Biomechanical Strain and Morphologic Changes with Age in Rat Calvarial Bone and Sutures

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Background: The role of calvarial sutures in transmitting biomechanical forces within the head is unclear.

Methods: To examine the biomechanical characteristics of sutures, the authors measured bite force changes in rats and tested for alterations in strain across intrafrontal and sagittal sutures and within parietal bone with age. To understand the effects of suture fusion on strain distribution in the head, the authors measured percentage fusion of the intrafrontal sutures with age ($n = 6$ per age group). The masticatory muscles in anesthetized 9-, 24-, and 70-day-old rats ($n = 15$ per group) were bilaterally stimulated. Stacked delta rosette gauges were fixed across the intrafrontal sutures and sagittal suture, or on the parietal bone. Strain and bite force were measured with a bite force transducer positioned at the incisors.

Results: Bite force increased significantly ($p < 0.05$) with age (9-day-old rats, 72.6 ± 20 gf; 24-day-old rats, 707.3 ± 150 gf; 70-day-old rats, 2425.6 ± 255 gf). Some significant differences were found between the volume and direction of strain among sites and age groups. Compressive strains of 230 $\mu\varepsilon$ on average were found across the intrafrontal sutures at all ages. In contrast, tensile strains less than 180 $\mu\varepsilon$ on average were found across the sagittal sutures of 9- and 24-day-old rats, increasing to 940 $\mu\varepsilon$ on average at day 70. Tensile strains in parietal bone tended to be less than 150 $\mu\varepsilon$.

Conclusions: The timing of sutural closure and patterns of transsutural strain do not suggest that strain patterns contribute to initial fusion in the intrafrontal sutures. Differences in strain are likely related to changes in rat skull kinetics with growth, perhaps resulting from fusion of the intrafrontal sutures. (*Plast. Reconstr. Surg.* 119: 2167, 2007.)

Sutures are the major sites of calvarial bone growth responsible for expanding cranial volume. This growth is thought to occur in response to extrinsic biomechanical signals induced by the underlying brain, translated into cell signaling at the sutural sites, and resulting in increased bone formation.[1–3] The cranial skeleton itself has proved to be plastic in its ability to adapt to the demands of the numerous functions it performs. Cranial form becomes modified under the influence of masticatory hypofunction[4,5] and the growth of the underlying brain.[6] This plasticity suggests that cranial form becomes optimized for brain size and muscle function as the animal grows. Limiting cranial adaptation, such as occurs with craniosynostosis (premature obliteration of sutures), can elevate intracranial pressure and inhibit brain growth.[7,8] However, the effect of suture obliteration on cranial adaptation that occurs with muscle function is unknown.

Although cranial sutures form in the absence of muscle activity,[9] a number of studies support Moss's[10] hypothesis that the fine details of suture morphology are secondary responses to extrinsic forces. The development and growth of calvaria and the effect of mastication on calvarial suture

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morphology have been studied in several mammals.[11–14] Masticatory muscle resection surgical isolation and mechanical immobilization of portions of sutures result in the loss of sutural complexity.[14–18] Previous workers have reported strains from cranial sutures and bones of miniature pigs during stimulation of their masticatory muscles.[19,20]

How the masticatory forces influence calvarial and sutural morphology is not known. Some previous work has focused on how the cranium is constructed to resist the forces acting on it,[21] and how sutures grow to transmit force.[22] Our working hypothesis is that sutures are plastic, allowing adaptation to extrinsic forces such as brain expansion and forces of mastication that are resisted by bone.

The present study was undertaken to investigate interrelationships between morphology and patterns of loading of calvarial bone and sutures during growth. We used electrical bilateral stimulation of the masticatory muscles to produce incisor bites on a force transducer in anesthetized rats as our method of skull loading. Strains were recorded from three-element rosette gauges placed across the intrafrontal and sagittal sutures and on the periosteal surface of the parietal bone. Our overall aim was to examine these relationships in a group of normal animals before further experimental studies in which growth factors are used to alter the patterns of suture closure. For the current study, we had three specific objectives. The first objective was to measure the changes in stimulated incisor bite force with age in growing rats. These data would provide us with the knowledge of maximum masticatory muscle forces during growth. The second objective was to examine growth changes in the pattern of transsutural strains and parietal bone strains during stimulated incisor biting. The third objective was to delineate the degree of intrafrontal suture fusion and to examine how this fusion relates to changes in transsutural and parietal bone strain. We hypothesized that patterns of cranial bone and transsutural strains and the mechanisms of resisting masticatory loads would change with sutural fusion during growth.

MATERIALS AND METHODS

Animals

Sixty-three Sprague-Dawley rats (Harlan, Indianapolis, Ind.) were used in this study. The protocol for surgery and postsurgical observation and care followed the guidelines dictated by the Baylor College of Dentistry Institutional Animal Care and Use Committee. All animals for this study were housed in the Animal Research Unit at Baylor College of Dentistry. Fifteen each of 9-day-old (immature), 24-day-old (young adult), and 70-day-old (mature adult) rats were anesthetized by intramuscular injection (1 ml/kg body weight) of a solution containing 13 mg xylazine (Rompun; Bayer Corp., Pittsburgh, Pa.) and 87 mg ketamine per milliliter of cocktail, before measurements of strains and bite forces. The remaining 18 rats were euthanized in groups of six each at 9, 24, and 70 days of age using isoflurane (Butler, Dallas, Texas) overdose, and their calvariae were prepared for histologic sectioning and hematoxylin and eosin staining of the intrafrontal and sagittal sutures.

Masticatory Muscle Stimulation Procedure

Bite force was generated by electrical stimulation of the masticatory muscles with two unipolar needle electrodes (Platinum Subdermal Electrode Type E2; Grass Instrument Co., Mass.). One electrode was inserted through the skin into each bilateral masseter muscle. A super maximal train of pulses (120 Hz, 40 V, single train) was delivered to the muscles using a SIU5 Stimulus Isolation Unit/Solid-State Square Pulse Stimulator (Grass Instrument Co.). The level of stimulation was determined experimentally in a small subgroup of rats by increasing the stimulation voltage until maximum incisor bite force was obtained. This stimulus causes contraction of all masticatory muscles and the resulting bite force should be near a maximum possible bite force for each animal, although it is unlikely that an animal would bite this hard when alert, because of the feedback of periodontal mechanoreceptors.

Bite Force Measurement

The bite force transducer consisted of two differential strain beams, 4.0 mm in thickness, mounted with four strain gauges in a full bridge configuration.[23] After calibrating the bite force transducer, all bite force measurements were recorded during muscle stimulation by placing the bite force transducer centrally between the incisors. Incisors were trimmed to a length of several millimeters before placement of the transducer so that the mandible was in a more physiologically normal position for biting and so that large torques were not placed on the incisor roots during stimulation. The bite force transducer was calibrated by suspending weights from the upper beam while the lower beam rested against a stable surface. The calibration procedure included measurement of transducer response to a range of loads exceeding those measured in the stimulated bite force experiments.

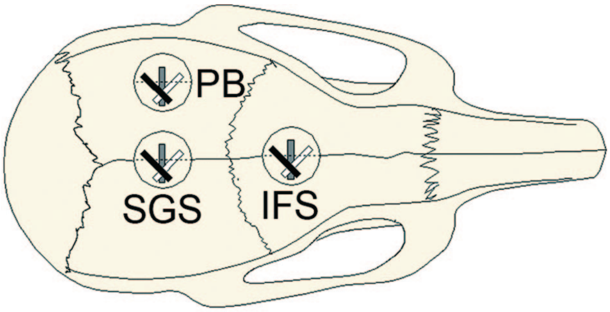

Fig. 1. Position of three-element rosette strain gauges fixed across the intrafrontal sutures (*IFS*) and sagittal suture (*SGS*), and on the periosteal surface of the parietal bone (*PB*) after removal of periosteum. Only one strain gauge was placed per rat.

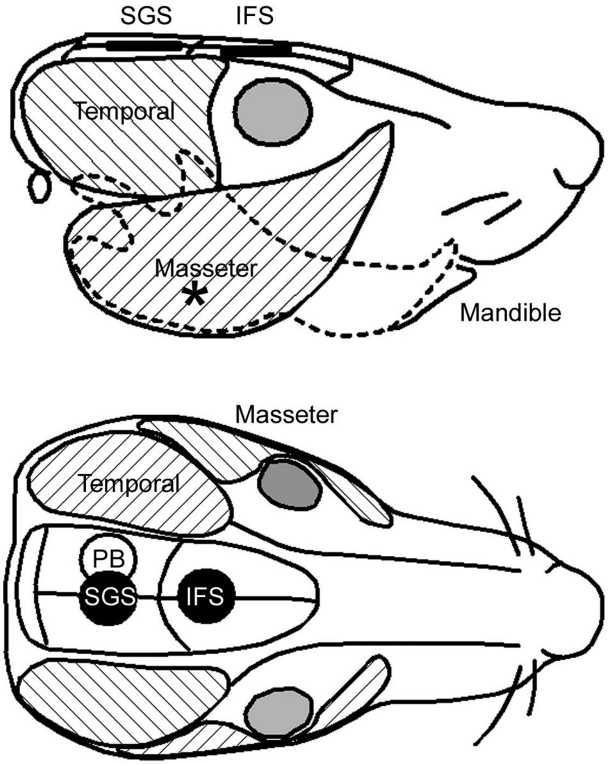

Fig. 2. Diagrams of lateral (*above*) and dorsal (*below*) views of a rat head showing the position of the strain gauges placed across the intrafrontal suture (*IFS*) and the sagittal suture (*SGS*) and on the parietal bone (*PB*) and their relationship to the muscles of mastication. The direction of hatching on the muscles approximates the average direction of muscle fiber orientation and therefore the general orientation of forces during muscle contraction.

Intrafrontal Suture, Sagittal Suture, and Parietal Bone Strain Measurement

Strain gauges were used to measure deformations of materials or tissues of interest (bones and sutures) in response to mechanical load. One strain gauge per rat was used for each experiment. The skin and periosteum were incised over the strain gauge sites on intrafrontal sutures, sagittal sutures, or parietal bone. The exposed bony surfaces were smoothed, degreased, and dried with acetone.[23] Stacked delta rosette gauges (UFRA-1-11; Tokyo Sokki Kenkyujyo Co., Ltd., Tokyo, Japan) were bonded across the posterior part of the intrafrontal sutures or the anterior part of the sagittal sutures closest to the coronal sutures, or to the parietal bone using the method of Dechow and Carlson[23] (Fig. 1). The anatomical relationship between the muscles of mastication and the points at which strain are measured is shown in Figure 2. During muscle stimulation, strain signals were digitized, sampled, and stored through the use of strain gauge conditioners and amplifiers (PCD-300A; Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan), and collected directly through a microcomputer interface. Values from each element of the rosettes were used to calculate principal maximum and minimum strain magnitudes and orientations using DAS-100A software (Kyowa Electronic Instruments Co.). Although minimum and maximum principal strains and their orientations are reported for all sites, these calculated values are unreliable for transsutural sites because the gauges are fixed to both bone and suture, which have different elastic properties. At transsutural sites, channel 2 of the gauge was always perpendicular to the average long axis of the suture and thus compression and tension directly across the suture could be measured. The other two channels (channel 1 and channel 3) are oriented at 45 degrees to channel 2. Differences in strain between channel 1 and channel 3 of the gauge can be used as an indication of the magnitude and direction of shear along the suture. According to convention, tensile strains were expressed as positive values and compressive strains as negative values. A minimum of 10 muscle stimulations were performed in each experiment, and values were consistent for each animal except for a minor decrease in strain during the later stimulations in some animals, which can be accounted for by decreased muscle force caused by muscle fatigue. Values for analysis were taken from the third stimulation record of the 10 total stimulations in each animal. After strain and bite force readings were obtained, all animals were euthanized with an isoflurane (Butler) overdose. The heads of the animals were kept in the freezer with the strain gauges intact for future reference.

Histology

All 18 rats used for histologic studies were decapitated and their scalps were removed. The heads were fixed overnight in 4% paraformaldehyde and decalcified in 0.5 M ethylenediaminetetraacetic acid for 14 days (9- and 24-day-old rats) or for 174 hours with a PELLCO Biowave 3470 microwave (70-day-old rats; Ted Pella, Inc., Redding, Calif.). After decalcification, the heads were cut along the coronal suture, separating them into two cranial specimens, one with frontal bones and intervening intrafrontal sutures and the other with parietal bone and intervening sagittal suture. Specimens were processed for paraffin embedding and sectioning. Finally, 6-μm sections were cut through tissues to display either intrafrontal sutures or sagittal suture in cross-section, and sections were stained with hematoxylin and eosin.

Scoring of Percentage Intrafrontal Suture Fusion

To examine the patency of each suture, every third section was taken, providing 18 sections from each suture. These sections were collected from the suture regions directly under the strain gauges, so that suture morphology could more closely be correlated with measured strain patterns. Sections were stained with hematoxylin and eosin and viewed with polarized microscopy, and the percentage of suture fusion was calculated as a ratio of the thickness of bone spanning the suture on the dural side of the fused intrafrontal sutures to the total bone thickness at the suture (Fig. 8, *above*).

Statistical Analysis

Descriptive statistics of the data are presented as means ± SD. One-way and two-way analysis of variance and Kruskal-Wallis tests as appropriate were calculated through the use of the Minitab Statistical Analysis Program Version 14 (Minitab, Inc., State College, Pa.). Levene's and Barlett's tests for equal variances were also used for selected comparisons.

RESULTS

Changes in Bite Force with Age

The response of the bite force transducer was linear, with an output of 0.37 μgf^{-1} (Fig. 3). Bite force, induced by bilateral stimulation of the masticatory muscles, increased with age. Using a two-way analysis of variance, no significant differences were seen among intrafrontal suture, sagittal suture, and parietal bone groups (Fig. 4), but age differences were significant ($p < 0.001$). Variances were unequal between groups, with 9-day-old rats having significantly ($p < 0.01$) less variance than 24- and 70-day-old rats. Despite the fact that unequal variances have only a slight effect on the results of analysis of variance with fixed factors, we also used a Kruskal-Wallis test to look for significant differences in bite force between age groups. Results here also indicate that differences were significant ($p < 0.001$). Bite force increased as age increased from 72.6 ± 20 gf at 9 days to 707.4 ± 150 at 24 days, to 2425.6 ± 255 gf at 70 days.

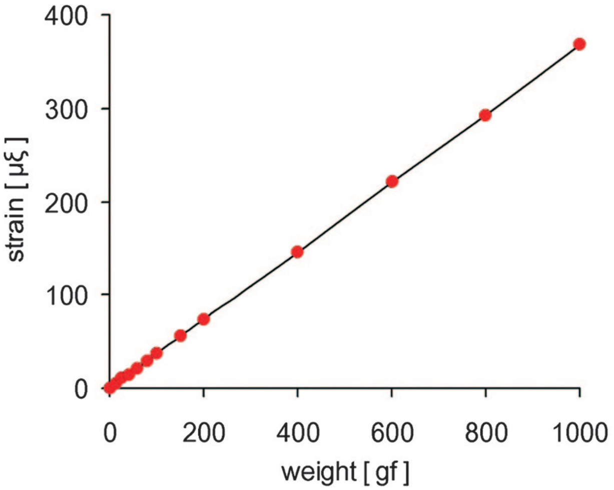

Fig. 3. Calibration of the bite force transducer.

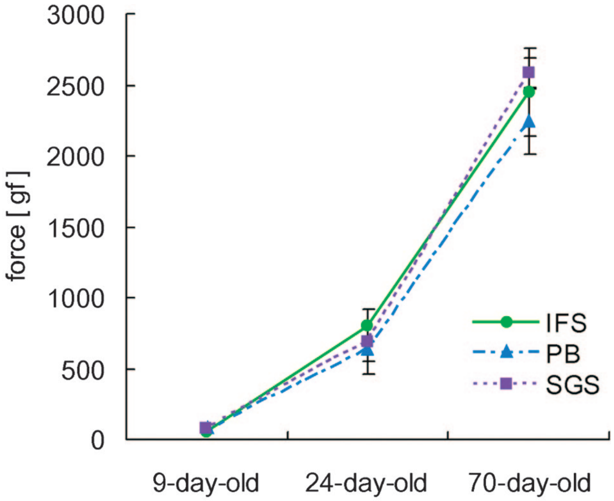

Fig. 4. Changes in stimulated bite force with age, presented as means ± SD. Stimulated bite force increased 10-fold from 9 to 24 days, and then again by 3.5-fold between 24 and 70 days.

Suture and Bone Strains during Biting

Interfrontal Suture

Strains were measured perpendicular to (channel 2) and at 45 degrees to (channels 1 and 3) the sutures. A small compressive strain of approximately 100 $\mu\varepsilon$ was observed perpendicular to the intrafrontal sutures at all ages (Fig. 5, *above*, *right*, and Table 1). At days 9 and 24, smaller values of absolute strain were also found for channels 1 and 3, indicating minimal strain at the intrafrontal sutures in the younger age groups (Fig. 6). Our histologic studies indicated that the intrafrontal sutures were largely fused by day 70, so it is appropriate to consider calculated minimum principal (compressive) and maximum principal (tensile) strains in this group at this age. These results showed that this region is experiencing larger compressive forces (-408 ± 237 $\mu\varepsilon$), which are nearly parallel to the fused suture at day 70, than at other ages (Table 1).

Sagittal Suture

Channel 2 of the strain gauges indicated that there were low levels of compressive strain perpendicular to sagittal suture at day 9 and day 24 (Fig. 5, *below*, *left*). Likewise, channels 1 and 3

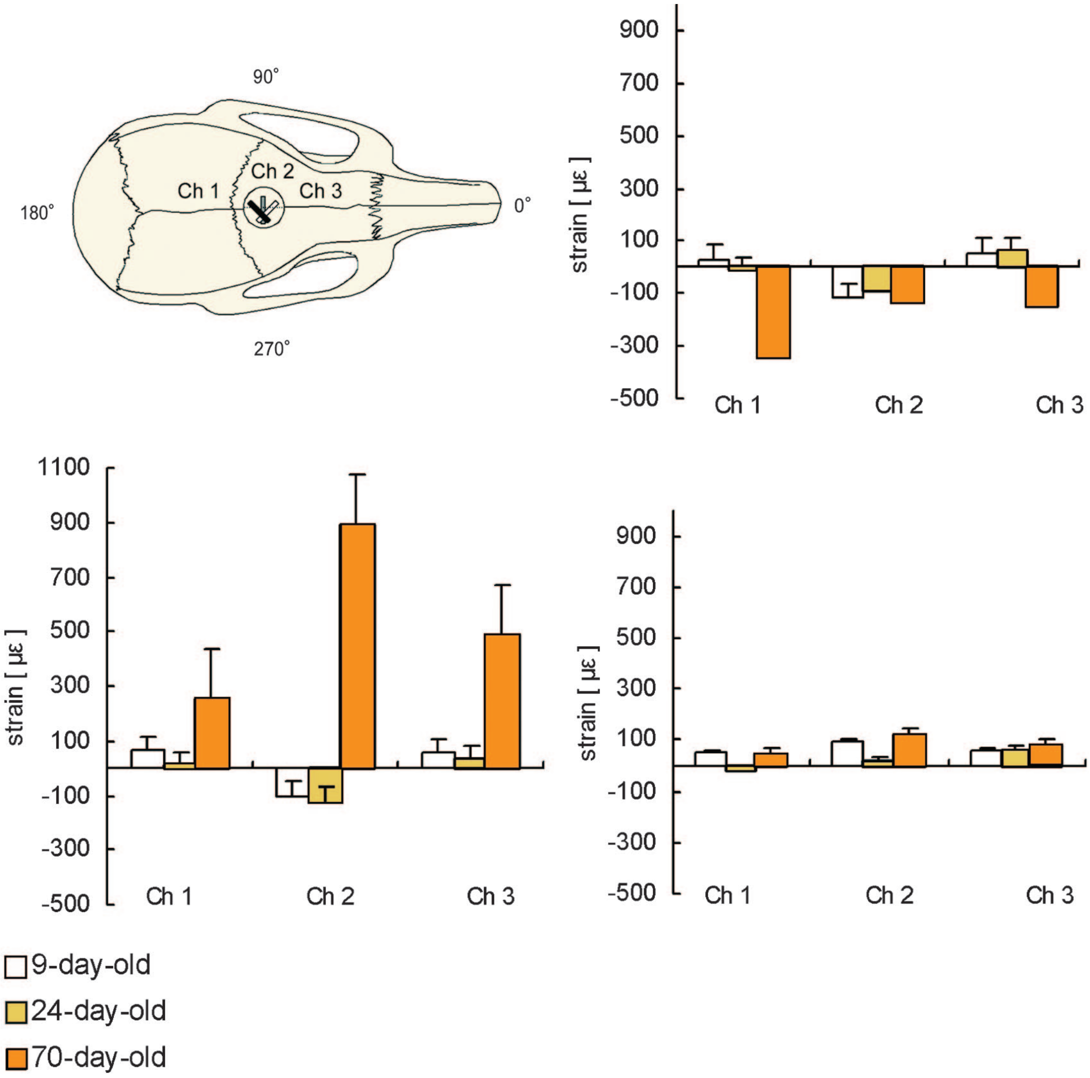

Fig. 5. Changes in strain across the intrafrontal sutures and sagittal suture and within parietal bone. (*Above*, *left*) Diagram showing strain gauge placed across a suture, and indicating the numbering of the channels, with channel 1 and channel 3 of the strain gauge at a 45-degree angle across the suture, and channel 2 perpendicular to the suture. (*Above*, *right*) Intrafrontal sutures; strains were small and not significantly different between 9- and 24-day-old groups. Compressive strains perpendicular to the suture were larger in the 70-day group. (*Below*, *left*) Sagittal suture; strains perpendicular to the sutures were small in the 9- and 24-day-old groups but were larger and predominantly tensile in the 70-day-old group. (*Below*, *right*) Parietal bone; small strains were found at all ages, with no significant differences between groups.

Table 1. Mean ± SD of Peak Strain and Orientation of Strain in Sutures and Bone Perpendicular to (Channel 2) and at 45 Degrees to (Channels 1 and 3) the Midline Sutures*

Age	Channel 1 ($\mu\varepsilon$)	Channel 2 ($\mu\varepsilon$)	Channel 3 ($\mu\varepsilon$)	Maximum ($\mu\varepsilon$)	Minimum ($\mu\varepsilon$)	∝ (°)
Interfrontal suture						
9 days	30 ± 97	–118 ± 74	55 ± 39	—	—	—
24 days	–5 ± 98	–90 ± 23	64 ± 59	—	—	—
70 days	–338 ± 200	–128 ± 55	148 ± 92	–78 ± 33	–408 ± 237	106 ± 7
Sagittal suture						
9 days	62 ± 25	–105 ± 64	55 ± 42	—	—	—
24 days	11 ± 36	–124 ± 110	30 ± 52	—	—	—
70 days	251 ± 134	892 ± 485	486 ± 343	—	—	—
Parietal bone						
9 days	51 ± 32	91 ± 34	58 ± 43	101 ± 51	–28 ± 66	94 ± 11
24 days	–17 ± 29	17 ± 24	57 ± 11	58 ± 10	–18 ± 30	138 ± 7
70 days	45 ± 48	125 ± 39	80 ± 46	139 ± 38	–15 ± 32	101 ± 16

*Maximum principal strain (Max), minimum principal strain (Min), and the angle of the maximum principal strain (∝) relative to the midline sutures across the fused intrafrontal sutures and on the parietal bone are also given.

registered low strain and did not indicate shear along this suture. At day 70, strain across the suture was much larger (892 ± 485 $\mu\varepsilon$). Strains measured at channels 1 and 3 were smaller and not significantly different from each other, indicating that this suture was primarily being tensed perpendicular to its long axis or being bent by flexing at the suture, with the parietal bones being depressed inferiorly.

Parietal Bone

Overall, the magnitudes of principal maximum and minimum strains were small in all age groups, with the largest values found at day 70 (139 ± 38 $\mu\varepsilon$) (Figs. 5, *below*, *right*, 6, and 7). Absolute values of tensile strain averaged approximately three (days 9 and 24) and nine (day 70) times greater than absolute values of compressive (minimum) strain, indicating that the periosteal surface of the parietal bone is primarily under tension in a direction perpendicular to the long axis of the sagittal suture.

Suture Morphology

At day 9, no fusion was detected in either the intrafrontal sutures or the sagittal suture. At day 24, frontal bones were fused across the intrafrontal sutures, starting from the dural side, whereas the sagittal suture remained opened (Fig. 8). Using our scoring system to calculate the degree of suture fusion (Fig. 9, *above*), we found that the intrafrontal sutures were 0 percent fused in day-9 animals, 18.7 ± 13.1 percent fused at age 24 days, and significantly ($p < 0.05$) more fused at day 70 (71.1 ± 22.6 percent) (Fig. 9, *below*).

DISCUSSION

Changes in Bite Force with Age in Growing Rats

The results showed that maximum stimulated bite force in rats increased by close to a factor of 10 between 9 and 24 days of age. These ages represent periods before and just after weaning, and likely reflect changes in bite force induced by the change to a hard diet. Copray and Liem[24] show that the superior aspect of the condylar cartilage undergoes pronounced ultrastructural changes shortly after weaning in rats, and they argued that these changes reflected an increase in articular loading on the superior surface of the condyle occasioned by the changeover to hard food. This indicated that not only aging but also changes in mastication attributable to changes in diet might affect bite force.

It is also notable that skull length increases by only approximately one-third between 9 and 24 days of age (Fig. 6), suggesting a large positive allometry of muscle force to skull size during this period of growth. Between 24 and 70 days, skull length increases by approximately one-fourth, whereas bite force increases by approximately 3.5 times, indicating a reduction in positive allometry in this later growth period. This greater increase in force than the increase in skull size could have noticeable effects on the biomechanics of the cranial bone and sutures, and is discussed in more detail below.

Bite force itself has been correlated with the degree of mouth opening or gape.[25,26] These authors showed that the maximum incisor bite force of 1-year-old Sprague-Dawley rats is 45.14 N (4603 gf) measured at a mean intermolar distance of 2.4 mm, when their masseter muscles are stimulated bilaterally (130 Hz at 25 V). The bite force is lower at a mean intermolar distance greater than 2.4 mm. We measured the incisor bite force of 70-day-old rats with a bite force transducer system similar to that used by Nemeth et al.,[26] and demonstrated an average force of 2426 gf at a similar

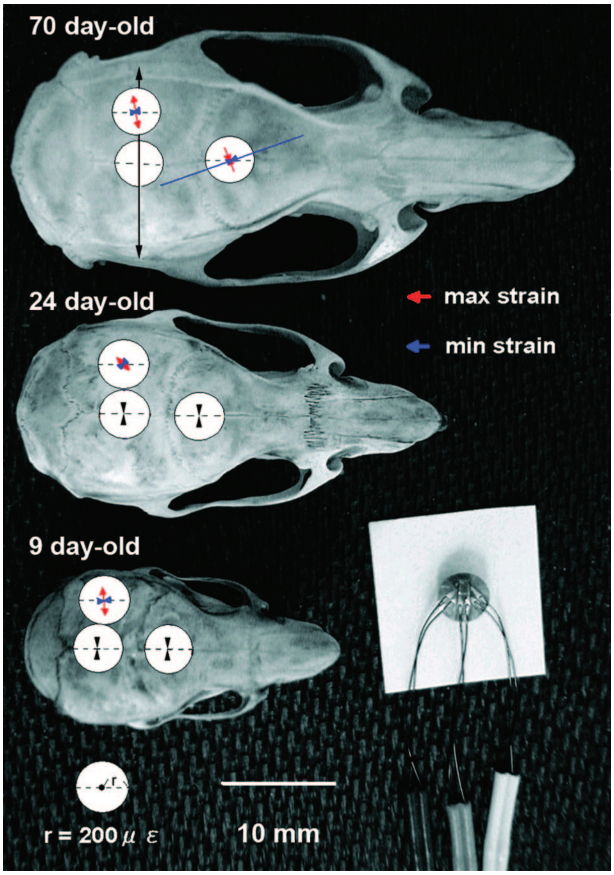

Fig. 6. Summary of strain changes in rat calvarial sutures and bone. The images were prepared from rat skulls after taking strain measurements and are shown at actual size relative to one another. The magnitude of strain for the central strain gauge (channel 2) is shown across patent sutures by the *black arrows*, with the direction of the *arrowheads* indicating compression (in) or tension (out). On the parietal bone at all ages and across the fused intrafrontal sutures at 70 days, the magnitude and direction of the maximum principal or tensile strains (*red*) and minimum principal or compressive strains (*blue*) are shown. The length of the radius of a *white circle* corresponds to a scale of 200 $\mu\varepsilon$ for all strains. Note that a separate scale bar is given for the dimensions of the skulls. A rosette strain gauge is shown to scale in the lower right portion of the figure.

intermolar distance. The differences in maximum stimulated bite forces between the rats used by Nemeth et al.[26] and our rats is likely because of the age difference between their 1-year-old and our 70-day-old rats.

Changes in Pattern of Strain and Its Orientation with Age

The results showed low strains in response to biting at all sites at 9 and 24 days. At 70 days, the predominant strain at the sagittal suture was larger, tensile, and perpendicular to the suture. This suggests that the temporalis muscle may be larger by the age of 70 days in rats and therefore exerts a larger outward and downward pull on the parietal bones during muscle contraction. The sagittal suture is situated between the parietal bones, and during biting the parietal bone is pulled laterally and downward by the temporalis muscle, which is attached to the nuchal crest. As a result, tensile force was seen perpendicular to the sagittal suture. Behrens[11] noted sagittal suture strain in juvenile *Macaca mulatta* monkeys during masticatory movements, and also suggested that the parietal bones were probably "pulled" downward, before moving back into their original position.

Another possibility is that temporalis muscle contraction may bend the cranium at the sagittal suture by pulling the parietal bones downward, resulting in a tensile strain perpendicular to the suture on the ectocranial surface. Further mechanical analysis is needed to resolve this issue. At the intrafrontal sutures, the predominant compressive strain was parallel to the fused posterior portion of the suture. This is likely to result from a bending upward of the rostrum of the rat during incisor biting.

A question raised by our results is why strains remained comparably low at 9 and 24 days of age. Given the large increase in bite force between these ages, an increase in strain should be expected. However, a better mechanical analysis of the cranial skeleton is needed to understand possible mechanical changes during growth. Usually such analyses ignore the effects of facial sutures in dampening the impact of occlusal and muscular forces. We suspect that mechanical analyses that include information on sutural and other craniofacial soft-tissue components are needed to understand this lack of strain differences in these early age groups.

It is also possible that there are aspects of our experimental design that could have an untoward effect on our results, although it is difficult to interpret them as important for creating the specific findings that we have described. One problem is that it is possible that measured strains in the cortical bone and sutural tissue in the youngest rats are influenced by the presence of the strain gauges themselves. The gauges are much thinner than the bone, but they are closer in size to the materials being tested than in the larger animals. However, if this were an important factor here, we would expect to see larger strains in 24-day-old animals, where the bone is larger in size relative to the gauges, but this is not found. In fact, the strain on the external surface of the parietal bone remained low in all age groups. This was surprising,

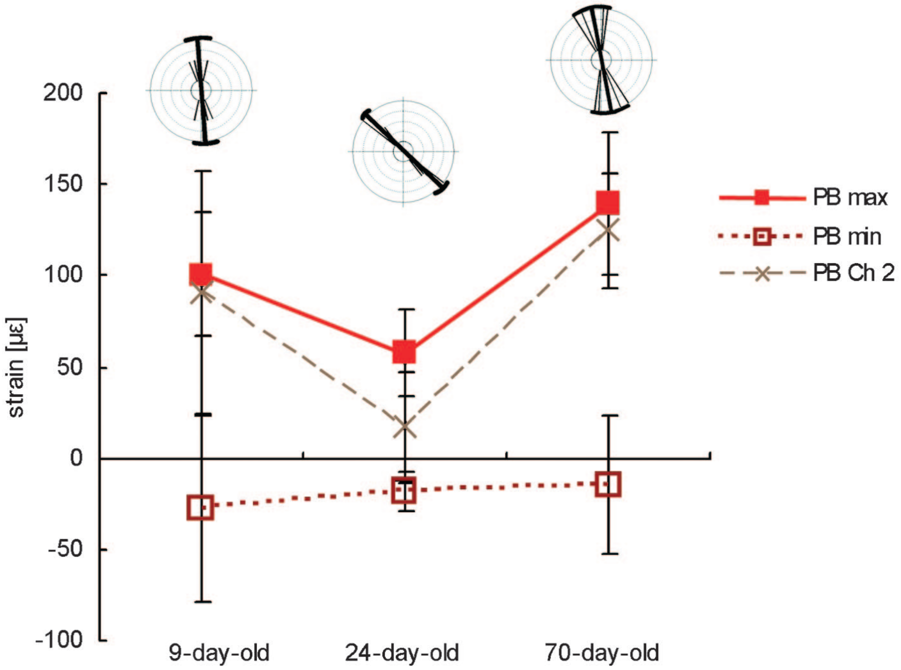

Fig. 7. Graph showing variations in minimum and maximum principal strains within the parietal bone with age, along with the strain measured by channel 2, which was positioned on the parietal bone perpendicular to the sagittal suture. The *circles* show the direction of maximum principal strain at each age.

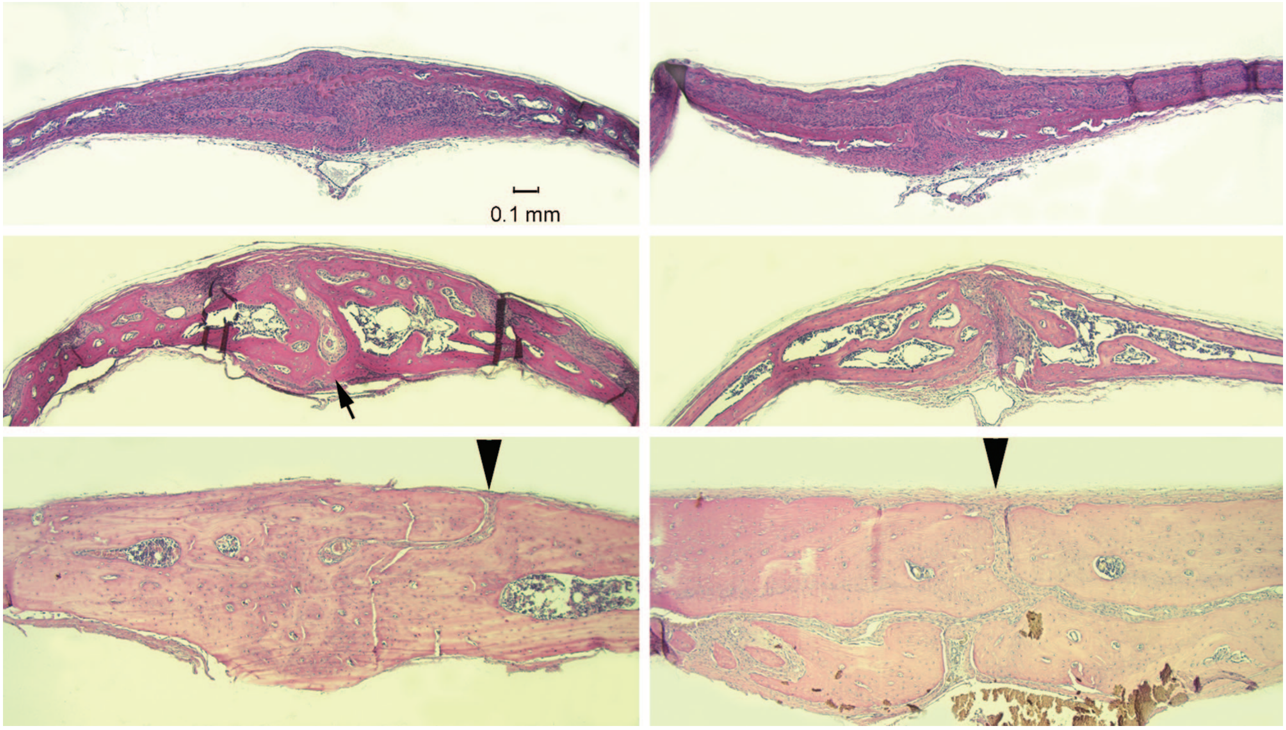

Fig. 8. Micrographs of hematoxylin and eosin–stained sections through intrafrontal (*left*) and sagittal sutures (*right*). (*Above*) Day 9: note the patent suture between bone fronts in both intrafrontal and sagittal sutures. (*Center*) Day 24: note the fused region at the endocranial surface of intrafrontal suture (*arrow*) and the lack of fusion in sagittal suture. (*Below*) Day 70: note residual remains of intrafrontal suture and the presence of highly complex, patent sagittal suture (*arrowheads*).

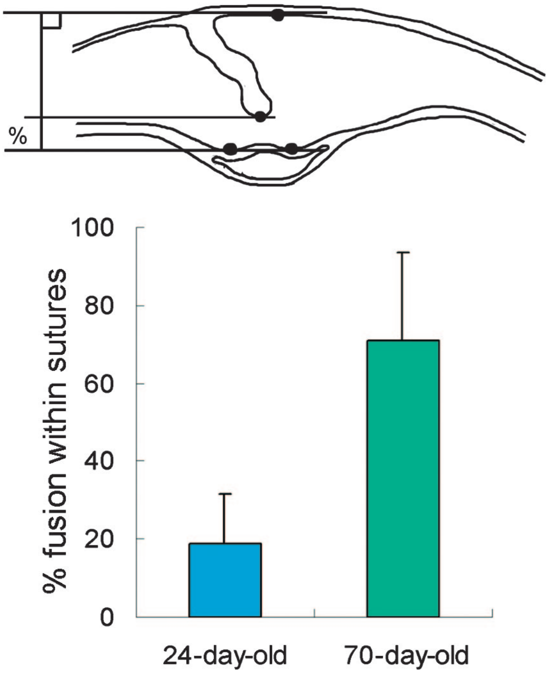

Fig. 9. (*Above*) Diagram showing the positions from which suture measurements were taken, and used to calculate percentage of suture fusion. Measurements between the top and bottom lines indicate the total suture width, and measurements between the bottom two lines indicate the total fused widths. The percentage of suture fusion was calculated as total fused width/total suture width. (*Below*) Graph showing increasing percentage suture fusion with increasing age. Mean ± SD: 24-day-old rats, 18.694 ± 13.098 percent; 70-day-old rats, 71.111 ± 22.640 percent. Nine-day-old sutures showed 0 percent fusion (single-factor analysis of variance, $p < 0.05$).

given the change in allometry, which predicted measurable effects on the biomechanics of the cranial bone and sutures. Thus, even if the gauges themselves have an influence on the strain, this factor is likely not the most important one for the small strains found at days 9 and 24.

Another factor is the effects of mouth opening or gape on occlusal force. Nordstrom et al.[27] showed effects on sarcomere lengths of the masseter and temporal muscles of the rat. As the sarcomere lengths increased beyond an optimum position, muscle force decreased. The proportion of expansion in sarcomere length of the anterior masseter muscle was greater than that for the posterior masseter and temporal muscles, and the amount of muscle stretch was greater in younger rats. This suggests that the effects of changes in masticatory muscle force caused by mouth opening are greater in younger and smaller rats. Likewise, it is important to consider that changes in the mechanics of the jaws with increased gape may alter the transfer of muscle force to occlusal force. However, this potential problem, like that regarding the influence of the strain gauge on strain described above, is unlikely to influence the main focus of our findings, as this effect would also predict larger strains in 24-day-old animals than in 9-day-old animals, which we did not find.

Another interesting question is how muscle loading, reaction force from the dentition or temporomandibular joint, or some combination of these factors induces the calvarial and sutural strains. Teng and Herring[20] suggested that the main effects are direct loads applied by the muscles, because jaw joints and the teeth are more distant from the sutural sites than are the attachments of the masseter and temporalis muscles. There is experimental support for this view at some sutures. For instance, Behrents et al.[11] found tensile strain at the sagittal suture after stimulating the temporalis muscle in a rhesus macaque. Jaslow and Biewener[12] found compressive strains on all calvarial bones and sutures in goats, and hinge-like bending in the interfrontal suture during masticatory function, and interpreted some of these as resulting directly from muscle function.

In contrast, the strain in sutures close to a loaded region at the temporomandibular joint or along the tooth row undoubtedly results from these loads. Mao et al.[28] found strains that seem to relate to occlusal loading in growing rabbit maxillary sutures. They suggested that the oscillatory component of cyclic masticatory force, or more precisely the resulting cyclic strain experienced in sutures, is a potent stimulus for sutural growth, and both microscale tension and compression induce an anabolic sutural growth response. Thomason et al.[21] suggested the possible importance of temporomandibular joint loading for the production of calvarial strain in sheep. This issue is not one that can be resolved easily for all cranial and sutural sites. The dampening effects of sutures on load transference in the cranium probably increase the importance of proximal forces for regional patterns of strain. This topic is one that has been largely unexplored in the literature.

Correlation of Suture Fusion to Observed Strain

In the rat, all of the cranial sutures remain patent throughout life, except the posterior part of the intrafrontal sutures.[1] This region of the suture undergoes osseous obliteration by 21 days of age.[10] Opperman et al.[29] and Roth et al.[30] showed that the posterior intrafrontal sutures from 15- to 20-day-old rats are actively fusing, and that in animals older than 20 days, the suture is largely fused with fibrous connective tissue replaced by calvarial bone. However, at 30 days of age, this suture still has remnants of sutural connective tissue on the periosteal (ectocranial) side. Our current results are consistent with these findings but extend them by calculating a percentage of total sutural fusion from the endocranial to ectocranial surface. In our 24-day-old animals, the percentage of total fusion was actually less than 20 percent, although endocranial bony bridges could be found throughout the area of fusion. Even by 70 days of age, the suture was not completely obliterated, but rather approximately 70 percent fused as measured by our current quantitative techniques. Because of this incomplete fusion, it is likely that deformations across the fusing sutural site remain greater than those found in the adjacent cortical bone and that remaining fibrous components of the suture may still play a mechanical role.

The relationship between mechanical factors and suture fusion/morphology has been discussed by Teng and Herring[20] and Sun et al.[22] Sun et al.[22] reported that in the interparietal suture of miniature pigs, the suture first fuses anteriorly and that fusion then extends posteriorly over several months. Fusion occurs first on the ectocranial side of the suture at approximately 7 months of age. Sun et al.[22] suggested two possibilities to explain this pattern. The first is that bending of the parietal bones at the suture would result in higher strains on the ectocranial side, which would promote bone formation. The second is that the fusion may just be the result of rapid ectocranial bone apposition, incidentally sealing the suture on the ectocranial surface, leaving the endocranial section as it was. The problem with these scenarios is that it is not apparent why bone formation itself should lead to sutural fusion. In fact, there is good paleontologic evidence in one group of dinosaurs with highly thickened (domed) cranial bones (pachycephalosaurs), that rapidly thickening bone carried the suture with the growing bone, such that a patent suture remained present throughout the thickness of the bony dome.[31] In fossils of more mature individuals with larger domes, these domes also show suture obliteration occurring at the ectocranial surface. The morphology of the thickened pig parietal bones with its marked reversal lines resembles to some extent the thickened dinosaur domes. It does not appear likely that thickening of dinosaur domes and ectocranially fusing sutures were attributable to masticatory influences, because there is no evidence of muscle scars on the dinosaur domes. The role of these thickened domes remains to be elucidated. It is therefore possible that the thickened morphology of pig crania and the ectocranial fusion of their interparietal sutures may also not be related to high strains generated by masticatory activities. In support of this idea, our results in rats indicate that strains in the intrafrontal sutures are low and that this suture becomes fused, whereas strains in the sagittal suture become highly elevated by 70 days, yet this suture remains patent. It must be kept in mind that rat sutures, like human sutures, fuse from the endocranial surface. The significance of sutures fusing either ectocranially or endocranially is as yet unclear, as is the effect of a partially fused suture on strain in the surrounding bone. However, it is unlikely that there will be significant strain on one side of a bone and not the other, even if there are inequalities of strain from one side to the other. From the present data, it is apparent that mechanical forces may be less responsible for the regulation or maintenance of sutural patency than has previously been thought. Furthermore, the results of Sun et al.[22] show consistently low strains in the bones surrounding the sutures, which tends to refute the notion that the thickening of the bones is related to increased levels of strain.

Whether these differences among species relate to differences in patterns of mechanical loading in the cranium and across sutures remains an open question. One goal of this investigation was to examine the relationship between transsutural strains and sutural fusion. Indeed, our results showed that the magnitude and directions of strain found across the intrafrontal sutures of 70-day-old animals were quite different from those of 9- and 24-day-old rats. Intrafrontal suture strains were small and compressive perpendicular to the suture for the younger age groups; however, in 70-day-old animals, the predominant compressive strains were much larger and oriented parallel to the suture. A similar pattern was seen in the nonfusing sagittal suture, in which small compressive strains were found perpendicular to the suture in the younger age groups. However, at 70 days of age, the predominant strain was much larger and tensile perpendicular to the suture.

Our lack of sufficient understanding of cranial mechanics in the rat skull makes it difficult to explain the strain patterns themselves, let alone their possible effects on sutural fusion or vice versa. It is important to note that our method of loading the skull—muscle stimulation—is not a natural activity. However, it does provide an indication of skull deformation when all masticatory muscles are being contracted maximally within the context of their normal anatomical positions. Because of this, the loading patterns likely resemble naturalistic deformations for hard incisor biting and other orofacial activities where cranial deformations result from occlusal loads and masticatory muscle contraction. In any case, the strains generated by such maximal masticatory muscle stimulation are likely to be at the upper range of the potential for in vivo strains.

In this context, it is significant that intrafrontal suture strains remain low at 24 days of age despite a dramatic 10-fold increase in bite force from 9 days of age. Further analysis of the biomechanics of cranial growth in rats is needed to resolve this issue. However, in terms of what is actually happening at the intrafrontal sutures, strains at the suture do not increase dramatically until sometime between 24 and 70 days of age, but it is clear that sutural fusion has begun at an age when transsutural strains remain low. One might view these low strains as a factor in fusion except that stains are also comparably low across the sagittal suture, and this suture remains patent.

CONCLUSIONS

Overall, these results do not lend support to the importance of mechanical factors in the initial fusion of the intrafrontal suture and the patency of the sagittal suture. However, it is not possible from our results to make a categorical statement that mechanical factors have no impact, especially at ages between 24 and 70 days, in which larger strains occur at both intrafrontal suture and sagittal suture sites, and the orientation and mode of the predominant strains at each site is different. Further conclusions would require a better understanding of changes in both cranial mechanics and sutural structure and physiology during these stages of growth.

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