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Research Report

Vibrotactile detection thresholds for chest skin of amputees following targeted reinnervation surgery

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ARTICLE INFO

Article history:
Accepted 10 November 2008
Available online 21 November 2008

Keywords:
Targeted reinnervation
Psychophysics
Vibrotactile
Amputee
Threshold
Sensory

ABSTRACT

Recent advances in the design of prosthetic arms have helped upper limb amputees achieve greater levels of function. However, control of upper limb prostheses is limited by the lack of sensory feedback to the user. Targeted reinnervation, a novel surgical technique for amputees, offers the potential for returning this lost sensation. During targeted reinnervation surgery, truncated nerves are directed to reinnervate new muscle and skin sites. Contractions of reinnervated muscles generate electrical signals that are used to control prosthetic arms. In addition, stimulation of reinnervated skin is perceived on the missing limb. Vibration detection thresholds were measured at four frequencies on the reinnervated chest skin of three shoulder-level amputees following targeted reinnervation surgery. Thresholds were also measured on the contralateral chest and arm skin of these amputees, as well as on the chest and arm skin of a control population. Vibrations applied to reinnervated skin were perceived at various locations on the missing arm and hand. Thresholds for the reinnervated chest skin were generally within the range of values measured on the chests of the control population. For the two unilateral amputees, these thresholds were similar to measures on their contralateral chests, but greater than measures on their contralateral hands. Targeted reinnervation appears to result in near-normal vibration-detection ability with respect to the target tissue, suggesting the functional reinnervation of mechanoreceptors by the reinnervating afferents. The functional limb sensation following targeted reinnervation could be used to provide prosthesis users with a sense of touch.

1. Introduction

The loss of an arm impairs the ability of amputees to perform a variety of tasks and to interact with their environment. Although advancements in the design and control of motorized prostheses continue to increase their functional capacities, control is limited by the absence of sensory feedback to the user. Targeted reinnervation (TR) is a recently developed surgical technique aimed at improving myoelectric control for upper limb amputees and has opened the possibility of

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Abbreviations: TR, targeted reinnervation; SIAM, Single Interval Adjustment Matrix

0006-8993/$ – see front matter
doi:10.1016/j.brainres.2008.11.039

providing intuitive sensory feedback to the prosthesis user (Kuiken et al., 2007a). TR involves rerouting the truncated nerves of the missing arm to unused muscles adjacent to the amputation site (Kuiken et al., 2004). These nerves and muscles develop functional connections, allowing an amputee to control a prosthesis with alternative muscles which respond to neural commands intended for the missing limb. The afferents of these nerves reinnervate the skin overlying the targeted muscles, causing stimulation of this skin to result in percepts on the missing limb (Kuiken et al., 2007a), subsequently referred to as "transfer sensation." The potential exists for sensory feedback applied to reinnervated skin to be incorporated naturally into prosthesis use, improving control and dexterity as well as the subject's quality of life.

An important step toward providing sensory feedback is to quantify the sensory capabilities of the reinnervated skin and compare it to the skin of the missing limb. It has already been shown that subjects are able to feel temperatures and constant pressures applied to reinnervated skin at near-normal thresholds (Kuiken et al., 2007a) as well as able to distinguish between sharp and dull stimuli (Kuiken et al., 2007b). In this study, we use vibrotactile detection thresholds as a means of assessing skin sensation function. Vibration information is important for our ability to feel textures (Hollins and Risner, 2000), use tools (Brisben et al., 1999; Johnson, 2001), and manipulate objects. Assessment of vibrotactile thresholds is well-established (Bolanowski et al., 1988, 1994; Merzenich and Harrington, 1969; Mountcastle et al., 1972; Verrillo, 1962), and demonstrates differences between the hairy and glabrous skin types (Mahns et al., 2006; Talbot et al., 1968) as well as regional variations within skin types (Lofvenberg and Johansson, 1984). It may also aid in detecting sensory neuropathy (Armstrong et al., 1998).

Vibration detection is a distinct capability of the sensory system made possible by specialized mechanoreceptive end organs (Johnson, 2001). It is reflective of the underlying physiology of the skin, specifically the skin receptors and their afferent fibers (Bolanowski et al., 1988, 1994; Gescheider et al., 2004). The measurement of vibrotactile thresholds has previously been used to create models of vibrotaction that attribute threshold-level detection of different frequencies to specific mechanoreceptor and fiber types and to assign them specific percepts, such as "pressure," "flutter," and "vibration" (Talbot et al., 1968; Vallbo, 1981). Different receptors are tuned to different frequency ranges, and typically, only one mechanoreceptor type is responsible for generating percepts at the threshold of detection (Bolanowski et al., 1988, 1994). For glabrous skin (the hairless skin of the palms of the hands and soles of the feet) threshold-level stimuli at low (1–4 Hz), mid (5–30 Hz) and high (30+ Hz) frequencies are thought to be detected by Merkel cells, Meissner's corpuscles and Pacinian corpuscles, respectively (Bolanowski et al., 1988). For hairy skin, the responsible mechanoreceptors are likely hair follicle receptors (1–4 Hz), Ruffini endings (5–30 Hz), and Pacinian corpuscles located in deep tissue layers (30+ Hz) (Bolanowski et al., 1994). Vibrotactile detection thresholds in hairy skin have been shown to be orders of magnitude greater than those of glabrous skin (Mahns et al., 2006; Merzenich and Harrington, 1969). This is likely a reflection of the different receptor compliments, differing densities of sensory innervation (Gardner et al., 2000), and the fundamental structural differences of the two skin types.

In this study, we investigate vibration detection thresholds at four frequencies on the reinnervated chest skin of three amputee subjects who have undergone the TR procedure. These measures are compared to measures on the contralateral chest, arm, and hand skin of these subjects, where applicable. In addition, baseline values for the chest, upper arm, forearm, thenar eminance, and fingertip were measured on a population of non-amputee (control) subjects.

2. Results

2.1. *Comparison of reinnervated and contralateral chest thresholds*

Thresholds of vibration detection on the reinnervated and contralateral chest skin of all three TR subjects were measured using a 9 mm-diameter probe (Fig. 1). Threshold measures from the reinnervated chest of amputee subject 1 (S1) were significantly higher than measures from the contralateral

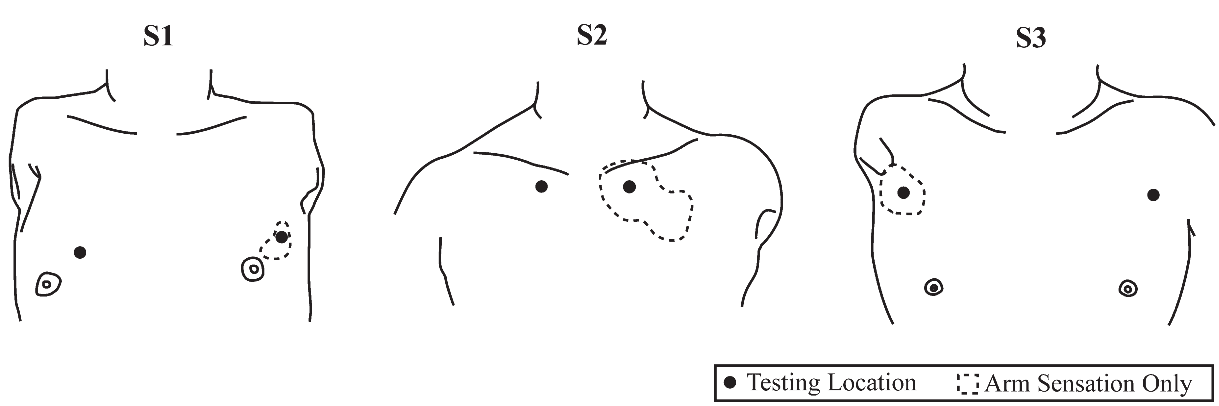

Fig. 1 – Vibrotactile testing locations on the chests of the three TR subjects. Each of the three TR amputee subjects was tested on the reinnervated and contralateral side of the chest (black dots). Testing on the reinnervated side was done in the center of an area on which light pressure was exclusively perceived in the missing limb (dotted outline). Testing on the contralateral side was done in an area free from scarring.

chest at 30 and 250 Hz ($p<0.001$ and $p=0.022$, respectively) (Fig. 2A). Threshold measures from the reinnervated chest of amputee subject 2 (S2) were significantly higher than measures from the contralateral chest at 5 Hz ($p=0.026$) (Fig. 2B). Threshold measures from the reinnervated chest of amputee subject 3 (S3) were not significantly different from measures from the contralateral chest at any frequency (Fig. 2C). Threshold measures at 400 Hz for all three subjects were potentially limited by the dynamic range of the stimulator, as subjects were not always able to feel the stimulus at maximum amplitude.

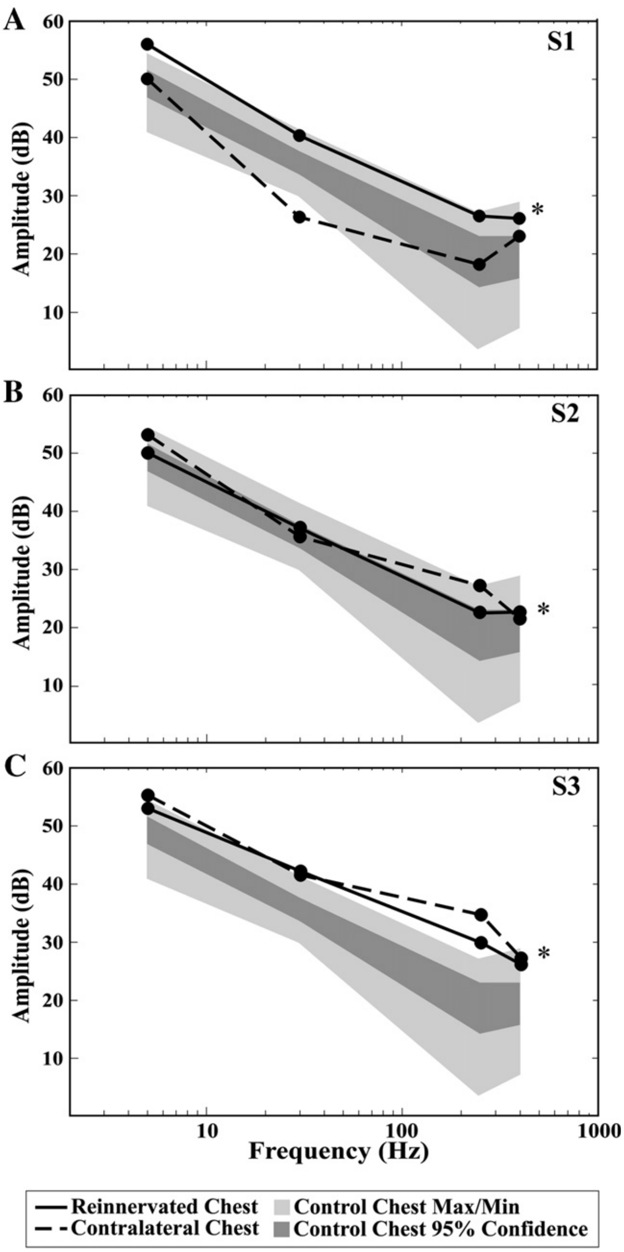

Fig. 2 – Vibrotactile detection thresholds on reinnervated and contralateral chests of TR amputees. Vibration detection thresholds were measured with the 9 mm probe on the reinnervated and contralateral chest sides of subjects S1 (A), S2 (B), and S3 (C). Maximum range (light gray) and 95% confidence intervals (dark gray) of the control population are included for reference. (Stars (*) denote potential underestimates and indicate where threshold measures are equal to the maximum range of the stimulator.)

2.2. *Comparison of amputee chest thresholds to control population thresholds*

Thresholds of vibration detection measured on the chest skin of amputees were compared to thresholds measured on the chests of 14 control subjects with the 9 mm probe (Fig. 2). Threshold values measured at all four frequencies on the reinnervated chest of S1 were above the 95% confidence intervals of the means of the control data but within the range of the data for all frequencies except 5 Hz (Fig. 2A). Thresholds measured on the reinnervated chest of S2 were within the 95% confidence intervals at all four frequencies (Fig. 2B). Thresholds measured on the reinnervated chest of S3 were well above the 95% confidence intervals, but within the range of the control data at 5 and 400 Hz (Fig. 2C).

Thresholds from the contralateral chests of S1 and S2 were within the 95% confidence intervals of the means of control data at all four frequencies, with the exception of the 30 Hz threshold for S1, which was lower than the control data (Figs. 2A, B). Threshold measures from S3's contralateral side were above the range of control data at all frequencies except 400 Hz (the low values for both chest sides at 400 Hz were likely due to the limited dynamic range of the stimulator) (Fig. 2C).

2.3. *Locations of referred sensations*

Application of suprathreshold vibrations to the reinnervated chests of the three amputee subjects elicited perceptions of vibrations on their missing limbs. For S1, stimulation at 30, 250 and 400 Hz produced sensations perceived on the missing hand and forearm (Fig. 3A). At 5 Hz, S1 also reported feeling the stimulus in his chest. For S2, stimulation at 30, 250, and 400 Hz produced sensations perceived at similar regions on the palm of the missing hand (Fig. 3B). S2 did not feel suprathreshold vibrations at 5 Hz on the missing limb. The quality of sensation described by S2 in response to the suprathreshold 400 Hz stimulus was that of a light tingling sensation. For S3, stimulation at all four frequencies produced sensations perceived on the missing hand, forearm and upper arm (Fig. 3C).

2.4. *Comparison of reinnervated chest thresholds to contralateral chest, arm and hand thresholds*

Thresholds of vibration detection measured using a 2 mm-diameter probe on the reinnervated chests of subjects S2 and S3 were compared to thresholds measured on their contralateral chests and arms (upper arm, forearm, thenar eminance and fingertip for S2; fingertip only for S3). Vibration detection thresholds from the reinnervated chest of S2 were closest in value to thresholds from the contralateral chest, upper arm, and forearm (Fig. 4A). Independent samples *t*-tests showed no significant differences between values for the reinnervated chest and the contralateral chest and forearm. Reinnervated chest thresholds were significantly higher than thresholds from the fingertip and thenar eminance at all frequencies

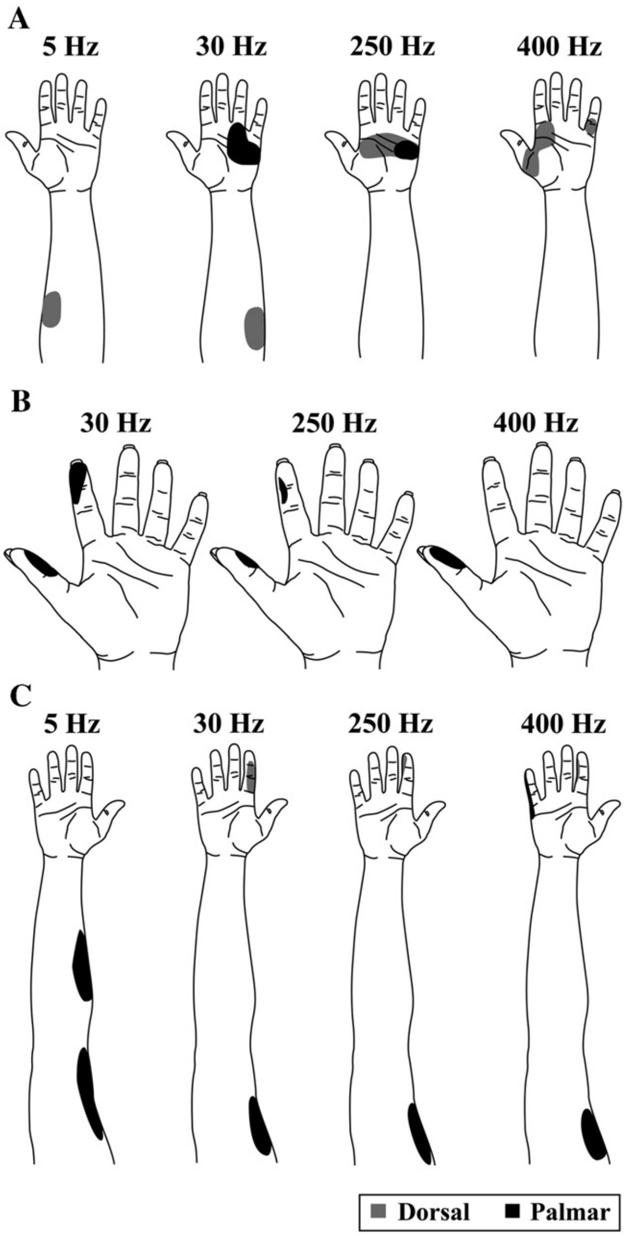

Fig. 3 – Localization of transfer sensation. Locations of perceived vibrations in the missing limbs of S1 (A), S2 (B) and S3 (C) following the application of vibration stimuli to the reinnervated chest testing site.

($p \leq 0.001$ for all), and upper arm at 5 and 30 Hz ($p = 0.005$ for both). Vibration detection thresholds from the reinnervated chest of S3 were closest in value to thresholds from the contralateral chest. An independent samples t-test revealed no significant differences between these chest measures (Fig. 4B). Thresholds measured on the reinnervated chest were significantly higher than those measured on the fingertip at 30, 250 and 300 Hz ($p < 0.01$ for all).

2.5. *Control population*

For the control population, average threshold measures for all three regions of hairy skin (the chest, upper arm and forearm) were significantly higher than threshold measures for the two regions of glabrous skin (the fingertip and thenar eminence) at all four frequencies (repeated-measures two-factor ANOVA with factors location and frequency, $p \leq 0.024$ for all) (Fig. 5). The only exception was that the average threshold for the forearm was not significantly greater than the threshold for the thenar eminence at 5 Hz.

Thresholds measured on locations of the same skin type were also compared. There were no significant differences between thresholds measured on the three regions of hairy skin ($p > 0.05$). The only significant difference between the two glabrous skin regions occurred at 5 Hz ($p = 0.004$).

Subjects were unable to consistently perceive the 400 Hz stimulus at maximum amplitude on hairy skin regions. This was likely due to the dynamic range of the stimulator, which was limited to an amplitude of 30 μm at 400 Hz. Only 72%, 61% and 61% of chest, upper arm and forearm trials at 400 Hz resulted in valid threshold measures. We consider the

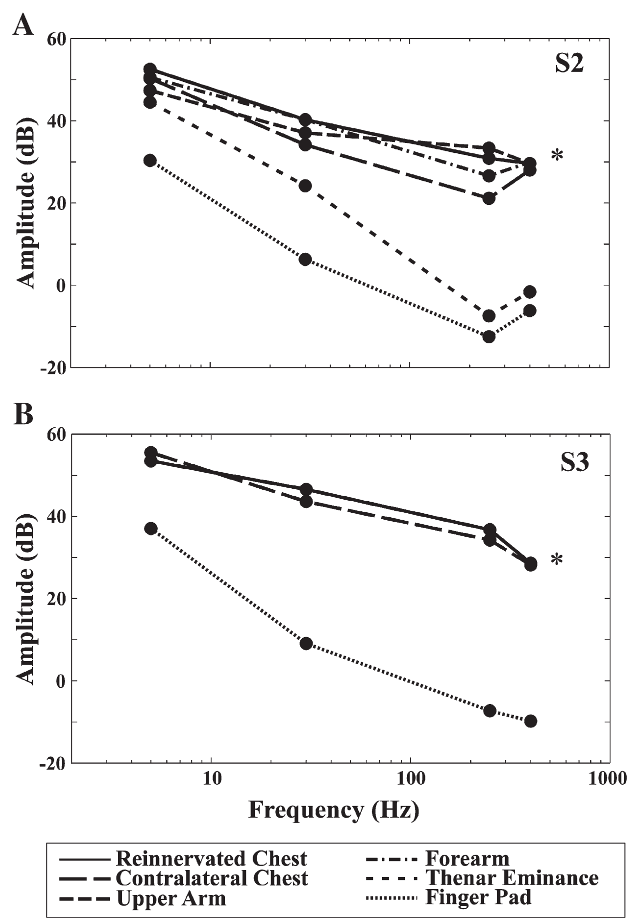

Fig. 4 – Vibrotactile detection thresholds on the chests and contralateral arms of amputees. Vibration detection thresholds were measured with the 2 mm probe on the reinnervated chest and contralateral chest, upper arm, forearm, thenar eminance and fingertip of S2 (A), and the reinnervated chest and contralateral chest and fingertip of S3 (B). (Stars (*) denote potential underestimates and indicate where threshold measures are equal to the maximum range of the stimulator.)

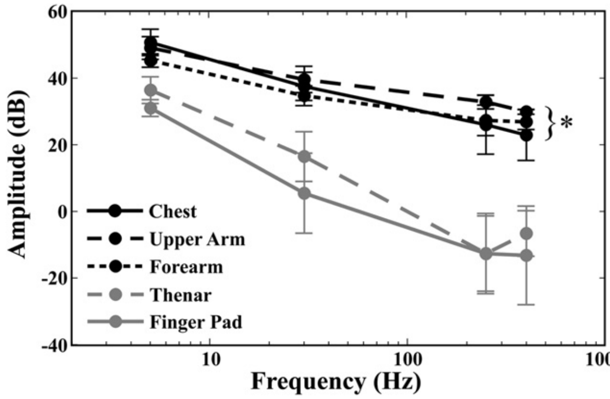

Fig. 5 – Vibrotactile detections thresholds on the chests and arms of control subjects. Vibration detection thresholds measured with the 2 mm probe on the five tested locations of control subjects. (Star (*) denotes potential underestimates and indicate where threshold measures are equal to the maximum range of the stimulator.)

resulting average threshold values at 400 Hz to be underestimates (star in Fig. 5).

2.6. *Comparison of amputee intact arm thresholds to control population thresholds*

Thresholds from the intact arms of subjects S2 and S3 measured using the 2 mm probe were then compared to thresholds measured on the control population. Median threshold values from subject S2 were within the 95% confidence intervals of the means of the control population thresholds for the fingertip and upper arm at all frequencies. Thresholds for S2 were greater than the 95% confidence intervals of the control population on the thenar eminence at 5 Hz and 30 Hz and the forearm at 5 Hz, 30 Hz and 400 Hz. For subject S3, fingertip values were within the 95% confidence intervals of the means of the control population at all but 5 Hz.

3. Discussion

The three tested TR amputees did not consistently demonstrate abnormal or impaired vibration detection thresholds on reinnervated skin. In general, thresholds from the reinnervated chest skin were elevated with respect to control data, but largely within the maximum range. In addition, two of the three subjects showed little to no difference in thresholds between their reinnervated and contralateral chest regions. This suggests that TR surgery and the subsequent reinnervation of the skin do not result in decreased vibration sensation ability. In contrast, spontaneous reinnervation of denervated chest skin following breast reconstruction has been found to result in decreased levels of sensory recovery (Shaw et al., 1997). The lack of impairment following TR surgery may be a result of the sheer density of afferents available to reinnervate the skin, as the rerouted nerves contain many times more afferents than originally innervated the skin of the chest (Kuiken et al., 1995). It may also be reflective of an increased accuracy of central processing devoted to behaviorally significant sensory input—in this instance from the redirected afferents of the hand (Azzopardi and Cowey, 1993; Catania, 1995; Johansson and Vallbo, 1979). Despite the fact that some of the applied vibrations were perceived on the missing hand, threshold data from the reinnervated skin of the two unilateral amputee subjects were more reflective of chest or arm thresholds (hairy skin) than hand thresholds (glabrous skin). It would appear that the environment into which the hand afferents were transferred had considerable influence on vibration detection thresholds (Dykes et al., 1984; Lewin and McMahon, 1991).

Two subjects (S1 and S3) demonstrated reinnervated chest thresholds that were elevated with respect to one of the two control measures. Subject S1 showed an increase of vibrotactile thresholds on reinnervated chest skin in comparison to contralateral chest skin; however, these thresholds were within the range of thresholds measured on the control population. Subject S3 showed thresholds on the reinnervated chest that were higher than those of the control population; however, these thresholds were virtually indistinguishable from thresholds on the contralateral chest. It is interesting to note that both of these subjects had amputations resulting from electrical burns; the observed thresholds may reflect sensory damage from these injuries. In contrast, Subject S2—whose reinnervated chest thresholds were similar to both control measures—suffered amputation in a motor vehicle accident and was more likely to be free of confounding factors from extensive tissue damage.

The measurement of thresholds on the hand, arm and chest of control subjects was largely intended to validate the experimental methodology and provide a framework for viewing results from TR subjects. In addition, normative thresholds from the chest and upper arm were not available in the literature. The results from hand, arm and chest regions of control subjects demonstrated the well-established difference in magnitude between glabrous and hairy skin (Mahns et al., 2006; Merzenich and Harrington, 1969), although this difference was greater than previous reports, due to the lower fingertip thresholds measured in this study. However, our fingertip thresholds were comparable to those measured by other investigators (Verrillo, 1962). While the experimental conditions used in this study were not identical to those used in any single past study, our experimental approach was designed to be reflective of previous studies and our results were largely comparable to those of the literature. Our average results for control subjects in the three previously tested locations were generally within the range of highest and lowest data collected from prior researchers, where data was available (Bolanowski et al., 1988, 1994; Mahns et al., 2006; Merzenich and Harrington, 1969; Talbot et al., 1968).

The ability of TR subjects to detect high-frequency vibrations at near-normal thresholds suggests the functional reinnervation of mechanoreceptors. Based on the current understanding of which mechanoreceptors are responsible for threshold-level vibration detection at the tested frequencies, this suggests the successful reinnervation of hair follicle receptors and Pacinian corpuscles (Bolanowski et al., 1994). It is unclear how likely Pacinian corpuscles are to reinnervate, as previous reports state both the presence (Jirmanova et al., 1994; Koshima et al., 1993; Terzis and Dykes, 1980) and absence (Mackel, 1985) of Pacinian reinnervation following nerve transection, transplantation, and grafting. Because Pacinian corpuscles have high sensitivities and large receptive fields, it is possible that those in neighboring skin could be responsible for detecting vibrations applied to reinnervated skin. However, because high-frequency vibrations were perceived on the missing limb, it is likely that they were detected by reinnervating afferents. It is also possible that Merkel Cells are responsible for vibration detection on reinnervated skin. These receptors have been shown to have a high capacity for reinnervation (Dubovy and Aldskogius, 1996), and are capable of detecting high-frequency vibrations at thresholds comparable to the high-amplitude thresholds measured on reinnervated chest skin (Bolanowski et al., 1988).

Demonstration of intact vibration sense in reinnervated skin following the TR procedure is promising for the application of sensory feedback from prostheses. Currently, amputees must visually monitor object manipulation and grasping with their prostheses. However, if touch information was fed back to the amputee by a device placed on reinnervated skin, sensory information would be returned to the original modality, and this information would likely be relayed to regions of the brain that normally process the tactile information for the missing limb. One impediment to this technique is that the receptors likely lose their original topography due to the random regeneration of the redirected afferents. This may disrupt the ability of TR amputees to integrate and process complicated tactile input delivered to reinnervated skin. However, since the redirected afferents are likely connected to regions of the brain specialized to process touch information from the arm and hand, a greater recovery of the tactile sense may be possible. While a sense of touch may be returned to the amputee through this procedure, the level at which tactile function could be restored is still unknown. Also undetermined is how such function could be improved by long-term practice with a prosthetic device incorporating sensory feedback.

Future work will focus on finding the best methods for returning touch sensation to the prosthesis user. In particular, the utility of different modes of sensory feedback with varying complexities will need to be examined. For example, will simpler modes of sensory feedback (such as goal confirmation and grip force) or more complex abstractions (such as texture) be advantageous to the user? In exploring the capabilities of TR amputees to sense, process and utilize this information, we hope to gain additional insights into the processes and mechanisms of sensory reinnervation following TR surgery and apply this knowledge to the continued improvement of prosthetic devices.

4. Experimental procedures

Vibratory stimuli were applied with a voice coil actuator (Equipment Solutions, VCS-10, Sunnyvale, CA). The voice coil consisted of a solenoid and sliding stage that was closed-loop position-controlled via an analog servo controller (Equipment Solutions, SCA-824, Sunnyvale, CA). Command voltages and displacement readings were communicated between the servo controller and the PC using Matlab XPC (MathWorks Inc., Natick, MA) and a data acquisition card (Measurement Computing, PCI-DAS1602/16, Norton, MA). Displacements were determined by an optical linear displacement sensor with a resolution of 150 nm which was built into the sliding stage. A probe with a cylindrical tip was mounted to the sliding stage, and the voice coil was attached to a micromanipulator (MM-3 Micromanipulator, Narishige, Tokyo, Japan) mounted on an adjustable swing-arm stand (Leica Microsystems Inc., Standard Horizontal Swingarm Stand, Bannockburn, IL). Chest thresholds were measured with a 9 mm-diameter acrylic probe tip. A 2 mm-diameter aluminum probe tip was used to apply stimuli to the hand, arm and chest. The 9 mm probe tip was needed to obtain clear results on the chest: preliminary trials with the 2 mm probe showed that the maximum achievable amplitude of the voice coil at 400 Hz was below the threshold of detection in several cases, and detection thresholds at high frequencies are lowered by the use of a larger contact area (Verrillo, 1963). The 2 mm probe tip was necessary for measuring thresholds on the hand, as vibrations at threshold generated by the 9 mm probe were too small to measure.

Vibration detection thresholds were measured at four stimulus frequencies: 5, 30, 250, and 400 Hz, which were chosen to specifically activate Merkel cells (5 Hz), Meissner's corpuscles (30 Hz) and Pacinian corpuscles (250, 400 Hz) at threshold level on glabrous skin (Bolanowski et al., 1988), and hair follicle receptors (5 and 30 Hz) and Pacinian corpuscles (250 and 400 Hz) at threshold level on hairy skin (Bolanowski et al., 1994). This study was performed on three amputee subjects who had undergone the TR procedure as well as 15 healthy control subjects. Testing locations included the chest, the ventral upper arm midway between the axilla and elbow, the ventral forearm midway between the elbow and wrist, the approximate center of the thenar eminance, and the distal volar pad of the index finger. Locations on the forearm and hand were chosen for comparison to previously published values (Bolanowski et al., 1988, 1994; Lofvenberg and Johansson, 1984; Merzenich and Harrington, 1969; Mountcastle et al., 1972; Talbot et al., 1968). All tests were performed with informed consent and IRB approval at the Rehabilitation Institute of Chicago.

Subject S1, a 60-year-old man with bilateral shoulder amputations, had undergone TR surgery in which the residual musculocutaneous, median, radial and ulnar nerves were transferred to the pectoralis major and minor muscles (Kuiken et al., 2004). Within five months, subject S1 developed an area of transfer sensation on the skin of the reinnervated chest (Kuiken et al., 2007a). He was tested on both the reinnervated and contralateral sides of the chest with the 9 mm probe.

Subject S2, a 26-year-old woman with a unilateral left-arm high-level transhumeral amputation, had undergone TR surgery in which the residual musculocutaneous, median, radial and ulnar nerves were transferred to separate regions of the pectoralis major and serratus anterior muscles (Kuiken et al., 2007b). In addition, the supraclavicular and intercostobrachial cutaneous nerves were cut and end-to-side anastomosed to the median and ulnar nerves, respectively, in order to encourage the development of sensory reinnervation. Sensory reinnervation was noticeable four months following surgery (Kuiken et al., 2007a). S2 was tested on both sides of the chest with the 9 mm probe. She was also tested on the reinnervated chest, contralateral chest, upper arm, forearm, thenar eminance, and fingertip with the 2 mm probe.

Subject S3, a 38-year-old man with a right-arm shoulder amputation, had undergone TR surgery in which the residual musculocutaneous, median, radial and ulnar nerves were transferred to separate sections of the pectoralis major, pectoralis minor, and latissimus muscles. Within five months, the subject exhibited transfer sensation on the skin overlying the reinnervated muscles. He was tested on both sides of the chest with the 9 mm probe. He was also tested on the reinnervated and contralateral chest sides as well as on the fingertip with the 2 mm probe.

The testing site on the reinnervated chest of each TR subject was located near the center of an area in which light pressure was perceived exclusively in the missing limb. Testing locations on the contralateral sides of the chest were chosen in regions free from scarring. In addition to testing for vibration detection thresholds, suprathreshold vibration stimuli were applied to the reinnervated chest to determine where the subjects perceived the stimuli. Following the application of the vibration, subjects outlined the location of the perceived stimulus on a drawing of their missing limb. Subject S1 reported the locations orally by referring to a drawing overlaid with a numbered grid, and then verified the areas outlined by the experimenter. Vibration testing on amputee subjects was carried out over a six-month period. No appreciable difference in thresholds was measured between testing sessions.

Vibration detection thresholds were also measured on the chest, arm and hand of non-amputee control subjects. Fourteen subjects (seven males, average age 37 SD 11, and seven females, average age 33 SD 13) were tested with the 9 mm probe on the right-hand chest, approximately 4 cm below the clavicle and midway between the acromium and sternoclavicular joint. Thresholds were also measured with the 2 mm probe on the right-hand chest, upper arm, forearm, thenar eminance and fingertip of six control subjects (three males ages 19, 27 and 39, and three females ages 28, 29 and 62), five of whom also participated in testing with the 9 mm probe. Testing on all locations was carried out in random order.

Subjects were seated with their arm placed on a layer of foam padding (Sunmate, Extra-Soft, Dynamic Systems Inc., Leicester, NC); this, along with a layer of sorbothane gel (1/8″ Shock Absorbing Sorbothane, Edmund Scientifics, Tonawanda, NY) clamped between the voice coil mount and the tabletop prevented additional vibrations from being transmitted to the subject. Subjects did not touch the table during chest trials. The testing site was shaved if necessary. Subjects wore earplugs and noise-canceling headphones (Bose QuietComfort® 2) to mask the sound of the voice coil. The headphones played gray noise over which masking tones were added for 250 and 400 Hz tests. Velcro straps affixed to the tabletop were used to secure the testing region on the upper arm. A wrist splint secured with Velcro straps was used to stabilize the forearm and thenar eminence. A finger splint glued to the back of the fingernail and embedded in a plasticine block was used to stabilize the fingertip. Due to breathing motion, it was not possible to completely stabilize the chest during testing; as such, subjects were instructed to remain still and breathe shallowly.

The probe was positioned normal to the skin at the testing site and lowered until it made light contact. For all trials on the arm, contact was indicated by a drop in the electrical impedance between the aluminum probe and the skin. Contact was determined visually for all trials on the chest. Contact was maintained throughout each trial either visually or by monitoring the impedance measure. A thermocouple (Omega Engineering, 5TC-TT-J-40-36, Stamford, CT) was used to monitor skin surface temperature during testing on the hand to ensure that it did not change dramatically over the test duration.

A baseline indentation of 1 mm was applied to the skin at the start of each stimulus presentation. The indentation followed a sigmoidal profile and reached the desired baseline value in 0.2 s. Vibration stimuli were superimposed on this baseline indentation in 50% of the trials following the first incorrect response (prior to the first incorrect response, vibrations were present in 75% of the trials). The duration of the stimulus (vibration or blank trial) was 1 s, therefore, the entire stimulus presentation period was 1.4 s. The vibration amplitude was initially set at a level that was assumed to be well above the threshold of perception based on pilot trials. Application of the stimulus was visually indicated to subjects on a command screen. After each presentation, subjects reported whether they had perceived a vibration using a two-button mouse. (Subject S1, the bilateral amputee, gave his responses orally and the experimenter operated the mouse for him.) Following each stimulus presentation, the amplitude of the applied vibration was adjusted based on whether subjects responded correctly or incorrectly and whether a vibration had been present. These adjustments are shown as a number of positive (increasing amplitude) or negative (decreasing amplitude) steps in Table 1, in accordance with the Single Interval Adjustment Matrix

Table 1 – Adjustment matrix for SIAM procedure

	75% stimulus presentation		50% stimulus presentation	
	Signal (0.75)	Noise (0.25)	Signal (0.5)	Noise (0.5)
Yes	−1	+6	−1	+2
No	+1	0	+1	0

Adjustment matrix used in staircase routine from Kaernbach (1990) for a target correct detection rate of 75% for two stimulus presentation probabilities, one in which the stimulus is presented in 75% of trials and one in which it is presented in 50% of trials. Integer values represent the direction (– decrease, + increase) and number of steps (in dB) of stimulus amplitude adjustment following each stimulus and response condition. Stimulus conditions include the presence (Signal) or absence (Noise) of a vibratory stimulus with the indicated probability of presentation. Responses are either "yes" (Y), indicating the detection of the vibratory stimulus, or "no" (N).

(SIAM) staircase procedure for a target correct-detection rate of 75% (Kaernbach, 1990). The 75% correct-detection rate was defined as threshold level in this study. In this manner, the testing procedure iteratively adjusted the stimulus amplitude to approach the threshold level. An initial step size of 2 dB (1 dB$=20\times\log_{10}$ (amplitude in μm)) was used until the first incorrect response, after which it was decreased to 1 dB. After each stimulus presentation, subjects were given feedback on their response as well as a score which was adjusted inversely to the stimulus amplitude, e.g. each time the stimulus level was decreased by one step, the subjects received one point (Kaernbach, 1990). This was intended to motivate subjects to achieve the lowest possible threshold while remaining unbiased in their responses (Kaernbach, 1990). Three trials were run at each location; each trial consisted of one staircase for each frequency (5, 30, 250 and 400 Hz), presented in random order. Two additional trials were performed on the chest regions of reinnervated amputee subjects. A typical staircase track included approximately forty to sixty stimulus presentations and lasted from 3 to 5 min. The staircase tracks terminated after twelve reversals. The final eight reversals were averaged to estimate the threshold command. The threshold command was then applied to the voice coil and the amplitude of the voice coil was recorded in order to directly measure the threshold-level stimulus.

The amplitude of the stimulus was determined by fitting a pure sign wave of the same frequency to the recorded signal. For 30, 250 and 400 Hz data, the sign wave was fit to the central 20 cycles. The sum of the squared error between the fit and the raw data was minimized using amplitude and baseline offset as the optimization parameters. Data from 250 and 400 Hz trials were band-pass filtered (sixth-order Butterworth filter with cutoff frequencies of 150 and 350 Hz and 300 and 500 Hz, respectively) if the initial correlation coefficient between the data and fit was less than 0.5; this was necessary for a number of trials on the fingertip and thenar eminance, as the amplitude of vibration at threshold level was small compared to the noise. Values were recorded in μm and converted to dB, where 1 dB$=20\times\log_{10}$(μm).

For TR subjects, the reported values are the medians of three trials for the arm and hand and five trials for the chest. For control subjects, representative thresholds for each subject, determined as the median value of three trials, were averaged across the subject pool ($n=6$ or $n=14$). All statistical analyses were performed using the software package SPSS (SPSS, Chicago, IL). Repeated measures 2-factor ANOVAs (frequency and location, and frequency and probe size) were used to analyze the results from control subjects. The 95% confidence intervals of the means of control data from hand ($n=6$), arm ($n=6$) and chest ($n=14$) regions were calculated using SPSS and used to compare to the median values of reinnervated subjects for the same regions. Comparisons of reinnervated to control skin were analyzed using two independent samples t-tests.

Acknowledgments

The authors thank Dr. Joseph Barton for his help with preliminary research and experimental design. We also thank Drs. J. Edward Colgate and Michael Peshkin for insightful feedback on the testing apparatus and experimental paradigm. This work was supported by the Defense Advanced Research Projects Agency (DARPA) and the generous support of the Rehabilitation Institute of Chicago (RIC) Women's Board.

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