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Experimental Forearm Immobilization in Humans Induces Cold and Mechanical Hyperalgesia

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Background: **Complex regional pain syndrome is a painful condition of unknown etiology. Clinical and experimental observations suggest that limb immobilization may induce symptoms and signs characteristic of complex regional pain syndrome. This study examined the effect of forearm immobilization on regional sensory and autonomic functions in healthy subjects.**

Methods: **Thermal and mechanical sensitivity, skin temperature, and vasoconstrictor responses were measured in 30 healthy subjects before and 0, 3, and 28 days after scaphoid cast immobilization. Fifteen subjects served as nonimmobilized controls.**

Results: **At cast removal, 27 subjects experienced pain at joint movement. Cast immobilization induced cold hyperalgesia in glabrous and hairy skin on the immobilized hand and induced significant skin temperature differences between the control and the immobilized hand at cast removal and after 3 days. Immobilization also reduced pain threshold at skin fold testing at all time points after cast removal. All measures except pain threshold at skin fold testing were normalized after 28 days. Immobilization did not affect thermal detection, heat pain, and pressure pain thresholds; resting skin perfusion; or vasoconstrictor responses induced by mental stress or deep inspirations.**

Conclusions: **Four weeks of forearm immobilization caused transient changes in skin temperature, mechanosensitivity, and thermosensitivity, without alteration in the sympathetically mediated vascular tone.**

COMPLEX regional pain syndrome (CRPS), formerly known as *reflex sympathetic dystrophy*, is a condition of unknown etiology characterized by spontaneous pain, allodynia, hyperalgesia, skin blood flow changes, edema, abnormal sudomotor activity, motor, and trophic changes in the painful extremity.[1,2] Clinical and experimental findings have shown that sympathetically mediated skin responses may be affected in CRPS, in which the affected extremity is either cold and vasoconstricted or warm and vasodilated.[3,4] In the acute phase of CRPS, vasoconstrictor responses induced by mental stress and deep inspirations may be reduced.[5,6]

The mechanisms responsible for inducing and maintaining CRPS are still unclear, but several observations suggest that limb immobilization could be a contributing factor.[7,8] First, complications to casting of extremities include joint contractures, compression neuropathy, dystonia, regional osteoporosis, movement-induced pain, and swelling, findings commonly seen in CRPS patients.[9–15] Second, CRPS patients have often been immobilized before the development of CRPS.[7,16,17] Third, CRPS patients often keep the affected extremity immobile and maintain it in a protective posture to avoid evoked pains.[7,18,19] Finally, mobilizing physiotherapy is reported to relieve signs and symptoms of CRPS.[19,20] Experimental observations in animals also suggest that immobilization may play a role in CRPS. Therefore, immobilization of rat limbs induces mechanical allodynia, increases skin temperature, and induces plastic changes in dorsal horn neurons with an increased number of wide-dynamic-range neurons and an increased amount of neurons responding to movement.[21–23] Preliminary findings in healthy subjects have suggested that limb immobilization may give rise to pain, neglect, and changes in skin temperature and sensitivity.[13,14]

Taken together, both experimental and some clinical observations suggest that immobilization may induce symptoms and signs characteristic of CRPS. Despite the potential role of immobilization in CRPS, no studies have systematically assessed the effect of immobilization on the autonomic and sensory nerve function in humans.

The aim of this study was to examine the effect of cast immobilization in healthy subjects on (1) thermosensation and mechanosensation and (2) efferent sympathetic activity as evaluated by skin temperature, skin perfusion, and vasoconstrictor activity.

Materials and Methods

Participants

The study was approved by the local ethics committee (No. 20020219). Right-handed, white volunteers were recruited from the University of Aarhus, Aarhus, Denmark, and the subjects' consent was obtained according to the Declaration of Helsinki. Thirty-one subjects were immobilized. Fifteen subjects (8 men, 7 women; mean age, 23 yr [range, 21–26 yr]; mean body mass index, 22.9 kg/m^2 [range, 19.5–27.1 kg/m^2]) served as nonimmobilized controls to examine for a possible time effect in

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thermal and mechanical sensory measures at repetitive testing. One subject was excluded 14 days after cast application because of repetitive damage to the cast. The remaining 30 volunteers (15 men, 15 women; mean age, 23 yr [range, 18–27 yr]; mean body mass index, 22.6 kg/m^2 [range, 19.4–29.2 kg/m^2]) completed the immobilization. Volunteers were considered healthy based on medical history, physical examination, arterial blood pressure less than 140/90 mmHg, and a normal 12-lead electrocardiogram.

To minimize external autonomic influences, subjects consumed a light meal not later than 2 h before the experimental sessions, emptied their bladders before the test, and were supine and not allowed to talk during testing. They refrained from smoking, alcohol, and coffee for the last 12 h and from excessive physical activity and medicine for the last 24 h before the experimental sessions. Examinations were performed by the same technician in a quiet room with a mean temperature of 23.1°C (range, 22.7–24.2°C).

Forearm Immobilization

The left forearm was immobilized in a circular scaphoid cast (Cellona Plaster of Paris Bandages; Lohmann, Neuwied, Germany) for 4 weeks, with the wrist kept in 30° extension. The cast extended from the interphalangeal joint of the thumb and the metacarpophalangeal joints of digits 2–5 distally to 2.5 cm below the elbow proximally, thereby allowing full flexions of the metacarpophalangeal joints. The cast was checked at day 1 and then on a weekly basis and was changed in six subjects because of looseness and in one subject because of pressure pain. During cast replacement, the extremity was kept immobile in the same position.

Active Range of Movement and Movement-induced Pain

At baseline and 0, 3, and 28 days after cast removal, active range of movement of joints from the elbow and distally was measured with a plastic 180° pocket goniometer measuring joint angles in steps of 5°. Movement-induced pain (yes/no) was reported for each joint. Subjects also reported duration of movement-induced pain 3 and 28 days after cast removal.

Thermal and Mechanical Sensory Testing

Thermal quantitative sensory testing (QST) was performed using a computerized thermal tester (TSA-2001; Medoc, Ramat Yishai, Israel) with a Peltier device of 32 × 32 mm. Baseline temperature was set at 30°C. The stimulus intensity gradually increased until the subjects pressed a response button at a specific thermal sensation. This returned the stimulus intensity to baseline and recorded the temperature at which the subject responded.[24] Warm and cold detection thresholds were defined as the smallest change from baseline temperature (warm or cold) that the participant could feel. Heat and cold pain thresholds were defined as the lowest and highest perceived painful temperature, respectively. For detection and pain thresholds, a stimulus change rate of 1°C/s and stimulus return rates of 3°C/s and 10°C/s were used, respectively. Thermal thresholds were calculated as an average of three stimuli. Thermal QST was performed on the dorsum of the hand between the first and second metacarpals (hairy skin) and on the radial palm in the area from the metacarpophalangeal joint of the index finger distal to the thenar eminence proximally (glabrous skin).

Mechanical QST was performed with a pressure algometer (Somedic AB, Hörby, Sweden) consisting of a pistol grip and a circular 1-cm^2 rod with a pressure-sensitive strain gauge at the tip. The rod tip is flat and covered with 2 mm of rubber to avoid painful skin stimuli due to sharp metal edges and securing a preferential activation of deep afferents.[25,26] A scale indicating the rate of pressure force increase enabled the examiner to keep a fairly constant pressure increase of 30 kPa/s. The subject indicated the pain threshold by pressing a button. During testing, the palm of the hand rested on a wooden tabletop. For joint pain threshold testing, the rod was pressed perpendicularly against the skin above the proximal interphalangeal joint of the middle finger. For skin fold testing, a skin fold area of 1 cm^2 between the thumb and the index finger was squeezed. Mechanical thresholds were calculated as an average of three stimuli.

Skin Temperature and Skin Perfusion

Skin temperature measurements were performed with a contact thermometer (accuracy, ±0.1°C; Omega HH42; Omega Engineering Inc., Stamford, CT) in the first 15 volunteers. Because of a long response time before reaching steady state skin temperature, an infrared thermometer (resolution, 0.1°C; Omega OS91) was used in the last 15 subjects. All measurements at all four data collection points were performed with the same thermometer for the 15 patients. Single measurements were performed on the hand at different sites (fig. 1).

Skin blood flow and temperature on the pulp (central volar part of phalanx distalis) of the thumb were continuously (1 Hz) measured with a laser Doppler perfusion monitor (DRT4; Moor Instruments Limited, Axminster, Devon, United Kingdom) with combined optic/temperature probes. Thermometer accuracy was 0.2°C. The skin was cleaned with alcohol, and the probes were placed in light contact with the skin. Fingers were kept immobile and unheated during measurement to assess skin perfusion during physiologic conditions.

To quantify sympathetically mediated vasoconstrictor responses, tonic (mental stress) and phasic (deep inspirations) vasoconstriction was induced while measuring cutaneous blood flow on the pulps of the thumbs. Mental arithmetic stress was induced by a paced auditory serial

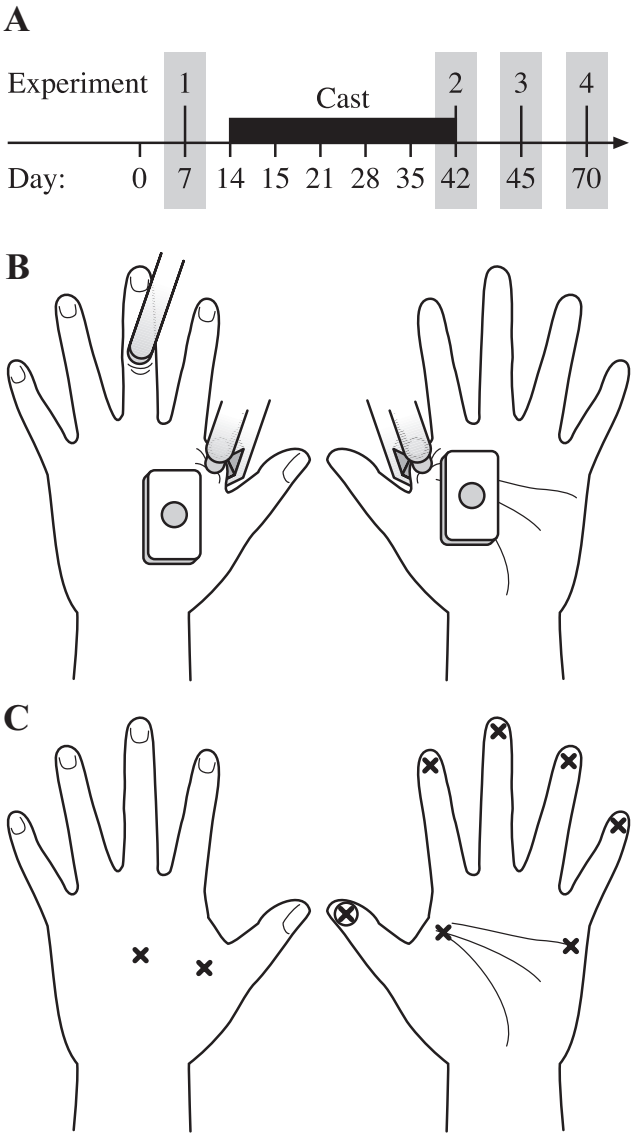

Fig. 1. Experimental setup. In the control subjects, thermal and mechanical testing was performed on both hands on 4 consecutive days with the same time intervals as the immobilized subjects (days 7, 42, 45, and 70). Immobilized subjects were included for periods of 70 days and participated in 10 sessions as illustrated in *A*: day 0 (inclusion); day 7 (experiment 1, baseline); day 14 (cast application); days 15, 21, 28, and 35 (control of cast); day 42 (experiment 2, cast removal); day 45 (experiment 3, 3 days after cast removal); and day 70 (experiment 4, 28 days after cast removal). On experimental days 1–4, sensory (*B*) and autonomic (*C*) tests were performed at different sites on the hands: Thermal testing was performed in hairy and glabrous skin. Skin fold testing was performed on the skin fold between the thumb and the index finger, and joint pressure was performed on the proximal interphalangeal joint of the middle finger (*B*). Single skin temperature measurements (×) were performed on the pulp of each finger (sympathetic tone), the radial part of the proximal palmar crease (median nerve innervation territory, glabrous skin), the hypothenar (ulnar nerve innervation territory, glabrous skin), the central part of dorsum of the hand, and the dorsum hand between the first and second metacarpals (radial nerve innervation territory, hairy skin). Skin perfusion and skin temperature (○) were measured on the pulp of the thumb during rest, mental stress, and deep inspirations (*C*).

addition task (PASAT) as described previously.[27,28] During deep inspirations, subjects were asked to breathe deeply (high tidal volume), at a low frequency (5 min^{-1}), and with an inspiration:expiration ratio of 1:3. Vasoconstrictor responses during PASAT and deep inspirations were calculated as mean perfusion during PASAT/5 min baseline perfusion and (mean of minimal perfusion during inspirations 2 to 4)/5 min baseline perfusion, respectively.

Heart Rate Variability

In a continuous 5-min electrocardiographic record sampled at 1,000 Hz (lead II), QRS complexes were inspected visually to exclude ectopic beats, artifacts, and missed beats.[29] Heart rate variability was estimated as previously described[28] and was expressed in the time domain as the mean time between consecutive normal R waves in the QRS complexes and the SD of all normal RR intervals (time duration between two consecutive R waves of the electrocardiogram).[29] To determine whether PASAT effectively stressed the subjects, power spectral analyses were performed.[29–32] High-frequency power (0.15–0.4 Hz) is considered an index of pure cardiac vagal activity.[31,33,34] Low-frequency power (0.04–0.15 Hz) is a baroreflex-mediated response affected by both sympathetic and parasympathetic activity.[32,33]

Experimental Setup

Immobilized volunteers were included for 70 days and participated in 10 sessions and 4 experiments (fig. 1A). On day 0 (inclusion), subjects were informed orally and in writing and went through all the procedures. On day 7 (experiment 1), baseline measures were obtained. The cast was applied on day 14 and was checked after 1 day and on a weekly basis (days 15, 21, 28, and 35). On day 42 (experiment 2), the cast was removed after 4 weeks of application, and postcondition measures were obtained. All tests were repeated 3 days (experiment 3, day 45) and 28 days (experiment 4, day 70) after cast removal.

In the 15 control subjects, thermal and mechanical testing was performed on both hands on 4 consecutive days with the same time intervals as the immobilized subjects (days 7, 42, 45, and 70).

All measurements were performed on the right and the left hands, selected randomly. Tests were performed in the same succession: joint pressure; skin fold testing; 30-min rest in supine position with forearms and hands uncovered; skin temperature measurements; skin temperature, perfusion, and heart rate measured during 5 min of rest, during mental stress, and during deep inspiration; and thermal QST (figs. 1B and C).

Statistical Analysis

For each group, measurements were summarized by computing arithmetic mean and SD for each hand on experimental days 1–4. To accommodate the assumptions of normal distributions, the vasoconstrictor responses were

Table 1. Cold and Warm Detection Thresholds Measured on Glabrous and Hairy Skin of the Hands

	Baseline	Cast Removal	3 Days After Cast	28 Days After Cast
Cold detection threshold, glabrous skin, $P = 0.49$				
n = 30				
L hand (cast)	28.6 (0.6)	28.7 (0.9)	28.8 (0.6)	28.8 (0.6)
R hand	28.6 (0.6)	28.3 (1.0)	28.6 (0.6)	28.5 (0.6)
n = 15				
L hand (control)	28.7 (0.5)	28.7 (0.6)	28.8 (0.5)	28.5 (0.7)
R hand (control)	28.6 (0.7)	28.7 (0.6)	28.6 (0.5)	28.3 (0.7)
Cast effect		$P = 0.27$	$P = 0.50$	$P = 0.43$
		0.4 [−0.4 to 1.2]	0.2 [−0.3 to 0.6]	0.2 [−0.3 to 0.7]
Cold detection threshold, hairy skin, $P = 0.57$				
n = 30				
L hand (cast)	28.9 (0.5)	28.8 (1.0)	28.9 (0.8)	29.0 (0.5)
R hand	28.9 (0.7)	28.8 (0.8)	28.9 (0.6)	29.0 (0.5)
n = 15				
L hand (control)	29.0 (0.5)	28.9 (0.9)	28.9 (0.8)	28.6 (0.8)
R hand (control)	28.8 (0.6)	28.9 (0.7)	28.9 (0.6)	28.7 (0.7)
Cast effect		$P = 0.27$	$P = 0.33$	$P = 0.13$
		0.2 [−0.2 to 0.7]	0.2 [−0.3 to 0.7]	0.3 [−0.1 to 0.7]
Warm detection threshold, glabrous skin, $P = 0.59$				
n = 30				
L hand (cast)	31.7 (1.1)	31.7 (0.8)	31.9 (1.0)	31.5 (0.7)
R hand	31.7 (0.7)	31.8 (0.7)	31.6 (0.7)	31.8 (0.8)
n = 15				
L hand (control)	32.0 (0.9)	32.0 (0.9)	32.3 (1.4)	32.4 (1.3)
R hand (control)	32.2 (1.0)	31.9 (1.4)	32.2 (1.6)	32.6 (1.5)
Cast effect		$P = 0.33$	$P = 0.95$	$P = 0.32$
		−0.4 [−1.1 to 0.4]	−0.02 [−0.8 to 0.8]	−0.4 [−1.0 to 0.4]
Warm detection threshold, hairy skin, $P = 0.97$				
n = 30				
L hand (cast)	31.9 (1.1)	31.9 (1.0)	31.8 (1.0)	31.8 (1.0)
R hand	31.9 (1.3)	31.8 (1.3)	31.8 (1.1)	31.8 (1.4)
n = 15				
L hand (control)	31.8 (1.1)	32.1 (1.3)	32.3 (1.2)	32.4 (1.2)
R hand (control)	31.7 (0.9)	31.8 (1.0)	32.2 (1.4)	32.2 (1.2)
Cast effect		$P = 0.85$	$P = 0.81$	$P = 0.78$
		−0.1 [−0.8 to 0.7]	0.1 [−0.6 to 0.8]	−0.1 [−0.9 to 0.7]

Values are presented as arithmetic mean (SD). For each group, the mean changes in the differences between left and right hand from baseline to cast removal and to 3 and 28 days after cast removal are estimated, and the cast effect is the difference between these means (cast group − control group). The cast effects are reported with two-sided 95% confidence intervals. All values are in °C.

log transformed before analysis and summarized by geometric means and coefficients of variation.

For thermal and mechanical measures, the change in the differences between left and right hand from baseline to the three time points after cast removal was estimated. For each of the 3 days, the cast effect was defined as the difference between the average of this change in the cast group and the control group. The cast effects were reported with two-sided 95% confidence intervals. An overall assessment of the cast effect, *i.e.*, a comparison of the two patient groups on all 3 days, was obtained from a mixed analysis of variance.[35] This analysis included day, hand, and group, and their interactions, as fixed effects. The random effects were interindividual variation and three types of intraindividual variation (between hands, between days, and between hands by days). The cast effects were assessed against the random variation within groups defined by experimental day, hand, and group. The overall assessment was supplemented by a separate *t* test on each day to assess the significance of the cast effect. Unequal variance *t* test was used in skin fold pain threshold.

To assess any time effect due to repetitive testing, right and left hand data in the control group and right hand data in the cast group and the control group were analyzed separately by a mixed analysis of variance similar to the one used for the main analyses, but omitting the factor group in the former situation and the factor hand in the latter situation. The same analysis was performed for the cast group alone to ensure that any significant effects were not due to changes in the control group.

For skin temperature and skin perfusion, the change in the differences between left and right hand from baseline to 0, 3, and 28 days after cast removal was estimated. A mixed analysis of variance with day, hand, and hand by day as fixed effects was used to simultaneously assess the average change on all three occasions against the random variation with groups defined by hand and day. On each experimental day, the average change in the left–right difference was assessed by a paired *t* test. Log-transformed

Table 2. Cold and Heat Pain Thresholds Measured on Glabrous and Hairy Skin of the Hands

	Baseline	Cast Removal	3 Days After Cast	28 Days After Cast
Cold pain threshold, glabrous skin, $P = 0.005$				
n = 30				
L hand (cast)	13.9 (7.6)	13.5 (8.2)	13.1 (6.9)	10.4 (6.7)
R hand	12.9 (7.5)	9.6 (8.0)	9.7 (7.4)	9.0 (7.6)
n = 15				
L hand (control)	16.9 (8.0)	14.1 (8.7)	13.8 (8.1)	14.3 (8.1)
R hand (control)	15.2 (8.6)	13.8 (7.3)	12.9 (8.8)	12.8 (8.5)
Cast effect		$P = 0.01$	$P = 0.049$	$P = 0.63$
		4.4 [1.1 to 7.8]	3.2 [0.01 to 6.3]	0.6 [−2.0 to 3.3]
Cold pain threshold, hairy skin, $P < 0.001$				
n = 30				
L hand (cast)	15.0 (8.8)	16.3 (9.4)	13.1 (7.4)	10.6 (7.3)
R hand	14.7 (8.5)	11.5 (8.1)	9.4 (7.4)	9.2 (7.3)
n = 15				
L hand (control)	17.8 (9.0)	14.8 (8.6)	14.3 (8.5)	15.5 (8.2)
R hand (control)	16.7 (9.2)	16.7 (8.9)	14.4 (8.6)	14.2 (9.0)
Cast effect		$P = 0.001$	$P = 0.02$	$P = 0.51$
		7.8 [3.1 to 12.1]	4.7 [0.9 to 8.4]	1.1 [−2.1 to 4.3]
Heat pain threshold, glabrous skin, $P = 0.28$				
n = 30				
L hand (cast)	43.6 (4.0)	44.2 (3.8)	44.8 (2.5)	44.8 (3.0)
R hand	44.4 (3.5)	45.0 (3.9)	45.8 (3.2)	45.8 (3.3)
n = 15				
L hand (control)	43.6 (4.9)	44.3 (4.1)	45.0 (4.0)	44.5 (4.1)
R hand (control)	43.5 (4.8)	43.0 (4.5)	44.7 (4.3)	44.8 (4.4)
Cast effect		$P = 0.15$	$P = 0.45$	$P = 0.92$
		−1.3 [−3.1 to 0.5]	−0.7 [−2.5 to 1.1]	0.1 [−1.7 to 1.9]
Heat pain threshold, hairy skin, $P = 0.16$				
n = 30				
L hand (cast)	41.8 (3.5)	42.2 (3.9)	42.9 (2.9)	44.2 (3.1)
R hand	42.1 (3.5)	44.0 (3.0)	44.7 (2.4)	45.1 (2.4)
n = 15				
L hand (control)	41.8 (4.2)	42.5 (4.0)	43.2 (4.0)	43.4 (4.5)
R hand (control)	42.0 (4.4)	42.6 (4.3)	44.2 (3.9)	44.1 (4.2)
Cast effect		$P = 0.11$	$P = 0.35$	$P = 0.93$
		−1.6 [−3.6 to 0.4]	−0.8 [−2.5 to 0.9]	−0.1 [−1.5 to 1.3]

Values are presented as arithmetic mean (SD). For each group, the mean changes in the differences between left and right hand from baseline to cast removal and to 3 and 28 days after cast removal are estimated, and the cast effect is the difference between these means (cast group − control group). The cast effects are reported with two-sided 95% confidence intervals. All values are in °C.

data were back transformed to the original scale and reported as a ratio (left hand relative to right hand).

All statistical tests were two-sided, and the level of significance was 5%. Stata 9 (StataCorp. 2005, Stata Statistical Software: Release 9; StataCorp LP, College Station, TX) was used for the basic statistical calculations. GENSTAT version 7 (VSNi, Hemel Hempstead, United Kingdom) was used for the mixed model analyses.

Results

Movement-induced Pain and Increased Hair Growth

No subjects had spontaneous pain on cast removal. However, 27 subjects experienced movement-induced pain on dorsal/palmar flexion of the hand (n = 24; mean duration, 6.4 days; range, 1–14 days), ulnar–radial deviation of the hand (n = 10; mean duration, 6.3 days; range, 1–11 days), movement of carpometacarpal and metacarpophalangeal joints of the thumb (n = 11; mean duration, 4.8 days; range, 1–9 days), or elbow flexion (n = 8; mean duration, 2.1 days; range, 1–9 days). In three subjects, increased hair growth started 2 weeks after cast removal. This was not a primary outcome measure but was reported by the subjects and documented by photos. In one subject, the thumb trembled at cast removal. Goniometry did not detect limited active range of movements in any of the volunteers.

Thermal QST

Immobilization did not affect thermal detection and heat pain thresholds in glabrous and hairy skin (tables 1 and 2).

Cold pain threshold was changed in glabrous and hairy skin on the immobilized side with cold hyperalgesia at cast removal and after 3 days followed by normalization after 4 weeks (table 2 and figs. 2A and B). Separate analysis for the cast group alone also showed cold hyperalgesia in hairy and glabrous skin at cast removal and after 3 days.

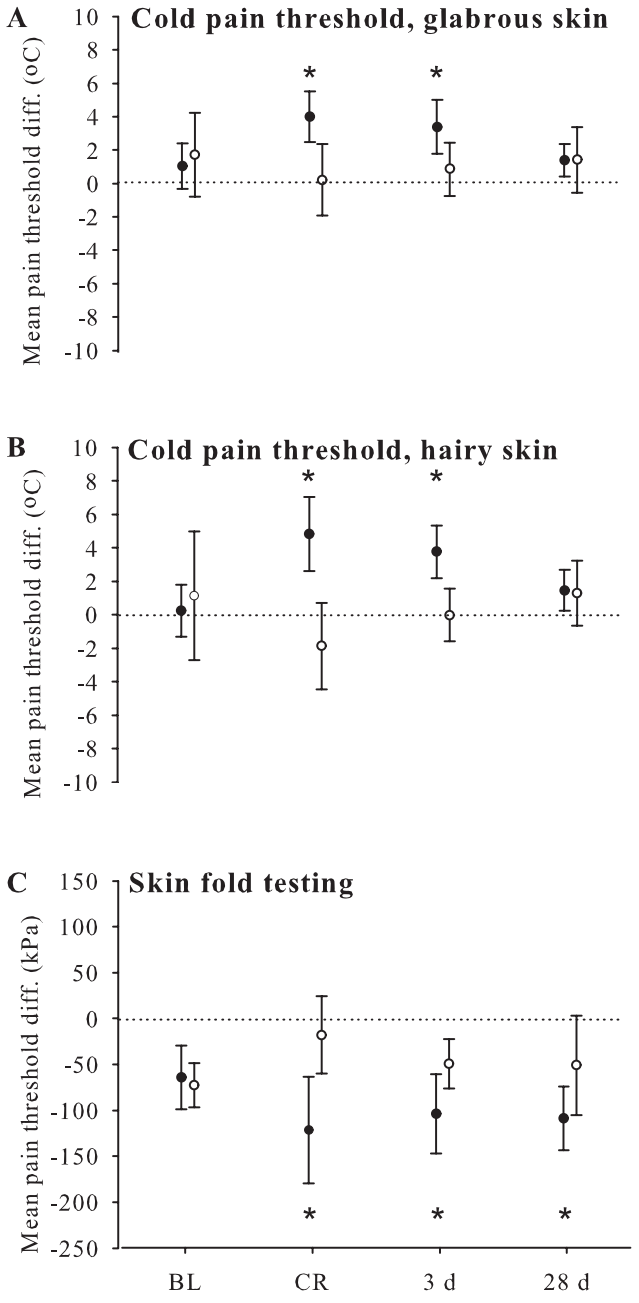

Fig. 2. Cold and mechanical hyperalgesia. In glabrous (*A*) and hairy (*B*) skin, cast immobilization induced cold pain hyperalgesia at cast removal (CR) and 3 days after cast removal (3 d). For all measures, no differences were seen 28 days after cast removal (28 d). Positive values indicate cold hyperalgesia. Pain thresholds at skin fold testing (*C*) of the immobilized hand were reduced at CR, 3 days, and 28 days after cast removal. The *circles* and *error bars* represent arithmetic means and 95% confidence intervals, respectively. The difference (diff.) is left − right hand. *Closed circles*: immobilized group (n = 30). *Open circles*: control group (n = 15). * Statistically significant inhibition. BL = baseline.

Mechanical QST

Joint pressure pain threshold was not changed by immobilization. Skin fold pain threshold was changed by cast immobilization with mechanical hyperalgesia at all time points after cast removal (table 3 and fig. 2C). Separate analysis for the cast group alone also showed reduced skin fold pain threshold. For the control group, there was a significantly higher skin fold pain threshold on the dominant hand ($P = 0.002$).

Analysis for Time Effects

For cold pain threshold in glabrous skin, a separate analysis of data from the control group showed a reduced pain sensitivity with time ($P = 0.02$) and no difference in time effect between the hands ($P = 0.54$). Right hand analysis in the control and cast group also detected this reduced pain sensitivity with time ($P < 0.001$) with no difference in time effect between the groups ($P = 0.60$). For cold pain threshold in hairy skin, data from the control group showed no time effect ($P = 0.11$) and no difference in time effect between the hands ($P = 0.14$). Analysis of right hand in the control and cast group detected a reduced pain sensitivity with time ($P < 0.001$) with no difference in time effect between the groups ($P = 0.16$). For pain at skin fold testing, data in the control group showed a time effect ($P = 0.03$) with no difference in time effect between the hands ($P = 0.10$). Analysis of right hand in the control and cast group also detected a time effect ($P = 0.04$) but no difference between the groups ($P = 0.28$).

Spatial Skin Temperature

Mean skin temperature based on nine single measurements of the hand (digits, palm, and dorsum) was changed on the left immobilized hand at cast removal, but not 3 and 28 days after cast removal (table 4 and fig. 3A).

The measurements with the contact as compared with the infrared thermometer showed a higher variability, but the thermometers showed the same pattern of the cast effect.

Skin Temperature on the Thumb

Mean baseline skin temperature on the pulp of the thumb, measured during 5 min of rest, was changed on the immobilized hand at cast removal and after 3 days but not 4 weeks after cast removal (table 4 and fig. 3B).

Skin Perfusion at Rest and during Efferent Sympathetic Activation

Mean baseline skin perfusion on the pulp of the thumb, measured during 5 min of rest, was not changed by cast immobilization (table 5).

Paced auditory serial addition task and deep inspirations induced similar vasoconstrictor responses on the hands (table 5).

Heart Rate Variability during Mental Stress

On all days, PASAT reduced mean and SD of all normal RR intervals, high-frequency power, low-frequency power, and total power in accordance with other studies using mental arithmetic to stress subjects[36–38] (table 6).

Table 3. Skin Fold and Joint Pressure Pain Thresholds

	Baseline	Cast Removal	3 Days After Cast	28 Days After Cast
Pain threshold at skin folding, $P = 0.03$				
n = 30				
L hand (cast)	449 (157)	396 (129)	431 (159)	444 (154)
R hand	513 (196)	518 (216)	535 (189)	553 (217)
n = 15				
L hand (control)	440 (103)	465 (143)	423 (120)	486 (140)
R hand (control)	513 (103)	483 (130)	472 (137)	536 (161)
Cast effect		$P < 0.001$	$P = 0.049$	$P = 0.02$
		−112 [−173 to −51]	−63 [−126 to −0.2]	−66 [−120 to −13]
Joint pain threshold, $P = 0.59$				
n = 30				
L hand (cast)	518 (177)	480 (177)	535 (189)	550 (193)
R hand	517 (191)	513 (178)	579 (206)	581 (210)
n = 15				
L hand (control)	529 (121)	528 (122)	525 (171)	551 (140)
R hand (control)	549 (144)	542 (152)	582 (149)	575 (159)
Cast effect		$P = 0.19$	$P = 0.79$	$P = 0.35$
		−40 [−102 to 21]	−9 [−77 to 59]	−28 [−87 to 31]

Values are presented as arithmetic mean (SD). For each group, the mean changes in the differences between left and right hand from baseline to cast removal and to 3 and 28 days after cast removal are estimated, and the cast effect is the difference between these means (cast group − control group). The cast effects are reported with two-sided 95% confidence intervals. All values are in kPa.

Discussion

This study demonstrates changes in skin sensitivity and temperature after immobilization in healthy subjects, changes that were not confined to the innervation territories of single peripheral nerves or roots. Such changes have been reported in a similar preliminary study in healthy subjects[13,14] and have also been reported after trauma, after surgical procedures,[39] and in CRPS.[40] Although the current experimental forearm immobilization does not mimic all features of CRPS, the findings indicate that this model could be used to investigate symptoms seen in CRPS as discussed in the following sections.

Movement-induced Pain and Reduced Pain Threshold at Skin Fold Testing

While none of the subjects reported spontaneous pain, cast immobilization caused movement-induced pain in 27 of the subjects lasting up to approximately 2 weeks and increased pain at skin fold testing. For the control group, skin fold pain thresholds were higher on the dominant hand as reported by other groups for blunt pressure.[41]

The mechanisms underlying pain at movement of the joints and increased pain at blunt skin pressure are not known. One possibility is that immobilization sensitizes mechanosensitive nerve fibers. Previous studies have shown that immobilization and experimentally induced joint arthritis increase ongoing and evoked afferent discharges from nociceptors and recruit silent nociceptors.[42–44] Other pain mechanisms such as connective tissue and joint capsule changes not requiring neuropathic sensitization could also be involved.[9] We did not find joint pain hyperalgesia despite movement-induced pain. One reason may be that pressure algometry was performed on a nonimmobilized joint.

Table 4. Skin Temperature Measured during 5 min of Rest with a Laser Doppler Perfusion Monitor Placed on the Thumb and Mean Skin Temperature Measured with a Contact Thermometer (Subjects 1–15) and an Infrared Thermometer (Subjects 16–30) on Pulps, Palms, and Dorsum of the Hands

	Baseline	Cast Removal	3 Days After Cast	28 Days After Cast
Mean skin temperature on pulps, palm, and dorsum hand, $P = 0.006$				
L hand (cast)	30.0 (3.3)	30.4 (3.0)	29.8 (3.5)	30.0 (3.5)
R hand	30.1 (3.3)	29.8 (3.2)	29.4 (3.8)	30.0 (3.4)
Cast effect		$P = 0.006$	$P = 0.07$	$P = 0.76$
		0.7 [0.2 to 1.1]	0.5 [−0.0 to 1.0]	0.1 [−0.3 to 0.5]
Skin temperature on pulp of the thumb during 5 min of rest, $P < 0.001$				
L hand (cast)	29.3 (3.3)	29.2 (3.0)	29.2 (3.5)	28.9 (3.5)
R hand	29.1 (3.3)	28.2 (3.2)	28.2 (3.8)	28.5 (3.4)
Cast effect		$P < 0.001$	$P < 0.001$	$P = 0.60$
		0.8 [0.5 to 1.2]	0.8 [0.4 to 1.1]	0.1 [−0.2 to 0.4]

Values are presented as arithmetic mean (SD). The cast effect is the change in the differences between left and right hand from baseline to cast removal and to 3 and 28 days after cast removal. The cast effects are reported with two-sided 95% confidence intervals. All values are in °C.

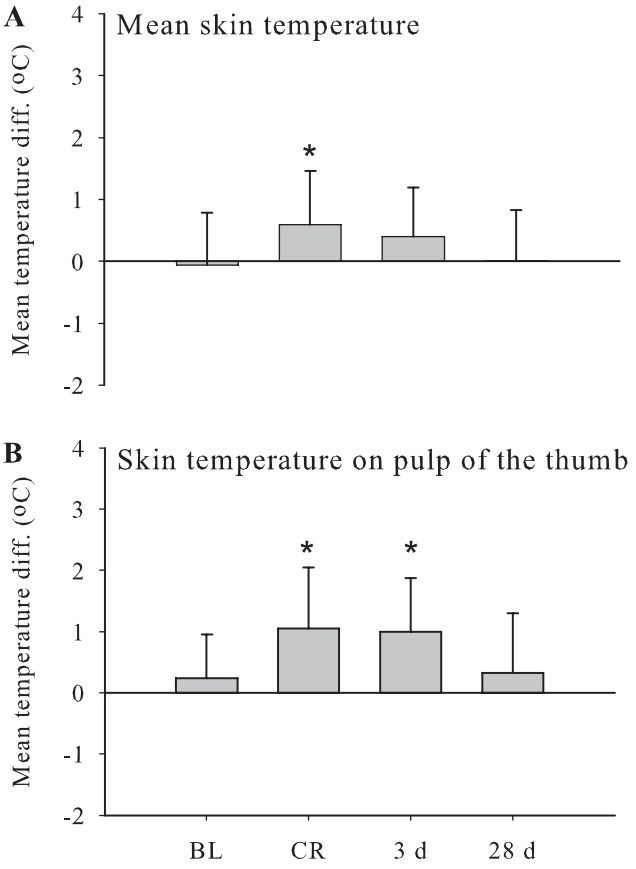

Fig. 3. Cutaneous temperature. Mean skin temperature of nine single measurements (*A*) differed significantly between hands at cast removal (CR). For mean skin temperature measured at digit 1 during 5 min of rest (*B*), there was a significant skin temperature difference between the hands at cast removal (CR) and 3 days after cast removal (3 d). For all measures, no differences were seen 4 weeks after cast removal (28 d). *Boxes* and *error bars* represent arithmetic means and SDs, respectively. The difference (diff.) is left − right hand. * Statistically significant inhibition of mean difference as compared with baseline (BL).

Immobilization-induced Cold Hyperalgesia and Increased Hair Growth

Guo *et al.*[23] postulated that tibial immobilization in a rat model causes substance P release. The current cold hyperalgesia may be related to such a mechanism.[45] Moreover, substance P is known to increase hair growth,[46,47] a phenomenon also seen after bone fractures.[13,48] We did not find heat hyperalgesia, which is consistent with the animal immobilization model[23] and also consistent with clinical reports that heat hyperalgesia is rarely seen in CRPS type I. [49,50]

In the current study, hyperalgesia was defined according to Meyer *et al.*[51] as a reduced pain threshold on the immobilized left hand compared with the right hand assuming that measurements on the nonimmobilized hand represent normal values. This notion has been questioned by Wahren *et al.*[52] in neuropathic pain patients. They suggested that the subjective criterion for pain may differ between patients with chronic pain and pain-free subjects, but none of the immobilized subjects in the current study had ongoing pain. The fact that detection and heat pain thresholds were unaffected indicates that immobilization did not give rise to a generalized hypersensitivity.

In hairy skin, cold pain threshold was reduced when pain thresholds at cast removal were compared with baseline, suggesting absolute hyperalgesia. In glabrous skin, pain sensitivity was reduced with time on both the immobilized and nonimmobilized hands. Therefore, it may be argued that the current cold hyperalgesia in glabrous skin does not reflect a real increase in sensitivity on the immobilized left hand, but rather a more pronounced decrease in pain sensitivity on the control side. It is unlikely that this explains the current hyperalgesia because separate analyses of left and right hand data in the control group and right hand data in the control group and cast group showed a time effect with no difference in time effect between the hands and the groups. Such a time effect was not seen on the immobilized hand. The change in the cast group was also seen when pain thresholds for cold and skin fold testing were analyzed separately for the cast group. Therefore, the tendency of the control data to change in opposite direction of the cast data (fig. 2) does not explain the

Table 5. Cutaneous Blood Flow on the Thumb during Rest, Paced Auditory Serial Addition Task, and Deep Inspirations

	Baseline	Cast Removal	3 Days After Cast	28 Days After Cast
Baseline skin perfusion, arbitrary units, $P = 0.09$				
L hand (cast)	118 (73%)	97 (75%)	110 (74%)	97 (82%)
R hand	119 (67%)	93 (70%)	92 (77%)	100 (76%)
Ratio	1.0 (32%)	1.0 (32%)	1.2 (41%)	1.0 (35%)
PASAT-induced vasoconstrictor response, $P = 0.26$				
L hand (cast)	51% (92%)	61% (98%)	63% (84%)	64% (105%)
R hand	57% (92%)	62% (73%)	69% (68%)	64% (81%)
Ratio	0.9 (37%)	1.0 (34%)	0.9 (28%)	1.0 (26%)
Inspiration-induced vasoconstrictor response, $P = 0.34$				
L hand (cast)	24% (117%)	32% (96%)	31% (85%)	29% (70%)
R hand	25% (95%)	34% (66%)	38% (74%)	32% (65%)
Ratio	0.9 (43%)	0.9 (39%)	0.8 (25%)	0.9 (28%)

Values are presented as geometric mean (coefficient of variation). The ratio is left hand/right hand.

PASAT = paced auditory serial addition task.

Table 6. Heart Rate Variability Measured during Rest (Baseline) and Paced Auditory Serial Addition Task

	Mean RR, ms	SDNN, ms	LF Power, ms^2/Hz	HF Power, ms^2/Hz	Total Power, ms^2/Hz
Day 1					
Baseline	1,014 (147)	76 (25)	1,609 (1,208)	2,377 (1,979)	4,107 (2,935)
PASAT	691 (128)	63 (34)	705 (594)	539 (642)	1,386 (1,260)
Day 2					
Baseline	1,029 (138)	82 (30)	1,809 (1,541)	2,560 (2,349)	4,503 (3,638)
PASAT	707 (145)	70 (34)	749 (867)	531 (649)	1,408 (1,488)
Day 3					
Baseline	989 (147)	81 (32)	1,702 (1,277)	3,032 (4,388)	4,889 (5,346)
PASAT	706 (146)	62 (32)	718 (730)	602 (853)	1,456 (1,580)
Day 4					
Baseline	1,012 (161)	78 (32)	1,682 (1,431)	2,354 (2,240)	4,148 (3,394)
PASAT	743 (188)	62 (40)	752 (976)	863 (1,940)	1,752 (2,966)

Heart rate variability of 5-min segments was expressed as high-frequency power (HF power), low-frequency power (LF power), the mean time between consecutive normal R waves in the QRS complexes (mean RR) and SD of all normal RR intervals (SDNN), and total power. Values are presented as arithmetic mean (SD).

PASAT = paced auditory serial addition task.

significant effect but is a consequence of a time effect at repetitive testing that does not appear in the casted hand because of hyperalgesia.

Previous studies have shown differences in pain sensitivity between dominant and nondominant extremities.[41] For ethical reasons, the nondominant hand was casted in all cases to ensure reasonable functioning during a 4-week immobilization. Although we cannot exclude that dominance may play a role for the current hyperalgesia, we consider this unlikely because thermal detection and heat pain threshold were not changed between the hands.

Immobilization-induced Skin Temperature Differences but Normal Function of Efferent Sympathetic Vasoconstrictor Nerves

From the current study, it is not possible to conclude whether the skin temperature difference is due to a higher skin temperature on the immobilized hand, a lower skin temperature on the nonimmobilized side, or a combination of both.

By measuring skin temperature, skin perfusion, and cutaneous vascular responses to deep inspirations and PASAT-induced mental stress, we assessed the function of efferent sympathetic vasoconstrictor nerves to the skin vessels. PASAT inhibited heart rate variability in accordance with other studies using mental arithmetic to stress subjects.[36–38] Vasoconstriction did not differ between the hands during mental stress and deep inspirations, indicating that skin vessel reactivity and the function of efferent sympathetic vasoconstrictor nerves were preserved.

Glabrous skin is predominantly innervated by sympathetic vasoconstrictor nerves, which in thermoneutral environments are tonically active.[53] Perfusion did not differ between the hands, suggesting that the efferent sympathetic activity was identical on the immobilized and the control hand. So, the higher skin temperature on the immobilized hand is probably not due to a reduced vasoconstrictor tone, a mechanism that is suggested to be responsible for the warm and vasodilated skin in acute CRPS.[3–6,54] Whether altered sensitivity of peripheral adrenergic receptors may play a role for the higher skin temperature on the immobilized limb is not clear.[4,55]

Skin perfusion measured with a laser Doppler perfusion monitor reflects changes in the microcirculation of the outer layers of the skin, whereas skin temperature reflects changes in the blood vessels below this layer.[53] Therefore, immobilization-induced skin temperature differences could be due to changes in blood flow in deeper tissue structures. Recent studies suggest that in the acute stages of CRPS, the pain component that is influenced by the sympathetic innervation of deep somatic structures is more important than the cutaneous activation.[56]

Other mechanisms such as neurogenic inflammation with release of neuropeptides from C nociceptors[57] or sweat glands[58] may also play a role for the observed skin temperature changes.

Guo *et al.*[23] found in a rat model that tibial immobilization increases skin temperature and induces mechanical allodynia and spontaneous protein extravasation. Because of reversal of these changes by a substance P receptor (NK_1) antagonist, they postulated that these changes are caused by substance P release. This neuropeptide binds to the NK_1 receptor on the vascular endothelium, resulting in vasodilatation and increased vascular permeability.[59] Along the same lines, substance P release might be involved in the current skin temperature changes.

Forearm Immobilization: A Human Model of CRPS?

In CRPS, the pain is typically elicited by joint movement as in the current study. The current immobiliza-

Table 7. Symptoms and Signs in Complex Regional Pain Syndrome and in the Current Immobilization Study

	CRPS	Immobilization
1. Sensory	Yes/no*	Yes (reported + observed)
2. Vasomotor	Yes/no*	Yes (observed)
3. Sudomotor/edema	Yes/no*	No
4. Motor/trophic	Yes/no*	No (in four: reported + observed)

* To make the clinical complex regional pain syndrome (CRPS) diagnosis,[60] the following criteria must be met: (a) continuing pain, which is disproportionate to any inciting event; (b) must report at least one symptom in three of the four categories listed above (1–4); (c) the examiner must observe at least one sign at time of evaluation in two or more of the categories listed above (1–4); and (d) no other diagnosis explains the signs and symptoms.

tion-induced cold hyperalgesia, mechanical hyperalgesia, and skin temperature differences between the hands are also common findings in CRPS.[40] In contrast, CRPS patients often present with spontaneous pain, abnormal blood flow regulation, sweating, edema, and trophic changes.[40] Therefore, the current subjects do not fulfill the new diagnostic criteria for CRPS[60] as illustrated in table 7, and forearm immobilization does not represent a complete human model of acute CRPS. This is not surprising because CRPS cannot be explained by disturbance in one system or mechanism only.[40]

Nevertheless, the overlapping features between CRPS and immobilization-induced findings in normal volunteers clearly raise the possibility that immobilization may play a role in at least elements of CRPS.

Although fairly mild sensitization and temperature changes with rapid spontaneous resolution were found, none of the patients developed what would likely be diagnosed as CRPS. This is fortunate because there would be a substantial ethical issue associated with creating a condition as potentially devastating as CRPS in volunteers. If this study had produced something closer to CRPS, it would have been unethical, but because it did not, its relevance to CRPS can only be inferred. If this model is to be adopted and exposed to many subjects, it is highly important that the cast be carefully checked and removed immediately if subjects report pain because of the risk of developing full-blown CRPS.

Effects of immobilization on sensory and autonomic functions is a largely unexamined issue, with no possibility *a priori* to point out an obvious primary outcome parameter. The current results should be considered exploratory and used to guide future research. Therefore, confirmatory studies are recommended. Future similar studies should be strengthened by randomly assigning patients to the immobilization or control group to control for unknown confounders, although confounders probably do not have any effect on the difference score between the hands. Blinding of the investigator to the patient status (casted/control) would be advantageous, but we did not consider this possible because of skin color changes (dryness/sunburn/odor/increased hair growth) and because of finger trembling and movement-induced pain in the immobilized subjects. Bias of the investigator, however, is less likely because measures were results of patients response (pressing a button), and the investigator and control subject were not talking during measurements.

In the future, it will be of interest to study the effects of immobilization in patients, *e.g.*, after fractures. Of special importance would be the investigation of sudomotor function, changes in blood flow to deeper structures, substance P release, and more specific changes of peripheral nociceptors.

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