Research Paper

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Sympathetic skin response following thermal, electrical, acoustic, and inspiratory gasp stimulation in familial dysautonomia patients and healthy persons

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To determine whether sympathetic skin response (SSR) testing evaluates afferent small or efferent sympathetic nerve fiber dysfunction, we studied SSR in patients with familial dysautonomia (FD) in whom both afferent small and efferent sympathetic fibers are largely reduced. We analyzed whether the response pattern to a combination of stimuli specific for large or small fiber activation allows differentiation between afferent and efferent small fiber dysfunction.

In 52 volunteers and 13 FD patients, SSR was studied at palms and soles after warm, cold and heat as well as electrical, acoustic, and inspiratory gasp stimulation. In addition, thermal thresholds were assessed at four body sites using a Thermotest device (Somedic; Stockholm, Sweden).

In volunteers, any stimulus induced reproducible SSRs. Only cold failed to evoke SSR in two volunteers. In all FD patients, electrical SSR was present, but amplitudes were reduced. Five patients had no acoustic SSR, four had no inspiratory SSR. Thermal SSR was absent in 10 patients with abnormal thermal perception and present in one patient with preserved thermal sensation. In two patients, thermal SSR was present only when skin areas with preserved temperature perception were stimulated.

In patients with FD, preserved electrical SSR demonstrated the overall integrity of the SSR reflex but amplitude reduction suggested impaired sudomotor activation. SSR responses were dependent on the perception of the stimulus. In the presence of preserved electrical SSR, absent thermal SSR reflects afferent small fiber dysfunction. A combination of SSR stimulus types allows differentiation between afferent small or efferent sympathetic nerve fiber dysfunction.

Keywords: familial dysautonomia, small nerve fiber testing, sympathetic skin response.

Familial dysautonomia (FD), or Riley-Day syndrome, is a rare, autosomal recessive disorder affecting the development and survival of sensory, sympathetic, and to a lesser extent parasympathetic neurons [1–4]. Among the cardinal signs are absent or diminished deep tendon reflexes, absence of fungiform papillae, lack of corneal sensitivity, lack of axon flare response to intradermal histamine, and increased sweating at the armpits, head, and occasionally the back [5]. During autonomic crises, patients show excessive sweating and skin blotching. In contrast, sweating is reduced at the palms of the hands and the soles of the feet [5]. Decreased function of small nerve fibers and sympathetic dysfunction are prominent and contribute to clinical manifestations such as decreased pain and temperature sensation, postural hypotension as well as hypertensive crises, gastroesophageal dysmotility, alacrimia, skin blotching with stress and acrocyanosis [1,2,4,5]. Biopsy specimens show significantly reduced numbers of peripheral small nerve fibers and sympathetic ganglia and neurons [6–10].

The reduction of peripheral small fibers accounts for the impaired temperature and pain perception, since sensory group III (Aδ) fibers subserve cutaneous cold receptors and nociceptors, and group IV (C) fibers mediate impulses from warmth and heat pain receptors [11–18]. Clinically, thermal sensation can be assessed by means of psychophysical quantitative sensory testing. However, this method requires patient cooperation[19,20] and is not applicable to many patients such as very young children.

The purpose of this study was to analyze whether testing of sympathetic skin response (SSR), a method that reflects peripheral small nerve fiber dysfunction independently from patient cooperation, might be suited to assess peripheral small fiber dysfunction in patients with FD.

The SSR results from transient and interacting electrical activity of sweat glands and adjacent epidermal tissue induced by any stimulus appropriate to elicit arousal [21,22]. The voltage change of the skin surface is attributed to an activation of eccrine sweat glands mediated by volley discharges of sympathetic preganglionic B-fibers and postganglionic unmyelinated C-fibers [23–27]. The central pathway of the reflex arc is thought to involve polysynaptic central circuitry influenced from the medullar reticular formation, midbrain, hypothalamic, limbic, and cortical structures but is still not fully understood [28–33]. The receptors and sensory fibers involved in the afferent pathway depend on the type of arousal stimulus.

Most commonly, electrical stimuli are used to elicit SSR as they are easily standardized and delivered [34–37]. However, electrical stimuli are primarily mediated via large myelinated group II sensory fibers [26,32,36,38]. As small group III

(Aδ) and IV (C) afferent fibers have no major bearing on the electrical SSR generation [38], it is unlikely that electrical stimuli are suited to evaluate small nerve fiber neuropathies.

So far, thermal stimuli have not been used to elicit SSR, although a specific stimulation of afferent group III and IV fibers seems more appropriate to diagnose sensory small fiber neuropathy. Moreover, temperature receptors and nociceptors are involved in the nervous control of sweat glands [38,39]. Therefore, this study tested whether warm, cold or heat pain stimuli are generally suited to elicit SSR in healthy persons as reliably as do electrical stimuli. The study further evaluated whether peripheral small fiber dysfunction in FD can be objectified by a combination of successive stimulus modes that induce SSR by selectively activating small or large nerve fiber afferences.

Material and methods

Subjects

Sympathetic skin response was studied in 52 healthy volunteers, 25 men and 27 women (age range, 4–71 y; mean, 31.0 ± 13 y), and in 13 patients with FD, seven men and six women (age range, 9–38 y; mean, 17.1 ± 9.4 y). Informed consent was obtained according to the declaration of Helsinki, with a parent signing for persons less than 21 years old. All patients were ambulatory; the initial diagnosis of FD had been established by Dr. F.B. Axelrod, Director of the Familial Dysautonomia Treatment and Evaluation Center, New York University, New York, New York, United States.

Experimental procedure

All FD patients underwent physical and neurologic examination. In control subjects and patients with FD warm (WT), cold (CT), and heat pain (HPT) perception thresholds were assessed, and the presence of SSR was evaluated after electrical, warm, cold, heat pain, and additional acoustic and inspiratory gasp stimulation according to the techniques described below. All tests were performed with the subject supine in a quiet room with an ambient temperature of 22 to 24°C. Participants were encouraged to relax but remain awake. Superficial skin temperature at the recording sites and the sites of thermal and electrical stimulation was measured with a skin surface thermometer (Physitemp TH8; Physitemp Instruments, Inc., Clifton, NJ). The test order was randomized for the various SSR stimuli. To allow for adjustment to the ambient temperature, thermal thresholds and SSRs were tested after history taking and physical and neurologic examination, a time period of at least 35 minutes. To avoid iatrogenic local changes of sweat gland activity, the skin was not warmed to a standard temperature by heat radiators [26].

Quantitative thermal threshold testing

In all control subjects and FD patients, quantitative WT, CT, and HPT perception thresholds were assessed psychophysiologically by means of a Thermotest (Somedic, Stockholm, Sweden), a modification of the "Marstock" device [41]. Thresholds were determined by the method of limits [41–43] using a baseline temperature of 32°C [42] and a temperature change rate of 1°C/sec for warming and cooling and of 3°C/sec for heating. The rectangular thermode operating on the Peltier principle [40] was attached to the tested skin area with constant pressure. A thermocouple affixed to the stimulating surface continuously registered instantaneous temperature changes within 0.1°C. Limits of stimulation were preset at 5°C and 50°C. Warm or cold stimuli were steadily increased until the subject indicated stimulus perception by pressing a button. The study participants were instructed to press the button as soon as they perceived a temperature change of the thermode surface. This ended stimulation and reversed the thermode temperature to the baseline. The difference between the signaled peak and the baseline temperature was registered. New stimuli were generated at 4 to 10 second intervals. Thresholds were automatically averaged from the peak-to-baseline differences of five warm and cold and three heat pain stimuli (Senselab, Somedic, Sweden).

Thresholds were determined at four body sites: at the distal volar forearm 3 centimeters proximal to the wrist, at the thenar eminence, at the distal medial calf 4 to 5 centimeters above the medial malleolus in the L4 dermatome, and at the lateral dorsum of the foot in the area innervated by the sural nerve. A 2.5 centimeter × 5.0 centimeter thermode was used for testing at the forearm, calf, and foot. A 1.5 centimeter × 2.5 centimeter thermode was used at the thenar eminence.

Sympathetic skin response

After skin preparation, SSR was simultaneously recorded from the palms and dorsum of the hands and from the soles and dorsum of the feet, using 10 millimeter diameter stainless steel electrodes (Nicolet) and commercial electrode paste.

The recording electromyograph (EMG) (Nicolet, Viking IV, Madison, WI, USA) displayed responses to electrical stimuli on a split screen in a triggered mode with a sweep duration of 5 seconds. The sweep was triggered via the electrical stimulator. With thermal stimulation, there was an individually variable interval between thermode activation and the moment when the temperature change was sufficient to elicit arousal. To assure that the sweep duration of 5 seconds was sufficient to record thermal SSR, the sweep was triggered after the start of thermal stimulation with a delay of 2 to 8 seconds depending on the temperature change rate. Simultaneously, changes of skin potentials were recorded on the split screen with a free running sweep and a sweep duration of 10 seconds. Filters were set at 0.2 Hz and 30 Hz [44].

Electrical SSR stimulation. For electrical stimulation, single square pulses of 0.1 to 0.5 millisecond duration were applied to the skin at the volar wrist. Four stimuli were delivered at randomized 50 to 90 second intervals and increasing voltage steps between 20 and 279 Volts [21,26,37].

Thermal SSR stimulation. For thermal SSR stimulation the Thermotest was used. The thermal stimulator operated independently from the recording EMG. Therefore, recording had to be started manually. To clearly differentiate SSR due to thermal stimuli from spontaneously fluctuating skin potentials, we monitored the spontaneous activity of skin potentials for 2 minutes before delivery of thermal stimuli. Four warm and cold stimuli and three heat pain stimuli were applied to the dorsal lateral foot in a randomized order and at 90 to 180 seconds interstimulus intervals. Starting from a baseline temperature of 32°C the thermode temperature changed continuously. As soon as SSR was registered, the examiner manually stopped the increasing temperature change, otherwise the thermode temperature advanced to the preset upper (50°C) and lower (5°C) limits before reversing to the 32°C baseline. Temperature change rates of 5°C/sec were applied for cold and 3°C/sec for warm and heat pain stimulation.

Whenever the first stimulus failed to elicit a SSR, temperature change rates were raised to 8°C/sec in volunteers as well as patients with FD. If the 8°C/sec stimulation at the foot still did not evoke SSR, 8°C/sec stimulation was repeated at the volar midforearm, then at the cheek, in one patient at the lateral neck and in another patient at the forehead.

Acoustic and inspiratory gasp stimulation

Responses to acoustic stimulation and to deep voluntary inspiration were also tested. Stimuli, however, were not standardized and required manual triggering of the recording EMG shortly after they were applied. Similar to thermal stimulation, we assessed a 2-minute baseline before acoustic or inspiratory stimulation to ascertain that any change of skin potential was due to stimulation and not spontaneous fluctuations. For inspiratory gasps the tested person was asked to take a deep breath, for acoustic stimulation the examiners used a 1-second burst of a 90 decibel horn held next to the person's head. Both stimuli were repeated four times at randomized intervals of 60 to 120 seconds.

Analysis of SSR parameters

Latency from stimulus onset to the first, usually negative SSR deflection was measured for electrical stimuli only. Onset latencies were not evaluated for SSR following thermal, acoustic, and inspiratory gasp stimulation, as these modalities required manual triggering of the recording EMG and thus implied a slight but undefined delay between the onset of stimulation and recording.

To compare waveforms of the various stimulation modalities, we measured peak-to-peak amplitudes between the maximal negativity and the maximal positivity. We determined the difference between the latencies of the first and the second SSR peak as an additional parameter allowing comparison of SSR wave forms despite the different stimulus modalities. This latency difference reflects the slope of skin potential changes. Differences between results of the various stimulation modalities were evaluated using a two-sided Friedman test.

Two-sided Wilcoxon signed ranks test analyzed differences between the upper and lower extremities and the left and right body side. Amplitudes and first-to-second-peak latency differences of thermal as well as electrical SSRs recorded in controls were compared to those of patients with FD using the two-sided Mann-Whitney U-test.

In control subjects, correlation of age, SSR onset latency, first-to-second-peak latency differences, and amplitude and stimulus intensity were calculated by the two-sided Spearman rank test. Since acoustic bursts and inspiratory gasps were not standardized, only the presence or absence of SSR was evaluated with these maneuvers. For all tests, significance was assumed at a level of $p < 0.01$. A commercially available statistics software (SYSTAT Inc., Chicago IL, USA) was used for computations.

Results

SSR in 52 controls with electrical, inspiratory gasp, acoustic, and thermal stimulation

The 52 control subjects had reproducible SSR at their hands and feet with electrical, acoustic, inspiratory, warmth, and heat pain stimulation (Figs. 1–4). Cold stimulation was effective in 50 controls only.

Four consecutive SSRs were obtained with 20 to 279 volts electrical stimulation (mean intensity, 66 V ± 39 V) and with 3°C/sec heat pain stimulation of the dorsal foot.

Warming of 3°C/sec was effective in 43 of 52 controls (83%). Cooling of the foot produced SSR in 26 of 52 controls (55%) with a 5°C/sec cooling rate. With an 8°C/sec change rate, all 52 controls had warm SSRs (100%) and 41 controls had cold SSRs (79%). In two control subjects cold stimulation failed to elicit SSR, in nine controls cold SSR was obtained only after 8°C/sec stimulation at the volar mid-forearm (7 of 9) or the cheek (2 of 9).

SSR wave forms with the different types of stimulation

SSR amplitudes and the differences between the latencies of the first and second SSR peak were similar with the different types of stimulation (Friedman test: $p > 0.1$; Table 1). Most of the responses showed an initially negative deflection. With thermal and inspiratory stimulation, SSR wave forms were more frequently tri- or polyphasic than with electrical stimulation.

With electrical stimulation, mean onset latencies at the feet (1958 ± 674 msec) were significantly longer than onset latencies at the hands (1411 ± 441 msec; Wilcoxon: $p = 0.000$). The first-to-second interpeak latencies also were longer at the feet (1736 ± 537 msec) than at the hands (1443 ± 428 msec; Wilcoxon: $p = 0.000$). Peak-to-peak amplitudes were higher at the hands than at the feet only with electrical or thermal stimulation (Wilcoxon: $p < 0.01$), but not with acoustic or inspiratory stimuli. Onset latencies, interpeak latencies, and amplitudes did not differ between both body sides, regardless of the stimulus modalities.

Associations with age

The age of the volunteers had little impact on SSR waveforms. With increasing age, amplitudes of hand responses

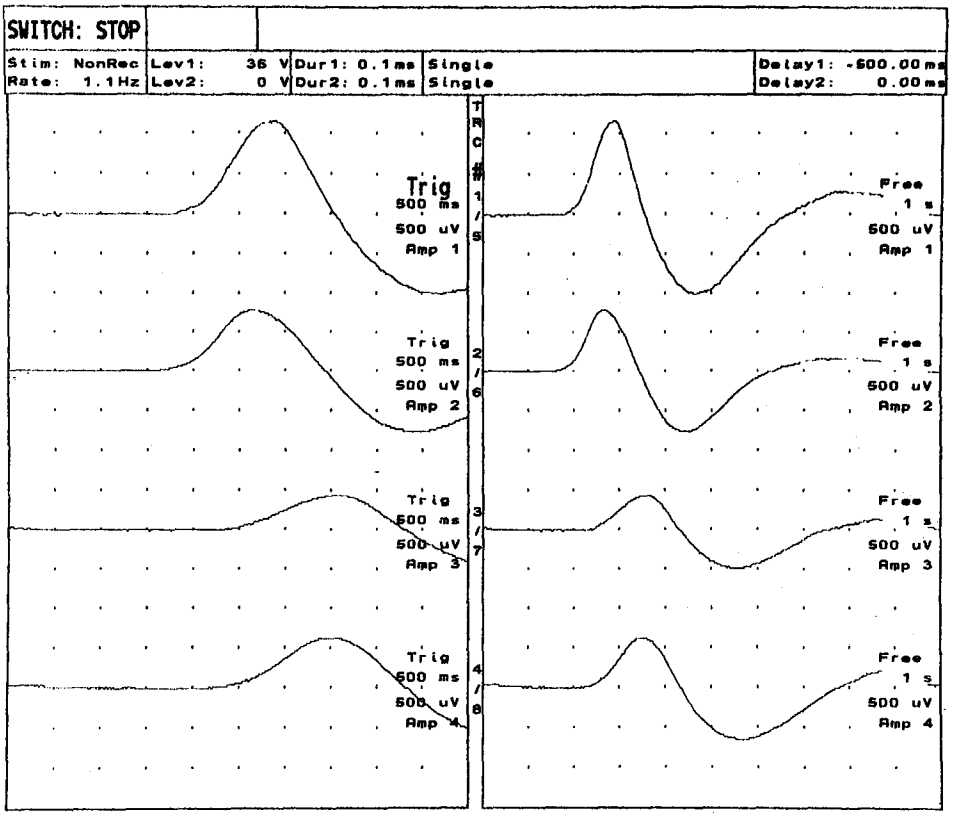

Figure 1. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 28-year-old female control person with electrical stimulation.

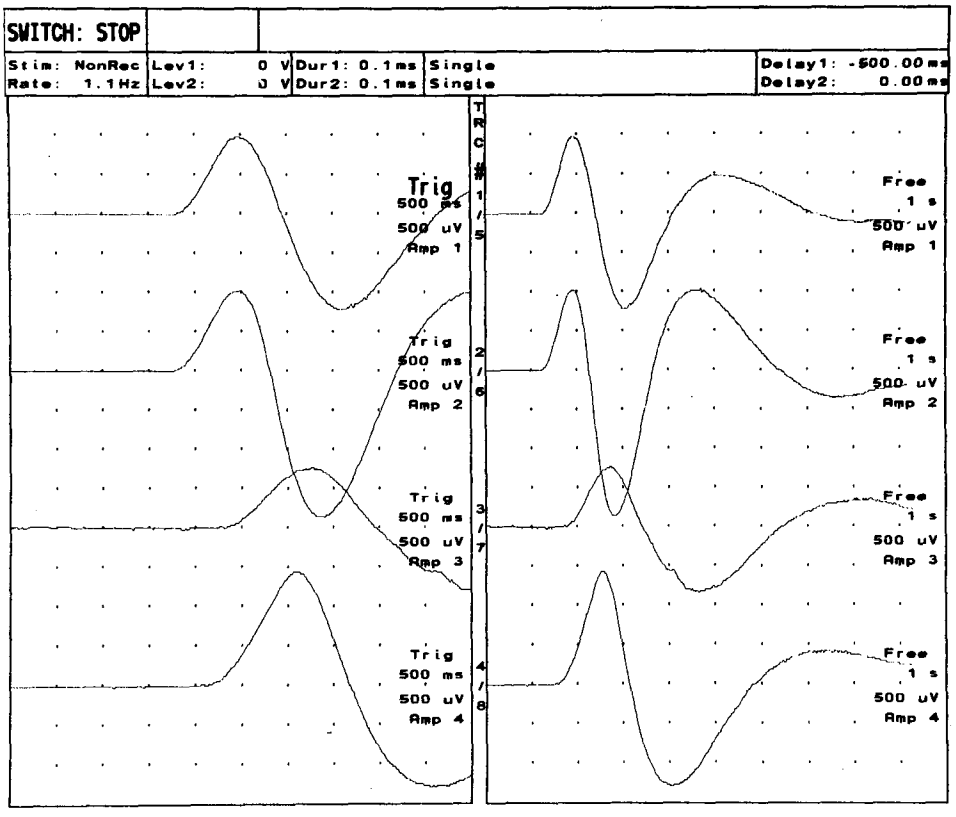

Figure 2. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 10-year-old boy with 5°C/sec cold stimulation at the left dorsal lateral foot.

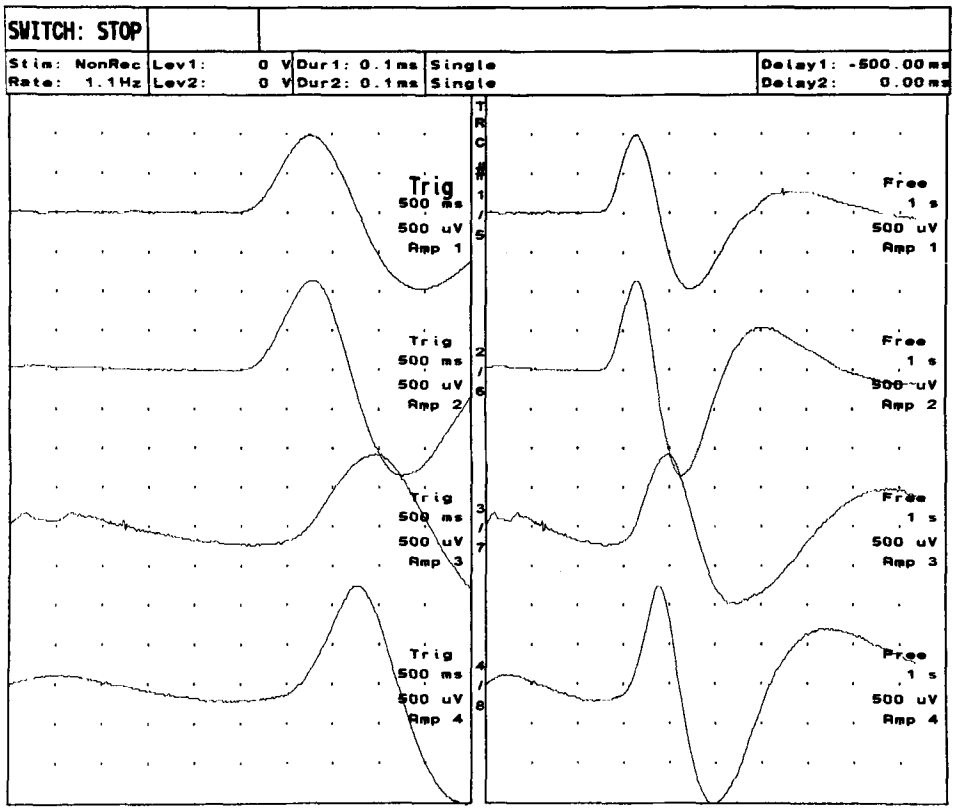

Figure 3. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 24-year-old male control person with acoustic stimulation.

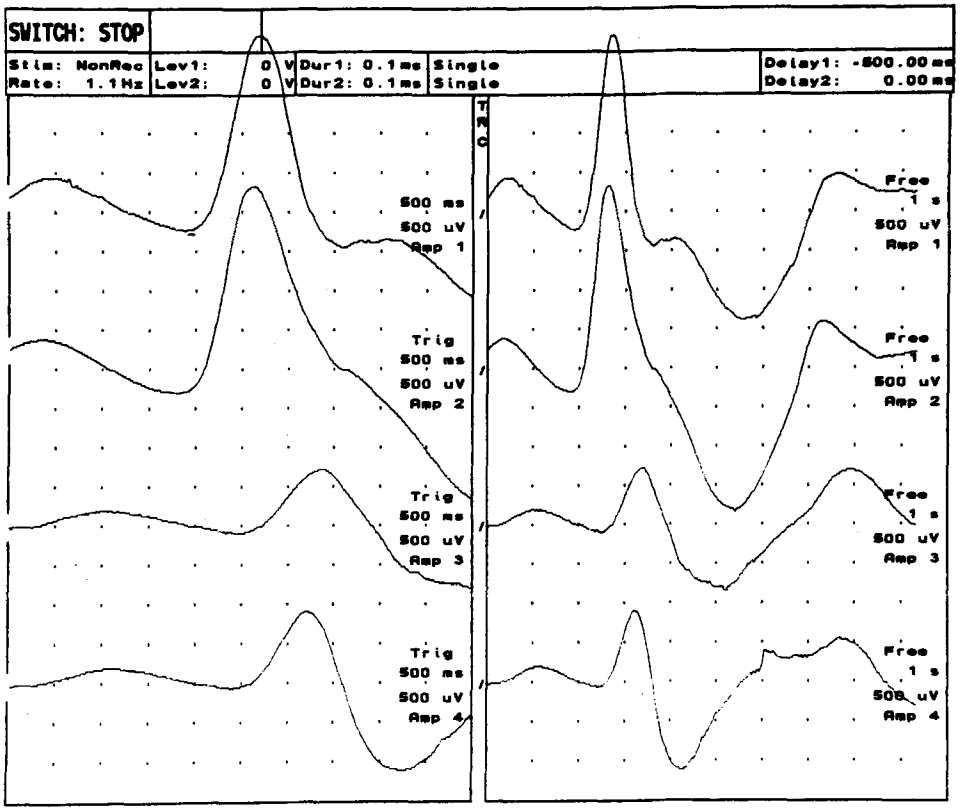

Figure 4. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 28-year-old male control person with inspiratory gasp stimulation.

Table 1. SSR evoked by different types of stimulation in controls*

Type of stimulation	Electrical	Acoustic	Inspiratory	Cold	Warmth	Heat pain
Amplitudes (μV)	(mean [SD])	(mean [SD])	(mean [SD])	(mean [SD])	(mean [SD])	(mean [SD])
At hands	1454 (804)	1261 (755)	1577 (831)	1131 (733)	1266 (663)	1938 (1020)
At feet	1027 (611)	1076 (1270)	1497 (580)	937 (568)	870 (539)	1715 (781)
First-to-second-peak latency						
At hands	1443 (428)	1592 (641)	1532 (749)	1875 (613)	1547 (401)	1590 (986)
At feet	1736 (537)	1795 (587)	1677 (890)	1582 (489)	1696 (467)	1447 (587)
Number of phases	%	%	%	%	%	%
Biphasic						
At hands	51	38	22	32	46	11
At feet	58	41	27	34	52	11
Triphasic						
At hands	30	22	13	15	8	23
At feet	25	24	10	34	7	19
Polyphasic						
At hands	19	40	65	53	46	66
At feet	17	35	63	32	41	70
Initial deflection						
Negative						
At hands	99	96	100	96	100	100
At feet	90	98	93	93	94	100
Positive						
At hands	1	4	0	4	0	0
At feet	10	2	7	7	6	0

*Shows the amplitudes and wave forms of SSR elicited with the different stimulus modalities. The data represent the responses generated in all 52 volunteers with electrical, acoustic, and inspiratory gasp stimulation. For thermal stimulation, the table shows the results obtained in 43 controls with 3°C/sec warm stimulation, in 26 controls with 3°C/sec heat stimulation, and in 26 controls with 5°C/sec cold stimulation at the foot.

decreased with inspiratory and cold stimulation only (Spearman: $p < 0.01$; Rs-values between -0.35 and -0.31). Amplitudes at the feet showed an age dependency with all types of stimulation, but coefficients of correlation were low (Spearman: $p < 0.01$; Rs-values between -0.48 and -0.28).

Temperature perception thresholds

Table 2 shows warm and cold perception thresholds of the 52 controls determined at the thenar eminence, the distal volar forearm, the distal medial calf, and the lateral dorsum of the foot. Skin temperature assessed prior to testing did not differ between patients and control subjects.

Familial dysautonomia patients

Clinical findings Common to all FD patients were histories of slow weight gain and growth and delayed developmental milestones. In addition, all had experienced, at some point and to varying extents, other common clinical problems such as the dysautonomic crisis (characterized by hypertension, irritability, vomiting, and tachycardia) and pulmonary infection secondary to aspiration from either gastroesophageal reflux or misdirect swallows. Autonomic perturbations were also seen in all patients as evidenced by propensity to excessive sweating and erythematous blotching of the skin with excitement and postural hypotension without compensatory

Table 2. Warm and cold perception thresholds in 13 patients with familial dysautonomia and 52 age-matched controls at four different body sites

	FD patients n = 13; aged 9–38 yrs mean: 17.1 (9.4) yrs	Controls n = 26; aged 4–24 yrs mean: 11.2 (5.4) yrs	Controls n = 26; aged 25–72 yrs mean: 42.1 (15.1) yrs
Cold thresholds			
Site	Mean (SD)	Mean (SD)	Mean (SD)
Thenar	13.5 (6.6)	2.4 (1.1)	2.4 (1.0)
Forearm	7.4 (5.2)	1.2 (0.4)	1.4 (0.3)
Calf	10.3 (5.6)	2.0 (1.4)	2.6 (1.0)
Foot	7.6 (4.3)	2.9 (1.0)	4.2 (2.0)
Warm thresholds			
Site	Mean (SD)	Mean (SD)	Mean (SD)
Thenar	8.1 (2.5)	1.5 (0.6)	1.8 (1.0)
Forearm	6.4 (3.4)	1.3 (0.3)	1.6 (0.5)
Calf	9.0 (4.3)	2.2 (1.0)	3.7 (2.1)
Foot	9.0 (4.3)	2.7 (1.3)	4.0 (1.9)

tachycardia. Decreased pain and temperature sensation resulted in unrecognized burns in two patients, in Charcot joints of the knees in two patients, and in bone fractures with minimal or no pain reaction in 10 patients.

The physical and neurologic examinations were also consistent with the diagnosis of FD. In addition to the expected consistent lack of lingual fungiform papillae, decreased tear flow, lack of deep tendon reflexes, and diminished corneal reflexes, other findings included kyphoscoliosis (10 patients), corneal scars from healed ulcerations (5 patients), dysarthric scanning hypernasal speech (10 patients), weakness of small foot and distal leg muscles (2 patients), present pyramidal tract signs (2 patients), pallesthesia (11 patients), impaired position sense (3 patients), decreased pin prick sensation (7 patients), and decreased light touch (1 patient). Coordination was slowed and dysmetric in eight patients. Gait and stance showed cerebellar and spinal ataxia in 12 patients.

Temperature perception thresholds. In 12 of 13 patients, thermal thresholds at the four tested body sites were higher than in the control group (Mann-Whitney U-test: $p < 0.005$). Threshold elevation varied from one tested body site to the other and there was no consistent pattern of distribution of impaired thermal sensation (Table 2). Only one patient, a nine-year-old boy, had warm and cold thresholds at all tested body sites that were within the normal limits of his age group [45].

Sympathetic skin response in familial dysautonomia patients. Out of the different stimulus types, electrical stimulation elicited SSR most easily and frequently in the FD patients (Figs. 5–8). The voltage needed to elicit SSR in FD patients (26 V to 400 V; mean, 94 V ± 68 V) was significantly higher than in controls (Mann-Whitney: $p < 0.005$).

In 10 subjects, the four electrical stimulations produced reproducible SSR at the palms and soles. In one patient, only the first two stimuli evoked SSR. Two patients had four reproducible hand responses but only three of four responses at the feet.

Acoustic SSR was present in 8 FD patients, but two of them had only hand and no feet responses. Inspiratory gasp evoked SSR in nine patients, one of whom had responses at the hands only.

Warm, cold, and heat pain stimulation failed to elicit SSR in 10 of the 13 patients (Tables 3 and 4). Only the nine-year-old boy with normal temperature perception thresholds had reproducible SSRs at palms and soles with all types of stimulation including warm, cold, and heat pain stimuli. This patient presented with only mild clinical symptoms and minimal sensory loss, preserved though diminished corneal and deep tendon reflexes, with no ataxia.

A 12-year-old boy not only had present SSR with electrical, acoustic, and inspiratory stimulation, but he also had SSR present at the hands, though not at the feet, following warm or heat pain stimulation of his lateral neck. This was the only body site with preserved warm, heat pain, light touch, and pinprick sensation. Cold sensation was impaired at the neck. Cold stimulation failed to elicit SSR. Regardless of the applied temperature change rates, SSR was also absent when thermal stimuli were applied to other body sites such as the feet, thighs, calves, arms, cheeks, and various ventral or dorsal trunk areas. The boy manifested severe FD with lack of tear flow, corneal and deep tendon reflexes, pronounced hypaesthesia, hypalgesia, pallhypaesthesia, and ataxia.

In a 24-year-old woman, SSR was present with acoustic, electrical, and inspiratory stimulation but also with warm stimulation of the cheeks or forehead. At both sites, she had elevated but preserved warm perception thresholds while cold perception thresholds were highly abnormal. Cold stimulation of the cheeks or forehead did not elicit SSR. Thermal perception was absent at any other body site and thermal stimuli did not evoke SSR (Tables 3 and 4).

Sympathetic skin response wave forms in familial dysautonomia patients. In FD patients—as in controls—SSR onset latencies, amplitudes and differences between the latencies of the first and the second SSR peak did not differ between the left and right extremities (Wilcoxon). Amplitudes and first-to-second peak latency differences elicited with the different stimulus modalities are shown in Table 3. In FD patients, amplitudes of electrical, acoustic, and inspiratory SSRs were significantly smaller than those of the controls (Mann-Whitney: $p < 0.001$), although the superficial skin temperature at the hands (mean, 29.8°C ± 3.2 °C) and feet (mean 30.2°C ± 1.7 °C) of the patients did not differ from the temperature at the hands (mean 31.2°C ± 1.8 °C) and feet (mean 30.7°C ± 2.0 °C) of the controls (Mann-Whitney). In contrast to the control subjects, amplitudes at the hands were similar to those at the feet (Wilcoxon). There was no correlation between latencies or waveforms and patient age.

Discussion

Assessment of small nerve fiber function is usually based on quantitative sensory testing, which is well established and mostly provides reliable information on thermal perception [20,42,46–48]. In previous studies, we demonstrated that thermal thresholds can be determined even in children as young as 3 years of age [45]. However, the psychophysical methods require patient cooperation and cannot be used in severely ill and non-cooperative patients. In these patients, an objective method of assessing small fiber dysfunction which does not require patient cooperation would be desirable and useful.

Several authors consider SSR a parameter reflecting afferent small fiber dysfunction [34,49]. Others interpret diminished or absent SSR as an indicator of only efferent sympathetic dysfunction [44,50] or a dysfunction that is not sensitive in early detection of small fiber sensory neuropathy [21].

We believe that the conclusions are due to inadequate or at least incomplete stimulation modalities. Electrical stimulation is the most common mode of SSR activation, but the

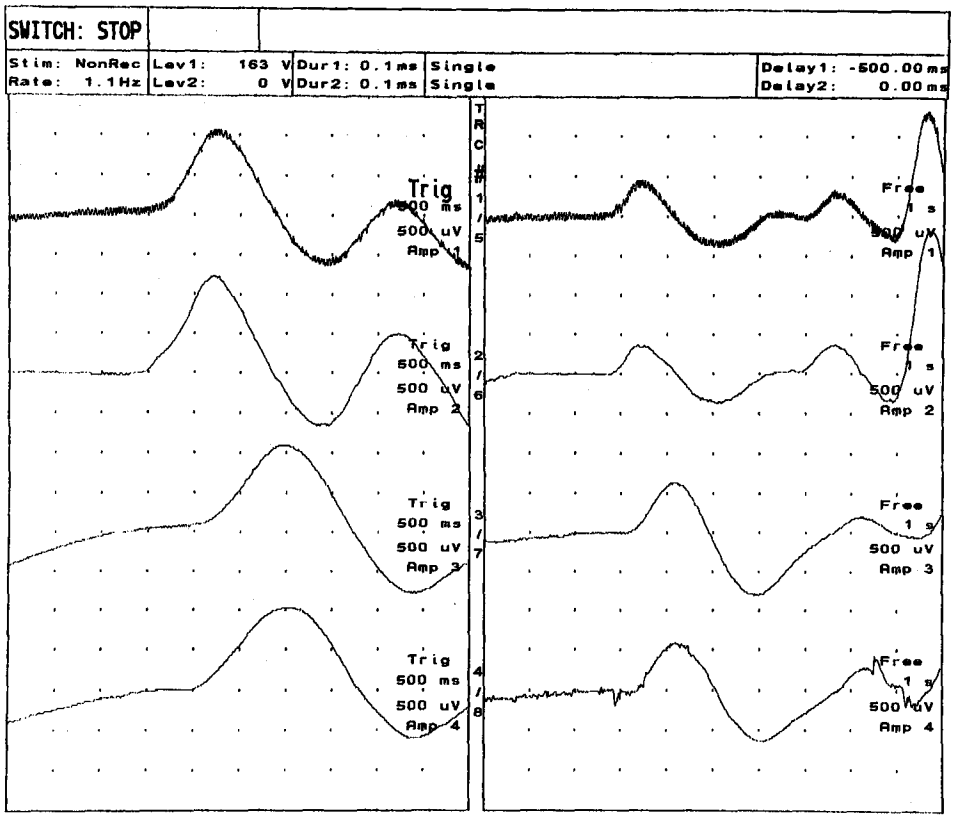

Figure 5. Sympathetic skin response in a 24-year-old male with familial dysautonomia elicited at the palms of the hands and the soles of the feet with electrical stimulation.

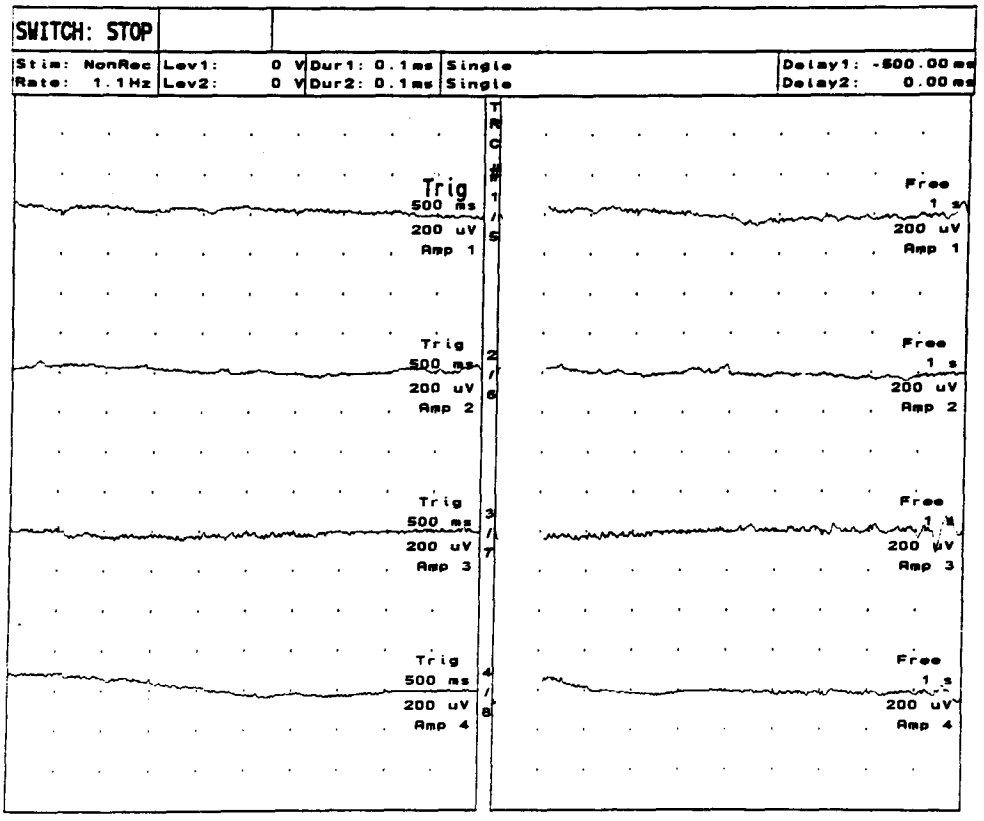

Figure 6. Absent sympathetic skin response at the palms of the hands and the soles of the feet in a 10-year-old boy with familial dysautonomia despite 8°C/sec cold stimulation at various body sites.

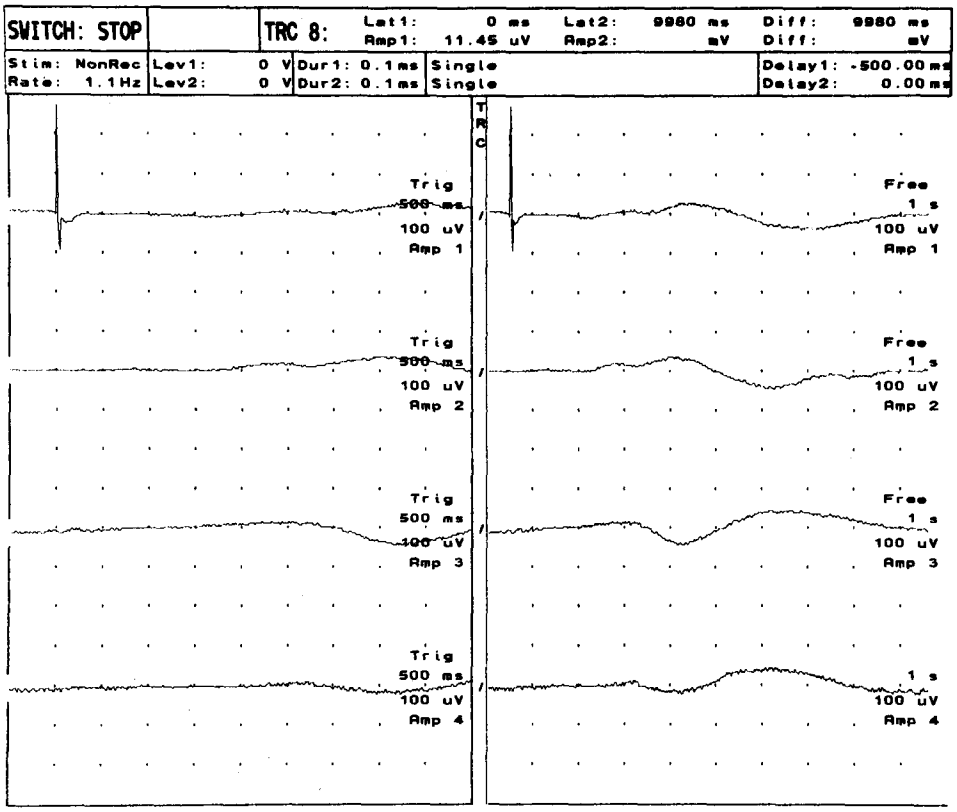

Figure 7. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 31-year-old female with familial dysautonomia with acoustic stimulation.

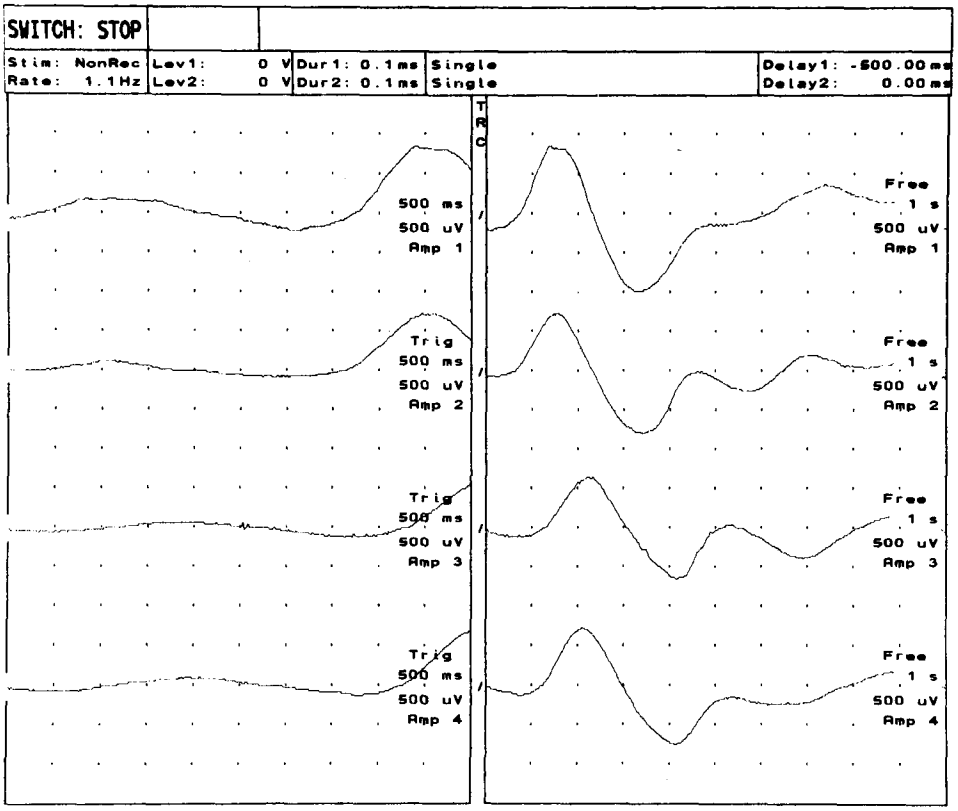

Figure 8. Sympathetic skin response at the palms of the hands and the soles of the feet elicited in a 38-year-old female with familial dysautonomia with inspiratory gasp stimulation.

Table 3. Sympathetic skin response evoked by different types of stimulation in patients with familial dysautonomia

Types of stimulation	Electrical	Acoustic	Inspiratory	Cold	Warmth and heat
Amplitudes (μV)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
At hands	573 (358)	595 (357)	407 (185)	166 (81)	277 (242)
At feet	381 (260)	189 (101)	215 (113)	125 (70)	131 (59)
First-to-second-peak latency					
At hands	1838 (301)	2073 (1220)	1979 (893)	1744 (642)	1973 (945)
At feet	1596 (428)	1380 (832)	1522 (482)	886 (769)	2283 (1302)
Number of phases	%	%	%		
Biphasic					
At hands	65	86	75	(biphasic)	(biphasic)
At feet	71	70	75	(biphasic)	(biphasic)
Triphasic					
At hands	30	14	25		
At feet	24	30	25		
Polyphasic					
At hands	5				
At feet	5				
Initial deflection					
Negative					
At hands	95	86	97	(absent)	(absent)
At feet	82	86	97	(absent)	(absent)
Positive					
At hands	5	14	13		
At feet	18	14	13		

SSR afferents mediating electrical stimuli are large diameter myelinated fibers and not small nerve fibers [33,36,38]. According to Karl *et al.* [38], electrical stimulation elicits synchronous group II nerve fiber volleys that occlude the action of group III and IV afferent volleys. Therefore, electrical stimulation is quite unsuited for the evaluation of afferent group III and IV nerve fibers.

Thermal sympathetic skin response in control subjects

This study demonstrates for the first time the feasibility of eliciting SSR by means of thermal stimulation. SSR was present in all 52 volunteers with warmth and heat pain stimulation just as with electrical, acoustic, or inspiratory stimulation. Similar to electrical activation, thermal stimulation required individually varying intensities to induce adequate arousal responses. Elie and Guiheneuc [26] point out that a positve SSR largely depends on the "novelty" or "surprise effect" of a stimulus. Therefore, slowly increasing thermal stimuli were insufficient to induce an SSR in some volunteers. Aδ and C fibers mediate thermal stimuli using the effects of spatial and temporal summation. Nerve impulse volleys show disproportionate overshoot activity when higher temperature gradients are applied to the skin [11–13,15,18,52,53]. The higher the temperature change rates and the higher the density of thermal receptors at the stimulated body site, the greater is the "surprise effect" and the likelihood of an arousal. Thus, thermal SSR was present in all volunteers when temporal summation was sufficient, *ie*, after the temperature change rates of the thermode had been raised to 8°C/sec or after the thermode had been moved to

Table 4. Sympathetic skin responses to different types of stimulation: findings in patients with familial dysautonomia

Patient ID	Gender	Age	Electrical stimulation		Acoustic burst		Inspiratory gasp		Cold stimulation		Warmth/heat pain stimulation	
			Hands	Feet	Hands	Feet	Hands	Feet	Hands	Feet	Hands	Feet
1	M	11.83	+	+	–	–	–	–	–	–	–	–
2	M	12.08	+	+/3	–	–	–	–	–	–	–	–
3	M	10.41	+	+	+	+	+	+	–	–	–	–
4	M	24.33	+/2	+/2	–	–	–	–	–	–	–	–
5	F	14.91	+	+	+	–	+	+	–	–	–	–
6	F	38.41	+	+	–	–	–	–	–	–	–	–
7	M	13.41	+	+	–	–	+	+	–	–	–	–
8	M	9.16	+	+	+	+	+	+	+	+	+	+
9	F	31.33	+	+	+	+	+	+	–	–	–	–
10	F	18.00	+	+	+	+	+	+	–	–	–	–
11	F	9.50	+	+/3	+	–	+	–	–	–	–	–
12	M	12.25	+	+	+	+	+	+	–	–	+	–
13	F	24.00	+	+	+	+	+	+	–	–	+	+

skin areas with higher temperature spot densities than at the foot, *eg*, to the forearm or the cheek [54].

Only cold stimulation failed to evoke SSR in two volunteers despite 8°C/sec stimulus velocity and stimulation at the cheek, a body site with high cold spot density [54]. For hygienic reasons we did not stimulate at the lips, where cold spots are even denser than at the cheeks. However, cold stimulation is known to be an inconsistent eliciter of pain. Verdugo and Ochoa [42] report cold pain hypoalgesia to be a frequent finding in normal individuals when tested with the Thermotest. Obviously, two of the 52 volunteers did not experience a sufficient "surprise effect" with a steady cooling from a baseline temperature as high as 32°C to a temperature only as low as 5°C. A more abrupt and cooler stimulus might have evoked an SSR in all volunteers. Still, we do not recommend to start cold stimulation from a low baseline temperature, *eg*, a 10°C reference temperature. This would require touching the skin with a 10°C cold thermode. The touch sensation by itself might elicit an SSR and one could not differentiate whether the SSR was due to the thermal or the touch stimulus. To clearly identify SSR as a result of thermal and not mechanical, *ie*, large fiber stimulation, the thermode has to be placed on the tested skin area prior to thermal stimulation. The tested person has to be accustomed to the touch sensation of the thermode before thermal stimulation is started. This approach prevents simultaneous large and small fiber stimulation. Our results suggest warmth and heat stimuli are preferable for thermal SSR studies.

Thermal stimulation elicited responses with amplitudes and first-to-second peak latency differences, *ie*, SSR durations, similar to those of the other types of stimulation. Only the waveforms of thermal SSR were more frequently tri- or polyphasic than with electrical stimulation. We speculate that this finding might be due to differences between the short electrical or acoustic stimuli and the continuous and increasing thermal stimuli.

Otherwise, thermal SSR findings conform with those of other stimulus responses and reports in the literature: responses at the hands were higher than at the feet and there were no left-right differences [21,26,33,35–37].

In contrast to the findings reported by Drory and Korczyn [33], we only found slight correlations between the age of our volunteers and the SSR amplitudes. Most likely the discrepancy is due to the lower mean age of our volunteers. Knezevic and Bajada [35] did not find any age correlations in their control group, which had a mean age similar to our group.

Sympathetic skin response in familial dysautonomia patients

In all 13 FD patients, SSR was present with electrical stimulation, although the responses were lower and less consistent than in control subjects. This finding indicates that the SSR reflex arc is principally functional provided large myelinated fibers are used as the afferent pathway. The lower amplitudes with electrical as well as inspiratory and acoustic stimulation can hardly be ascribed to an afferent large fiber dysfunction. These fibers have been reported to be intact in FD patients [5,7,9,55–57]. Sural nerve biopsies of FD patients showed only a minimal reduction of the overall number of myelinated nerve fibers and internodal length as compared to controls [6]. Consequently, nerve conduction velocities are only slightly reduced [6,58].

Most likely, low SSR amplitudes and inconsistent responses are due to deficiencies of central processing pathways and particularly efferent sympathetic sudomotor activity. One of our patients had preserved hand responses but incomplete foot responses with electrical stimulation. The afferent large fiber stimulation was sufficient to induce an arousal and a sudomotor hand response, but the efferent sympathetic output was insufficient to activate foot sweat glands [50].

The decreased SSR amplitudes are consistent with the clinical observation of reduced sweating at the palms and soles of FD patients [5] and reflect the sympathetic dysfunction, one of the most prominent clinical findings in FD patients [56,57,59]. The diminished sympathetic sudomotor output is due to the insufficient development and survival, primarily of sympathetic fibers and neurons in FD patients [1,2]. In FD patients, there is a depletion of neurons in the spinal cord intermedio-lateral gray columns. The size of sympathetic ganglia and the number of neurons as well as peripheral sympathetic nerve terminals are reduced [5,9,60].

Apart from sympathetic failure, impaired temperature and pain perception is the second most prominent finding in FD patients [56,57,59]. The sensory dysfunction is due to a reduction of unmyelinated nerve fibers to 5% to 15% of the normal number and of neuron somas in the Gasserian and spinal ganglia to 50% of normal [6–9]. In cooperative FD patients, quantitative thermal perception testing is the most adequate method to assess afferent small fiber dysfunction [20]. Our patients cooperated sufficiently in the psychophysical test, and almost all of them had highly abnormal thermal thresholds. In many, especially young and severely affected FD patients, results of quantitative thermal threshold testing are biassed due to inadequate cooperation. In these patients, thermal SSR testing adds to the psychophysical thermotest as demonstrated by the most important result of our study.

Although electrical stimulation demonstrated a preserved SSR reflex arc, thermal SSR was absent when thermal stimuli were applied to body sites with impaired thermal perception. In contrast, thermal SSR was present after stimulation of sites with preserved warm or cold perception. The result of warm or cold SSR stimulation closely correlated with the impairment of warm or cold perception. In the 12-year-old boy and the 24-year-old woman, thermal SSR was present only when warm and heat but not cold stimuli were applied to those body sites with preserved warmth and heat perception but absent cold perception.

Conclusions

A combination of different stimulus modalities activating primarily large or small nerve fibers improves the diagnostic value of SSR studies. A sequence of large and small fiber

stimuli better specifies the type of afferent fiber dysfunction than does electrical stimulation only.

In FD patients, electrical stimulation shows reduced response amplitudes but demonstrates the overall integrity of the SSR reflex. The reduced SSR amplitudes might be attributed to the reduction of efferent sympathetic nerve fibers. The absence of SSR with thermal stimulation is consistent with the neuropathological findings of afferent small fiber reduction.

In contrast to methods such as the quantitative sudomotor axon reflex test (QSART), SSR is only a semi-quantitative technique and does not allow accurate assessment of sudomotor or sweat gland activity. However, QSART is rather time consuming and technically demanding [63,64]. Therefore, SSR is more widely used. Almost any standard electromyography machine and surface electrodes allow one to measure changes of skin resistance in response to an arousal [21,34,35]. Our results show that SSR is present in FD patients with electrical stimulation despite a pronounced afferent small fiber neuropathy and autonomic dysfunction. We conclude that SSR testing with electrical stimulation only is too insensitive to evaluate autonomic or afferent small fiber neuropathy. SSR might fail to provide diagnostic information unless the technique is applied with a refined and distinct methodology. Stimulus parameters should be standardized and SSR amplitudes or latencies have to be compared to age related normative control values. Most importantly, SSR should be tested with a combination of stimuli; among them are stimuli specific for the afferent fiber types to be evaluated.

Further studies have to show whether the combined application of electrical and thermal stimulation might allow the use of SSR for small fiber evaluation in patients with limited capacity to cooperate in psychophysical quantitative sensory testing procedures.

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