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Age-dependent residual tensile strains are present in the dura mater of rats

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The objectives of this study were to determine whether residual tensile strains exist in the dura mater of mammals *in vivo*, and whether the strains are age-dependent. We made incisions in the parietal dura mater of immature and mature rats, and measured the retraction of the dura mater from each incision. We then used a finite-element model to calculate the strain present in the parietal dura mater of each rat. We found that age-dependent residual tensile strains are present in the dura mater of rats. The mean average residual strain of the immature rats was significantly larger than that of the mature rats ($4.96 \pm 1.54\%$ (s.d.) versus $0.39 \pm 0.13\%$, $p < 0.0001$), with the mean strain calculated in the mature rats of the order of the minimum measurement that could be made using our experimental approach. In addition, in the immature rats mean residual strain in the longitudinal direction was significantly larger than mean residual strain in the transverse direction ($6.11 \pm 3.62\%$ versus $3.82 \pm 2.64\%$, $p = 0.0218$). Our findings show that age-dependent residual tensile strains exist in the dura mater of rats. We speculate that these strains may reflect the rate and direction of cranial growth and may also influence cranial healing.

Keywords: calvarial healing; mechanobiology; cranial growth and development; growth-generated strain; prestress

1. INTRODUCTION

Both the human cranium and the rat cranium grow rapidly during early postnatal development, as approximately 90% of intracranial volume growth has occurred by two years of age in humans and by two months of age in rats (Abbott *et al.* 2000; Fong *et al.* 2003; Henderson *et al.* 2004b). Growth during development can generate residual strains and stresses (or prestresses) in connected tissues that grow at different rates (Henderson & Carter 2002). Residual strains and stresses are the strains and stresses that remain in a tissue or organ after all external loads have been removed (Gregersen *et al.* 2000).

Residual strains and stresses have previously been found in the tissues of a number of organ systems, including the cardiovascular system (Vaishnav & Vossoughi 1983; Fung 1984; Han & Fung 1996; Vaishnav & Vossoughi 1987), the gastrointestinal tract (Gregersen *et al.* 2000) and the musculoskeletal

system (Bertram *et al.* 1998; Popowics *et al.* 2002). It has been proposed that residual strains and stresses may influence not only the biomechanical behaviour of tissues, but also the local cell biology of the tissues (Gregersen *et al.* 2000). For example, in the duodenum of the gastrointestinal tract, the prestress owing to residual strain may contribute to increased resistance to luminal pressures (Gregersen *et al.* 2000). In addition, the residual strains may influence local cell biology and thereby influence the height of the mucosal villi (Gregersen *et al.* 2000). In the perichondrium/periosteum of developing bones, the prestress owing to residual strain may play a structural role in some locations, such as the zygomatic arch (Popowics *et al.* 2002). In addition, the residual strains may influence the growth of the associated bones (Warrell & Taylor 1979; Bertram *et al.* 1998; Popowics *et al.* 2002).

The objectives of this study were to determine whether residual tensile strains are present in the dura mater of mammals *in vivo*, and whether the residual tensile strains are age-dependent. To achieve these objectives, we harvested the calvariae of immature and mature rats, made incisions in the parietal dura mater and measured the retraction of the dura mater from the incisions. Using a finite-element model, we then

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calculated the residual tensile strain present in the parietal dura mater of each rat.

2. METHODS

2.1. Animals

Time-dated Sprague–Dawley rats were purchased from Charles River, Inc. (Wilmington, MA). All rats were housed in a temperature- and light-controlled environment and provided with food and water *ad libitum*. All experiments were performed in accordance with Animal Care and Use Guidelines for Stanford University.

2.2. Calvarial harvest and dural incision

A total of 34 immature rats were euthanized via CO_2 asphyxiation at the following ages: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26 postnatal days ($n=1$ –4 rats per age group). An additional seven mature rats were sacrificed between 95 and 105 postnatal days. Calvariae were carefully dissected free (leaving intact the dura mater underlying and attached to the frontal, parietal and occipital bones), and were washed in phosphate-buffered saline (PBS). Great care was taken to maintain the dural attachments at the borders of the parietal bone, specifically the coronal suture (anterior border), sagittal suture (medial border), occipital suture (posterior border) and sphenoparietal suture (lateral border), as these sutural attachments of the dura mater to the cranium maintained the dura mater in a strained state after the calvaria was dissected free of the surrounding tissues.

Surgical incision of the dura mater was performed under a dissecting microscope with the calvariae immersed in PBS. A longitudinal (anterior–posterior) incision was made with a No. 15 blade scalpel in the dura mater over the flattest part of one of the parietal bones (figure 1); the anatomical side of the longitudinal incision was randomized for each rat. A separate transverse (medial–lateral) incision was then made perpendicular to the first incision in the dura mater over the contralateral parietal bone. For each rat, the length of each incision was made in approximate proportion to the size of the cranium. As such, the length of the incisions tended to increase with the age of the rat.

Following incision, the dura mater was allowed to relax for 5 min to permit the tissue to reach a new equilibrium state. During incision or the subsequent relaxation period, the edges of the incision often retracted, causing the incision to assume an elliptical shape (figures 1 and 2a). Images of the incisions were then captured at a minimum resolution of 1600×1200 pixels using a Nikon Coolpix 5700 digital camera (Nikon; Melville, NY) attached to the dissecting microscope. Image dimensions were calibrated to a known standard and the length (a) and maximum width (v) of each incision was measured by a single trained operator using IMAGEJ software (NIH; Bethesda, MD; figures 1 and 2a). For each length or width measured, two independent measurements were made and then averaged. There was a relatively low difference between

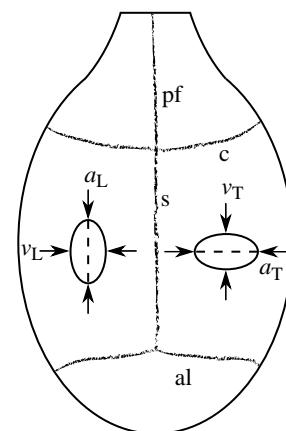


Figure 1. Schematic of the rat cranial vault, showing the approximate location of the longitudinal and transverse incisions. This dorsal view shows the posterior frontal (pf), sagittal (s), coronal (c) and anterior lambdoidal (al) sutures. The anterior–posterior and medial–lateral dashed lines indicate the approximate locations at which the respective longitudinal and transverse incisions were made in the dura mater. The anatomical side of the incisions was randomized for each rat. The solid ovals illustrate how the edges of the incision often retracted, causing the incision to assume an elliptical shape. The length (a) and maximum width (v) of each incision was measured as shown.

the two measurements, with an average absolute difference of 5.65% for all immature measurements and 11.96% for all mature measurements.

2.3. Calculation of residual tensile strain

A two-dimensional, plane stress, linear elastic, finite-element model with two axes of symmetry and non-linear geometry was created to represent retraction of the edges of an incision made in rat dura mater under varying levels of simulated residual tensile strain (figure 2b). The initial side length of the unstrained model was $L_0=10$ mm. The initial dimensions of the unstrained incision were 0.8333 mm long by 0 mm wide. Longitudinal (δ_L) and transverse (δ_T) displacements were applied to the edges of the model to simulate straining of the dura mater in both the longitudinal and transverse directions (figure 2c). The longitudinal (ε_L) and transverse (ε_T) strains applied by these displacements were calculated as $\varepsilon_L=\delta_L/L_0$ and $\varepsilon_T=\delta_T/L_0$. Analysis of the model was performed for 11 different magnitudes of δ_L (0–1 mm with increments chosen manually as 0.1 mm), with 11 magnitudes of δ_T (0 to ~ 1 mm with increments chosen automatically during solution by the finite-element program (MSC.Patran, MSC.Software; Santa Ana, CA)) for each magnitude of δ_L , resulting in an analysis of a total of 121 combinations of δ_L and δ_T corresponding to 121 combinations of ε_L and ε_T . The maximum strain applied in either the longitudinal or transverse direction was therefore 10%. For each combination of δ_L and δ_T , and the associated ε_L and ε_T , applied to the model, we recorded the half-length ($a/2$) and the maximum half-width ($v/2$) of the incision (figure 2d). The assigned elastic modulus of the dura mater was 1 MPa (a parametric analysis showed that increasing

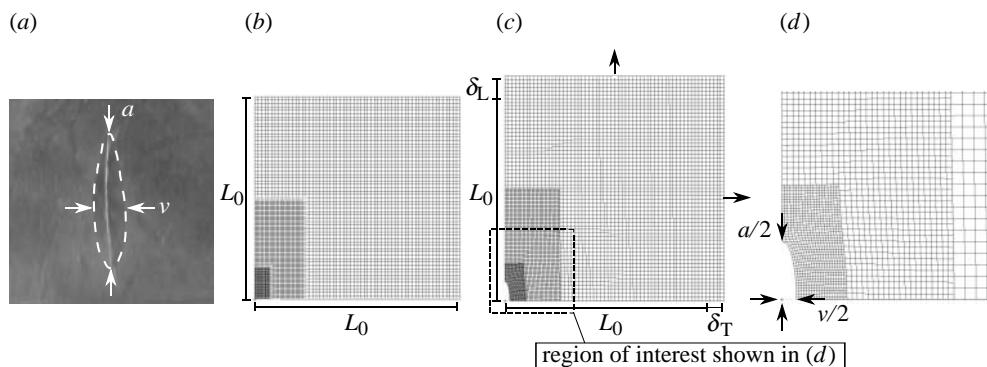


Figure 2. A longitudinal incision in the dura mater of a 2-day-old rat and the finite-element model used to calculate the strains present in the dura mater. (a) Following incision, the length (a) and maximum width (v) of each incision was measured. The dashed line denotes the edge of the incision after being allowed to relax for 5 min. (b) The finite-element model used to calculate retraction of the edges of an incision made in rat dura mater under varying levels of tensile strain. The unstrained model had an initial side length of L_0 . (c) Longitudinal (δ_L) and transverse (δ_T) displacements were applied to the edges of the model to simulate straining of the dura mater in both the longitudinal and transverse directions. (d) For each combination of δ_L and δ_T applied to the model, we recorded the half-length ($a/2$) and the maximum half-width ($v/2$) of the incision.

or decreasing the elastic modulus by one order of magnitude had a negligible effect on the results; data not shown). The assigned Poisson's ratio of the dura mater was 0.45, based on previous finite-element modelling of human dura mater (Voo *et al.* 1996). The closed-form Westergaard solution (Westergaard 1939) for the displacement of a through-thickness crack in an infinite plate loaded in biaxial tension was not used for this analysis, as the large displacements observed in the incised dura mater violated the small displacement assumption of the Westergaard solution. To accommodate the large displacements, the finite-element analysis was solved for nonlinear geometry. To verify the finite-element analysis results for small strains, the results were compared with the Westergaard solution results and, as expected, for small displacements, the finite-element results closely matched the Westergaard solution results (data not shown). For example, for a ratio of incision width to incision length (v/a) of $0.03553 \text{ mm mm}^{-1}$, the Westergaard solution (0.976% strain) differed from the finite-element analysis solution (1.000% strain) by only 0.023% strain.

To calculate the residual tensile strains present in the parietal dura mater of each rat, we used the finite-element results to determine the mathematical relationship between the ratio of incision width to incision length (v_L/a_L) of a longitudinal incision made in dura mater under tensile strains of ε_L and ε_T . The equation describing this relationship was found by fitting a polynomial surface to the 121 values of v_L/a_L as a function of ε_L and ε_T that were produced by the finite-element analysis. To fit the polynomial surface, first a second-order polynomial was fit to the 11 values of v_L/a_L for which $\varepsilon_T=0$. Then, 11 first-order polynomials were fit to each set of values of v_L/a_L corresponding to a constant ε_L . A third-order polynomial was then fit to the slopes of the first-order polynomials. The equation for the polynomial surface was formed by combining the second and third-order polynomials. All polynomial fitting was performed in EXCEL (Microsoft; Redmond, WA). The polynomial surface closely fit the data: excluding the value of v_L/a_L produced by the

combination of ε_L and ε_T closest to zero, the average absolute error between the value of v_L/a_L predicted by the polynomial surface and the value of v_L/a_L produced by the finite-element analysis was 0.29% (for the combination of ε_L and ε_T closest to zero, which was $\varepsilon_L=0\%$ and $\varepsilon_T=0.01\%$, the error between the value of $v_L/a_L=0.0005451$ predicted by the polynomial surface and the value of $v_L/a_L=0.0002470$ produced by the finite-element analysis was 121%). The resulting equation for the polynomial surface,

$$\frac{v_L}{a_L} = (-1.3225\varepsilon_L^2 + 1.0966\varepsilon_L + 0.0003) + \varepsilon_T(51.243\varepsilon_L^3 - 3.5804\varepsilon_L^2 - 2.2536\varepsilon_L + 2.451), \quad (2.1)$$

allows calculation of v_L/a_L for known values of ε_L and ε_T . To determine the corresponding equation for the ratio of incision width to incision length (v_T/a_T) of a transverse incision, we used the symmetry of the finite-element model and exchanged the longitudinal and transverse strain direction, which yielded

$$\frac{v_T}{a_T} = (-1.3225\varepsilon_T^2 + 1.0966\varepsilon_T + 0.0003) + \varepsilon_L(51.243\varepsilon_T^3 - 3.5804\varepsilon_T^2 - 2.2536\varepsilon_T + 2.451). \quad (2.2)$$

The above equations allow calculation of v_L/a_L and v_T/a_T for known values of ε_L and ε_T . However, the present study required calculation of ε_L and ε_T from the known values of v_L/a_L and v_T/a_T measured in the dura mater of each rat. Therefore, for each rat, we determined the residual longitudinal and transverse tensile strains present in the dura mater from the measured incision dimensions (v_L/a_L , v_T/a_T) by simultaneously solving equations (2.1) and (2.2) for the combination of ε_L and ε_T that uniquely satisfied the two dependent equations. The MATHEMATICA (Wolfram; Champaign, IL) software package was used for solution of the dependent equations. We then calculated the average residual strain (ε_{AVG}) in the dura mater of each rat as $\varepsilon_{AVG}=(\varepsilon_L+\varepsilon_T)/2$.

3. RESULTS

We found that residual tensile strains are present in the dura mater of rats. As measured from the images of the incisions, the average longitudinal and transverse incision lengths were 3.24 ± 1.22 mm (s.d.) and 2.95 ± 0.78 mm for the immature rats and 5.31 ± 0.98 and 4.04 ± 0.79 mm for the mature rats. The increase in length of the incisions with the age of the rat resulted from each incision being made in approximate proportion to the size of the cranium. The average longitudinal and transverse incision widths were 0.44 ± 0.17 and 0.55 ± 0.27 mm for the immature rats and 0.05 ± 0.02 and 0.07 ± 0.03 mm for the mature rats.

The mean average residual strain in the dura mater of immature rats was significantly larger than the mean average residual strain in the dura mater of mature rats ($4.96 \pm 1.54\%$ versus $0.39 \pm 0.13\%$, $p < 0.0001$; figure 3). Both means were significantly different than zero ($p < 0.0001$ for immature rats and $p = 0.0002$ for mature rats). However, the mean strain calculated in the mature rats is of the order of the minimum measurement that could be made (see §4), and indicates that the strains in the mature rats were too small to measure accurately using our experimental approach. Average residual strain (ε_{AVG}) in the immature rats (postnatal age 2–26 days) exhibited a weak but statistically significant decrease with age ($r^2 = 0.1827$, $p = 0.0117$; figure 4).

When we examined the longitudinal and transverse components of the residual strain of each immature rat, we found that the longitudinal strain (ε_L) showed no dependence with age ($p = 0.6598$) and the transverse strain (ε_T) exhibited a weak but statistically significant decrease with age ($r^2 = 0.1536$, $p = 0.0219$; figure 5). When the mean longitudinal and transverse strains for the immature rats were compared, we found that mean longitudinal strain was significantly larger than the mean transverse strain ($6.11 \pm 3.62\%$ versus $3.82 \pm 2.64\%$, $p = 0.0218$), and both means were significantly different than zero ($p < 0.0001$; figure 5). As discussed above, we determined that the residual strains in mature rats were too small to measure accurately using the current technique, and the longitudinal and transverse residual strains of the mature rats were not compared.

4. DISCUSSION

The objectives of this study were to determine whether residual tensile strains are present in the dura mater of mammals *in vivo*, and whether the residual tensile strains are age-dependent. Our findings show that residual tensile strains are present in the dura mater of rats and that the residual tensile strains are age-dependent. We also found that the residual tensile strain in the dura mater of immature rats is on average larger in the longitudinal direction than in the transverse direction.

The main limitation of this study is that the method used to calculate the residual tensile strain present in the dura mater cannot calculate small strains that do not retract the incision edges more than the initial

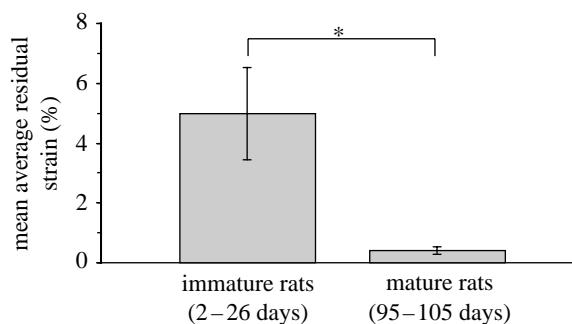


Figure 3. Mean average residual strain in the dura mater of immature rats (postnatal age 2–26 days) versus mean average residual strain in the dura mater of mature rats (postnatal age 95–105 days). The mean average residual strain for immature rats was significantly larger than the mean average residual strain for the mature rats ($4.96 \pm 1.54\%$ versus $0.39 \pm 0.13\%$, $p < 0.0001$). However, the mean strain calculated in the mature rats is of the order of the minimum measurement that could be made (see §4), and indicates that the strains in the mature rats were too small to measure accurately using our experimental approach.

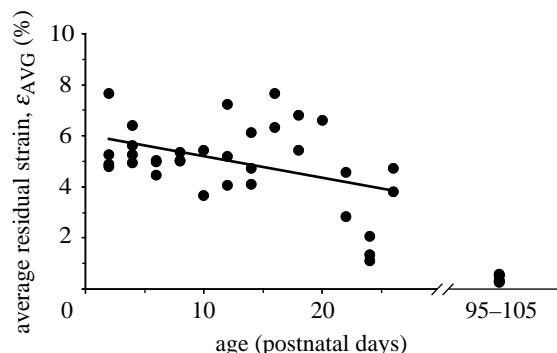


Figure 4. Average residual tensile strain versus age in the dura mater of immature and mature rats. The average residual strain (ε_{AVG}) in the immature rats (postnatal age 2–26 days) exhibited a weak but statistically significant linear decrease with age ($\varepsilon_{AVG} = 6.00 - 0.08 \times \text{age}$, $r^2 = 0.1827$, $p = 0.0117$). There are seven rats in the mature group. The exact age of each mature rat was unknown, but all were between postnatal age 95 and 105 days. Note that there is a discontinuity on the x-axis (age).

displacement of the edges caused by the width of the incising scalpel blade. The maximum width of the No. 15 blade scalpel used to make the incisions is 0.37 mm, as measured by digital callipers. Therefore, we assume that the blade would create an incision width of up to 0.37 mm, and that incisions measured to have a width of 0.37 mm or less may not reliably indicate the residual tensile strains present in the dura mater of that rat. For the immature rats, both the average longitudinal incision width (0.44 ± 0.17 mm) and average transverse incision width (0.55 ± 0.27 mm) were greater than 0.37 mm. In contrast, for the mature animals, both the average longitudinal incision width (0.05 ± 0.02 mm) and average transverse incision width (0.07 ± 0.03 mm) were substantially smaller than 0.37 mm. Consequently, our analysis may overestimate the strains present in the dura mater of mature rats, and so the analysis cannot determine whether there is no strain or low strain in the dura mater of mature rats.

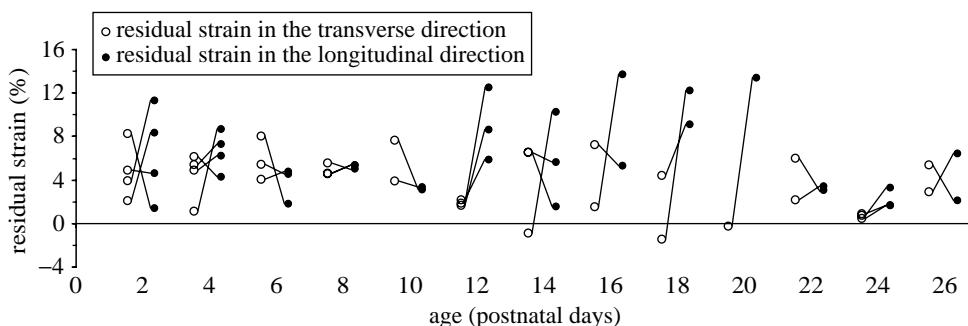


Figure 5. Longitudinal and transverse residual strain versus age in the dura mater of immature rats. Longitudinal strain (filled circle, ε_L) showed no dependence with age ($p=0.6598$), and transverse strain (open circle, ε_T) exhibited a weak but statistically significant decrease with age ($\varepsilon_T = 5.45 - 0.13 \times \text{age}$, $r^2=0.1536$, $p=0.0219$; regression line not shown). The mean longitudinal residual strain was significantly larger than the mean transverse residual strain ($6.11 \pm 3.62\%$ versus $3.82 \pm 2.64\%$, $p=0.0218$). A line connects the longitudinal strain and transverse strain for a given rat.

It is also worth noting that the relative error associated with the image-based measurement of incision length and width increased with decreasing magnitude of the dimension being measured. The average error, given as a percentage of the total measurement, associated with a change of one pixel was 0.28 and 1.86% for measurement of length and width of immature incisions, and 0.14 and 10.45% for measurement of length and width of mature incisions.

The study also included a number of assumptions. First, we modelled the rat dura mater as structurally isotropic (uniform material properties in all directions). The results of a study by Hamann *et al.* (1998) using small-angle light scattering indicated that, in general, adult human cranial dura mater is structurally isotropic for size scales greater than 1 cm^2 , but with localized regions of highly aligned fibres in the vicinity of the coronal sutures. It is not known whether the size scale for isotropy in immature and mature rat dura mater is proportionally smaller than that in adult humans, or whether there are similarly localized regions of highly aligned fibres in certain regions of the rat dura mater. If the rat dura mater is not isotropic at the location of the incisions used in the current study, and if this anisotropy is on a sufficiently large scale to affect the retraction of the dura mater from the incisions, then the incorrect assumption of isotropy could affect the accuracy of the calculated residual strains. Whether or not there is anisotropy in the rat dura mater, large residual strains must be present in the dura mater of immature rats to account for the large retraction observed. However, anisotropy could (at least partly) account for the finding that residual tensile strain in the longitudinal direction was larger than the residual tensile strain in the transverse direction. Therefore, future work is required to determine whether and to what extent the longitudinal and transverse strains differ. The second assumption was that we modelled the dura mater using a plane stress analysis, which disregarded the slight curvature of the dura mater where the incisions were made. That the length of the incisions was small relative to the curvature of the dura mater at the location of the incisions suggests that this is a reasonable assumption. Third, for modelling purposes, we assumed that the longitudinal and transverse directions are the directions

of principal strain in the dura mater. The relatively symmetric retraction of the dura mater perpendicular to the incisions observed during the experiments suggests that this was a reasonable assumption, and that large shear strains were not present along the incisions. Fourth, we assumed that the attachment of the dura mater to the parietal bone did not interfere with opening of the incision and did not vary with age. Although we did not quantitatively measure the strength of the adhesion, the qualitative experience of the surgeon who performed the incisions was that the dura mater of rats is not firmly attached over the flat portion of the parietals, that there were not detectable differences in the strength of adhesion of the immature and mature rat dura mater, and that the immature and mature rat dura mater are equally prone to peel off the flat portion of the parietal in response to manipulation. Fifth, we assigned a Poisson's ratio of 0.45 to the rat dura mater, based on previous finite-element modelling of human dura mater (Voo *et al.* 1996), and we assumed that the Poisson's ratio did not change with age and that residual strains were the same in the two parietal dura mater samples from each rat. These assumptions could have affected the accuracy of the calculated strains (see appendix). For example, the mean longitudinal strain and mean transverse strain are $6.11 \pm 3.62\%$ and $3.82 \pm 2.64\%$ for a Poisson's ratio of 0.45 and $9.82 \pm 4.11\%$ and $8.12 \pm 2.83\%$ for a Poisson's ratio of 0.0. Although the present analysis was based upon the assumptions listed above, we believe that the results are suitably robust to contribute to our understanding of the mechanical environment of the dura mater. In addition, the need for these assumptions highlights the relative scarcity of information on the material properties and mechanical environment of the dura mater.

The finding that, in the dura mater of immature rats, the residual tensile strain in the longitudinal direction is larger than the residual tensile strain in the transverse direction may reflect the fact that the growth of the rat skull favours longitudinal growth during the period of development that we analysed. In a longitudinal radiographic study on the growth of the skull of black-hooded rats, Hughes *et al.* (1978) found that calvarial length increased more rapidly than calvarial width from before birth to postnatal day 5 and then

again from postnatal day 14 to postnatal day 119. We suggest that our finding that, on average, strain in the longitudinal direction is larger than the strain in the transverse direction in immature rats between postnatal days 2 and 26 ($6.11 \pm 3.62\%$ versus $3.82 \pm 2.64\%$, $p=0.0218$) is a result of this differential growth. We hypothesize that as the cranium grows more in length than in width, the parietal dura mater, which is tethered to the cranium at the sutures, is strained more in the longitudinal direction than the transverse direction.

Cranial sutures are the primary sites of cranial growth during early postnatal development (Mednick & Washburn 1956; Duterloo & Enlow 1970). It has long been hypothesized that brain growth generates quasi-static tensile strain across the sutures (Herring 1993) and that this quasi-static tension influences the rate of bone deposition and, therefore, growth at the sutures (Enlow 1990). The mean average residual tensile strain that we have measured in the dura mater of immature rats in the present study ($4.96 \pm 1.54\%$) is larger by two to three orders of magnitude than the tensile strains resulting from intracranial pressure that we have calculated to exist in the sutures of humans (approximately 0.0021 – 0.0410% at 1 month of age) and rats ($0.0270 \pm 0.0190\%$ between 2 and 60 days) (Henderson *et al.* 2004a). Based on this finding, the mechanosensitivity and osteogenic nature of the dura mater, and the fact that the dura mater is integrated into the fibrous tissue at the sutures, we speculate that the large residual tensile strains present in the dura mater during early postnatal development may promote osteogenic signalling from the dura mater at the site of its attachment to the sutures. If and how such signalling could influence the bone deposition rate of osteoblasts in the sutures, and therefore influence the rate and direction of cranial growth, is unclear.

The adult skeleton possesses amazing regenerative potential, being capable of healing defects and deficiencies via the formation of new bone. However, while immature animals and children under the age of 2 years maintain the capacity to heal large calvarial defects, adult animals and humans are incapable of healing the smallest of injuries (Glaser & Blaine 1940; Sirola 1960; Aalami *et al.* 2004). This inability of adults to heal calvarial defects secondary to trauma or disease constitutes a significant biomedical burden, as a number of techniques (including grafting of autogenous bone, application of synthetic pastes or scaffolds and insertion of alloplastic materials) must be employed to correct the defects resulting from the nearly 13 000 craniotomies and craniectomies performed annually. The estimated cost of procedures to repair these defects in the US alone was over 400 million in 2001 (Bostrom & Mikos 1997; Steiner *et al.* 2002). By understanding the molecular, cellular and biomechanical factors that enable immature animals to re-engineer calvarial bone, we may be able to develop clinically applicable strategies with which to repair adult calvarial defects.

Although the discrepancy in calvarial healing between children and adults has been recognized for centuries, it is only relatively recently that

investigators have begun to gain insight into the biology underlying the age-dependent phenomena of calvarial regeneration. This knowledge has been gained by studying the contributions of the anatomic components that comprise a healing calvarial defect. These components include the bony edges of the defect, the overlying periosteum, and the underlying dura mater. While all of these structures have been postulated to contribute to or orchestrate the regeneration of calvarial bone in immature animals and children, data from modern *in vivo* studies suggest that the immature dura mater is the key regulator of calvarial regeneration (Berezowsky 1899; Hobar *et al.* 1993, 1996; Wang & Glimcher 1999; Mardas *et al.* 2002). For example, as early as the 1800s, Berezowsky (1899) reported on the inability of rabbit calvarial defects to heal if the dura mater was disturbed. That immature dura mater is required for calvarial healing has perhaps been demonstrated best by Hobar *et al.* (1993), who demonstrated that critical sized calvarial defects in adult guinea pigs could be rescued and converted to healing defects by the transplantation of immature dura mater. Conversely, transplantation of adult guinea pig dura mater to immature guinea pigs resulted in a loss of regenerative capacity, thus strengthening the theory that immature dura mater is a key mediator of calvarial re-ossification.

Based on this evidence suggesting that the dura mater and its stage of development are critical in ensuring successful calvarial healing, several investigators have examined dura mater *in vitro*, and have looked for age-related differences in the molecular and cell biology of this tissue. Greenwald *et al.* (2000a) have demonstrated that immature dura mater from 6-day-old rats is significantly more biologically active *in vitro* than dura mater from mature (60-day-old) rats, producing greater amounts of protein and/or mRNA for numerous osteogenic factors including FGF-2, TGF- β 1, collagen I α I and osteocalcin. In addition, immature dura mater was shown to proliferate at an accelerated rate, and was the only dura mater capable of forming bone nodules *in vitro* (Greenwald *et al.* 2000a). Greenwald and colleagues have also demonstrated the presence of greater numbers of alkaline phosphatase-positive cells (an early marker of osteoblast differentiation) in cultured immature dura mater than in cultured mature dura mater (Greenwald *et al.* 2000b). These data suggest that immature dura mater, but not mature dura mater, may provide both humoral and cellular elements required for calvarial re-ossification.

Noting that the window of calvarial healing competency in humans corresponds roughly with the period of most rapid cranial growth, and that rapid growth of the brain has been hypothesized to produce tensile strain in the calvaria (Herring 1993), Fong *et al.* (2003) investigated the effect of equibiaxial tensile strain on gene expression in immature rat dura mater *in vitro*. They demonstrated that application of 10% static equibiaxial tensile strain to cultured non-suture dura mater cells derived from immature rats increased cell proliferation and expression of osteogenic growth factors, including FGF-2.

Our findings show that there are residual tensile strains in the dura mater of immature rats *in vivo*, and that these strains decrease by maturity. However, it is unclear whether residual strain decreases gradually, as suggested by a weak but statistically significant linear decrease with age (figure 4), or suddenly, as might be suggested by the strain data from the 22, 24 and 26 day rats (figure 4). The average residual tensile strain present in the dura mater of immature rats (postnatal age 2–26 days) was $4.96 \pm 1.54\%$. The study by Fong *et al.* (2003) showed that application of strains of the same order of magnitude (10% strain) to cultured non-suture dura mater cells derived from immature rats increased cell proliferation and expression of osteogenic growth factors, including FGF-2. In contrast, our findings suggest that there are no strains or low strains in the dura mater of mature rats, as discussed above.

Based on our current findings documenting the presence and decrease with age of residual tensile strain in rat dura mater *in vivo*, we speculate that large residual tensile strains present in the dura mater during growth may influence local cell biology and facilitate calvarial healing in immature animals, whereas the absence of large strains during maturity may impede healing in adult animals. Although this preliminary measurement of the residual tensile strains present in the dura mater does not provide conclusive evidence that residual tensile strain in dura mater regulates calvarial healing, the overlap between the time periods of calvarial growth, the presence of *in vivo* strain and the window of competency for calvaria re-ossification suggests that these processes may be intimately linked. It should be noted, however, that experiments such as those by Hobart *et al.* (1993) indicate that immature guinea pig dura mater remains capable of healing calvarial defects, even when transplanted into adult guinea pigs. This finding suggests that, even if residual tensile strains influence the healing capacity of immature dura mater, the residual tensile strains may not need to be present at the time of healing since they may have already exerted their effects on the differentiation of constituent cells.

Because the endosteal outer layer of the dura mater is analogous to the periosteum of the bony skeleton, it is worth noting that previous investigations have recorded large residual strains in the periosteum of a number of bones. During mechanical testing of the periosteum from tibiotarsi of 4- to 14-week-old chicks, Bertram and colleagues (1998) allowed samples to retract 10–15% before testing, indicating *in vivo* residual strains within that range. During testing of the periosteum from the mandibular bodies, zygomatic arches and metacarpals of immature pigs, Popowics and colleagues (2002) measured mean *in vivo* residual strains ranging from 3.1% for the long-axis of the zygomatic arch to 17.2% for the long-axis of the metacarpals. A number of surgical experiments have used circumferential incisions to release periosteal strains *in vivo* and thereby ‘decompress’ the growth plate of the associated bone (Crilly 1972; Warrell & Taylor 1979). The results of these experiments suggest that growth plate decompression accelerates new bone

formation. However, based on the findings of their mechanical testing, Bertram and colleagues (1998) suggest that circumferential incision of periosteum cannot have any direct mechanical effect on the cells of the growth plate and must instead result from the release of growth-modulating factors that stimulate the growth plate. There may be similarities between the residual strains present in the dura mater and periosteum, but further work is necessary to determine if, how and to what extent the residual strains in these analogous structures influence bone growth.

In summary, we have shown that residual tensile strains exist in the dura mater of rats and that the strains are age-dependent. In addition, we found that in the dura mater of immature rats tensile strain in the primary direction of cranial growth is larger than the tensile strain in the orthogonal direction. Based on these findings, we speculate that residual tensile strains in the dura mater may influence both cranial healing and cranial growth.

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APPENDIX. EFFECT OF THE ASSIGNED POISSON'S RATIO AND THE BILATERAL STRAIN ASSUMPTION ON CALCULATED STRAINS

As mentioned in §4, in our analysis, we assigned a Poisson's ratio of 0.45 to the rat dura mater, based on previous finite-element modelling of human dura mater (Voo *et al.* 1996), and we assumed that residual strains were the same in the two parietal dura mater samples from each rat. Because we calculated longitudinal and transverse strain (from which average strain was calculated) using a set of dependent equations (equations (2.1) and (2.2)), if the assigned Poisson's ratio or the assumption of the strains being the same was not correct, then the calculated strains would be affected. It was not possible to determine whether the residual strains were the same using the current technique, but it is customary to assume bilateral symmetry in biomechanical experiments.

To determine the effect of the assigned Poisson's ratio on the calculated strains, we repeated the finite-element analysis using a Poisson's ratio of 0.0, determined a new set of equations analogous to equations (2.1) and (2.2), and then recalculated the longitudinal, transverse and average strains present in the dura mater of the rats. We found that using a Poisson's ratio of 0 did change the calculated strains, but did not change any of the results we reported from statistically significant to not significant. For example, when we used a Poisson's ratio of 0.45, we had calculated that longitudinal strain (ϵ_L) showed no dependence with age ($p=0.6598$), transverse strain (ϵ_T) decreased with age ($p=0.0219$), mean longitudinal strain was significantly larger than the mean transverse strain ($6.11 \pm 3.62\%$ versus $3.82 \pm 2.64\%$, $p=0.0218$), and both means were significantly different than zero ($p<0.0001$; figure 5 and redrawn in

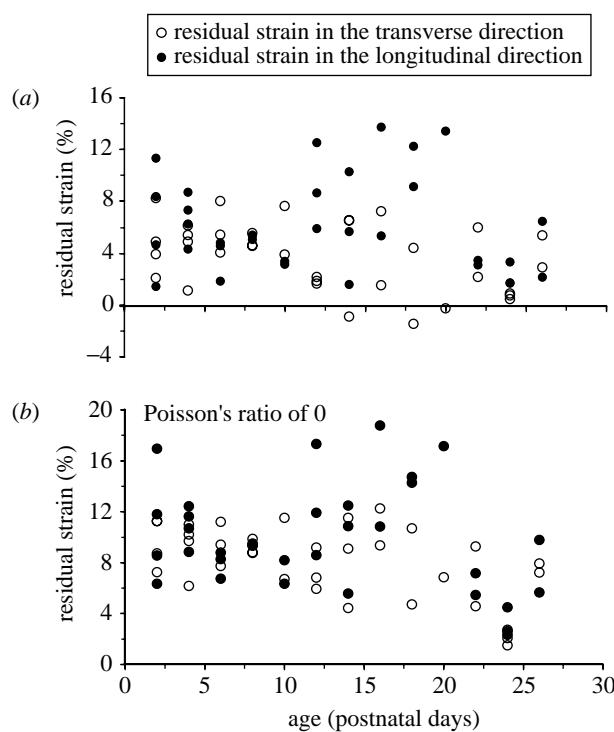


Figure 6. Longitudinal (filled circle) and transverse (open circle) residual strain versus age for the immature rats calculated using a Poisson's ratio of (a) 0.45 and (b) 0. Using a Poisson's ratio of 0 did change the calculated strains but did not change any of the results we reported from statistically significant to not significant. Note that the data in (a) are the same as those presented in figure 5.

figure 6a). When we repeated the analysis using a Poisson's ratio of 0, we again calculated that longitudinal strain (ε_L) showed no dependence with age ($p=0.1884$), transverse strain (ε_T) decreased with age ($p=0.0014$), mean longitudinal strain was significantly larger than the mean transverse strain ($9.82 \pm 4.11\%$ versus $8.12 \pm 2.83\%$, $p=0.0207$), and both means were significantly different than zero ($p<0.0001$; figure 6b). In addition, for a Poisson's ratio of 0, we again calculated that the mean average residual strain of the immature rats was significantly larger than the mean average residual strain of the mature rats ($8.97 \pm 2.87\%$ versus $2.28 \pm 4.34\%$, $p<0.0001$), and the mean strain calculated in the mature rats was again of the order of the minimum measurement that could be made.

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