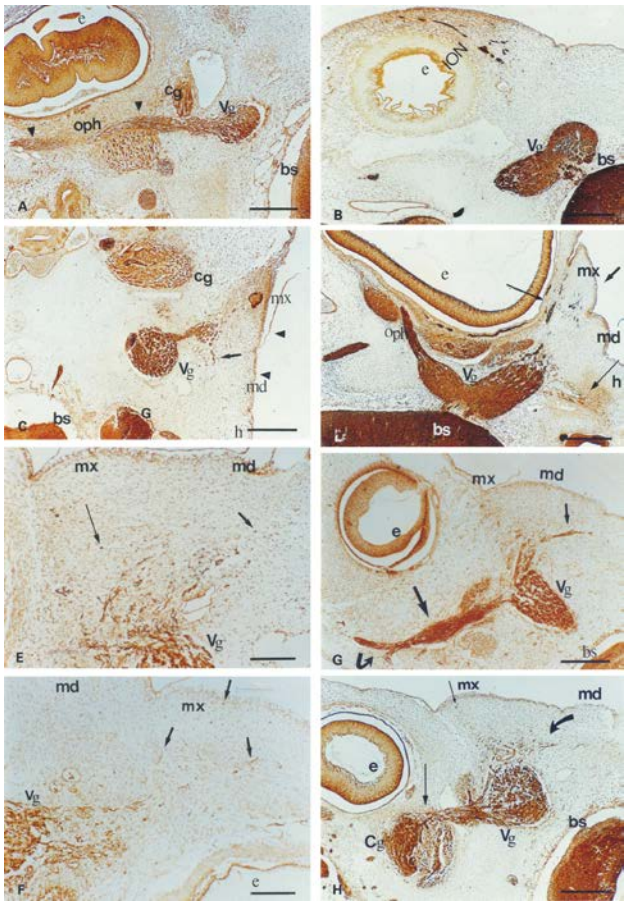

Fig. 6. Sections of chick embryo through the first branchial arch region 80 hours post-operation. Scale bars A-D, G-H = 200μm (x40). Scale bars E-F= 100μm (x100). For abbreviations see Figure 1.

Operated

The ophthalmic nerve was long (~700-1800μm), highly fasciculated and developed normal (Fig. 6A, arrowheads). In those embryos (n=27) where the cauterization resulted in a significant damage to the eye region, this nerve branch was reduced to ~400μm or even 200μm thick protuberance (Fig. 5B, arrow on right side). The eyes showed abnormal development resulting in obliteration, reduction and abolition of lens formation (Fig. 6A, 7A, C and E). Trigeminal ganglia and nerve connections with the brainstem developed normally (Fig. 6B and D).

Short thick nerve fibres formed the maxillo-

mandibular trunk (Fig. 6B and D). The maxillary nerve initially developed ventral to the eye as 100-500μm long scattered axons of the infraorbital branch (Fig. 6B). This nerve branch was more pronounced on the operated side because of reduction in the eye size (diameter, ~600μm) compared to the control side eye (~1.5-2.0 mm). Axons of rostrally present supraorbital branch were inconspicuous on both operated and control sides. The maxillary nerve formed short nerve branches towards the operated maxillary process where short clusters of axons were evident (Fig. 6D, long thin arrow). A similar pattern was observed for the mandibular nerve that showed up as a bundle of thick axonal fibres (Fig. 6G, thin arrow and Fig. 7G, long thin arrow, arrowheads show reduced maxillary and mandibular processes). In those embryos where the mandibular processes were smaller indicating reduction or redevelopment, mandibular nerve fibres showed lateral deflections towards the hyoid process (Fig. 6, arrow and arrowheads in C, long thin arrow in D, curved arrow in H).

In a large number of embryos (n=53) the pattern of nerve outgrowth was comparable to that of control sides for major nerve branches. Axons were found close to epithelia of removed or reduced maxillary and mandibular processes. A reduction in axon density, nerve branching, axon arrest and a non-directed pattern of axons was noticeable on the operated sides depending on the degree of reduction of target tissue regions (Fig. 6C and E) compared to control sides (Fig. 5D). The arrest of nerve fibres was noticeable at distances of ~150-200μm from the maxillary and mandibular epithelium. In only one embryo, an intensely stained and thick axonal branch was observed on the medial side (Fig. 6G, thick arrow). It showed a high degree of deflection and gave off small thick side branches (Fig. 6G, curved arrow). A merger of the trigeminal ganglion's maxillo-mandibular lobe with the ciliary ganglion and abducens axons was also observed in four embryos (Fig. 6H, long thick arrow). In those embryos (n=27) where the intensity of electro-cauterisation caused significant damage to the maxillary and mandibular processes which appeared either highly reduced, abnormally fused or completely absent axons were highly reduced. The maxillo-mandibular nerve trunk was abnormally short (Fig. 7 E and F, arrow); in a few it did not develop (Fig. 7A and B), showed a complete halt with some degree of reduction in its size or developed as a short bundle or its further extension into maxillary and mandibular nerves was arrested (Fig. 7A-F). None of the axons made contact with the maxillary or mandibular process epithelia but halted a short distance (10μm) from the epithelium (Fig. 7G, long arrow).

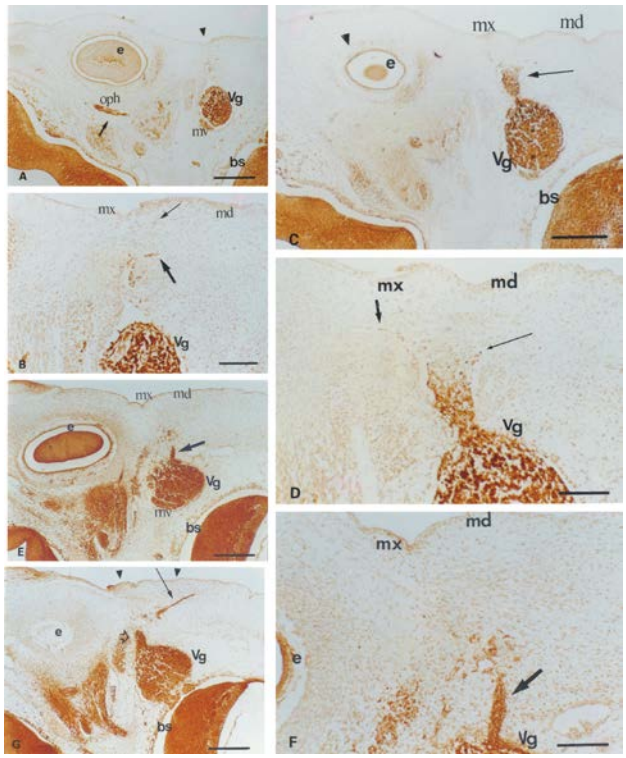

Fig. 7 Sections of chick embryo through the first branchial arch region 80 hours post-operation. Scale bars A, C, E and G = 200μm (x40), Scale bars B, D, F = 100μm (x100).
For abbreviations see Figure 1.

DISCUSSION

In vitro cultures of mouse trigeminal ganglia provided evidence that the outgrowth and extension of axons was dependent on maxillary and mandibular target tissues (Lumsden and Davies, 1983, 1986). To determine if the same occurs *in*

vivo these peripheral trigeminal targets were removed in chick embryos by electrocauterization before their innervation by trigeminal axons. Results showed that targeted ablation neither affected the formation of maxillo-mandibular nerve trunk nor the maxillary and mandibular nerve fibre outgrowth. Target-oriented outgrowth of trigeminal ganglion axons towards removed targets was difficult to assess, since in a large number of embryos the nerve fibres could be traced to the vicinity of the ablated targets even though their target tissues were completely removed or highly reduced, while in others, axons formed major nerve trunks but did not approach the epithelium. More pronounced effects observed were reduction in nerve fibre density, fasciculation and misrouting of axons.

Technical considerations

The electrocauterizing technique adapted here despite having advantages over removal of tissues using tungsten needles and microscissors damaged the neighbouring regions and most importantly the blood supply to the branchial arch even though the current was applied to the area to be operated upon only for a few seconds (the whole operation took less than 40 seconds). Although a complete removal of the target tissues was achieved in fairly good number of embryos, but due to curvature of the embryos in some, it was not possible sometimes to completely remove the target tissues whilst ensuing preservation of the trigeminal ganglion, and minimal damage to the blood supply. This obviously was a limitation of the experimental method used and has also been demonstrated by Wahl and Noden (2001) that surgical manipulations in avian embryos lead to bizarre effects. A case has been described at 36 hours with excessive damage that occurred in an attempt to completely remove the target tissues but in such embryos assessment of normal nerve outgrowth and directionality of axons was not possible.

Looking at illustrations from 48 and 80 hours post-operative ganglia the epithelium is evident covering the reduced arches. Either the epithelium was not completely removed by the operation or it regenerated from regions which escaped cauterization. The latter appears conceivable because the maxillary and mandibular processes just appear at stages 19-20 (Hamburger and Hamilton, 1951). An in-growth from either the ophthalmic region above or from the hyoid region below or from proximal parts of epithelium covering maxillary and mandibular arch is also a likely possibility. Grafting of target tissues to ectopic locations (Honore and Hemmati-Brivanlou, 1996) could provide the answer but ectopic grafting was not performed because pinning an extra arch without causing damage to the blood vessels supplying the branchial arches could not be achieved.

Despite these limitations, the study bears important implications mainly because axons neared the target tissues where a significant removal of targets was achieved. Differences in routing and distribution should have been expected if growing axons were solely dependent on target-derived factors. Instead, it was observed that the maxillo-mandibular trunk emerged and branched off into maxillary and mandibular nerves correctly which, by 48 and 80 hours had further progressed into the first branchial arch mesenchyme as far as contacting the epithelium. Moreover, infraorbital axons grew as far as the distal tip of the eye on the ventral side and were present in remnants of the ablated arch as thick short clusters.

Noticeably, less number of axons approached and contacted the ablated targets compared to the control non-ablated half of the embryos. Axon density appeared affected, with some degree of misrouting and fasciculation near the targets while they showed defasciculation near the ganglia. Misrouting near the ablated targets, axon halting, lateral deflections and absence of branch formation all point out that the target tissues regulate the arrest and branch formation only when growing axons have reached in their vicinity. This observation seems parallel to those of muscle nerves of chick embryos where nerve branches to individual muscles are lost following X-irradiation of somitic mesoderm but the main muscle nerve trunks develop normal (Lewis *et al.*, 1981). Together these and present observations suggest that the target tissues influence axons over a short range.

It is known that the neurite morphology near the target is governed by the target tissues (Erzurumlu *et al.*, 1993; Erzurumlu and Jhaveri,

1995). Similarly *Xenopus* trigeminal nerves while growing towards the ablated cement gland target fail to defasciculate, do not form terminal arbors and follow aberrant routes as they approach within 50μm of ablated target (Honore and Hemmati-Brivanlou, 1996). Vestibuloacoustic neurons have also been shown recently to reach their cerebellar targets even if transplanted to the position of the trigeminal ganglion (Tashiro *et al.*, 2000). The present study questions the role of target tissues in the outgrowth and long range guidance of developing chick trigeminal axons under the action of chemotropic substances as was proposed for mouse trigeminal axons (Lumsden and Davies, 1983, 1986). The present observations however cannot be compared directly with those studies because of species difference and theirs were conducted *in vitro*.

Axons grew in waves of neurogenesis

Birth dating studies in chick embryos have shown that placode-derived axons of the trigeminal ganglion are generated between E2-5 (stages 15-27) and neural crest-derived axons do not undergo terminal mitosis until E4-7 (stages 22-31), (D'Amico-Martel and Noden, 1980; Covell and Noden, 1989). Because the operation was performed at stages 19-20 (E3.0), the placode-derived neurons had started axonal extension while the neural crest-derived neurons were presumably not post-mitotic at the time of operation. Thus, either the nerve fibres which managed to reach the vicinity of ablated targets within 24 hours after operation emerged from the remaining placodal cells which were not post-mitotic at the time of operation and extended axons after becoming post-mitotic during the first 24 hours; or axons re-grew from the proximal portions of fibres which were present very close to the ganglia thus escaped the operative procedure; or they were derived from the second wave of neurogenesis and were presumably neural crest-derived axons. Both possibilities are likely because trigeminal axons are generated by an earlier and a late wave of neurogenesis in both chick and mouse embryos (D'Amico-Martel and Noden, 1980; Covell and Noden, 1989; Stainier and Gilbert, 1990; Mody *et al.*, 1989). From birth dating studies, it is evident that only 50% placodal neurons would have extended axons while the remaining placodal cells were undergoing terminal mitosis. The first neurons of neural crest origin become visible at stage 19 (E4). These neurons are located deep in the ganglion and shortly afterwards extend their first axons (Covell and Noden, 1989). Thus a few axons which were found in the remnants of the maxillary and mandibular targets 24 hours post operation possibly consisted of both placodal and neural crest-derived axons.

Possible guidance by trigeminal pioneers

The possibility that pioneer axons had already laid down the pathways for the followers is unlikely because pioneer neurons grow out very early in the development of the chick trigeminal system. The first axons of the maxillo-mandibular lobe emerge at stages 13 and 14 and approach the base of mandibular arch by stage 15. The maxillo-mandibular trunk breaks up into nerve bundles between stages 17 and 18 (Covell and Noden, 1989; Moody *et al.*, 1989; Riggott and Moody, 1987) Since the operation was performed at stage 19 and no axons were found in the vicinity of ablated targets immediately after the operation, thus all pioneers neurons were successfully removed and thus could not have guided later migrated neurons which were found 24 hours after the operation. This appears parallel to earlier observations where later generated trigeminal mesencephalic axons ignored pre-existing fibres in reaching their hindbrain targets (Stainier and Gilbert, 1990).

Moreover, the maxillary and mandibular processes appear late by stages 19-20 correlating with the arrival time of the first trigeminal fibres which contact the maxillary and mandibular epithelium at stages 23-24 (Hamburger and Hamilton, 1951; Riggott and Moody, 1987). Because the maxillary and mandibular process were removed as soon as they formed at stages 19-20 thus they could not have guided incoming axons which had already started their journey towards these targets after emerging from the ganglion very early at stage 13. Several candidate molecules that may orient axons have been isolated and characterized but to date these factors could not have been directly linked with chemotropically-guided migration of developing trigeminal axons (Tessier-Lavigne and

Goodman, 1996; O'Connor and Tessier-Lavigne, 1999; Kennedy, 2000; Araijo and Tear, 2003).

Among cell adhesion molecules PSA-NCAM has been recently been predicted to have a role in neuronal migration and axonal growth (Ultig and Chan, 2004). Extracellular matrix molecules are very well known to lay down the pathways for developing axons and trigeminal axons may be guided by absolute and relative permissiveness of the substratum (Miyahara *et al.*, 2004).

The study concludes that initial outgrowth and migration of developing trigeminal neuron toward targets is intrinsically determined. However, reduction in axon density, misrouting, lateral deflections, halting, and fasciculation near the targets suggests that if target tissues do secrete chemotropic factors, such factors play a role in fine-tuning of axons and branch formation.

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