Title Page
Cyclic Loading Effects on Craniofacial Strain and Sutural Growth in Pigs

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Susan Herring: contributed to the study design and development, data analysis and inscription of the project.

Abstract

Introduction: Current craniofacial growth modification devices utilize static forces but cyclic forces are believed by some to be more effective. The latter has not been evaluated in large animal models, and it is not known how such forces are transmitted to distant parts of the skull. This study aims to**:** 1. Develop a portable loading system capable of delivering reliable cyclic loads to the porcine nasofrontal suture (NFS), 2. Explore strain transmission to distant sutures and 3. Characterize the sutural growth effects in a small pilot study.

Methods: After validating the device, cyclic (2.5Hz) tensile loads were applied unilaterally to the NFS of abattoir pig heads (n=6), with strain gages on multiple sutures. Similar loading was applied to 3-month old live pigs (*Sus scrofa*, n=4 and 1 sham) 30 minutes/day for 5 days. These animals received fluorescent markers of mineralization on loading days 1 and 3. Suture strains were recorded on day 5. Histomorphometric analysis quantified suture width and mineral apposition rate (MAR).

Results: A wearable loading system was developed to produce an average of +900 με at the targeted NFS. Substantial strains were seen at the contralateral NFS and midline sutures, but bone strains were low. Strain patterns were similar *ex vivo* and *in vivo*, with the latter generally having higher magnitudes. Preliminary evidence demonstrates wider sutures with higher MARs in the loaded sutures.

Conclusions: Daily spurts of cyclic load caused sutural strain throughout the skull. The regime likely enhances suture growth and may be therapeutically useful.

INTRODUCTION AND LITERATURE REVIEW

Underdevelopment of the craniofacial skeleton due to early sutural fusion can lead to severe deformities with impairment in feeding, respiration, and esthetics. Current dentofacial orthopedic growth modification devices use static forces, but studies in small animal models suggest that cyclic forces may be more efficient in enhancing suture growth.[1] However, such studies need to be replicated in large mammals because of size effects on skeletal biology.[2,3] Moreover, the small size of previous models has prevented an understanding of how suture loading affects the rest of the skull. If these loads are transmitted through the skull, the growth of neighboring sutures could potentially be altered as well.

The present work aims to develop a large animal model for the study of cyclic loading and to assess the degree to which such loads extend to non-targeted sutures. This pilot study uses a pig model to investigate how cyclic loading of the nasofrontal suture (NFS) affects strain distribution and growth at multiple sutures in the midface and cranium. The pig (*Sus scrofa domesticus*) was chosen as it has an adult size comparable with that of a human and has similar patterns of embryology and ossification.[4] The NFS was chosen because of its accessibility and major contribution to midfacial length in normal pigs. The NFS is also affected in syndromic craniosynostosis.[5] The aims of this pilot study are: 1. To develop a wearable cyclic loading system for large animals, 2. To characterize the strains produced by direct cyclic loading of the NFS on targeted and other craniofacial sutures, 3. To compare such strains in fresh *ex vivo* pig heads with those in live pigs, and 4. To conduct a preliminary test of whether cyclic loading can increase sutural widths and mineral apposition rates (MAR).

MATERIALS AND METHODS

Development and Testing of the Loading System

The custom-engineered loading device used a voice coil connected to a power supply and function generator to produce cyclic forces (Figure 1). A load cell on the device measured the magnitude of force output. The device was placed superior to the NFS parallel to the mid-sagittal axis. Two vertically extended plates were fitted with holes and screws that engaged loading attachments on the skull. Calibration with known loads allowed conversion of output voltage into kilograms.

Loading attachments were installed on the frontal and nasal bones. To provide sufficient contrast with physiological strain during mastication (-1600 με[6]), the imposed cyclic strain was tensile, but the system is capable of compressive loading as well. Frequency was set at 2-3Hz to mimic the rate of pig chewing. To avoid injuring the sutural tissue, the desired NFS strain magnitude was 800-1000 με, about the mean for sutures during mastication.

Four pig heads of varying age and provenance (D-1 to D-4, Table I) were used to establish the load output necessary to achieve the targeted strain at the NFS. Initially, stainless steel screws were used as the loading attachment. However, these screws lacked adequate retention and were changed to stainless steel loading plates with a central loading post (Figure 2). The heads were instrumented with single-element strain gages (EP-08-125BT-120, Micro-Measurements, Wendell, NC) over the loaded and contralateral NFS. Bilateral NFS were exposed, periosteum removed and the sites prepared using the Micro-Measurements BAK-200 Basic Application Kit before the strain gages were affixed with cyanoacrylate glue. The lead wires from the strain gages were connected to the strain conditioner/amplifiers (Model 2110, Micro-Measurements, Vishay Precision Group, Wendell, NC), which were linked to a computer for data acquisition. Strain information was collected at intervals throughout the loading session and digitized using the BIOPAC MP150 system and Acqknowledge 3.9 software (BIOPAC Systems, Inc., Goleta, CA). The load output from the loading device was also sent through a strain gage conditioner/amplifier as a separate channel. Strain and load output values were simultaneously collected in Acqknowledge in voltage, transferred into Microsoft Excel and converted to microstrain and kilograms respectively. Magnitude of the strain and load output was obtained by subtracting baseline values from the peak values.

Ex vivo Study of Strain Transmission through the Skull

Six heads (H-1 to H-6) from freshly slaughtered pigs (Kapowsin Meats Inc., Graham, WA), sex unknown and assumed to be about 6 months old, were used. Loading attachments (2-screw plates) were fastened on both sides of the right NFS. Single-element strain gages were placed bilaterally on the NFS and 4 coronal sutures, and on the internasal (INS) and interfrontal (IFS) sutures as described previously. Three-element 45o stacked rosette gages (WA-13-060WR-120, Micro-Measurements, Vishay Precision Group, Wendell, NC) were placed on the right nasal and frontal bones (Figure 3), with the middle element perpendicular to the mid-sagittal plane. Cyclic loading was carried out for 30 minutes, with mean load output and strains for the beginning, middle, and final minutes of the session determined from averages of 20 waveforms each.

In vivo Study of the Suture Response to Cyclic Loading of the Nasofrontal Suture

Five (4 male, 1 female) mixed breed pigs (Sus scrofa) approximately 3 months old (Progressive Swine Farms, Woodinville, WA) were used (P-1 to P-5, Table II). All experimental procedures followed the National Institutes of Health guide for the care and use of laboratory animals and were approved by the University of Washington Institutional Animal Care and Use Committee. Animals were acclimated for approximately 1 week. Pigs P-1 and P-2 received the 2-screw plate attachments but because these became loose, the design was changed for Pigs P-3 to P-5. The new design (Figure 4) featured a custom titanium plate with 4 flanges, which could be contoured to the natural skull curvature. Each flange was stabilized by 2 stainless steel cortex self-tapping 2 mm screws (SYNTHES Vet, West Chester, PA). These produced comparable load output to the 2-screw design in trials using dry skulls.

To affix the attachments, anesthesia was induced with a cocktail consisting of midazolam, xylazine and butorphanol delivered intramuscularly and maintained with isoflurane inhalation. Under aseptic conditions, arcuate incisions were made unilaterally on the right frontal and nasal bones. The NFS was not exposed. Flaps were raised with the periosteum left intact. Pilot holes were made for the screws and the loading attachments were placed. The incisions were closed with interrupted sutures so that the loading posts projected from the skin. After 1 week of healing, cyclic loading sessions began. Pig P-2 had loose attachments and was considered a sham control. Daily for 5 consecutive days, the pigs were anesthetized as described previously, placed in a prone position, and the loading device was engaged to the loading attachments. On days 1 and 3, mineralization labels were administered (see below). Loading was conducted for approximately 30 minutes per day. In the sham control, the loading device was engaged but no loads were applied. On the final (5th) day, prior to loading, under anesthesia, periosteal flaps were elevated to expose the nasofrontal, internasal, interfrontal and coronal sutures. Strain gages were affixed as in the ex vivo study, except for the unloaded sham control. Because bone strain levels recorded in the ex vivo experiments had been very low, no gages were placed on the nasal and frontal bones. Load output and strain data were collected during cyclic loading as for the ex vivo study. Data were analyzed by a single investigator (S.H.S.).

Fluorochrome labels of mineral apposition were dissolved in sterile saline, adjusted to a neutral pH, filtered at 0.22 μm and administered IV on days 1 (calcein, SIGMA; 12.5 mg/Kg) and 3 (alizarin complexone, SIGMA; 12.5 mg/Kg) of loading.

After the loading session on day 5, the animals were injected IV with heparin and then euthanized with pentobarbital (Beuthanasia-D Special, Merck, Madison, NJ). This was followed by perfusion through the ascending aorta with approximately 4 liters of saline then approximately 4 liters of 20% glyoxal fixative (Prefer Concentrate, (Anatech Ltd., Battle Creek, MI) diluted with reagent alcohol and deionized water). Instrumented sutures were removed and separated into 2 blocks, one for assessment of sutural width and mineral apposition, and the other for future immunohistochemistry. In addition, one randomly chosen NFS was removed from each of four additional pig specimens of comparable breed mix (C-1 to C-4, histological controls, Table II). These animals had been used in an unrelated study involving tooth mobility and periodontal disease and had received bone labels on a slightly different schedule.

The undecalcified samples were dehydrated in an ethanol series and infiltrated with Micro-bed resin (Electron Microscopy Sciences, Hatfield, PA) before curing for approximately 21 days in a thermal oven set at 35-36 °C.

Sections perpendicular to the sutures at 50 μm intervals were obtained using a saw microtome (Leica SP1600) and mounted on slides. 1-3 sections were selected per suture based on clarity and completeness, and examined under epifluorescent illumination (Nikon Eclipse E400; Nikon Y-FL, Japan). Using a digital camera (SPOT RT3 2Mp Slider, Diagnostic Instruments, Inc, Sterling Heights, MI), the same microscopic field was separately captured for calcein and alizarin. Images were then merged to show images with double fluorescent labels via the MetaVue (Molecular Devices, LLC., Sunnyvale, CA) software. Histomorphometric analysis was performed with MetaMorph (Molecular Devices, LLC., Sunnyvale, CA) software. Suture width was calculated by dividing the area between both sutural margins over the average length of the two sutural margins. Mineral apposition rate (MAR) was calculated by measuring the area between the calcein and alizarin bone fronts and dividing it by the average suture length and the number of days between labels to give MAR in μm/day. MAR values from each side of the suture were averaged to give the mean MAR (Figure 5). Intra-examiner reliability was determined by re-measurement of 5 randomly selected samples. Selection of slides and measurements were all made by a single investigator (B.L.) blinded to the identity of the samples.

Mann Whitney U tests and unpaired t-tests were used for statistical analyses, and statistical significance was determined at $p<0.05$ (GraphPad Prism, La Jolla, CA).

RESULTS

Ex vivo, the 2-screw plate loading attachments remained intact and stable but they were unsuccessful *in vivo* (Pigs P-1 and P-2). The 8-screw plate loading attachments in Pigs P-3 to P-5 were stable except for the nasal attachment in Pig P-4 which was somewhat moveable, although still permitting loading and strain recording.

Ex vivo Study on Transmission of Strain in Pig Heads (H-1 to H-6)

The loading device produced a mean output of 1.55 ± 0.1 Kg. Strains matching the loading frequency of 2.5 Hz frequency were registered at working gage locations. Load output and strains tended to reduce in magnitude during the session. The highest level of strain was seen in the loaded right NFS, with a mean tensile strain of +898 ± 233 με (Figure 6, Table III). The contralateral NFS also showed substantial, but lesser tensile strain (+660 ± 205 με). Coronal sutures experienced very low compressive strain, averaging -47 ± 30 με on the loaded side and -17 ± 16 με on the contralateral side. The interfrontal suture (IFS) was usually in tension, with a single exception (H-6, -85 με). The internasal suture was less consistent, showing tension or compression in an equal number of pig heads, ranging from -210 to +230 με.

Recordings from all three elements (allowing calculation of principal strain magnitude and orientation) of the rosette gages were only achieved for 3 nasal bones and 2 frontal bones, with partial data for a few additional specimens. Bone principal strain magnitudes were low, with absolute values ranging from 15 to 96 με (Figure 6). For the nasal bone, rosette gages (except for H-5) indicated a predominance of compression (greater minimum than maximum principal strain), with the tensile axis running antero-medial to postero-lateral. Compression also dominated in the frontal bone and the tensile axis was roughly orthogonal to the sagittal plane.

In vivo Study of the Effect of Cyclic Loading on the Skull (P-1 to P-5)

The loose attachments in P-1 by day 2 of loading resulted in a failure to produce sutural strain on day 5, so it is unclear to what extent this animal actually experienced cyclic loading. Loading was successful in P-3 to P-5, and the mean load output (1.65 Kg) closely approximated the *ex vivo* study, indicating stable attachments, even though the nasal attachment in P-4 was slightly movable. As in the *ex vivo* study, there was a mild tendency for load output and strain magnitude to reduce during sessions.

In the three successfully loaded animals, tensile strain magnitudes at the loaded NFS averaged +1543 ± 636 με (Table IV, Figure 7). On the contralateral side, NFS strains averaged +814 ± 353 με. These values were larger than those seen *ex vivo* (Figure 8), but given the small sample size and high standard deviation, the differences did not reach statistical significance. The loaded-side coronal suture showed unexpectedly large compressive strain, averaging -764 ± 386 με, greatly exceeding that seen *ex vivo* (-47 ± 30με), yielding a statistically significant difference ($p = 0.02$). Strain at the contralateral coronal suture was inconsistent in polarity (Table IV) but magnitude (144 ± 108 με) was again greater than that seen *ex vivo* (17 ± 16 με, $p = 0.01$). As in the *ex vivo* study, the INS strains showed individual variation in magnitude and inconsistent polarity. Only P-5 showed strain magnitude (+251 με) outside of the *ex vivo* range (-192 to +190 με). IFS strains were uniformly tensile, as were most in the *ex vivo* study. Strain magnitudes although higher, were not statistically significant from *ex vivo* ($p = 0.10$). However, the relatively low values in Pigs P-4 and P-5 (+115 and +109 με respectively) were probably underestimates; P-4 because of the loose attachment and P-5 because the waveform was strongly truncated due to a malfunction.

At the termination of the experiment, the skulls of P-3 to P-5 showed periosteal apposition on the nasal and frontal bones around the attachments and an increase in inter-post distances by 3-4mm during the12-day interval between attachment placement surgery and day 5 of loading, indicating growth. Although sutural surfaces consistently showed mineralization, the suture gap was not always clearly visualized in the slides. Measurements were made only of the clear areas and therefore may not be fully representative of the entire suture; these cases are identified by asterisks in Table V, which summarizes suture widths and MARs. Intra-examiner error was low for suture width (1.6%) and acceptable for MAR (5.4%). The data for P-1 are included in Table V but were not used in calculations because of its uncertain loading history.

Despite the small sample size, the cyclically loaded pigs (P-3 to P-5) had strikingly larger NFS widths than the controls (P-2 plus C-1 to C-4), both on the loaded right side (327 vs 186 μm, $p = 0.001$ in an unpaired t-test, Table V) and on the contralateral left side (301 vs 186 μm, $p = 0.003$, unpaired t-test). Mineralization rate (MAR) was also associated with loading in the loaded right NFS (25 ± 6 μm/day) and contralateral left NFS (18 ± 4 μm/day), both higher than in controls (10 ± 2 μm/day, $p = 0.005$ and $p = 0.02$ respectively, unpaired t-tests). There were no histological controls for the other sutures tested, and the sham pig (P-2) had only coronal suture values available, but these did not differ appreciably from those of the cyclically loaded pigs (Table V).

DISCUSSION

Limitations

Although strain gage recording is the best empirical method for assessing bone response to loading, it is limited. Strain gages cannot distinguish planar strain from bending and torsion, and the single-element gages we used on sutures cannot distinguish between shear and axial strain. Thus, the three-dimensional pattern of strain transmission in the skull could not be completely determined. The technique also incurs experimental variability. Poor strain gage adhesion to the irregular, damp skull surface and/or insulation faults probably attenuated some recordings and contributed to gage failures.

The *in vivo* study constituted a feasibility pilot project for enhancing sutural growth in a large animal model, and hence sample size was small, limiting the conclusions. Also, the *in vivo* sample was younger than the *ex vivo* sample and 3 of the 4 histological controls. However, as pigs of this age grow at a constant or increasing rate,[7] the older animals would not be expected to have fused or more slowly-growing sutures.

Development of a Cyclic Loading System for Large Animals

The voice coil mechanism for loading proved to be successful in producing sutural strains of similar frequency and magnitude to those of mastication. Moreover, its small size and ‘wearability’ rendered it less clumsy in operation than the use of a mechanical testing machine as in previous studies[1]. Adapting it to human anatomy would not be too difficult.

Strain Transmission in the Skull

The strains applied to the NFS, although similar to mastication in frequency and magnitude, differed in polarity. We tensed the suture rather than compressed it, to provide a novel mechanical stimulus albeit still in a normal range. It has long been assumed that strain applied to one suture (e.g. the intermaxillary) would affect distant sutures as well, but the pattern of deformation has never been determined. The present study has quantified this effect.

Sutures at closer proximity to the site of loading generally registered higher strains. *Ex vivo*, mean recorded strains decreased in magnitude in the following order: loaded NFS > contralateral NFS > INS ≥ IFS > loaded-side coronal suture > contralateral coronal suture (Table III). In the smaller *in vivo* skulls, strain magnitudes at the loaded-side coronal sutures and IFS were generally larger than the INS but still lower than the NFS (Table IV). This trend corresponds with published literature that strain is a local phenomenon, with proximity of the applied force being important.[8]

The load was applied asymmetrically, and side differences were therefore expected. The tensile forces on the right NFS affected the left side, but with attenuated strain. The differential in strain magnitude between loaded and contralateral sides implies a “wedging” effect, with nasal and frontal bones more separated on the right than the left. This disparity should cause shearing forces at the INS and IFS, which would be recorded as tensile strain. Tension was in fact observed at 5/6 interfrontal and 3/6 internasal sutures. The wedging effect would also have tended to rotate the right nasal and frontal bones around their gravitational centers, with either compressive or tensile strain observed depending on the location of the strain gage with respect to the bone’s center of rotation, perhaps explaining the variation in strain at the midline sutures. Coronal suture strains on the cyclically loaded side were predictably compressive because of the loading forces that “pushed” the frontal bones towards the parietal bones. In summary, (1) sutures in close proximity to the targeted suture are most affected by loading, (2) sutures are flexible zones that allow a variety of small bone movements, and (3) loading of a single suture displaces bones to cause wide-ranging strain transmission across the skull.

Comparison of *In vivo* vs *Ex vivo* Loading Patterns

Similarities *in vivo* and *ex vivo* included strong tensile strain at both the loaded and the contralateral NFS (45-61% of loaded NFS values). In addition, coronal suture strain was compressive on the loaded side, interfrontal strain was tensile, and internasal strain polarity was inconsistent in both situations. However, strain magnitudes across the sutures were mostly higher *in vivo*. This could be attributed to several factors. First, the younger live pigs might have had less interdigitated sutures as is claimed for the human midpalatal suture.[9] Second, the 8-screw plate loading attachments used *in vivo* may have transmitted load more extensively and reliably than the smaller 2-screw design used *ex vivo*. Nevertheless, the attachments in the *ex vivo* pig heads all seemed secure during the experiments. Third, living, vascularized sutures may be more flexible than post-mortem material. In a related consideration, extended loading *in vivo* over 5 days could have loosened the sutures or caused them to remodel; this is our preferred hypothesis. Additional factors, however, may have caused the 10-fold difference in the loaded-side coronal suture strain. Conceivably, coronal sutures of the older *ex vivo* heads were partially fused. In addition, the larger loading attachments in the smaller skulls would have placed the point of force application closer to the loaded-side coronal sutures *in vivo*.

Can Cyclic Loading Increase Sutural Width and Mineralization Apposition Rate?

Although the *in vivo* study was meant only to test feasibility, the increased sutural widths and mineralization apposition rates of both the loaded and contralateral NFS were striking, especially given the brief loading period of 30 min/day for 5 days. These results strongly suggest that the loading regime promoted growth at these locations. Future work will investigate the underlying mechanism for the sutural response and whether sutures of midfacial hypoplastic pigs can be rescued through this means.

However, our controls were unloaded sutures, and therefore we have no evidence that cyclic loading is superior to continuous strain in stimulating sutural osteogenesis. Continuous tension is well known to increase sutural width and apposition.[10-12] In addition, we cannot confirm the claim[1,13] that cyclic loading stimulates sutural growth regardless of strain polarity. The only suture consistently compressed by our regime was the loaded-side coronal suture. The single sham specimen available for this suture did not differ from the loaded animals in either suture width or MAR.

CONCLUSIONS

1. Unlike prior use of mechanical testing machines, this study developed a less bulky, wearable loading device capable of delivering cyclic loads directly to the craniofacial skeleton.

2. The strain patterns confirm that loading of a single suture causes widespread strain transmission throughout the skull featuring rotation of skull bones with sutures as break points.

3. Strains were much larger *in vivo* than *ex vivo*, perhaps because of younger sutures, more stable attachments, and/or extended loading.

4. Preliminary evidence suggests that direct cyclic tensile loading has positive effects on suture width and mineral apposition rate at the targeted suture and its contralateral mate. The mean increase in MAR of 25 12

μm/day at the loaded NFS would translate to a clinically significant MAR of 9.1 mm/year. Short spurts of cyclic loading may hence be a more time-efficient growth modification treatment modality for patients with dentofacial deformities.

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Figure Captions

Figure 1: The loading device, showing how it engaged the loading attachments on the skull.

The loading device weighed 420 g and measured 14 cm x 4 cm x 6.3cm (diagram not drawn to scale). F-Att: Frontal bone attachment, N-Att: Nasal bone attachment.

Figure 2: Design of the 2-screw plate loading attachment.

This custom attachment, made of stainless steel and weighing 0.94g, was used in the *ex vivo* heads (H-1 to H-6) and *in vivo* Pigs 1-2 (P-1, P-2). The stainless steel screws were 1.5mm in diameter and 7.9mm long.

Figure 3: Pig head H-3 showing placement of the 2-screw plate loading attachments as well as single-element (white rectangles) and three-element 45_{o} stacked rosette (white squares) gages.

Figure 4: Design of the 8-screw plate titanium loading attachment.

This custom attachment, made of titanium and weighing 1.63g, was used for *in vivo* Pigs 3-5 (P-3 to P-5). The screws were self-tapping stainless steel, 2.0mm diameter and 6.0-8.0mm long (Synthes Vet, West Chester, PA).

Figure 5: Entire interfrontal suture of Pig P-3 under epifluorescent illumination.

The endocranial (more interdigitated) side is towards the bottom of the figure. A: Double-labeled interfrontal suture. The first label was calcein (green) and the second label was alizarin complexone (red); the labels were given on days 1 and 3 of loading. B: Suture gap highlighted in yellow. Width was calculated as gap area divided by the average true length of the sutural bony margins. C: Newly mineralized bone on each side of the suture highlighted in magenta and blue. Scale bar = 993 μm.

Figure 6: Strain patterns produced by cyclic loading of the right NFS on pig heads H-1 to H-6.

Tensile strains are indicated by divergent arrowheads and compressive strains by convergent arrowheads. The values are found in Table III. Arrows of type A indicate strain data from the single- 17

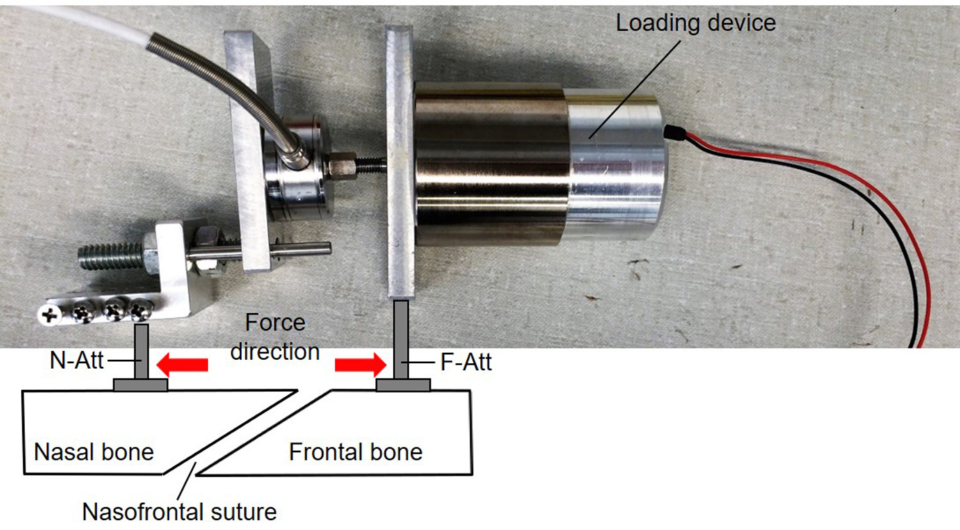
element strain gages. Arrows of type B indicate the rosette gage principal strains. Arrows of type C indicate strain data from individual working elements of rosette gages when principal strains were indeterminate. Note the difference in scale for arrow types.

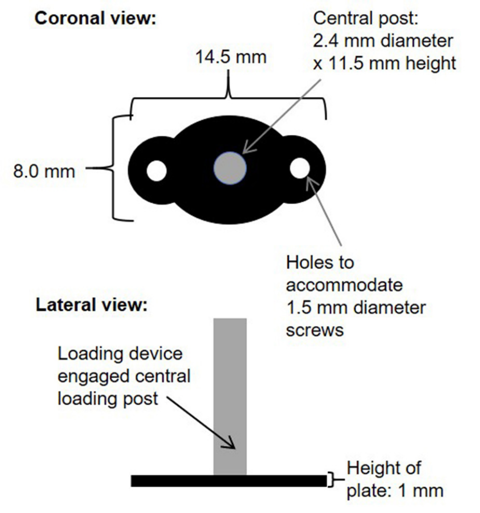
Figure 7: Strain patterns during day 5 of cyclic loading of the right NFS on Pigs 3-5 (P-3 to P-5).

Tensile strains are indicated by divergent arrowheads and compressive strains by convergent arrowheads. Arrow conventions are the same as in Fig. 6.

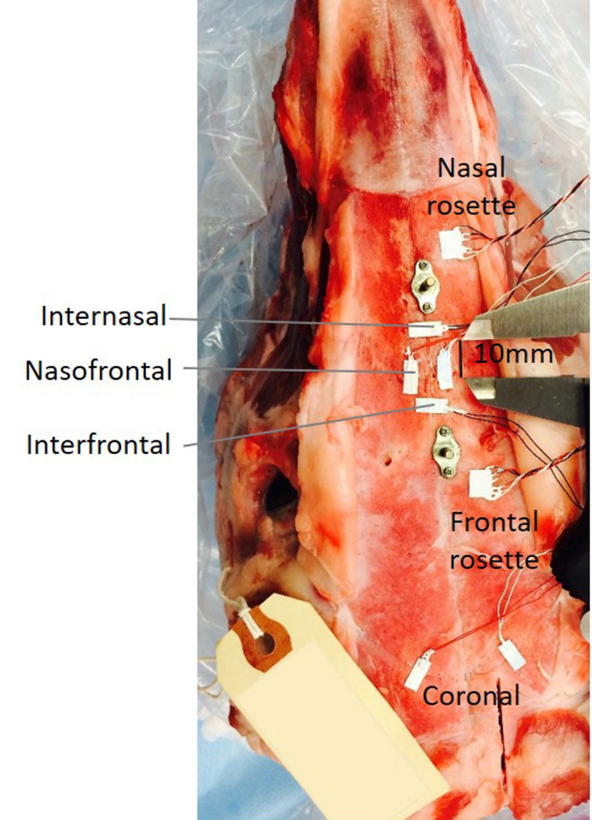
Figure 8: Comparison of *in vivo* and *ex vivo* strain magnitudes (mean and standard error).

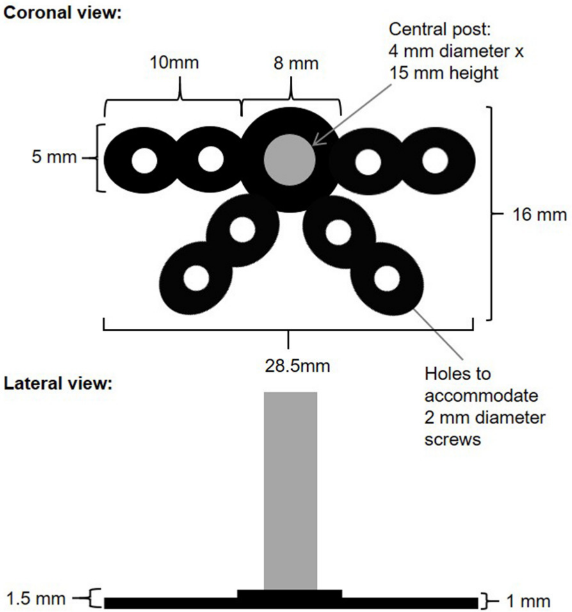
Absolute values of strain magnitudes were generally higher i*n vivo* than *ex vivo*, but only those of the coronal sutures reached statistical significance.

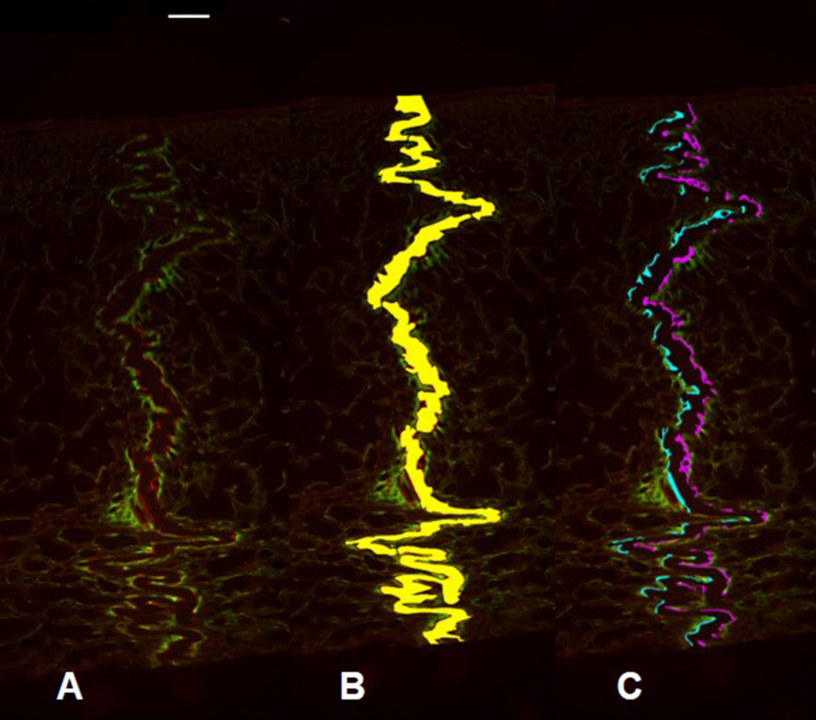

Loading device
Force
direction
N-Att
F-Att
Nasal bone
Frontal bone
Nasofrontal suture

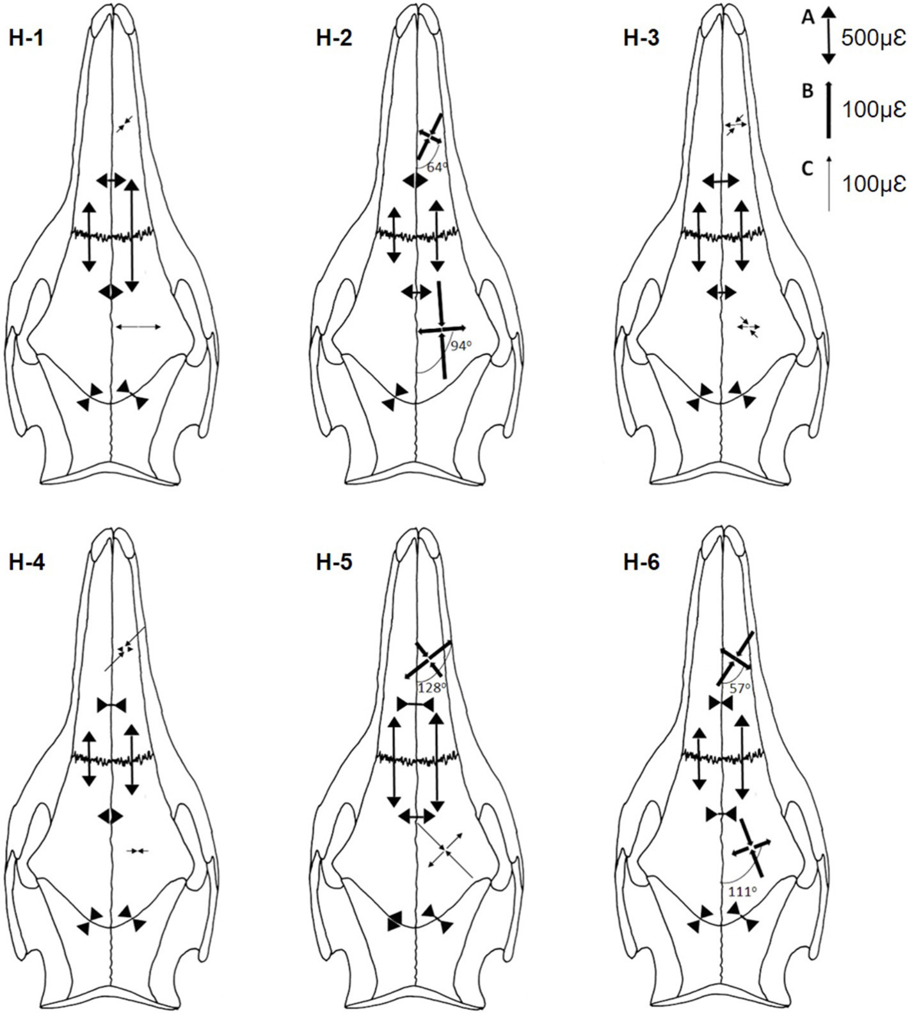


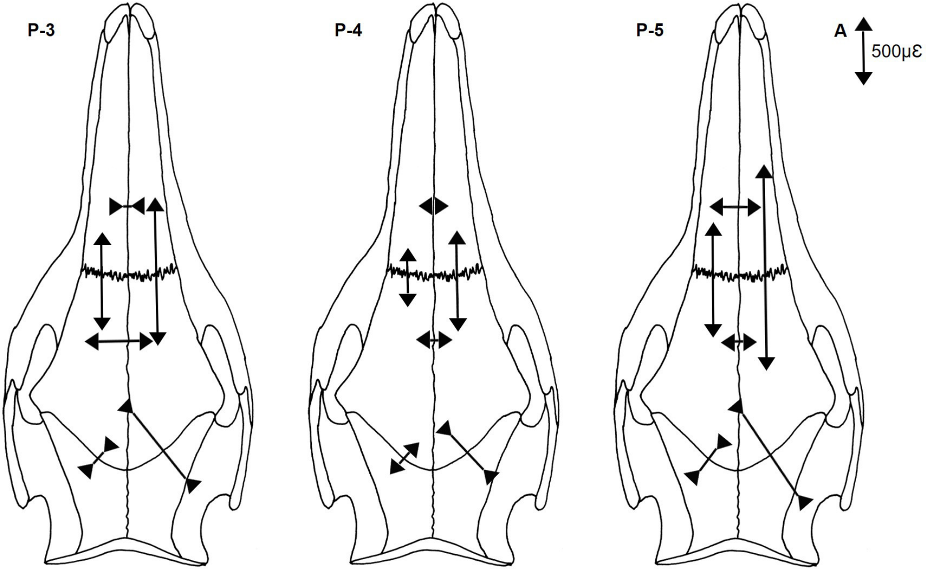
Coronal view:
Central post:
2.4 mm diameter
x 11.5 mm height
14.5 mm
8.0 mm
Holes to
accommodate
1.5 mm diameter
screws
Lateral view:
Loading device
engaged central
loading post
Height of
plate: 1 mm

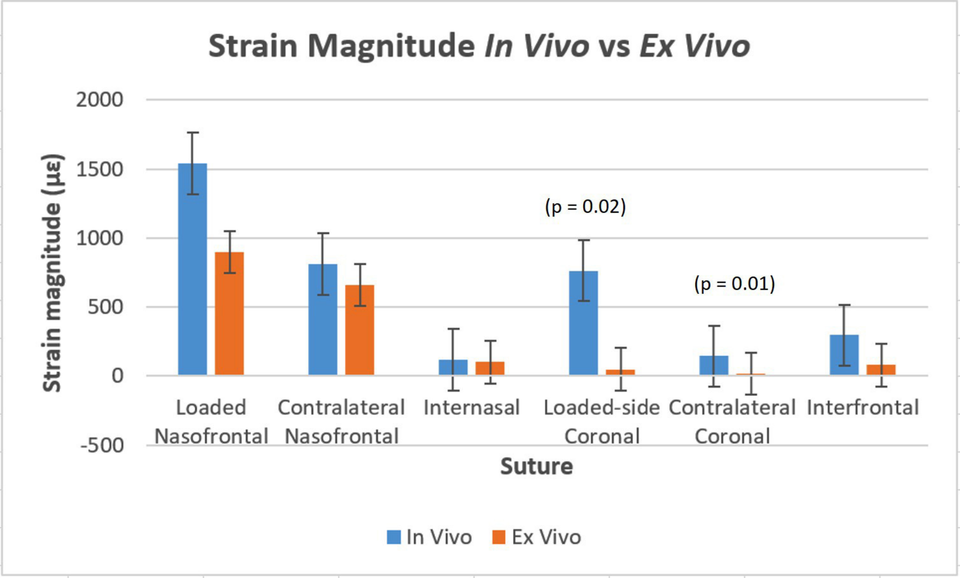

Nasal
rosette
Internasal
Nasofrontal
10mm
Interfrontal
Frontal
rosette
Coronal


Coronal view:
Central post:
4 mm diameter x
15 mm height
10mm
8 mm
5 mm
16 mm
28.5mm
Holes to
accommodate
2 mm diameter
screws
Lateral view:
1.5 mm
1 mm


A
B
C


H-1
H-2
H-3
A 500με
B 100με
C 100με
64°
94°
H-4
H-5
H-6
128°
57°
111°


P-3
P-4
P-5
A
500µε



Strain Magnitude *In Vivo* vs *Ex Vivo*
2000
1500
1000
500
0
-500
Strain magnitude (με)
(p = 0.02)
(p = 0.01)
Loaded Nasofrontal
Contralateral Nasofrontal
Internasal
Loaded-side Coronal
Contralateral Coronal
Interfrontal
Suture
In Vivo
Ex Vivo

Tables

Table I: *Ex vivo* pig heads: Summary of source, loading attachment type, strain gages placed and nasofrontal suture (NFS) strains (mean ± standard deviation [number of loading cycles measured])

No.	Source	Loading Attachment Type	Strain Gages Placed	Loaded NFS Strain (με)	Contralateral NFS Strain (με)
Heads used to develop and calibrate loading system					
D-0	Dry skull, estimated 3 months, unknown source	Stainless steel screws: 9.5mm length, 1.4mm diameter	Single-element: bilateral NFS	108 ± 3 [10]	29 ± 2 [10]
D-1	Fixative-perfused head, 5.5-month male minipig			2161 ± 4 [10]	307 ± 1 [10]
D-2	Previously frozen head, dentally mature (≥ 2years) female minipig			187 ± 14 [5]	-9 ± 2 [5]
D-3	Previously frozen abattoir head, estimated 6 months, unknown sex			241 ± 3 [20]	162 ± 2 [20]
D-4a	Previously frozen abattoir head, estimated 6 months, unknown sex			1369 ± 19 [5]	764 ± 14 [5]
D-4b		Stainless steel plates each stabilized by 2 screws (Fig. 2)		1856 ± 7 [3]	836 ± 2 [3]
Heads used in definitive *ex vivo* strain measurements					
H-1	Fresh abattoir heads, estimated 6 months, unknown sex.	Stainless steel plates each stabilized by 2 screws (Fig. 2)	Single-element: bilateral NFS and CS, INS, IFS Rosette: loaded-side nasal and frontal bones	1272 ± 190 [20]	693 ± 86 [20]
H-2				684 ± 104 [20]	490 ± 62 [20]
H-3				737 ± 137 [20]	664 ± 111 [20]
H-4				720 ± 27 [20]	508 ± 10 [20]
H-5				1063 ± 202 [20]	1045 ± 162 [20]
H-6				914 ± 70 [20]	558 ± 23 [20]

Legend: NFS: Nasofrontal suture, CS: Coronal suture, INS: Internasal suture, IFS: Interfrontal suture.

No.	Sex, Age (months), Weight (kg)	Treatment	Bone Labels	Single-element Strain Gages Placed	Histological Analysis
In vivo subjects					
P-1	Male, 4.0, 20.5	Right NFS: Stainless steel plates each stabilized by 2 screws (Fig. 2). Loaded 5 days but loose after day 1	Calcein and alizarin injected 2 days apart during loading period	Bilateral NFS and CS, INS, IFS	Bilateral NFS and CS
P-2	Male, 4.0, 21.0	Right NFS: Stainless steel plates each stabilized by 2 screws (Fig. 2). Sham control (no loading), loose attachments.		None	Bilateral NFS and CS
P-3	Male, 4.0, 20.0	Right NFS: Titanium plate stabilized by 8 screws (Fig. 4). Stable attachments, loaded 5 days.		Bilateral NFS and CS, INS, IFS	Bilateral NFS and CS, INS, IFS
P-4	Female, 4.0, 18.6	Right NFS: Titanium plate stabilized by 7 screws		Bilateral NFS and CS,	Bilateral NFS and CS, INS, IFS

Table II: Summary of *in vivo* pigs and histological controls (all Landrace mixed breed)

		(Fig. 4). Slightly movable nasal attachment on day 1, loaded 5 days.		INS, IFS	
P-5	Male, 4.0, 20.9	Right NFS: Titanium plate stabilized by 8 screws (Fig. 4). Stable attachments, loaded 5 days.		Bilateral NFS and CS, INS, IFS	Bilateral NFS and CS, INS, IFS
Histological controls					
C-1	Female, 5.4, 34.0	None	Calcein and alizarin injected 5 days apart	None	Right NFS
C-2	Female, 5.4, 30.8				Left NFS
C-3	Female, 5.4, 32.2				Left NFS
C-4	Female, 3.6, 22.2				Right NFS (only suture width)

Legend: NFS: Nasofrontal suture, CS: Coronal suture, INS: Internasal suture, IFS: Interfrontal suture.

Table III: Strain transmission in *ex vivo* heads: Mean strains and load output during 30 minutes of cyclic loading of the right nasofrontal suture (NFS)

No.	Right Nasal Bone Rosette Gage				Right Frontal Bone Rosette Gage				Load Output (Kg)	Suture Single-Element Gages (με)					
	Principal Strain (με)			Max Angle (°)	Principal Strain (με)			Max Angle (°)		Right NFS Loaded	Left NFS	Right CS Loaded-side	Left CS	IFS	INS
	Max	Min	Shear		Max	Min	Shear								
H-1	Indeterminate				Indeterminate				1.55	1272	693	-54	-15	15	81
H-2	15	-37	52	64	38	-84	121	94	1.52	684	490	0	-3	86	26
H-3	Indeterminate				Indeterminate				1.60	737	664	-28	-15	53	190
H-4	Indeterminate				Indeterminate				1.64	720	508	-47	-44	20	-100
H-5	46	-34	80	128	Indeterminate				1.64	1063	1045	-80	0	213	-192
H-6	27	-46	73	57	26	-55	80	111	1.37	914	558	-75	-27	-85	-17
Mean [S.D.]	29 [15]	-39 [6]	68 [14]	83 [39]	32 [8]	-69 [21]	101 [29]	103 [12]	1.55 [0.10]	898 [233]	660 [205]	-47 [30]	-17 [16]	*	*

*Means and S.D.s were not calculated for IFS and INS because of the inconsistency of strain polarity.
Legend: Values are means of 60 cycles, 20 each from the beginning, middle, and end of the loading period. Positive strain values indicate tension, and negative strain values indicate compression. Principal strains cannot be determined unless all three elements of a rosette gage produce measurable readings. CS: Coronal suture, IFS: Interfrontal suture, INS: Internasal suture, Max: Maximum principal strain (tension), Max Angle: Orientation of maximum principal strain, where 0^{o} represents the sagittal axis (see Fig. 6), Min: Minimum principal strain (compression), S.D.: Standard deviation.

Table IV: Strain transmission in *in vivo* loaded pigs on the 5^{th} day of cyclic loading of the right nasofrontal suture (NFS):

Load output and mean strains in comparison to *ex vivo* results and previous data on masticatory strain in pigs

No.	Load Output (Kg)		Mean Suture Strain (με)					
	Mean Days 1-5	Day 5 of Loading	Right NFS Loaded	Left NFS	Right CS Loaded-side	Left CS	IFS	INS
P-1	0.70	1.50	N/A	N/A	N/A	N/A	N/A	N/A
P-3	1.52	1.50	1521	932	-863	54	477	-81
P-4	1.30	1.08	917	417	-338	113	115	22
P-5	2.14	2.11	2189	1092	-1091	-265	109*	251
Mean [S.D.] P-3, P-4, P-5	1.65 [0.43]	1.56 [0.51]	1543 [636]	814 [353]	-764 [386]	-33 [203]	296* [256]	-81 to 251†
Ex Vivo Heads (from Table III)		1.55 [0.10]	898 [233]	660 [205]	-47 [30]	-17 [16]	-85 to 213†	-192 to 190†
Mastication[6,8]	N/A	N/A	-1583 [506]		-268 to 808†‡		1036 [400]	-440 [238]

*IFS strains in P-5 were truncated and therefore underestimated; they were excluded from calculations of IFS mean and S.D.

†Because the polarity of strain varied for the INS in this study, for the IFS *ex vivo*, and for the CS during mastication, range is presented instead of mean and S.D.

‡Unpublished raw data

Legend: Positive values are tensile strain, and negative values are compressive strain. CS: Coronal suture, IFS: Interfrontal suture, INS: Internasal suture.

Table V: Histological measurements of mean suture width and mineral apposition rate (MAR)

No.	Right NFS		Left NFS		Right CS		Left CS		IFS		INS	
	Width (µm)	MAR (µm/day)	Width (µm)	MAR (µm/day)	Width (µm)	MAR (µm/day)	Width (µm)	MAR (µm/day)	Width (µm)	MAR (µm/day)	Width (µm)	MAR (µm/day)
Right nasofrontal suture cyclically loaded in vivo												
P-1	187	26	197	22	103*	11*	ND	10*	ND	ND	ND	ND
P-3	332*	30*	266	19	147*	9*	103	6	210	13	163	8
P-4	361	26	341	13	168	4	155	10	293*	14*	150*	8*
P-5	290	18	297*	21*	186*	19*	207*	9*	139	8	184	9
Mean [S.D.] P-3, P-4, P-5	327 [36]	25 [6]	301 [37]	18 [4]	167 [20]	11 [8]	155 [52]	8 [2]	214 [77]	12 [3]	166 [17]	8 [0]
Controls	NFS†				CS†							
	Width (µm)		MAR (µm/day)		Width (µm)		MAR (µm/day)					
Sham control (loading attachments placed but no loading)												
P-2	238		13		146*		4*					
Histological controls												
C-1	157		8		ND		ND					
C-2	175		10									
C-3	180		8									
C-4	178		ND									
Mean [S.D.] P-2 plus C-1 to C-4	186 [31]		10 [2]									
p-value, Loaded vs Controls‡	0.001	0.005	0.003	0.02								

*The sutural space was sometimes obscured. These values are based on the measurable portions of each suture.
†Values for left and right NFS and CS were averaged for the sham control, P-2. For histological controls, one NFS was randomly selected for measurement.
‡ Unpaired t-tests
Legend: NFS: Nasofrontal suture, CS: Coronal suture, ND: No data, S.D.: Standard deviation.