A simple and objective marker for stress

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Abstract

Objective: To examine the association between pressure pain sensitivity (PPS) at sternum and various well established physiological stress measures among opera singers during a performance as a measure for transitional stress, and resting values in out-clinic patients as a measure for persistent stress.

Methods: Changes in PPS on the index finger and sternum, middle blood pressure (MAP), heart rate (HR), pressure-rate-product (PRP) and salivary cortisol (SCO) were recorded in 26 opera solo singers during a performance. Resting PPS, HR, MAP, PRP and presence of a noxious withdrawal reflex (NWR) were recorded in 181 out-clinic patients.

Results: During opera performance, the PPS on sternum changed concomitantly with MAP (correlation coefficient \( r = 0.42, p < 0.005 \)), HR (\( r = 0.55, p < 0.001 \)), PRP (\( r = 0.54, p < 0.001 \)) and SCO (\( r = 0.26, p = 0.066 \)). During rest, a significant correlation was found between PPS on sternum and HR, PRP and presence of noxious withdrawal reflex (all \( p < 0.01 \)).

Conclusions: The PPS measurement at sternum was associated with well established physiological stress measures and may represent a simple, objective and reliable measure of physiological stress used by both non-professional and professionals.

Key Words: Cardiovascular physiology, medical physiology, stress test, psychological stress, physiological stress reaction, blood pressure

Introduction

Stress is developing into a 21st century epidemic. It is not only a threat to the health of the individual but also of great concern for societies as a whole, and a lot of research has been done in the field [1]. However the results of the studies carried out in this field have been inconsistent, and therefore it is important to find reliable objective ways of measuring stress in order to standardize research [2].

It is essential to distinguish between transient and persistent stress. Transient stress is a physiological state of preparedness, a state that is automatically induced through neural/hormonal signals from the brain when a threat or work challenge is perceived. Thus it serves as a defence mechanism and helps to increase performance level whether this is to be used in a fight/flight situation or to solve a job task. When the threat/challenge is over, homeostasis is re-established. The process of activation and recovery back to normal levels is also known as allostasis. In contrast, persistent stress occurs due to a prolonged exposure of the hormones involved in transient stress causing an allostatic load, which leads to physiological dysfunctions that can be harmful to health [3,4].

Since stress is not directly measurable, researchers often use physiological markers (heart rate, blood pressure, hormone levels etc.) together with behavioural observations and personal questionnaires. The physiological markers are essential since they require a minimum of cognitive and emotional processing and thus are more objective [5]. However many of these physiological markers are influenced by several other factors than stress, i.e. physical exercise, tobacco, alcohol and the time of day [6,7]. Similarly, visionary input causing stress has been found to enhance hearing [8] and olfactory input to augment the startle reflex [9].

During clinical observations of patients with heart diseases and other conditions of stress-related origin, we have observed increased pain sensitivity on specific locations on the skin of the sternum. This observation is in concordance with the notion of a hyper analgesic effect and can be explained by the occurrence of cutaneous polymodal nociceptors, which responds
to mechanical stimuli, noxious heat, exogenous chemicals and inflammatory mediators and might result in a noxious withdrawal reflex (NWR) [10]. The NWR is regarded as a reliable and objective tool for exploring pain control systems in humans [11].

In a fight-flight situation it makes sense to prepare for a maximal defence reaction in order to protect the vital heart-region. The existence of points on the sternum with association to the heart is part of Oriental acupuncture tradition [12]. On this background, we hypothesize that transient and persistent stress increases the sensitivity in specific points located in an area of the skin on the sternum and that this sensitivity can be measured as the pressure pain sensitivity (PPS), which can be a useful marker for stress.

**Methods**

**Subjects and study design**

This paper describes a series of three studies where study 1 consisted of the following three sub studies: Opera chorus before and after a performance (1:A), Solo singers at a premier performance (1:B) and Opera students at a song and exercise test (1:C). Study 2 was of 181 out-clinic patients during rest, and study 3 compared the patients in study 2 with healthy subjects.

All subjects were given verbal and written information and were required to sign a written consent before the first measurement took place. The local ethical committee approved the study in opera singers and trainees. The remaining study was conducted as part of the daily medical work at a medical outpatient clinic in accordance with Danish medical regulations and ICH Guidelines for good clinical practice.

For measuring the pain pressure sensitivity of the sternum (PPS) we used a special algometric instrument (Ull Meter) designed for this purpose. The instrument measured a pressure pain threshold, and that value was transformed into a logarithmic scale and inverted to a sensitivity scale (high value = low threshold), in order to have a scale for which a low value (reading) can be mentally associated with a low level of stress and a high value with a high level of stress.

**Study 1. Changes in pressure pain sensitivity and physiological variables for transient stress in conditions with predictable short-term changes in stress**

Opera singers are likely to be exposed to performance-related psychological stress [13], especially solo singers at the peak of their solo performance, and even more so at a premier performance.

1:A. In 46 members of the Royal Opera Chorus (18 female, average age 42±8 and 28 men, average age 45±8) the PPS on the index finger and on the sternum were measured before and after a 3-hour opera performance. On each occasion two measurements were made, separated by 5 seconds.

1:B. The experimental set-up was arranged to increase the changes in recorded stress and decrease the influence from confounding factors: Only solo singers were included (n = 26; 9 female, average age 41±6 and 17 men, average age 47±9), and three measurements were recorded on the same day: (i) before the performance; (ii) just after the top of the performance at the expected peak level of their stress and; (iii) at the end of the evening, i.e. as late as possible after the top performance, allowing maximum restitution within the limitations of the study logistics. The first and third measurements were conducted in the singer’s dressing room, while the second measurement was conducted at a temporary back stage area in order to minimize the time period between peak performance and recording. The presence of NWR was included as an observation.

1:C. Twenty-six opera trainees (14 female, average age 27±2 and 12 men, average age 28±2), participated in two different tests on separate days: a singing audition and a bicycle exercise test. Further steps were taken to reduce a possible influence from confounding factors; the subjects were asked not to take any medication, or consume coffee, alcohol or tobacco during the last two hours prior to the tests and they were asked not to engage in heavy physical activity during the last hour before the tests. The presence of NWR was included to test the preliminary findings in opera solo singers.

The singing test consisted of two measurements: (i) after 10 minutes of rest but as close as possible to the audition, and (ii) right after the audition after 10 minutes of rest. Accordingly, any recorded changes in PRP and PPS between the first and second recordings were related to the restitution in transient stress following the audition.

The bicycle test was conducted on a Kettler Ergometer bicycle, model X3 with Siemens electronic cardio fitness/SD 4,8,5,9 (Freizeit Marke Kettler, Heins Kettler GmbH & Co, KG postfach 1020, D-59463 Ense-Parsit, Germany).

The test consisted of four successive recordings: (i) after 10 minutes of rest, (ii) after two minutes of exercise aimed at increasing PRP with 25%, when compared to resting PRP, (iii) after another two minutes of exercise with the aim of increasing PRP with a minimum of 40% when compared to resting PRP, but below a maximum workload of 80% of the age-estimated maximum workload (heart rate), and (iv) after 10 minutes of rest. The 1st, 3rd and 4th recordings were used in the calculation related to Figure 2B.

Depending on the estimate of the individual subject’s physical fitness, the initial workload was 50/75 Watt and 80 revolutions per minutes (rpm) for women and 75/100 Watt and 80 rpm for men. For the second exercise phase, workloads were increased.
to 75/100/125 Watt and 90/100/110 rpm for women and 100/150/200 Watt and 90/100/110 rpm for men, with the possibility of increasing bicycle time to three minutes. One subject was excluded from this test, as the PRP increased from 9.700 to 33.300 during the second exercise test with a heart rate of 197 (80% age-adjusted limit for heart rate = 154).

Study 2. Resting values of pressure pain sensitivity, physiological measurements, and presence of a noxious withdrawal reflex

A total of 181 consecutive patients (126 women and 55 men) at a private medical outpatient clinic were included. Their average age was 58 years. Their diagnoses were: cancer 55; heart disease 49; stress 19; and others 58. Resting values of PPS, blood pressure, heart rate and the presence/absence of NWR were conducted in the supine position after 10 minutes of rest. Measurements were conducted by two separate research teams: PPS and NWR by the one team; blood pressure and heart rate by another team. Registrations of NWR were missing in 28 patients (10 patients with PPS<30, 12 patients with PPS from 30–60, and 6 patients with PPS>60). These patients were excluded from this part of the study. For clinical use the PPS scale was made into a 3-step scale, with a 100% increase in sensitivity between each step: PPS<30; 30≤PPS<60, PPS=60. This same scale was used when comparing the PPS measurement and NWR.

Study 3. Reliability testing of the pressure pain sensitivity measurement

After the initial observation of significant correlation between repeated PPS measures in study 1:A, we conducted a study to test the reliability of the PPS measurements in three different subject groups: 103 healthy subjects who had not previously been measured; the 181 consecutive patients described in study 2 and 33 healthy opera singers from study 1 conducting self-measurements twice daily during a two week period. The measurements in situation A and B were performed by a healthcare professional (HCP). Reliability testing of the PPS measurement (PPS units) was conducted by taking two measurements with 5 seconds between measurements in three different situations.

Minimizing bias

The following precautions were taken to minimize bias.

The instrument’s reading was not visible during measuring for either subject or researcher; recordings of blood pressure, pulse and salivary cortisol were done after measuring PPS; the use of two measurement points: a hypothetically active and a hypothetical control point, and the hypothetical distinction between the two locations being unknown to the subject; repetition of the study on transient stress three times (study 1) including different subjects each time, and while eliminating possible confounding factors. As deviating results were found in study 1:B, a supplementary study (1:C) was conducted to explore if the observed deviations had a biological background; In study 2 the recordings of PPS and cardiovascular effect variables were conducted by separate research teams, with each team unaware of the other team’s findings. The inclusion of the noxious withdrawal reflex as an effect variable over which the subject or the observing researcher had no conscious control, did apply more objectivity to the reading.

Effect variables

The instrument for measurement of pressure pain sensitivity. This was a special algometric instrument (Ull Meter, patent numbers: PA 2004-00359; PA 2004-00550) with the following features:

(1) Algometric readings are hidden until the measurement is completed in order to blind the subject and the researcher. The applied algometric instrument pressure is mathematically transformed into a logarithmic scale of sensitivity levels similar to the Decibel scale, which uses air pressure threshold for the determination of hearing thresholds. The scale is inverted so that an increase in sensitivity is equivalent with an increase in value of the PPS measure. In order to prevent damage to the skin, an alarm sound is activated when pressure reaches a level of 14 kilograms.

(2) It has a special rubber measurement foot-plate to allow the determination of pressure pain sensitivity of the bone without applying noxious stimulation to the skin, as such stimulation might lead to determination of skin pain sensitivity instead.

The pressure pain sensitivity: All measurements were carried out with subjects in a supine position. Each trial started with two measurements on the dorsal part of the middle phalanx on the left index finger (control point), during which the technique and procedure were introduced. The point for measuring (active point) was identified by palpation by the researcher as the most tender point of the skin on the sternum within the area between the third, fourth and fifth intercostal space, reflecting the area of segmental innervations of the heart [14].

The measuring device (Ull Meter) was applied with a gradual increase of pressure, in total allowing...
3–4 seconds pressure time. The subject was instructed to say 'Stop' as soon as discomfort was felt. If the researcher observed a startle or withdrawal reflex, this was considered a stop signal as well. Subsequently, measurements on the sternum were conducted following the same procedure.

The procedure was repeated twice on both index finger and sternum except for the study of opera trainees (1:C), in which 4 recordings were made within 1–2 hours. In this study the first recording of the day consisted of two measurements, for the remaining recordings, only one measurement was used in order to prevent damage of the sensory receptor.

Physiological and biochemical measurements: Blood pressure (mmHg) and heart rate (HR, beats/min) were recorded by Thuasne automatic blood pressure monitor, model W0840 002 001 (Microlife ref. BP-3AA1-2, BP 243–92307 Levallois-Perret Cedex, France). For analysis, the mean of two consecutive measurements was used. Pressure-Rate-Product (mmHg×beats/min) was calculated as systolic blood pressure×heart rate. Mean arterial pressure (mmHg) was calculated as (2/3 diastolic blood pressure + 1/3 systolic blood pressure). Salivary cortisol (nmol/l) samples were obtained on location using standardized techniques and analysed by a radio-immunoassay method [15,16].

The noxious withdrawal reflex: The noxious withdrawal reflex (NWR) was recorded as involuntary muscle contractions around the eyes (=startle reflex), cheeks or in the flexor muscles of the neck and upper extremity.

Statistics

Statistics were analysed by an independent statistician, with the use of SAS version 9.1 for Windows. For regression analysis the regression ANOVA (least squares) was used. For comparison a t-test was used.

Due to the limited number of subjects, a skewed distribution of values, and a potential skewed influence from confounding factors, the nonparametric Wilcoxon rank test was used to compare performance situations.

In all analyses a significance limit 5, 1 and 0.1% was used (two-tailed).

Results

Study 1. Changes in pressure pain sensitivity and physiological variables during an opera performance

1:A. Among 46 members of the Royal Opera Chorus, PPS on the sternum was significantly higher before performance (mean 39), than after performance (mean 33) (p<0.01). Concomitantly the value of

| Table I. Changes in variables through the different stages. |
|----------------|----------------------|
| From before to just after performance | From just after performance to after rest |
| PPS | 20 subjects of 26 increase p<0.01 22 subjects of 26 decrease p<0.001 |
| HR | 22 subjects of 26 increase p<0.001 24 subjects of 26 decrease p<0.001 |
| PRP | 21 subjects of 26 increase p<0.001 25 subjects of 26 decrease p<0.001 |
| MAP | 17 subjects of 26 increase p<0.05 20 subjects of 26 decrease p<0.001 |
| SCO | 12 subjects of 26 increase p=0.43 17 subjects of 25 decrease p<0.01 |

PPS on the index finger was 0 for 88 and 80% of the singers in the two situations (p>0.1). There was a significant correlation between PPS measurements obtained at 5-second intervals (r=0.92; p<0.0001 for both finger and sternum).

1:B. For 26 solo singers, who were measured before, just after performance and after rest, significant changes were found in PPS on the sternum, heart rate (HR), pressure-rate product (PRP) and mean arterial pressure (MAP). The change in salivary cortisol (SCO) was only significant from ‘just after performance’ to ‘after rest’ (Table I, Figure 1)

Concomitantly no tenderness was found on the index finger for 77, 84 and 85% of the singers in the three situations (p>0.1). Changes in PPS on the sternum correlated significantly with changes in PRP (r=0.54, p<0.001, (see Figure 2A)), HR (r=0.55, p<0.001) and MAP (r=0.42, p<0.005) but not with SCO (r=0.26, p=0.066).

In the correlation between changes in PRP and PPS, two measurements deviated from the general pattern, where an increase in PRP was associated with a decrease in PPS (indicated by the arrows in Figure 2A). These two singers did have a high level of physical activity in the minutes up to the second measurement, which could explain the findings.

1:C. Subsequently 26 opera trainees attended a song audition and underwent a bicycle exercise test, simulating the exercise patterns of the two opera singers on stage. PPS changed significantly from before the song audition (mean 55) to after (mean 43) (p<0.01), reflecting the anticipated expectancy pressure. Changes in PRP and PPS was found to correlate positively (r=0.26; p<0.05). Figure 2B, shows the negative correlation between changes in PRP and PPS during the bicycle exercise (r=−0.70; p<0.001). The mean PPS values measured were: before biking 57, just after biking 35, and after rest 42.

Study 2. Resting values of pressure pain sensitivity, physiological measurements and presence of a noxious withdrawal reflex

Among 181 consecutive patients PPS on the sternum correlated significantly to PRP (r=0.23; p<0.01)
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Singing test (before and after) PPS: 55, 43 (mean PPS units) NWR: 68, 46%.

Bicycle test (before, during and after): PPS: 57, 35, 42 (mean PPS units) NWR: 56, 33, 50%.

**Study 3. Reliability testing of the pressure pain sensitivity measurement**

The reliability tests of the PPS measure performed by healthcare professionals showed a high correlation on both healthy subject and medical patients (Figure 4).

Figure 4A shows the PPS value in 103 healthy subjects ($r=0.94$, $p<0.001$). Figure 4B shows the PPS value in 181 consecutive patients ($r=0.97$, $p<0.001$). Likewise the correlation was high for the non-healthcare professionals who conducted self-measurements during a two week period ($r=0.95$, $p<0.001$) (Figure 4C).

**Discussion**

These studies suggest that when healthy people are exposed to an acutely stressful situation, it is associated

and HR ($r=0.25; p<0.01$). Females had a significantly higher PPS on the sternum (mean 48, $n=126$), compared to males (mean 32, $n=55$) ($p<0.001$). No correlations were found between HR, PRP, and PPS on the index finger ($r=0.0$).

An increase in PPS on the sternum was found to be linked to an increased frequency of NWR, as seen in Figure 3. PPS levels, separated into three groups with a 100% increase in pain sensitivity separating each group, showed an increase in frequency of NWR from 17–56% ending at 93% ($p<0.001$). Among the Opera trainees (1:C) the corresponding numbers were from 38–59% ending at 87% thus supporting the findings of study 2.

With respect to gender differences in pain perception among the 181 patients, the mean PPS values accompanied by NWR observed were 61 for women and 43 for men ($p<0.001$). For both sexes we found a positive correlation between PPS and NWR ($r=0.64$, $p<0.001$ for women, and $r=0.49$, $p<0.001$ for men).

In study 1:C the frequency of NWR was found to follow the dynamic pattern of PPS during the singing and bicycle test: Singing test (before and after) PPS: 55, 43 (mean PPS units) NWR: 68, 46%.

Bicycle test (before, during and after): PPS: 57, 35, 42 (mean PPS units) NWR: 56, 33, 50%.

**Figure 1. Recorded changes in 26 opera solo singers during an opera performance. (A) Pressure Pain Sensitivity (PPS) mean (SEM): 34 (3.5), 43 (4.4), 32 (3.6) (PPS units), (B) Heart Rate (HR) mean (SEM): 69 (2), 84 (3), 72 (3), (C) Pressure-Rate-Product (PRP) mean (SEM): 9828 (395), 12052 (673), 9865 (480) (mmHg×beats/min), (D) Mean Arterial Pressure (MAP) mean (SEM): 103 (2), 107 (3), 101 (2) (mmHg), (E) Salivary cortisol (SCO) mean (SEM): 3.8 (0.6); 5.0 (0.9); 2.6 (0.3) (nmol/l).**
While an opera performance contains a substantial element of mental stress [13] it also includes a certain level of physical activity due to the physical act of singing. However this form of physical activity differs from that of cardiovascular exercise like running. Exercise is known both to reduce pain sensitivity [6,17,18] and to reduce stress by improving allostasis [3,19]. By adding a physical exercise study (1:C) we found that the PPS measure responded differently during exercise and during a singing performance. Accordingly, we found that the PPS measure seems promising as a method to distinguish between the increases in sympathetic tone caused by mental performance from the increases in sympathetic tone due to physical exercise.

These findings indicate that physical exercise is a confounder for the measurement of physiological stress as it reduces PPS. During the study we did not register the level of the singer’s physical activity on stage, thus it is likely that the difference in PPS levels before and just after peak performance might have been greater for the subjects who did have some level of physical activity (i.e. dancing). We imagine other confounders to be any stimulating or calming substances like caffeine, alcohol or tranquillizers that all have an effect on the sympathetic nervous system. Therefore these potential confounders were eliminated in study 1:C. A decreased adaptive capacity due to an allostatic load could also be confounding. Study 2 shows a significant difference between genders, thus we found that gender is likely to be a confounding factor as well.

The studies have certain limitations due to being open non-blinded studies without control-groups. Blinding the subject was not possible as the subject always will know whether they are at performance or not. However we did attempt to mask the results from the researcher by using a specific sequence in measuring methods.

Figure 2. The link between PPS and PRP in opera performance and bicycle exercise. (A) Opera solo singers (N=26), changes in PRP (mmHg×beats/min) and PPS (PPS units) during an opera performance (r=0.54, p<0.001). (B) Opera trainees (N=26), changes in PRP and PPS during a bicycle exercise test (r=−0.70, p<0.001).

With an increased sensitivity of an area of the skin on the sternum, measured as pressure pain sensitivity with the presence of a noxious withdrawal reflex. When the stressful situation resolves, the sensitivity is normalized. The initial observations of opera chorus singers (study 1:A) were confirmed in both solo singers (study 1:B) and opera trainees (study 1:C). Change in pressure pain sensitivity of the sternum was found to correlate with known physiological measures. The measurements in these studies followed the expected pattern of allostasis with arousal and recovery during transitional stress [3].

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The small number of subjects and their individual biological variation (in PPS values) combined with the confounding factor is a limitation to study 1. We think it likely that the SEM values would have been lower if adjustments had been made to meet these limitations.

Resting HR and PRP have both been found to be useful variables in stress-related conditions and as
Reliability testing found that the correlation coefficient between first and second measurement was high for both professionals and non-professionals. This matched the level of contemporary non-chemical non-invasive diagnostic methods: repeated audiometric measurement ($r=0.70$) [26], armpit versus rectal temperature ($r=0.43$) and rectal versus core temperature ($r=0.94$) [27].

Modulation of pain sensation is initiated by cognitive and emotional processing in the brain [28], by attention [29], as well as by social factors [30]. The cellular site of modulation is the polymodal receptor, which is of an identical nature throughout the evolutionary chain: from fish to higher vertebrates to humans [31]. Two distinct receptors have been identified which are the A-delta receptor with a low pressure pain threshold, and the C-receptor with a high threshold [34]. It is possible that the molecular site of modulation is the Ca$^{2+}$ permeable channel subgroup TRPV 4 [32]. Recent research indicates that TRPV 4 can be stimulated by a variety of exogenous and endogenous substances, including sympathetic input [33]. Against this background, we find that a functional, structural and molecular basis for the observed modulation may be present.

The NWR has been a survival device throughout evolution as a defence response and represents the simplest centrally organized response to painful stimuli [22,23] and a variety of NWR exists in both animals and humans [24]. In observations with horses, a dose-dependent relationship between stimulus dose and NWR magnitude has been observed [25]. In the present study, a higher frequency of NWR was found at higher PPS (Figure 3) making a dose-dependent relationship between stimulus dose and NWR frequency probable. We imagine that a higher dose stimulus of stress activates a non-conscious defence-reaction in a dose-dependent way.

Figure 4. (A) First-time measures by HCP in 103 healthy people ($r=0.94, p<0.001$). (B) Clinical measures by a Health Care Professionals (HCP) in 181 consecutive patients ($r=0.97; p<0.001$). (C) Self-measures by 33 NON-HCP's conducted twice daily during a two week period ($r=0.95; p<0.001$).
patterns associated with prolonged elevated levels of stress hormones, i.e. persistent stress.

Conclusion
The present series of studies provides interesting observations with the method potentially very useful in record stress in an objective fashion. Presently, no international consensus exists concerning measurement of stress in general. The method measuring PPS is found to be reliable and easy to use, which may suggest a broad range of practical applications, whenever an objective marker for stress proves useful, for example in areas of work-related or performance-related stress, in post-traumatic stress disorders and in chronic disease where persistent stress has a significant prognostic impact.

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Declaration of interest: Dr Soeren Ballegaard is a share holder of the company that supported the study financially and that owns the instruments Ull Meter, which made the pressure pain sensitivity measurements possible. Therefore the statistical analyses were confirmed by a supplementary external unbiased statistician (Arne Haahr Andreasen).

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