# Evidence of connective tissue involvement in acupuncture[1]

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## SPECIFIC AIMS

Acupuncture needle manipulation gives rise to 'needle grasp', a biomechanical phenomenon characterized by an increase in the force necessary to pull the needle out of the tissue (pullout force). This study investigates the hypothesis that winding of connective tissue rather than contraction of muscle is the mechanism responsible for needle grasp.

## PRINCIPAL FINDINGS

### 1. Increased needle penetration of muscle does not result in greater pullout force than increased penetration of subcutaneous tissue

To determine whether muscle or subcutaneous tissue is responsible for needle grasp, we measured pullout force with and without needle penetration of muscle while controlling for depth of needle insertion in 60 healthy human subjects randomized to three types of needle manipulation: bidirectional rotation, unidirectional rotation, and needle insertion with no rotation. We used a computer-controlled acupuncture needling instrument to perform all needle insertions, manipulations, and pullout force measurements. Within subjects and at two locations (lumbar and sacral), right and left sides of the body were randomized to two different needle depths D and D+1. D was defined as the thickness of subcutaneous tissue at the lumbar location determined by ultrasound and D+1 was equal to D plus 1 cm (**Fig. 1*a***). The difference in pullout force between the two lumbar needles ($\Delta_L$) thus corresponded to the effect of 1 cm of muscle penetration, whereas the difference in pullout force between the two sacral needles ($\Delta_S$) corresponded to the effect of 1 cm of subcutaneous tissue penetration. With both no rotation and bidirectional rotation, there was no evidence that $\Delta_L$ was greater than $\Delta_S$ (linear contrast, $F_{1,57}=1.19$, $P=0.28$; $F_{1,54}=0.03$, $P=0.86$ for no rotation and bidirectional rotation, respectively). With unidirectional rotation, $\Delta_S$ was significantly greater than $\Delta_L$, indicating that the difference in pullout force between depths D and D+1 was greater when both needles were not in muscle (linear contrast, $F_{1,57}=5.7$, $P=0.02$) (Fig. 1*b*).

### 2. Acupuncture needle rotation increases pullout force and subcutaneous tissue volume in the vicinity of the needle

We measured pullout force bilaterally in 13 live anesthetized rats. Right and left sides of the body were randomized to needle insertion, followed by unidirectional needle rotation vs. needle insertion only without rotation. Pullout force measurements were followed by rapid tissue fixation and measurement of dense subcutaneous connective tissue volume in serial-sectioned histological specimens. Mean (±SE) pullout force was 2.48 ± 0.39N with needle rotation compared with 0.17 ± 0.01N with no rotation (paired *t* test, $t_{12}=5.9$, $P<0.001$). Histological examination of tissue sections revealed marked thickening of dense subcutaneous connective tissue around the rotated needle. Relative dense connective tissue volume was significantly greater with rotation ($1.01\pm0.11$ mm$^3$) than with no rotation ($0.5\pm0.06$ mm$^3$; paired *t* test, $t_{10}=5.0$, $P<0.001$). With rotation, pullout force and volume were positively correlated ($r=0.78$, $P<0.01$); no significant correlation was found with no rotation ($r=0.47$, $P=0.14$).

### 3. Acupuncture needle rotation results in deformation of subcutaneous tissue characterized by increased periodic architectural order

We used high frequency (50 MHz) ultrasound scanning acoustic microscopy to obtain images of rat abdominal wall tissue explants with and without unidirectional needle rotation. This in vitro ultrasound technique yields high-resolution images of fresh tissue without need for fixation or staining. We found prominent spiral patterns centered on the needle in ultrasound images with needle rotation (**Fig. 2*b***). Fourier analysis performed on radial scan lines centered on the needle showed significantly increased periodic order with rotation compared with no rotation (Fig. 2*c*).

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[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01–0925fje; to cite this article, use *FASEB J.* (April 10, 2002)10.1096/fj.01–0925fje

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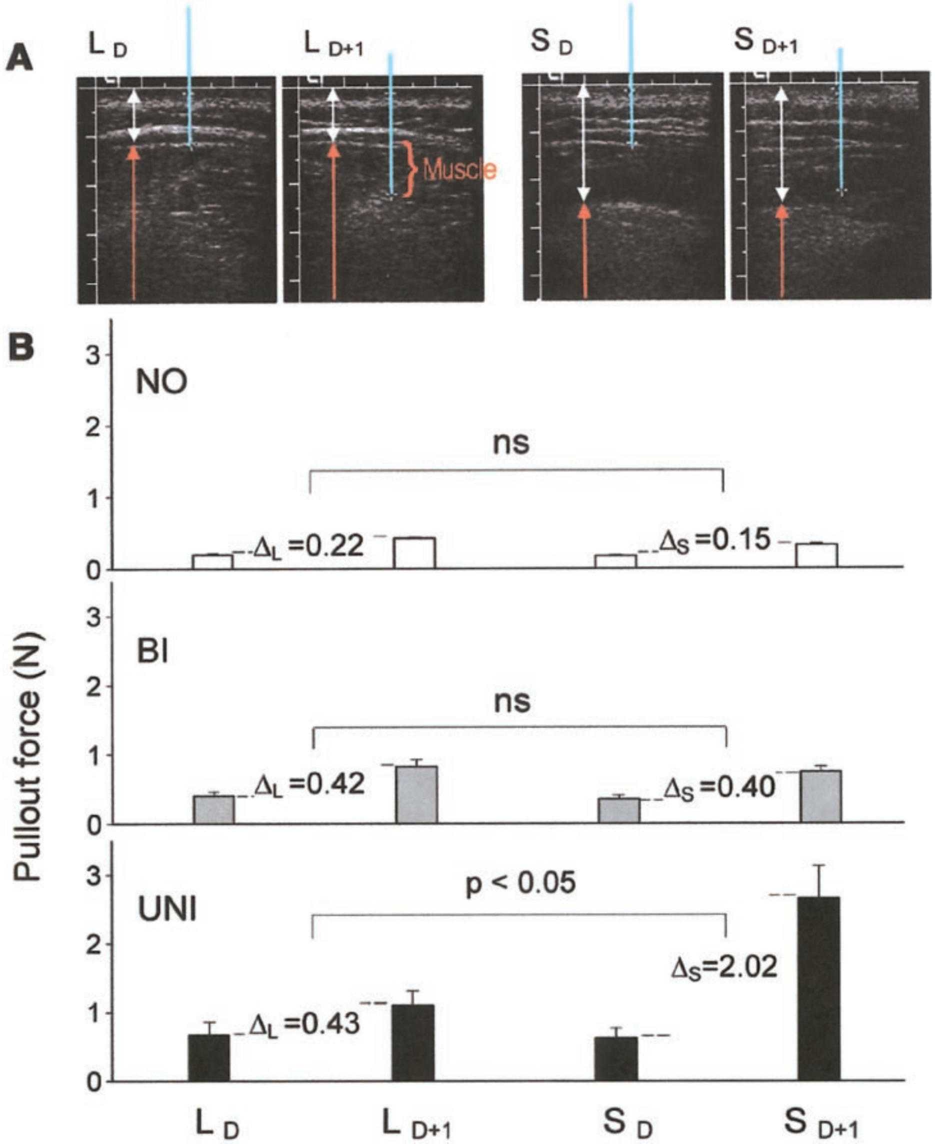


**Figure 1.** Pullout force measurements in humans. *a)* Sample ultrasound images for lumbar and sacral needling locations bilaterally. Red arrows indicate muscle, and white arrows indicate subcutaneous tissue. In each image, an acupuncture needle (drawn in blue) indicates depth of needle insertion. At the lumbar location, needle $L_D$ was inserted to the perimuscular fascia. On the contralateral side, needle $L_{D+1}$ was inserted 1 cm beyond the perimuscular fascia (i.e., into muscle). At the sacral location, needles $S_D$ and $S_{D+1}$ were inserted to the same depths as needles $L_D$ and $L_{D+1}$, respectively. Because subcutaneous tissue thickness was at least 1 cm greater at the sacral than at the lumbar location in all subjects, neither needle $S_D$ nor needle $S_{D+1}$ penetrated muscle. *B)* Mean (± SE) pullout force with no needle rotation (NO), bidirectional needle rotation (BI) and unidirectional needle rotation (UNI). $\Delta_L$ indicates difference in pullout force between the two lumbar needles and $\Delta_S$ the difference between the two sacral needles. Pullout force results are expressed in Newtons (N).

## CONCLUSION AND SIGNIFICANCE

These results support the conclusion that connective tissue winding is the mechanism responsible for the increase in pullout force induced by needle rotation. These findings have important implications for the field of acupuncture. For the past 30 years, a general assumption of acupuncture research has been that the effects of acupuncture essentially take place via the nervous system. This study, by providing evidence of subcutaneous tissue involvement in needle grasp, suggests that the mechanism of action of acupuncture also involves extraneural tissues and paves the way for further investigations of local cellular and molecular effects of acupuncture needle manipulation.

Winding of connective tissue itself is a mechanism with relevance not only to the field of acupuncture, but also to the fields of mechanical signal transduc-

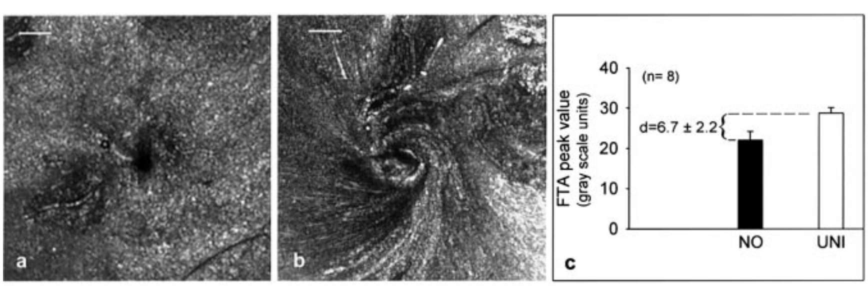


**Figure 2.** Ultrasound scanning acoustic microscopy of rat subcutaneous tissue. *a)* No needle rotation; *b)* unidirectional needle rotation. The plane of ultrasound scanning is parallel to the subcutaneous tissue plane and the needle (appearing as a black dot in the center of each image) is perpendicular to the tissue. Scale bars: 1 mm. *c)* Fourier analysis of radial scan lines centered on the needle. Mean Fourier transform peak value with no rotation (NO) and unidirectional rotation (UNI). *$P < 0.02$. Error bars represent mean ± SE of the differences between sample pairs.

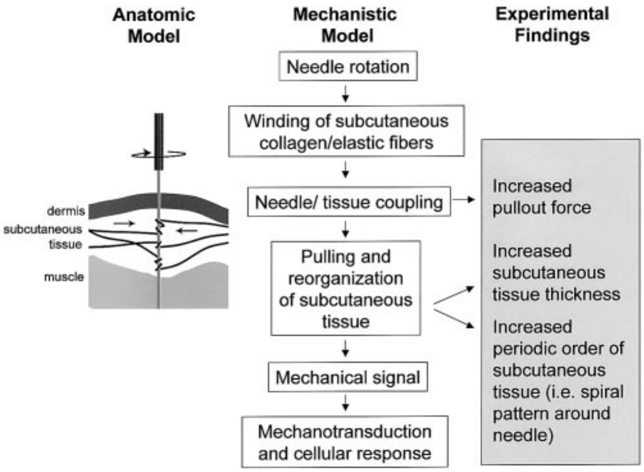


**Figure 3.** Summary of experimental findings and proposed mechanism. Winding of collagen and elastic fibers around the needle allows movements of the needle to deliver a mechanical signal to connective tissue. Possible cellular responses to this mechanical signal include cell contraction, signaling pathway activation, and gene expression.

tion and connective tissue physiology. The increase in friction force that occurs with progressive winding of material around a small diameter shaft makes winding an ideal mechanism for achieving a tight mechanical coupling between an instrument (needle) and tissue. Achieving this mechanical coupling allows movements of the needle to deliver a powerful mechanical signal to the tissue. The cellular and molecular effects of mechanical signal transduction have been shown to be widespread and powerful, ranging from cell contraction to signaling pathway activation and gene expression. So far, most studies of the effects of mechanotransduction have been performed in tissue culture environments. A study of the effects of mechanical forces on whole tissue is an important extension of this basic work, allowing an examination of these effects in the context of native tissue architecture. In this study, we have demonstrated that acupuncture needle rotation results in a measurable deformation of connective tissue. Pulling of collagen and/or elastic fibers and deformation of extracellular matrix during needle manipulation may have powerful and long-lasting effects on local cells via the mechanical link existing at focal adhesions between extracellular matrix and intracellular cytoskeleton. Knowledge derived from the study of acupuncture needle manipulation may therefore contribute to a better understanding of acupuncture and of the effect of mechanical forces in tissues.

The results of this study also highlight the potentially important role of interstitial connective tissue in neuromodulation. Techniques such as acupuncture may act not simply via neural stimulation, but also by producing changes in the connective tissue milieu surrounding sensory afferent nerve fibers. These connective tissue changes may be long lasting, which may explain the claims that acupuncture can have prolonged therapeutic effects. FJ