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# Electrically induced quantitative sudomotor axon reflex test in human volunteers

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ABSTRACT

Chemically-induced quantitative sudomotor axon reflex test (QSART) and quantitative sensory testing (QST) are established clinical tools to assess thin fiber function in humans. We investigated stimulus-response functions to transcutaneous electrical stimuli of different current intensity (3.75 to 10 mA) and pulse frequency (5 to 100 Hz) comparing sweat output (ml/h/m$^2$) and pain intensity (numeric rating scale [NRS], 0–10). Efferent sudomotor and afferent nociceptive responses were recorded after a 30 s electrical stimulation period of distal (hand and foot) and proximal (forearm and thorax) body sites with 3 repetitive measures per body site.
Sweat responses increased intensity dependently and peaked (~100 ml/h/m$^2$) at highest currents (10 mA) that had been administered. Similarly, pain ratings increased with an escalating current intensity. At a constant stimulus intensity of 7.5 mA, sudomotor activity was highest (~75 ml/h/m$^2$) at a stimulus frequency of 20 Hz without further increase at 50 or 100 Hz. In contrast, pain ratings increased frequency dependently and reached NRS 7 at 100 Hz. Sudomotor activity, but not pain ratings, was significantly different between the body sites ($p<0.05$, ANOVA) with maximum sweat responses obtained at the ventral forearm.
Varying response patterns for higher stimulation frequencies between sweating (peak maximum at 20 Hz) and pain (maximum at 100 Hz) might indicate differential axonal properties of sympathetic efferent and nociceptive afferent fibers. Electrically induced QSART could be a useful explorative and clinical method to indirectly study characteristics of frequency-dependent axonal excitability changes of sudomotor fibers.


## 1. Introduction

Functional investigation of thin fibers includes subjective tests, for instance quantitative sensory testing (QST), and objective methods such as measurement of sweat output. Baseline sweat production and responses to localized chemical or electrical stimuli represent functions of postganglionic sympathetic sudomotor neurons. The sweat output can be analyzed after iontophoresis of acetylcholine by imaging silicone sweat droplet impressions (Gibbons et al., 2008), recording the induced axon reflex area of sweat production (iodine-starch reaction (Schlereth et al., 2005; Namer et al., 2004; Riedl et al., 1998), or by measuring the total sweat output within this area (quantitative sudomotor axon reflex testing, QSART (Low et al., 1983)). The acetylcholine-induced QSART is widely used to assess impaired sudomotor function clinically in thin fiber neuropathy (Singer et al., 2004). Chemical stimulation with acetylcholine is robust and well-tolerated by the patients. For structural analysis, epidermal nerve fiber and sweat gland density quantified in skin biopsies is used as a diagnostic tool for e.g. diabetic neuropathy (Kennedy et al., 1996; Gibbons et al., 2009). Axonal excitability of sympathetic fibers would be another useful clinical parameter, especially for those diseases in which axonal sodium channels are involved, such as erythromelalgia (Waxman, 2007; Cummins et al., 2004) that is also linked to abnormal sympathetic skin responses (Kazemi et al., 2003).

In order to assess axonal excitability, transcutaneous electrical stimulation was used to induce a combined sudomotor and nociceptor activation. Thereby, both intensity of axon reflex sweating and area of axon reflex erythema can be quantified (Namer et al., 2004). The measurement of axon reflex erythema responses is mainly used under steady state conditions and therefore requires several minutes of electrical stimulation. Thus, systematic analyses of stimulus-response functions for current intensity or frequency would be problematic using this extended protocol. We therefore assessed transient sweat responses and pain to electrical stimulation of 30 s duration. Differential axonal excitability and activation profiles were explored and functionally described for sudomotor neurons and nociceptors by means of current intensity- and frequency-dependent response curves in different body sites.

## 2. Methods

The local Ethics Committee of the University Medicine Mannheim at the University of Heidelberg, Germany, approved the study according to

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the Declaration of Helsinki. Twenty volunteers (average 38 ± 12 years, 10 male and 10 female) were recruited, informed in detail about the purpose and time course of the study, requested to sign the informed consent form and to complete a medical history questionnaire.

### 2.1. Design of the sweat chamber

Quantitative measurement of sudomotor function was performed using a circular chamber made out of polycarbonate *makrolone®* (inner diameter 25.4 mm; height 8 mm). Each chamber had two lateral holes (3 mm Ø) serving for dry air supply (inlet) into the upper part of the chamber and humid air outlet from the lower part (Fig. 1A). The chambers were equipped with a polycarbonate separator disc that directed the flow of dry air from the upper into the lower part of the chamber, preventing the air from entering directly the outlet without passing the skin surface (Fig. 1B, C).

### 2.2. Humidity measurement

Filtered (FP10, Boge, Germany) and dried (MDK 6, KT 2016 M, Zander, Germany) air (1.5 bar, pressure gauge control unit, Heyer, Germany) was led into the upper part of the chamber (inlet) at a constant flow rate of 6 l/h adjusted by an air flow control unit (KDG 1113 V 000, Kobold Messring GmbH, Germany). After having entered the lower part, the air flow reached the skin surface absorbing the sweat. The sweat content was measured by a humidity sensor (HygroClip-SC04, Rotronic GmbH, Germany) placed into the outlet tube at a 5 cm distance to the lower chamber (Fig. 1A).

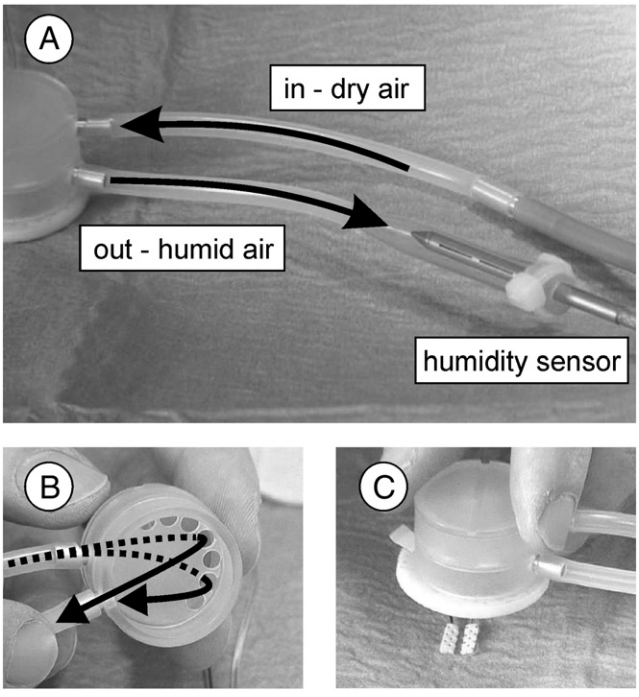


**Fig. 1.** Design of the sweat chamber and principle of measurement. Dry air is led into the upper part of the chamber towards the opposite wall. There it is directed to the lower part of the chamber via a separation disc containing seven openings. On the way back to the outlet the dry air passes the skin surface from which it takes up the evaporated sweat. The humid air leaves the lower part of the chamber and passes a humidity sensor for measurement (A). The direction of the air flow from the upper part (dotted line) to the lower part of the chamber (solid line) via the holes containing separation disc is shown in (B). The chamber is placed above a pair of adhesive contact electrodes used for electrical stimulation (C).

### 2.3. Humidity data recording and calculation

Values for absolute humidity (g/m$^3$), relative humidity (%), temperature (°C), and air pressure (hPa) were measured by the humidity sensor control unit (HygroLab 2, Rotronic GmbH, Germany) and stored at 0.5 Hz intervals on a computer by HW4 software (Rotronic GmbH, Germany). Sweat output was calculated offline in ml/h/m$^2$ according to the absolute humidity, flux and the surface area of the skin covered by the chamber.

### 2.4. Electrical stimulation

To induce axon reflex sweating, electrical stimuli were delivered transdermally by a pair of self-adhesive rectangular 3 × 10 mm surface electrodes (Pierenkemper, Germany) attached to the skin. Surface electrodes were connected to a constant current stimulator (DS7A, Digitimer, UK) delivering square wave pulses triggered by an external generator (Pulsgenerator PG1, Rimkus, Germany). Axon reflex sweating was assessed in response to 150 pulses delivered in 10 bursts with 15 stimuli each and 2 s intervals between the bursts. Responses to the *current intensity* and the *current frequency* were investigated. Constant current pulses were studied in randomized order at intensities of 3.75–5–6.25–7.5–8.25–10 mA delivered at 20 Hz frequency and 0.5 ms pulse duration. Frequency of current pulses was determined by the time interval between the 10 stimuli of delivered bursts. Frequencies of 5–10–20–50–100 Hz were investigated in randomized order at a current intensity of 7.5 mA and 0.5 ms pulse duration.

Maximum pain intensity perceived during the 30 s stimulation period was recorded by means of an 11-point numeric rating scale (NRS) with the endpoints “no pain” (0) and “maximum pain” (10).

### 2.5. Experimental protocol and set up

As outlined above, different current intensities were assessed at a frequency of 20 Hz and stimulation frequencies were tested at a stimulation intensity of 7.5 mA. In a second session we quantified the sweat response from different body sites, i.e. the volar forearm, the dorsum of the hand and the foot, the upper and the lower back, and in response to current frequencies of 5–20–100 Hz, respectively, administered at 7.5 mA and a pulse width of 0.5 ms.

Volunteers were laid comfortably on a bed in an air-conditioned (humidity 55%–65%, temperature 22–24 °C) room. The sweat chamber was attached air tight (Biatain® self-adhesive rings, Coloplast GmbH, Germany) to the skin, with its centre above the surface electrodes used for electrical stimulation (Fig. 1C), and held in position by flexible bandages (Urgoflex®, Urgo GmbH, Germany). As a control, a second sweat chamber was attached to the non-stimulated contra-lateral body site assessing the systemic sweat response.

Following to the fixation of the chambers on the skin, the entire system was flushed with dry air until a constant value of humidity was measured. Thereafter, a baseline period of 15 s was recorded, followed by the 30 s stimulation interval delivering 150 electrical pulses and a 105 s recording period. Sweat responses were assessed in triplicate for each current intensity and pulse frequency, and mean responses were used for statistical evaluation.

### 2.6. Statistics

Sweat volume per hour per square meter was calculated in Excel 2003 (Microsoft®, US) and analyzed by Statistica® 7.0 software package (Statsoft, US). Analysis of variance (ANOVA) of sweat output over time was performed between the factors “current intensity”–“pulse frequency”–“body site” and Fisher's Least Significance Test (LSD) as post-hoc tests to identify significant differences within factors ($p < 0.05$). Data are