Population Average T2 MRI Maps Reveal Quantitative Regional Transformations in the Degenerating Rabbit Intervertebral Disc that Vary by Lumbar Level

John T. Martin, M.S.\textsuperscript{a,b,c}, Christopher M. Collins, Ph.D.\textsuperscript{d}, Robert L. Mauck, Ph.D.\textsuperscript{a,b,c,e}, Kensuke Ikuta, M.D.\textsuperscript{a,b}, Dawn M. Elliott, Ph.D.\textsuperscript{f}, Yeija Zhang, M.D., Ph.D.\textsuperscript{a,g}, D. Greg Anderson, M.D.\textsuperscript{h}, Alexander R. Vaccaro, M.D., Ph.D.\textsuperscript{h}, Todd J. Albert, M.D.\textsuperscript{h}, Vincent Arlet, M.D.\textsuperscript{b}, and Harvey E. Smith, M.D.\textsuperscript{a,b,*}

Abstract

Magnetic resonance imaging (MRI) with T2-weighting is routinely performed to assess intervertebral disc degeneration. Standard clinical evaluations of MR images are qualitative, however, and do not focus on region-specific alterations in the disc. Utilizing a rabbit needle puncture model, T2 mapping was performed on injured discs to develop a quantitative description of the degenerative process following puncture. To do so, an 18G needle was inserted into four discs per rabbit (L3/L4 to L6/L7) and T2 maps were generated pre- and 4 weeks post-injury. Individual T2 maps were normalized to a disc-specific coordinate system and then averaged for pre- and post-injury population composite T2 maps. We also developed a method to automatically segment the nucleus pulposus by 2-D and 3-D curve fitting routines. Puncture injury produced...
alterations in MR signal intensity in a region-specific manner mirroring human degeneration.
Population average T2 maps provided a quantitative representation of the injury response, and
identified deviations of individual degenerate discs from the pre-injury population. We found that
the response to standardized injury was modest at lower lumbar levels, likely as a result of
increased disc dimensions. These tools will be valuable for the quantitative characterization of
disc degeneration in future clinical and pre-clinical studies.

Keywords
intervertebral disc; degeneration; rabbit; MRI; T2 mapping

Introduction

Low back pain accounts for $100-$200 billion dollars in economic losses in the United
States\(^1\),\(^2\), approximately 1\% of our gross domestic product.\(^3\) Lumbar intervertebral disc
degeneration has been implicated in back pain as the natural, age-related degenerative
process is closely related to deficiencies in disc function.\(^4\),\(^5\) Towards developing therapies
for disc degeneration, animal models are commonly used to assess degenerative changes and
the efficacy of proposed therapeutics. In these models, degeneration can be induced by
injuring the annulus fibrosus (AF) with a scalpel or needle, which depressurizes the nucleus
pulposus (NP) and triggers a physiological response that closely resembles human disc
degeneration. AF injury has been conducted and validated in many species\(^6\)–\(^9\), with the
rabbit model among the most common.\(^10\)–\(^13\)

Rabbit discs closely resemble human discs in composition and structure, and their response
to puncture injury closely resembles human degeneration. Glycosaminoglycan (GAG)\(^14\) and
collagen\(^15\) contents of both the NP and AF of healthy rabbit lumbar discs are similar to non-
degenerate human lumbar discs. In addition, puncture injury results in compositional,
cellular, structural, and mechanical changes that resemble human degeneration. Specific
examples of puncture-induced modifications similar to human degeneration include the loss
of NP GAG\(^16\);\(^17\), pro-fibrotic changes in mRNA expression\(^17\), structural changes inclusive
of a shift from bulging AF lamellae to serpentine lamellae\(^18\), and mechanical changes at
both the whole disc level\(^16\);\(^19\) and the tissue level.\(^20\)

Magnetic resonance imaging (MRI) allows for the quantitative, non-invasive assessment of
soft tissues like the intervertebral disc and, consequently, is used to identify pathological
changes in the disc. In the healthy disc, signal intensity on T2-weighted MR images is
highest in the central, hydrated NP and dissipates radially with transition to the
fibrocartilaginous AF.\(^21\) With degeneration, there is a characteristic loss of NP signal
intensity and consequently the NP and AF become indistinguishable.\(^21\) These abnormalities
are typically assessed by visual inspection of MR images or by qualitative evaluations on an
integer scale, like the Pfirrmann or Thompson grading frameworks.\(^21\);\(^22\) In addition,
clinicians routinely evaluate candidates for surgery using MRI to identify disc abnormalities
in the presence of radicular and/or low back pain, and, while these scoring systems provide
some level of discrimination between degenerative states, they do not provide quantitative

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information on T2 signal or positional information regarding the location of the compositional changes.

T2 relaxation time is a physical property related to tissue water content and can be quantitatively measured in vivo with MR imaging. This has been well described in articular cartilage\textsuperscript{23–26}, and more recently, the disc\textsuperscript{27, 28} Rigorous spatial quantification of T2 MR images may allow for improved discrimination between age-based sub-populations or degenerate sub-populations (populations with early versus advanced degeneration) by identifying changes in disc shape, structure and composition. Furthermore, the development of quantitative outcomes that enable non-invasive assessment of degeneration is critical for longitudinally evaluating therapeutics.

The objective of this study was to spatially map changes in T2 relaxation time as a result of puncture-initiated degeneration in the rabbit. To do so, rabbits were imaged before and after puncture to generate population average T2 maps of the healthy and post-injury state. Additionally, an auto-segmentation procedure was developed to enable objective observer-independent isolation of the NP. We hypothesized that population composite images would identify specific changes with degeneration as a function of disc level (i.e., change in NP area and T2 values), as well as morphologic changes such as the disappearance of the intranuclear cleft, a region of lower T2 signal intensity at the center of the NP\textsuperscript{29}.

**Experimental Methods**

**Surgical procedure**

With local institutional approval, 20 New Zealand White rabbits (3 mos., 2.5–3.0 kg, Charles River Laboratories, Wilmington, MA) underwent a procedure in which four lumbar discs (L3/L4 to L6/L7) were injured by needle puncture to induce degeneration\textsuperscript{12; 30–32}. Rabbits were tranquilized and anesthetized, and a retroperitoneal approach to the lumbar spine was achieved by incising the skin at the left flank and then the external oblique, and bluntly developing a plane between the paraspinal and psoas muscles. An 18G needle was inserted through the lateral AF a depth of 5 mm controlled by a stopper. Rabbits were returned to normal cage activity and medicated for pain (meloxicam, 0.2 mg/kg) and infection (cefazolin, 22 mg/kg).

**Radiography**

Lateral radiographs were acquired preoperatively and 4 weeks postoperatively for disc height and of spinal curvature analysis (Fig. 1a). Resultant images were digitally processed in Matlab to calculate Disc Height Index (DHI)\textsuperscript{10; 33} and the relative angles of rotation between vertebrae in the sagittal plane. First, each disc and its corresponding adjacent superior and inferior vertebrae were manually traced for area quantification (A\textsubscript{D}, A\textsubscript{VB1}, A\textsubscript{VB2}) (Fig. 1b,c). Then, a principal components analysis\textsuperscript{34} was performed on individual disc and vertebrae shapes. The principal axes generated by this analysis point in the direction of minimum distribution of pixels within the shape, and thus the widths of the vertebrae (W\textsubscript{VB1}, W\textsubscript{VB2}) and disc (W\textsubscript{D}) could be defined along the principal axes (Fig. 1c). Disc height (H\textsubscript{D}) and vertebral length (L\textsubscript{VB1}, L\textsubscript{VB2}) were defined as H\textsubscript{D} = A\textsubscript{D}/W\textsubscript{D} and L\textsubscript{VB} = A\textsubscript{VB}/W\textsubscript{VB}. DHI was defined as DHI = 2H\textsubscript{D}/(L\textsubscript{VB1}+L\textsubscript{VB2}).
MRI Acquisition

*In vivo* T2 mapping was performed pre-injury and 4 weeks post-injury on each rabbit. A custom MR coil (Fig. 2a) and a 3.0 T MRI spectrometer (Medspec S300; Bruker Instruments, Ettlingen, Germany) were used to generate coronal T2 maps as measured from a multi-slice, multi-echo acquisition (three 2mm-thick slices, 17 Echoes, TE/TR=7.55ms/2000ms, FOV=16.5x16.5cm², matrix=384x384, 2 averages).

Population Average T2 Maps

Week 0 and Week 4 T2 maps (Fig. 2b,c) were processed in Matlab to enable quantitative comparisons between groups. Discs were initially manually segmented from coronal slices and mapped to a coordinate system normalized to disc dimensions (Fig. 2d-f). The mapping process began with a principal components analysis of the manually segmented disc shape to identify the long and short axes. Then, the centroid of the disc was set as the origin and the disc axes were rotated to match the vertical and horizontal axes of a rectangular coordinate system. Finally, a grid was defined spanning −1 to 1 in both the axial and lateral disc dimensions, and the T2 map was linearly interpolated to these regularly spaced grid points. By mapping each disc to a grid, population average T2 maps could be developed by averaging the T2 values of discs from the Week 0 or Week 4 groups at each grid point. In addition, T2 difference maps were constructed by subtracting (and subsequently taking the absolute value of) the Week 0 population average map from (1) the Week 4 population average map (to identify the average change post injury), (2) from the Week 4 L3/L4, L4/L5, L5/L6 and L6/L7 population average maps (to identify average change as a function of level), or (3) from Week 4 individual disc maps (to identify deviations of individual discs from the population average). In case (3), for an individual disc, the T2 difference at each pixel was averaged to generate a single numerical quantity that described the degenerative changes following puncture injury. (Note: all analyses were performed in Matlab; the code is available as supplemental material.)

Auto-Segmentation of the NP by T2 Signal

An automatic procedure was developed to enable non-biased segmentation of the NP based on T2 signal. The area surrounding the NP was first manually segmented to provide an initial guess for curve fitting (lateral boundaries at mid AF, axial boundaries at vertebral endplates). This area was mapped to a normalized grid as described above. Then, to evaluate fitting techniques, four modified Gaussian distribution functions (Table S1), two 2-D functions (a *unimodal, univariate* function and a *bimodal, univariate* function) and two 3-D functions (a *unimodal, bivariate* function and a *bimodal, bivariate* function), were fit to T2 data at the disc mid-height along the lateral axis (2-D) or to the full T2 surface along the lateral and axial axes (3-D). This was done for each disc in the Week 0 and Week 4 groups (Fig. 3). The mean and standard deviation associated with the Gaussian functions are determined from the fitting procedure and describe the distribution of the NP signal. Using the concept of full width at half maximum, NP boundaries were defined as points where 50% of the max NP T2 signal had dissipated as defined by the curve fit (Fig. 3a,c). This concept was expanded to determine NP dimensions with bimodal or bivariate fits (Fig. 3b,d).
Calculation of Mean T2 Signal and T2 Volume

From the original raw T2 maps, the mean T2 signal was calculated for the whole disc, the AF, and the NP of all discs. The mean signal from the whole disc T2 map (isolated by manual selection) was quantified by averaging the T2 value at all pixels within the disc. The mean signal from the NP T2 map (isolated by bimodal, bivariate segmentation) was quantified by averaging pixels within the segmented region. The pixels within the disc excluded by auto-segmentation were used to define the AF and the mean AF signal was defined by the average of T2 values in the excluded region. The T2 volume (or volume under the T2 surface) of the whole disc, AF, and NP regions was determined using a method analogous to numerical integration, summing the volume at each pixel, where dimensions are: axial resolution by lateral resolution by T2 value. T2 volume is comparable to the MRI Index (the average T2 volume or the product of the total NP area and the mean NP signal intensity) which decreases following puncture injury as both the NP area and NP T2 signal decrease.

To examine inter-observer reliability of the automatic segmentation procedure, two researchers (a PhD student with 6 years experience in spine research and an attending orthopaedic spine surgeon) independently analyzed 10 sets of images from the pre-injury and post-injury groups. Pearson correlation coefficients were calculated to determine the linear correlation between each set of observations. To demonstrate reliability, correlations related to manual outlining were compared to those from automatic segmentation using the bimodal, bivariate Gaussian function, specifically for measurements of mean NP T2 values.

Measurement of Disc Geometry from MR Images

Area, width, and height for the whole disc, the AF, and the NP were determined from the original raw T2 maps for all discs (Figure S1). Whole disc area was defined as the number of pixels within the manually segmented whole-disc region multiplied by the scan resolution. Whole disc width was defined as the maximum distance across the lateral axis of the disc; whole disc height was defined as the whole disc area divided by the whole disc width. NP area, width and height were defined in the same way using the NP area isolated from bimodal, bivariate segmentation. AF area was defined as the difference between the disc area and NP area, AF width as the difference between the disc width and NP width, and AF height as the AF area divided by the AF width. AF width is reported as half the measured width to indicate the thickness of the right or left lateral AF.

Statistical Analysis

Measurements of four discs from each rabbit were averaged for Week 0 (n=80, 4 discs/rabbit, 20 rabbits) and Week 4 (n=76, 4 discs/rabbit, 19 rabbits) groups to determine the effect disc injury on disc geometry and T2 signal. Paired t-tests were used to determine significant differences (p<0.05). To evaluate NP segmentation functions, the mean coefficient of determination (R^2) for each of the 4 Gaussian functions was calculated for Week 0 (n=80) and Week 4 (n=76) groups and a two-way ANOVA was implemented. Post-hoc Bonferroni analysis was employed to determine differences between groups at each timepoint (p<0.05). To analyze discs by level, measurements from each disc level were averaged for Week 0 (n=20 discs/level) or Week 4 (n=19 discs/level) groups and a one-way
ANOVA was performed with post-hoc Bonferroni analysis used to determine differences between groups at each timepoint (p<0.05).

Results

All rabbits survived surgery and maintained healthy body weight postoperatively. One rabbit was removed from the study due to skin necrosis and resultant wound complication.

Quantitative T2 mapping was used to generate spatial maps of the T2 relaxation rate of each disc. Upon fitting these data, the bimodal, bivariate method was most effective for NP segmentation, as determined by the mean $R^2$ of all fits (Table 1). For Week 0 fits, there were no differences between the bimodal, bivariate method and the univariate methods, while the unimodal, bivariate method mean $R^2$ was less than the bimodal, bivariate method. For Week 4 fits, the bimodal, bivariate function fit data more accurately than all other methods. Thus, all reported NP dimensions and T2 values were determined by bimodal, bivariate segmentation.

There were no differences between independent observers in terms of mean NP T2 values both pre- and post-injury for either manual outlining or bimodal, bivariate segmentation. In addition, we found that Pearson coefficients were identical between manual outlining and auto-segmentation (Pre-injury: manual outlining $r^2 = 0.86$, auto-segmentation $r^2 = 0.83$; Post-injury: manual outlining $r^2 = 0.44$, auto-segmentation, $r^2 = 0.44$). Thus, automatic segmentation was equivalent to manual outlining, while having the additional benefit of a rigorously defining NP boundaries and potentially eliminating user bias.

Population average T2 maps showed quantitative differences in T2 values across healthy discs and revealed specific transformations following injury (Fig. 4a). Before injury, T2 relaxation time was lowest in the AF and increased gradually towards the NP. The pre-injury map identified that the intranuclear cleft (bilateral T2 peaks at the center of the NP) is a consistent anatomical feature preserved across all discs. At Week 4, NP T2 values decreased and the T2 difference map showed that this reduction occurred at the periphery of the NP.

The T2 relaxation time decreased in the NP but not in the AF. Between Weeks 0 and 4, the mean T2 decreased in the NP and the whole disc, while there was no change in the AF (Fig. 4b). T2 volume also decreased in the whole disc and NP, and increased in the AF (Fig. 4c).

Needle injury also resulted in changes in disc shape. Whole disc area remained constant from Week 0 to Week 4 while NP area decreased and AF area increased (Fig. 5a). Whole disc width increased, while NP width decreased and AF width increased (Fig. 5b). In addition, whole disc and AF heights decreased, while there was no change in NP height (Fig. 5c). While MRI measurements confirmed disc height decreased in the coronal plane, radiographs demonstrated that disc height decreased in the sagittal plane (Fig. 5d).

Population average T2 maps revealed differences in how discs at different lumbar levels responded to injury. Specifically, level-by-level analysis showed that the L4/L5, L5/L6 and L6/L7 discs were less responsive to injury than the L3/L4 discs (Fig. 6). With progression along the spine, the T2 difference in individual discs showed an increasing range, indicating
that the response to injury was not only less severe, but was less repeatable at these lower levels. The mean NP T2 signal in the L3/L4 and L4/L5 discs was significantly decreased between Weeks 0 and 4, while the mean NP T2 value in both the L5/L6 and L6/L7 discs did not change over the same time period and was significantly greater than the L3/L4 NP T2 (Fig. 7a). In addition, while DHI changed for all levels from 0 to 4 weeks, the amount it changed was significantly less for L6/L7 discs compared to L3/L4 discs (Fig. 7b). Interestingly, the mean NP T2 signal in the L6/L7 discs was significantly greater than the mean NP T2 signal in L3/L4 discs at Week 0 (Fig. 7c), and the mean AF width of the L5/L6 and L6/L7 discs was significantly greater than the mean AF width of the L3/L4 discs (Fig. 7d).

**Discussion**

Rabbit lumbar discs were injured using the needle puncture method established by Masuda and et al.\(^{10}\), and changes in T2 MRI maps were quantified. We generated population average T2 maps before and after injury which demonstrated that transformations in T2 signal occurred primarily in the NP, with the intranuclear cleft disappearing as a result of injury. This method also identified transformations in disc geometry, as quantified by MRI and radiographs, analogous to those observed in the human degeneration process. Finally, analysis by level showed that lower lumbar discs were protected from injury, illustrating how anatomical differences (AF thickness) mediate the response to a standardized injury.

Overall, this study showed that lumbar disc injury led to MRI and geometric transformations consistent with those previously reported in both animal models and human disc degeneration. A standard MR definition of disc degeneration, the loss of T2 signal in the NP, is consistently replicated in this and previous animal models.\(^{11; 35–37}\) Through quantitative analysis of MR images, this study also demonstrated the disappearance of the NP intranuclear cleft following injury, a feature of the rabbit puncture model that was not previously defined, but is consistent with human disc pathology.\(^{29}\) Geometric changes like disc height loss and the disappearance of hydrated tissue in the NP are well documented for human disc degeneration and are similarly exhibited in this rabbit puncture model.\(^{11; 36}\) This study revealed additional geometric transformations not previously described in the rabbit puncture model. Specifically, the increase in the coronal disc width following puncture supports the idea that disc bulging occurs in this model, a common finding in epidemiological studies of the human disc.\(^{38}\)

Another feature of this methodology was its sensitivity in identifying degeneration as a function of level. Level-by-level analysis illustrated the limits of set needle puncture depth (5mm) with respect to the increasing disc dimensions at lower lumbar levels. The heterogeneity in response to injury at L4/L5 and lower may suggest that needle insertion depth should be a function of disc level. Many studies support that structural perturbation is only apparent when the injury is of sufficient size relative to disc geometry.\(^{7; 9; 39; 40}\) The specific findings of Michalek and Iatridis, who demonstrated computationally that disc mechanics were sensitive to the ratio of needle diameter to AF width, is particularly relevant to our result that increased AF width was protective in the context of a standardized needle injury.\(^{41}\) Because initiating degeneration in the rabbit lumbar disc with an 18G needle is a
standard model in disc research, we believe this information is important to take into account for future experiment planning, especially in light of the NIH call for improvements in the experimental reproducibility in animal research. Careful selection of puncture depth and validation of the effects are critical to proper interpretation of experimental outcomes in multi-segment experiments.

Auto-segmentation of the NP using a modified Gaussian function was successful in both pre- and post-injury discs, with the bimodal, bivariate function providing the best fit at both timepoints. Univariate functions along the lateral disc coordinates fit the pre-injury data well, but failed to capture post-injury transformations in signal, likely because the spatial distribution of T2 signal was plateau-shaped (rather than peak-shaped) in the post-injury situation. Post-injury, the T2 signal transformed from bimodal T2 peaks along a left-right line at the disc mid-height to bimodal T2 peaks at the superior and inferior margins of the NP. Thus, the bimodal, bivariate function provided the best fits, as expected for a bimodal function fit to bimodal data. In all cases, Gaussian functions produced meaningful boundaries for defining the NP geometry.

Use of bimodal, bivariate NP segmentation was an objective approach to defining internal boundaries. This method has less potential for bias relative to other NP isolation methods (i.e. by the absolute position within the disc, by using the geometry of a predefined region of interest, or by tracing the NP at its boundaries). A comparable auto-segmentation procedure delineates the NP based on T2 pixel intensity by defining a T2 threshold based on the mean T2 signal within a region of interest in a control NP and selecting pixels above this threshold. This method warrants direct comparison to the bimodal, bivariate method in future studies.

The rabbit AF response to needle puncture was different from human degeneration. In human degeneration the AF T2 relaxation times decreases with increasing degeneration grade, while for the rabbit disc, there was no change in AF T2 following needle puncture. This suggests that biochemical changes that occur immediately following puncture are primarily in the NP and not the AF. Similar findings have been described for this model previously. Miyamoto et. al showed there were no changes in AF DNA, proteoglycan or collagen contents 8 weeks after needle puncture, while significant changes in composition occurred in the NP. We propose that in disc puncture models compositional modifications in the AF are preceded by structural modifications in the AF. Evidence for AF structural disorganization in the rabbit puncture model was provided by Sakai et. al who demonstrated lamellar disorganization 6 weeks after puncture, and also by Gregory et. al who reported a decrease in interlamellar shear strength at 12 weeks. Future work must evaluate later time points to confirm the effects of puncture on AF composition in the rabbit model and its relation to changes in the human AF.

The primary criticism of disc injury models is that the rapid advancement of degeneration does not replicate changes seen in human degeneration, which develop over the course of years. These models, however, have a number of features that mimic the human condition: disc height collapse and bulging, the disappearance of the NP T2 signal, and the presence of a number of molecular markers. Because the endpoints of both injury-
initiated degeneration and human degeneration are comparable, injury-mediated degeneration is a powerful technique to study the basic science of disc degeneration and develop therapeutic strategies to regenerate tissue. Another limitation of this study is the inability of the MR analysis to detect subtle changes in disc geometry due to limited scan resolution. For example, at the segmentation boundaries there is volume averaging within each voxel, and consequently, evaluating the AF dimensions by T2 signal may overestimate AF geometry. Relative changes in AF and NP geometry resulting from puncture injury were detectable despite limited resolution, and, in addition to changes in T2 signal, measurement of these parameters enables monitoring of the degeneration process quantitatively.

**Conclusion**

Population average T2 maps enable the quantification of the deviation of an individual disc from a population norm, providing new information on the degree of degeneration that is not available with standard ordinal grading methods. Furthermore, the spatial discrimination of changes implicit in this approach provides additional information on alterations in regional disc structure. This is exemplified here by the disappearance of the intranuclear cleft after injury, a potential early indicator of degeneration, which may be obscured in grading standard T2-weighted clinical MR images. Future work will determine whether these population average T2 and corresponding T2 difference maps are useful in a clinical population to assess the degree of degeneration, to predict pain/disability, and to quantify the adjacent segment degeneration.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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**References**


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Figure 1. Radiographic analysis of response to injury
(a) Representative lateral radiograph with the location of punctured lumbar discs identified.
(b) Manual outline of vertebrae for disc height analysis. (c) Features used for the analysis of
disc height, vertebral width ($W_{VB1}$, $W_{VB2}$) and disc width ($W_D$), vertebral area ($A_{VB1}$,
$A_{VB2}$) and disc area ($A_D$), and 1st and 2nd principal axes generated by principal components
analysis.

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Figure 2. MRI analysis of response to injury
(a) Custom MR coil for imaging the rabbit spine. (b) Week 0 and (c) Week 4 coronal T2 maps. Example of (d) manual segmentation (AF, annulus fibrous; NP, nucleus pulposus; VEP, vertebral endplate) and (e) rotation of a disc based on axes generated by principal components analysis. (f) Interpolation of a disc T2 map to a regularly spaced grid. The aspect ratio represents the average ratio of disc length to disc width.
Figure 3. Gaussian distribution functions for NP auto-segmentation
Gaussian distribution functions fit to the NP T2 signal: (a) unimodal, univariate, (b) unimodal, bivariate, (c) bimodal, univariate, and (d) bimodal, bivariate. On each plot, the max and ½ max points are labeled. These landmarks were used to determine the boundaries of the NP. Details on these functions are provided in Table S1. In (b, d) NP T2 maps are shown before (Initial NP T2 Map) and after (Auto-segmented NP T2 Map) application of a Gaussian function to determine the NP boundaries.
Figure 4. Population average T2 maps and T2 quantification by disc region

(a) Population average T2 maps were generated for discs from Week 0 (n=80, top) and Week 4 (n=76 discs, middle) groups. Map of T2 difference (bottom) between Week 0 and Week 4 population averages. (b) Mean T2 values for the whole disc, the NP, and the AF at each timepoint (*, p<0.05 vs. Week 0). (c) Mean T2 volume (volume under T2 surface) for the whole disc, the NP, and the AF at each timepoint (*, p<0.05 vs. Week 0). Data is shown as the mean ± standard deviation.
Figure 5. Disc geometry pre- and post-injury

MRI measurement of whole disc, NP, and AF (a) area, (b) width, and (c) height from Week 0 (n=80) and Week 4 (n=76) discs (*, p<0.05 vs. Week 0). Note: AF Width refers to the AF radial thickness; see Figure S1 for details. Radiographic measurement of (d) Disc Height Index (DHI) reported as % of Week 0 DHI and (e) vertebral offset angle in the sagittal plane. Data is shown as the mean ± standard deviation.
Figure 6. T2 difference as a function of level

T2 difference maps were used to identify the location of T2 transformation within each disc. (a) These were defined by subtracting the individual T2 maps of Week 4 discs from the Week 0 population average map (n=19 discs/level). (b) T2 difference maps of individual discs were averaged at each point on the grid to quantify changes in T2 values with injury. Individual data points represent the mean T2 difference for an individual disc and are displayed with the median and interquartile range (*, p<0.05 vs. L3/L4 and $, p<0.05 vs. L4/L5). (c) Population average T2 maps of discs at each level confirm the variability in response to needle puncture injury, with less difference observed at lower levels.
Figure 7. Effects of puncture by level and baseline differences
(a) Mean NP T2 and (b) %DHI₀ by level after injury. ($, <0.05 vs. corresponding Week 0 group and *, p<0.05 vs. Week 4 L3/L4). (c) Mean NP T2 signal and (d) AF width by level before injury (*, p<0.05 vs. Week 0 L3/L4).
Table 1

Coefficient of Determination (R^2) for 4 modified Gaussian distribution functions fit to NP T2 Maps

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<th>Unimodal, Univariate</th>
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<td><strong>Week 0</strong></td>
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<td><strong>Week 4</strong></td>
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**Bold font**, p<0.05 vs. Bimodal, Bivariate at corresponding timepoint

Values are listed as mean ± standard deviation