Wound Healing in the Hindbrain of the Chick Embryo

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ABSTRACT

In the fore and midbrain regions of the neuraxis, closure of the neural canal involves fusion of the neuroepithelial layers of the neural folds. In the hindbrain region, however, fusion of the neuroepithelial portion of the neural folds does not occur. Rather, following apposition of the tips of the neural folds, many of the neuroepithelial cells that should participate in neural fold fusion undergo apoptosis. The rest of the neuroepithelial cells in the region of the dorsal midline undergo rearrangement and flattening to form a single-layered neuroepithelium of the hindbrain roof plate. It is not clear whether in the light of the above, the hindbrain region may be a weakened portion of the neuraxis, and therefore be predisposed to the development of neural tube defects (NTDs). The present study uses wound healing to ascertain whether the hindbrain roof plate at the time it is undergoing apoptosis possesses the ability to close when it is reopened through wounding. Chick embryos at stages 11 and 12 of development were wounded in the dorsal midline of hindbrain rhombomeres r1/r2 and r1-r3, and reincubated for varying periods of time to allow healing to be effected. Complete healing occurred in both r1/r2 and r1-r3 wounds, implying that the length of the wound did not affect the ability of the hindbrain roof plate to repair itself. Acridine orange histochemistry revealed that apoptosis in the hindbrain occurred normally in the presence of wound healing leading to the normal morphogenetic thinning of the hindbrain roof plate. The implication is that the early embryo has in place reparative mechanisms that ensure that assaults to it are taken care of, thereby preventing the interference of normal morphogenesis. Healing of the neuroepithelium in the presence of massive apoptosis suggests that apoptosis may not likely predispose the hindbrain to the development of NTDs.

Keywords: Hindbrain, Neural tube defects, Wound healing.

INTRODUCTION

The neural tube is the primordium of the central nervous system. It is formed through organized morphogenetic movements of its precursor cells in a process known as neurulation. [1-3]. Further differentiation of the neural tube results in the formation of the main components of the central nervous system, namely, the brain and the spinal cord. In higher vertebrates (birds and mammals), neurulation occurs in two phases: primary neurulation, during which the prospective brain and most of the prospective spinal cord form, and secondary neurulation, during which the extreme caudal portion of the prospective spinal cord forms [4-6]. Primary neurulation occurs in four distinct but overlapping stages: Formation of the neural plate; shaping of the neural plate; bending of the neural plate to form a neural groove and bilateral neural folds; and finally, fusion of the neural folds to form a neural tube [4,6].

Briefly, at the start of primary neurulation, epiblast cells rostral to Henson's node and those flanking the cranial part of the primitive streak, undergo apico-basal (dorsoventral) thickening to form the neural plate. The neural plate so formed is relatively short anteroposteriorly and wide transversely. It is also pseudostratified and columnar with two main neuroepithelial cell

types, namely, spindle-shaped or fusiform and wedged-shaped or flask-like [2]. Shortly after the neural plate is formed, it undergoes shaping. Neural plate shaping is generated largely by cell behaviors that reside in the neuroepithelium (i.e., intrinsic factors), and involves convergent extension movements [7-9] that result in the anteroposterior lengthening and transverse narrowing of the neural plate [6,7]. The next stage which is neural plate bending, begins while neural plate shaping is underway and involves folding of the neural plate about three longitudinal furrows (hinge points) formed in the plate. As a result of neural plate bending, a gutter-like space, the neural groove, and bilateral neural folds are formed [10]. Additionally, the tips of the paired neural folds, which consist of an outer surface epithelial layer and an inner neuroepithelial layer, are brought into contact with each other in the dorsal midline of the embryo. Finally, the neural folds fuse in the dorsal midline, thereby closing the neural groove and the anterior (cranial) and posterior (caudal) neuropores [11-12]. Prior to neural fold fusion, the surface epithelial layers cap the neuroepithelial layers. During neural fold fusion, therefore, the surface epithelial layers of the neural fold fuse first and delaminate from the neuroepithelial layers, thus contributing ultimately to the dorsal epidermis of the embryo. Similarly, the neuroepithelial layers of the neural folds fuse deep to the surface epithelium, thereby closing the neural groove.

Until recently, it was presumed that complete fusion of both surface epithelial and neuroepithelial layers of neural fold occurred along the entire length of the neuraxis. A study by Lawson and England [13] has, however, demonstrated that in the hindbrain, fusion of the neuroepithelial layers of the neural folds does not occur. Rather, once the edges of the hindbrain portion of the neural folds become apposed at stage 10, massive apoptosis (programmed self-destruction of cells) occurs in the dorsal midline among neuroepithelial cells that should have participated in the fusion process [13,14]. By stage 11 when apoptosis becomes marked, the dorsal midline of the neuroepithelium is disorganised due to the presence of numerous rounded apoptotic cells [13,14]. The death of these cells significantly decreases the neuroepithelial cell population in the dorsal midline. The remaining neuroepithelial cells in the region of the dorsal midline of the hindbrain then undergo rearrangement and flattening to form a single layer of cuboidal neuroepithelial cells deep to the simple squamous surface epithelial layer. Therefore, apoptosis in the forming hindbrain of the chick embryo plays a significant role in the morphogenetic thinning of the hindbrain roof plate [13,14].

It is not clear whether in the light of thinning, the hindbrain roof plate becomes a weakened part of the neural tube, and probably becomes more predisposed to the development of neural tube defects. Neural tube defects are common congenital malformations, and may occur when the neural folds fail to fuse or reopen after fusion [15,16]. In the present study, we determined whether the hindbrain roof plate at the time it is undergoing apoptosis possesses the ability to close when it is reopened through wounding. The results demonstrated that (1) the hindbrain roof plate at the time it is undergoing apoptosis has the capability to close when it is reopened. (2) Wound healing occurs rapidly from the ends of the wound by a zipping-up mechanism in both surface epithelial and neuroepithelial layers. Longer wounds generally heal at a faster rate than shorter ones. (3) Apoptosis in the hindbrain roof plate occurs normally in the presence of wound healing, and results ultimately in the thinning of the hindbrain roof plate.

Fertilised eggs from White Leghorn chickens were obtained from Afariwaa farms in Tema, Ghana. These were set in a humidified Gallenkamp economy size incubator at 38˚C for between 32 and 36 hours to obtain embryos at stages 11-12 of development [17]. The embryos were mounted and maintained by new culture technique [18] for wound healing studies.

The embryos were wounded either in rhombomere r1/r2 of the hindbrain or in rhombomeres r1-r3. Wounding was carried out as follows: the well formed by the glass ring was flooded with saline and the cranial edge of the blastoderm was carefully detached from the vitelline membrane using fine forceps [19]. The detached part was folded onto the caudal half of the blastoderm to expose the dorsal side of the neural tube. Using a sharpened and sterilised tungsten needle, a single, straight, longitudinal wound was made in the dorsal midline of the hindbrain through the surface ectoderm and the underlying neuroepithelium. The wound was carefully examined under the microscope and the observations recorded. The detached part of the blastoderm was then folded back onto the vitelline membrane. Excess saline was removed from the ring, and thin albumen was placed beneath the vitelline membrane. The embryos were either fixed immediately, or re-incubated for periods between 30 minutes and 12 hours (overnight) before fixation. Control embryos were similarly treated except that they were not wounded.

MATERIALS AND METHODS

Embryos used for light microscopy and transmission electron microscopy were fixed for a maximum period of 24 hour in a mixture of 2% gluteraldehyde and 2% paraformaldehyde in 0.1M sodium cacodylate buffer with calcium chloride at a final concentration of 2.5 mM and a pH of 7.2. Next, they were washed in 0.1 M sodium cacodylate buffer for an equal period of time and post fixed with osmium tetroxide (1% in 0.1 M cacodylate buffer, pH 7.2) for 30 minutes. The embryos were then dehydrated in a graded ascending series of ethanol/water mixtures up to 100% ethanol, infiltrated with two changes each of propylene oxide, and propylene oxide-resin mixtures (Embed 812) respectively, before being finally embedded in 100% resin using 21- well embedding moulds. The moulds, with the embedded embryos were kept for at least 48 hours in an oven set at 60□C for the resin to polymerize.

Specimens for light microscopy were sectioned serially at two microns (2 µm) in a plane perpendicular to the longitudinal axis of the neural tube. The sections were picked up serially with a pair of jeweller's forceps onto clean glass slides, and stained with 1% toluidine blue in 1% sodium tetraborate. They were viewed under an Olympus BHB light microscope fitted with an Olympus C-35AD camera. For TEM, ultra thin sections of 1/r2 and r3 of the hindbrain were cut with a diatome diamond knife. The sections were then mounted on 150-mesh copper grids dipped in 100% acetone, and stained with 2% uranyl acetate for 5 minutes. They were left to dry and then contrast-stained with 2% lead citrate for 3 minutes. The sections were later viewed with a HITACHI H-600 transmission electron microscope.

Acridine orange histochemistry

Acridine orange histochemistry (AOH) was used to determine the presence and distribution of apoptosis in the hindbrain of both control and wounded embryos. Both control and wounded embryos used for AOH were immediately incubated at 38□C for 30 minutes in phosphate buffered saline (PBS) containing acridine orange at a concentration of 5µg/ml. Following incubation in PBS containing acridine orange, all embryos were further washed in PBS. Each embryo was then mounted in 20% glycerine on a glass slide and examined as whole mounts with a Leica MicroStar IV florescence microscope using a fluorescen (FITC) filter.

RESULTS

CONTROL EMBRYOS

Examination of control stage 11 embryos as whole mounts revealed that fusion of the neural folds had already occurred in the forebrain, midbrain and hindbrain. Transverse sections through these primary brain vesicles confirmed that there was complete fusion of both surface epithelial and neuroepithelial layers of the neural folds in the dorsal midline of the fore and midbrain. Additionally, in these parts the neuroepithelium of the dorsal midline was pseudostratified columnar. In the hindbrain, however, although the neuroepithelial layers of the neural folds were apposed to each other in the dorsal midline, they appeared to be disorganised here and comprised a lot of rounded neuroepithelial cells. Migratory neural crest cells were identified as a mass of cells sandwiched between the fused surface epithelium and the neuroepithelial layer in the first rhombomere (r1/r2). They were located dorsal to the basal surface of the neuroepithelial layer. These migratory neural crest cells were absent from the dorsal aspects of the basal surface of the neuroepithelial layer in r3. In the caudal part of r1/r2, the neuroepithelial layer in the dorsal midline had an eminence that projected into the neural tube lumen. Examination by transmission electron microscopy (TEM) showed that the rounded neuroepithelial cells were indeed undergoing apoptosis (Fig. 1b). They possessed fragmented nuclei and condensed chromatin material. Whole-mounts of control stage 11 embryos stained with acridine orange showed intense fluorescence in the dorsal midline of r1/r2 and r3 (fig. 1c). This showed as punctate fluorescence under the fluorescence microscope. There was a similar intensity of acridine orange fluorescence in r5 and the auditory pits adjacent to this rhombomere.

As whole mounts, control stage 12 embryos were larger in size than those at stage 11. Transverse sections through the embryos revealed that neuroepithelial layer in the dorsal midline of r1/r2 and r3 seemed reduced in its full thickness. The reduction in thickness was more marked in the caudal part of r3. Here, the reduction in thickness extended to include the dorsolateral aspects of the neuroepithelial layer. There were also fewer cells in the disorganised dorsal midline of the neuroepithelial layer of r1/r2 and r3 than observed at stage 11. Additionally, at stage 12, the midline neuroepithelial eminence seen in r1/r2 had significantly reduced in size in most embryos. However, migratory neural crest cells were still present between the surface epithelial and neuroepithelial layers in r1/r2. As in acridine orange-stained stage 11 embryos, there was intense fluorescence in the dorsal midline of r1/r2, r3 and r5 stage 12 embryos stained with acridine orange.

Transverse sections through control embryos at stages 13-15 showed that the full thickness of the neuroepithelial layer in the dorsal midline of r1/r2 and r3 was significantly reduced when compared with those at stages 11 and 12. Here, the roof plate comprised a simple squamous surface epithelium overlying a single layer of cuboidal neuroepithelial cells. Additionally, the midline neuroepithelial eminence, observed in r1/r2 at stage 11, had disappeared in all embryos. Thinning of the roof plate in embryos at stages 14 and 15 extended more laterally than in stage 13 embryos.

WOUND HEALING

The results indicated that the healing of neural tube wounds in stages 11 and 12 embryos followed a similar pattern. Therefore, they are described together. The wounds are described at three levels, namely, the rostral level (site A), middle level (site B), and caudal level (site C). The length of the wound is defined as the length along the longitudinal axis of the wound (i.e. A-C) (Fig. 2).

Zero hour (fresh) wounds

Fresh wounds appeared slit-like or gaped slightly when examined as whole-mounts. r1-r3 wounds were longer than those involving only r1/r2, but shared similar characteristics with the later. Examination of transverse sections of each wound revealed that it involved a full thickness of both surface epithelial and neuroepithelial layers of the roof plate. At its ends (i.e., at sites A and C), both surface epithelial and neuroepithelial wounds were slit-like (Figs 3A and C). The edges of the surface epithelial wounds curled towards the neuroepithelium in such a manner that they capped the edges of the adjacent neuroepithelial wound. Neuroepithelial cells in the immediate vicinity of the neuroepithelial wounds were rounded; they appeared similar to neuroepithelial cells in the dorsal midline of control (unwounded) embryos (cf. Figs. 3A with Fig. 1A). The morphology of both surface epithelial and neuroepithelial wounds at site B was similar to that at sites A and C except that at site B the wounds gaped slightly (Fig. 3B). The extent of gaping varied even among embryos of the same stage. In most of the embryos, the neuroepithelial eminence seen in the control embryos was split into two halves. In embryos with r1/r2 wounds, neural crest cells were identified between the surface epithelium and the basal surface of the neuroepithelium along the whole extent of the wounds. However, in embryos with r1-r3 wounds neural crest cells were seen sandwiched between the surface epithelial and neuroepithelial layers at sites A and B but were absent at site C, thus confirming that r1-r3 wounds involved both r1/r2, where neural crest cells migrate out of the neural tube, and r3 where neural crest cells do not migrate. With acridine orange staining, fluorescence was observed along the wound edges (Fig. 3D), and the intensity of the fluorescence was similar to what was observed in the control embryos.

Half-hour reincubation

Following thirty minutes of reincubation, the wounds showed signs of healing at their ends. The wound edges were in contact with each other at sites A and C. Here, transverse sections of the wounds revealed that the surface epithelial wound edges had fused, and therefore, the healed surface epithelium consisted of a continuous sheet of cells which curled towards the neuroepithelium, creating a “V” -shaped depression (Figs 4A and C). At site B, the surface epithelial wound edges were separated by a gap, with the cells at the wound edges capping the neuroepithelial cells as before (Fig. 4B). The gap between the wound edges appeared narrower than it was at zero hour in most embryos (cf. Fig. 4B with 3B). As in the surface epithelium, the edges of the neuroepithelial wounds were in contact with each other at sites A and C, but separated by a gap at site B (Figs. 4a,b,c). However, at all three sites, neuroepithelial cells in the immediate vicinity of the neuroepithelial wounds were still rounded. There was also no significant change in the size of the neuroepithelial eminence when compared to its size at zero hour. In embryos with r1/r2 wounds, migratory neural crest cells were sandwiched between the surface epithelium and the neuroepithelium at all three sites (Figs 4A to C) but the neuroepithelium seemed reduced in its full thickness at site C (cf. Figs 4C with 3C). However, in embryos with r1-r3 wounds neural crest cells were seen sandwiched between the surface epithelial and neuroepithelial layers at sites A and B.

One-hour reincubation

After one hour of re-incubation, the wounds had further decreased in length (about 61% for r1/r2 wounds and 50% for r1-r3 wounds). In transverse sections, the appearance of both

surface epithelial and neuroepithelial wounds at sites A and C was similar to that at corresponding sites of thirty-minute-old wounds. At site B, the edges of both surface epithelial and neuroepithelial wounds were now separated only by a slit-like gap. However, the edges of the surface epithelial wounds still capped the adjacent neuroepithelium. The neuroepithelial eminence was present in the caudal part of r1/r2 in only a few embryos but neuroepithelial cells in the immediate vicinity of the wounds were rounded at all three sites. The location of migratory neural crest cells was similar to that at half-hour. The intensity of fluorescence was similar to what was observed in the fresh wounds suggesting that apoptosis was still occurring (Fig. 5). The outline of the wounds, however, now appeared slit-like.

Two-hour reincubation

After two hours of reincubation, the length of r1/r2 wounds had decreased by about 95%, and that of r1-r3 wounds by about 90%. In both wounds there was complete healing at sites A and C so that it was difficult to identify these sites. At site B the wounds were represented by a shallow depression. Transverse sections of the wounds showed that at sites A and C the edges of the surface epithelium no longer curled towards the neuroepithelium (Figs 6A and C). The V" –shaped depression that formed above the surface epithelium had therefore disappeared. At site B, the edges of the surface epithelial wounds were now in contact with each other in embryos with r1/r2 wounds though they still curled slightly towards the neuroepithelium, creating a shallow V"–shaped depression above the fused surface epithelium (cf. Figs 6B with 4B). In embryos with r1-r3 wounds, the edges of the surface epithelial wounds were separated by a slit-like gap at site B, whereas neuroepithelial wounds at sites A and C were healed. Additionally, the neuroepithelial eminence had either disappeared or significantly reduced in size, and the full thickness of neuroepithelial layer in the dorsal midline of r3 had decreased. At site B, though a slit-like gap existed between the wound edges in the r1-r3 wounds, the edges of the r1/r2 wounds were now in contact with each other. Rounded neuroepithelial cells were seen in the immediate vicinity of the wound at all three sites, and migration of neural crest cells was as described for one-hour wounds. Acridine orange fluorescence was similar to what was observed in one-hour wounds. In embryos with closed wounds, acridine orange fluorescence was seen in the dorsal midline of r1/r2 as in the controls (Fig. 6D).

Twelve-hour (overnight) reincubation

By twelve hours of reincubation the wounds were healed. Transverse sections of the wounds revealed that there was complete healing of both the surface epithelium and neuroepithelium (Fig. 7). There was also a significant reduction in the full thickness of the roof plate in the whole extent of each wound (Fig. 7). The surface epithelium was made up of simple squamous cells, while the neuroepithelium now comprised simple cuboidal cells. In the r1-r3 wounds, thinning of the roof plate extended more laterally in r3.

SUMMARY OF WOUND HEALING

1. Fresh wounds were slit-like at sites A and C but gaped slightly at site B.
2. The edges of the surface epithelium capped the edges of adjacent neuroepithelium.
3. By thirty minutes of reincubation wound healing had begun at the ends (i.e., sites A and C) of the both r1/r2 and r1-r3 wounds, with the edges of both surface epithelial and neuroepithelial wounds making contact with each other in the dorsal midline.
4. After one hour of reincubation, the gap between the wound edges at site B had narrowed.
5. By two hours of reincubation some r1/r2 wounds were already completely closed.

6. Following twelve hours of reincubation, there was complete healing of both r1/r2 and r1-r3 wounds. Additionally, the roof plate had significantly reduced in its full thickness; it comprised a simple squamous surface epithelium overlying a simple cuboidal neuroepithelium.

Table 1: Comparison between the healing of r1/r2 and r1-r3 wounds.

Time	Length of wound (µm)		Length closed (µm)		Rate of healing (µm/hr)		% of wound healed	
	r1/r2	r1-r3	r1/r2	r1-r3	r1/r2	r1-r3	r1/r2	r1-r3
Zero hour	164	232	0	0	0	0	0	0
Half hour	122	176	42	56	84	112	25.6	24.1
One hour	64	115	100	117	99	117	61	50.4
Two hours	8	24	156	208	78	104	95.1	89.7

Rate of healing
Test of significance using student's *t* test ($p < 0.05$) $t = 0.0303$ (significant)

Percentage of wound healed
Test of significance using student's *t* test ($p < 0.05$) $t = 0.8434$ (nonsignificant)

Comment
The rate of healing of r1-r3 wounds was significantly faster than that of r1/r2 wounds (Table 1). However, the percentage of the original length of the two wounds (i.e. r1/r2 and r1-r3) closed at any given time did not differ significantly (Table 1).

DISCUSSION

Previous studies on embryonic wound healing have shown that the healing capability of a wounded tissue or an organ rudiment may be affected by the nature of morphogenetic processes occurring during wound healing [20,21]. For example, whereas midbrain neuroepithelial wounds heal completely during the formation of the neural tube [20], there is a marked reduction in the healing capability of the midbrain neuroepithelium at a time when rapid morphogenetic brain expansion occurs [17]. Lawson et al., [14], on the basis of their findings that massive apoptosis occurs in the hindbrain roof plate, hypothesized that apoptosis may render the hindbrain roof plate incapable of healing when damaged, leading to the formation of neural tube defects. The present study, which was partly aimed at testing this hypothesis has, however, clearly demonstrated that wound healing in the hindbrain neuroepithelium is not directly affected by the major morphogenetic processes occurring in this portion of the neuraxis, namely, apoptosis and thinning of the hindbrain roof plate. Indeed, the wounds ultimately healed completely despite the high levels of apoptosis in the hindbrain. It is, therefore, not likely that apoptosis had a detrimental effect on the healing process. Therefore, if neural tube defects should develop in the hindbrain through a reopening of its roof plate, the process is likely to be due to factors other than apoptosis. Taken together, the above results demonstrate that the nature of morphogenetic processes occurring in any part of the neuraxis plays a key role in determining whether an assault to that part will result in

neural tube defects or not. Morphogenetic processes that prevent the edges of a wound from coming together ultimately prevent wound healing from taking place, thereby leading to the formation of neural tube defects. On the other hand, if the morphogenetic processes permit the apposition of the wound edges, wound healing occurs, and neural tube defects are not formed.

There is also evidence to suggest that neural tube wound healing affects normal neural tube morphogenesis in embryos [17,21]. Lawson and England [17], in their studies on wound healing in the midbrain of the chick embryo at stages 18-22 demonstrated the effects of neural tube wound healing on morphogenetic brain expansion. Here, healing failed to occur, exposing the apical surfaces of the neuroepithelial cells to the external environment. Additionally, the cavity of midbrain was significantly reduced to an irregular slit by the intense folding of the walls of the neuroepithelium. As a result of this, morphogenetic brain expansion failed to occur and neural tube defects resulted. Clark and Scothorne [21] in a similar study demonstrated that in embryos at stages 17 and 18, the inability of wounds to heal in the spinal cord region of the neural tube has deleterious effects on the occlusion of the neural canal in this part of the neuraxis. The latter morphogenetic process, namely, spinal canal occlusion, is required for normal expansion of the cranial portion of the neuraxis [22]. In the hindbrain region however, the present study has shown that wound healing does not appear to influence the main morphogenetic process occurring in this portion of the neuraxis, namely, thinning of the roof plate. Indeed, acridine orange histochemistry showed that apoptosis occurred normally in spite of wound healing, resulting in the thinning of the hindbrain roof plate. It is clear from the results of the present study that apoptosis in the hindbrain roof plate is neither arrested nor reversed during wound healing. It is interesting that the early embryo has in place reparative mechanisms that ensure that assaults to it are taken care of, thereby preventing the interference of normal morphogenetic processes.

In the present study, closure of both surface epithelial and neuroepithelial wounds started from the ends of the wounds and proceeded in a zipper-like manner toward the middle portion of the wound. This pattern of wound healing observed in these tissues may be related to the level of tension existing in them at the stages used. At stages 11 and 12, tension in these tissues was not high enough to cause the edges of the linear wounds to be pulled far apart [20,21]. Thus, the wound edges gaped very slightly in the middle portion of the wound, while they were separated only by a slit-like gap at the ends of the wound. Therefore, wound healing began at the two ends of the wounds where physical contact between the wound edges was easily established at the start of the healing process, and then progressed in a zipper-like manner towards the middle portion of the wound. Healing of the surface epithelial wound occurred first, followed by healing of the neuroepithelial wound. A reason for this may be that the edges of the surface epithelium capped the edges of the neuroepithelium, making it impossible for apposition of the neuroepithelial wound edges to occur first. However, following the healing and delamination of the surface epithelium from the neuroepithelium, apposition and healing of the neuroepithelial wound edges ensued.

The nature of the cells that effected the healing of the hindbrain neuroepithelial wound is of interest. It has been suggested that at the early stages of development, progenitor neural crest cells have the ability to form neuroepithelial cells [23, 24]. One may thus be tempted to conclude that at the time that neuroepithelium in the dorsal midline of the hindbrain undergoes massive apoptosis, it is possible that progenitor neural crest cells would contribute the cells needed to effect wound healing in the hindbrain. However, there is evidence to suggest the involvement of a subpopulation of hindbrain neuroepithelial cells [13,14]. Lawson et al. [14] have demonstrated

the presence of a subpopulation of neuroepithelial cells in the hindbrain roof plate that do not undergo apoptosis. According to them, these cells rearrange themselves to reform the neuroepithelial layer of the hindbrain roof plate after apoptosis has ceased [13]. It is therefore likely that some or all of these neuroepithelial cells might have participated in the healing of the neuroepithelial wound in the present study.

The present study also demonstrates similarities in the processes of neural tube wound healing and neural fold fusion in the hindbrain of the chick embryo. In the zero hour wound, the cut edges of the surface epithelial layer cap the cut edges of the neuroepithelial layer in much the same way that the surface epithelial layers of the bilateral neural folds cap the neuroepithelial layers prior to neural fold fusion [13]. Like hindbrain neural fold fusion therefore, healing of the surface epithelial layer occurs first, followed by healing of the neuroepithelial layer once the former has delaminated from it. It has been suggested that the migration of cephalic neural crest cells may contribute to surface epithelial fusion during neurulation [13]. According to Lawson and colleague [13], the neural crest cells may help to push the edges of the surface epithelial layers of the neural folds medially as they migrate to enhance fusion. In the present study, the presence of neural crest cells immediately deep to the healing surface epithelial layer may in a similar fashion enhance surface epithelial wound healing at these stages.

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