# Project ES3: attempting to quantify and measure the level of stress

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**Introduction.** The WHO has qualified stress as a 'world epidemic' due to its increasingly greater incidence on health. The work described in this paper represents an attempt to objectively quantify the level of stress.

**Aim.** The aim of the method developed here is to measure how close or how far a subject is from a situation that can be considered 'normal' in medical and social terms.

**Subjects and methods.** The literature on the pathophysiology of stress and its methods of study in experiments on both animals and humans was reviewed. Nine prospective observational studies were undertaken with different types of subjects and stressors covering the different types of stress.

**Results.** The results of the literature review made it possible to identify the different types of stress, the indicators that yield significant results, the psychometric tests and the well-documented 'stressors'. This material was then used to design the general method and the details of the nine clinical trials. The preliminary results obtained in some of the studies were used to validate the indicators as well as the efficacy of the techniques used experimentally to diminish stress or to produce it.

**Conclusions.** The early results obtained in the experimental trials show that we are on the right path towards defining and validating multivariable markers for quantifying levels of stress and also suggest that the method can be applied in a similar way to the study of mental disorders.

**Key words.** Chronic stress. Electrophysiological, biochemical and psychometric parameters. Emotional stress. Multivariable biomarkers. Quantification of the level of stress. Traumatic stress.

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## Introduction

Stress is the response of an organism to harmful situations or to a hostile environment that the individual perceives as a 'threat'. The general adaptation syndrome –as it was named by Selye in 1950 [1]–, consolidates the meaning of stress in Psychology as an organism's response to a tension caused by one or several of the different ways of struggling for existence regardless of what the causal agent may be.

In fact, the word stress, in relation to the 'physical effort to which a material object is subjected', first appeared in Materials physics in the mid-15th century and became soundly established as of 1855. Not until the early 20th country did it take on the meaning that is most commonly used today [2].

When a material is subjected to a force, for example, stretching, it is deformed by the action of that force; the atoms that make up its structure are separated, which increases the cohesive force that holds them together, thereby generating a resistance to the process of deformation. This resistance to the action performed by the external agent was called stress. Deformation stops when the force of resistance generated equals that exerted from the exterior and therefore a new state of equilibrium is achieved.

It comes as no surprise that the idea was later applied to living beings, since it matches almost exactly the intuitive concept of stress that is generally held. When an external agent separates the subject from his or her state of homeostatic equilibrium, a reaction is produced immediately in an attempt to oppose the changes produced and thus reach a state of balance once again. If the action by the external agent is not interpreted as harmful, the reaction is limited and more or less specific to that external agent. In contrast, if the action is interpreted as being potentially harmful, other types of mechanisms are triggered that are not linked solely to one particular stressor but instead involve a general alert, in which a new point of equilibrium is temporarily reached so that the subject can attempt to ensure his or her survival.

In this paper we will use the terms stress factor or stressor to refer to any stimulus that is interpret-

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ed as harmful and alters the homeostatic equilibrium of the organism in the sense discussed above. Stressors will therefore include an aggression, a death threat, the feeling of suffocation due to lack of oxygen because of the altitude or being shut up in a closed space, an injury or traumatic event, an illness, and exposure to extreme heat or cold, among others. It should be noted that, for example, imagining a lack of oxygen will trigger stress, regardless of whether it is really happening or not. Finding oneself in an uncomfortable situation due to any kind of circumstance is also stressing and stressors therefore include being required to do work that is perceived as impossible to accomplish, creating the need to consume items that will always remain beyond our reach, forcing oneself to memorise a text that is too long or imposing cognitive or mechanical response times that are either too short or unachievable. From the foregoing comments it becomes clear that we can easily distinguish between two types of stress: that triggered by a physical agent, a physical force or pressure upon the subject, which is tangible and direct (this would be the case of an injury or illness), and that produced by psychological pressure, which is far less tangible and less direct (this will be the case of the tension caused by the death of a relative, fear of speaking in public, the apprehension felt prior to an exam, fear of making a fool of oneself, etc.). It is clear that these two types do not exclude one another. In particular, in the case of an injury not only the upsetting of the homeostatic equilibrium caused by the injury itself contributes to the general stress, but also the feeling of pain and/or uncertainty induced by the threat it may represent.

In the vast majority of cases, if the stressor is permanent, the individual adapts to the novel situation by reaching a new point of homeostatic equilibrium or if the external action ceases, then the subject gradually recovers his or her state of equilibrium. In some cases, however, after the external action has stopped the subject does not recover the original state of equilibrium but instead maintains a state of 'chronic' stress that is now pathological and may eventually become independent of the cause that initially triggered it. The same thing happens in Materials engineering: depending on the material and the force applied, once the external force stops the material regains its original form and this is what is known as elastic deformation. But if the force exceeds the elastic limit of the material, then the deformation persists even after the force that caused it has ceased or if the breaking strength of the material is exceeded, then the material fractures.

In today's world, stress, understood as a 'toxic' factor produced by psychologically traumatic experiences prolonged over time, has increased significantly as a result of the lifestyle of a changing society that is often perceived by its citizens as threatening. A large number of people in the world, of course, really are subjected physically and mentally to situations that produce a constant high level of stress and this is why the World Health Organisation calls it a 'world epidemic' [3]. Indeed, we are witnessing a phenomenon of generalisation of the problem that has gone from being very localised (helping survivors from the war to respond to the traumatic situations they have experienced), and which was studied and treated only by specialists, to a world that is far more immediate, in which those who are affected might be a child being psychologically abused by his or her peers, a teenager for whom studying at university means living in the hostile environment of a large city (with the added pressure of needing to get good grades to respond to the economic effort being made by his or her family) or the director of a large company whose lack of strategic vision is reducing his or her efforts to ashes, thus leaving the future as something that appears to be an insurmountable obstacle.

The increasing age of the population is also a factor that is helping to make the dimensions of the problem larger still. Following on with the parallelism, we could say that the elastic limit becomes lower as the material gets older. How many times have we broken the rubber band that held documents together in a filing cabinet? The same thing happens in psychological stress: the ease with which an equilibrium is recovered after being subjected to stressors becomes more reduced and slower as we get older. In fact it has been shown that after the age of 50 the capacity of resistance to stressing situations decreases, which is associated to a diminished response of cortisol at least in monkeys.

With regard to the indirect consequences, stress factors are known to be able to promote physiological and behavioural perturbations that range from dysfunctions in the immunological system to psychiatric disorders. For example, today there is evidence to prove that stress can enhance the neuropathological changes associated with neurodegenerative diseases such as Alzheimer [4] or play an important role in the development of certain pathologies such as diabetes [5], as well as in the outcomes of surgical interventions [6] or clinical emergencies such as heart attacks or strokes [7]. Some problems such as apnoea, depression or psychotic episodes such as schizophrenia may also be associated with stress. One hypothesis currently undergoing validation even claims that stress plays a certain role in the appearance of multiple sclerosis.

With regard to their impact, the so-called mental disorders such as anxiety, depression, dementia and also stress are currently revealing themselves as being a very important factor in global health. It is estimated that by the year 2020 neuropsychological disorders will have overtaken infectious diseases on global health maps in terms of importance [8]. From a different point of view, psychiatric and neurological illnesses could increase their contribution to the worldwide burden of morbidity by almost 50% [8], which represents a rate of growth that is higher than that of cardiovascular diseases. Their social impact is also important: according to [9], six working days are lost every year in the USA due to illness, whereas the average is 25 as a result of anxiety, stress and related disorders, giving rise to losses estimated at 50,000 million dollars per year [10].

Nevertheless, and despite the growing awareness of its medical, social and economic importance, there is no effective tool available today that allows the objective measurement of the intensity with which stress affects the individual. There is still a great deal of uncertainty which makes it difficult to determine whether the disorder exists or not and sometimes to even appreciate whether the situation is beginning to wear down the subject's health or, in contrast, it is being successfully overcome.

The method presented in this paper describes an attempt to objectively quantify the psychological component of the level of stress by trying to quantitatively appraise how far a subject is from the situation that is considered socially and medically 'normal'. The importance of this type of objective measure is not limited to stress itself. On the one hand, it is known that stress can activate the pro-inflammatory pathways, which makes a direct contribution to the genesis of depressive processes. On the other hand, it seems clear that the population affected by a depressive disorder is more sensitive to stressors and presents more pronounced reactions to them [11], which highlights the relationship between inflammatory-immune processes and clinical depression.

## Subjects and methods

In order to achieve an objective, reliable and repeatable quantitative valuation of the level of stress, work has been carried out on a method that aims to 'calculate' it from the value of certain electrophysiological, biochemical and psychometric parameters, its development over time, the characteristics of the subject, and the type and characteristics of the stressor.

To cover the different types of stress, while attempting to gain information that can be useful regardless of the particular type of stressor, the method is structured around nine pilot studies conducted in different samples of individuals that involve different types of stress, as well as varying types and intensities of stressors or application times. Each of the pilots is a prospective observational study with a specific protocol that was approved by the corresponding Ethics Committee. A priori estimations indicated that a sample that could be used as a statistically significant indicator for each individual trial and that was powerful enough to make a contribution to the development of the joint algorithm must consist of no fewer than 30 individuals. Forty volunteer subjects were selected for each trial, all of whom fulfilled the required eligibility criteria, in the hope that the minimum figure would be reached despite the dropouts and those excluded due to errors.

Each trial consists in studying the action of a particular stressor, which could be real or induced artificially. In each of these sessions, which correspond to different states of the subject, the action of the stressor upon the electrophysiological, biochemical and psychometric variables established in the corresponding protocol was evaluated while at the same time the temporal sequences of their values were also collected.

In most of the trials the reference was taken as being the actual individual under conditions that could be considered his or her normal state. In the trials in which this is not possible, the reference used was a subject with the same characteristics, paired by age, sex, etc., but without the presence of the stressor.

The method implemented attempts to avoid the errors and uncertainty due to the type or form of measurement. Hence, homogeneous protocols were designed for the taking of measurements and processing of samples for the different trials. The electrophysiological measurements are performed using the same calibrated commercial equipment with CE certification following a single procedure for placing the sensors and for recording. Each of the biochemical variables (analytes) are determined in the same specialised laboratory that will process the samples from all the pilots that include the valuation of this analyte. The same considerations were applied to the analyses to be carried out on saliva and hair.

Any identifying details were removed so as to make the data anonymous before being introduced into a database that was common for all the trials [12]. The database, located in a server in Spain, can be accessed on the Internet by researchers and authorised personnel.

This database includes the raw data obtained in all the cases. Nevertheless, if it is considered advisable and common practice to apply some form of data processing – filtering, elimination of noise, extraction of specific parameters – a single processing procedure is used and the processed data are than labelled and go on to form part of the database. However, this does not mean that other formulas and processing techniques cannot be used, and may or may not be incorporated into the common database.

## Results

Based on the results of the bibliographic analysis performed, the following insights were taken into account: the mechanisms described in the general adaptation syndrome [1], studies on stress induced by different procedures such as the Stroop Test, arithmetic tasks and social phobia following the so-called Trier Social Stress Test, or hyperventilation [13], stress tests under real conditions in professionals [14,15], and results reported in animal models of stress. Consequently, different groups of variables were selected and the specifications and requirements of the systems of measurement to be used were defined.

### Electrophysiological parameters

Responses to stress are coordinated through the hypothalamic centres and the hypophysis, so that the vegetative nervous system and the endocrine system are activated in an integrated manner, although with different timings. The activation of the sympathetic nervous system thus produces vasoconstriction and an increase in blood pressure, dilatation of the pupils, an increase in cardiac response, sweating and other secretions, as well as dilatation of the bronchial tubes and an increase in glycaemia. At the same time, certain responses associated to the activation of the parasympathetic system also take place.

Traditionally, a lot of work has been carried out on characterising the possible biological correlates of emotional situations and stress, but in recent years interest has been focused on obtaining non-invasive measurements. One of the first studies that attempted to establish a relationship between psychophysiological factors and electrophysiological variables was that conducted by Jing et al [16]. In that study the authors establish that the psychophysiological factors defining the level of stress are heart rate (HR) and its variations, HRV (HR variability). These are both mediated by the autonomous nervous system (ANS) in its sympathetic and parasympathetic branches, which is reflected in the appearance of low-frequency (LF) and high-frequency (HF) components, depending on whether one or the other branch was predominant, respectively. Even earlier works provide support for the idea by reporting variations in the HRV due to the actions of psychological/cognitive stressors, such as mental arithmetic or public speaking. In general in all cases it has been observed that there is a reduction in the HRV, an increase in the LF band parallel to a reduction in the HF band, and/or an increase in the LF/HF quotient, thereby reflecting an increase in sympathetic stimulation and parasympathetic inhibition [17-19]. There are also reports of an increase in the respiratory rate, RR, and its variability, RRV [20], and even an alteration affecting the synchronisation between them [21] as a response to the action of a stressor.

In other studies a relationship has been established between stress and blood pressure (BP), blood volume pulse (BVP), the electrical characteristics of the skin (GSR – galvanic skin response), skin temperature (ST), brainwaves (EEG – electroencephalography), and the size of the pupil (PD – pupil diameter) [13,14,22,23]. Yet, due to the difficulties involved in measuring some of these factors in clinical conditions, most of the studies have only taken into account those that are easily accessible, thereby forsaking the precision of the study.

Later results reported by Barretto provided evidence that the simultaneous processing of four physiological signs BVP, GSR, PD and ST offer acceptable results regarding the differentiation between 'relaxed' and 'stressed' states [24]. In fact, the vast majority of studies reported follow the same pattern [13,14,25] and conclude that the values of these parameters vary in the presence of the action of a stressor, but without coming to any conclusion as far as distinguishing between different intensities is concerned. Moreover, the MIT Affective Computing Group [26] has conducted several studies and concludes that there is no single or standard model for classifying the levels of stress, and what we commonly call stress is in fact composed of a variety of different situations that share certain external manifestations.

The analysis performed is the rationale that provides support for selecting the physiological signals that will be used to derive the indices or parameters that will determine the level of stress, if our hypothesis is confirmed. The signals are the following: GSR, ST (in finger and cheek), RR (by means of a chest band and impedance), ECG, EMG (in orbital and trapezius muscles) and photoplethysmography, PPG, (in finger and temple). The ECG signal is used to derive the HR and the HRV, which will be analysed using techniques that make it possible to characterise the sympathetic and parasympathetic branches of the ANS. The variability of the cardiac pulse extracted from the PPG will also be analysed and compared with the HRV, which is expected to provide useful information about the mismatch between the two and on their relationship with pulse transit time, PTT.

Some signals, such as $pO_2$ and $pCO_2$, were precluded due to the difficulty involved in implementing them and others, such as thoracic bioimpedance, used in some of the studies cited, were also ruled out due to their lack of specificity. The signals are measured simultaneously with the ABP-10 module of the Medicom device (Medicom MTD Ltd.) [27]. The parameters related to heart rate are extracted from the ECG, mainly by the GTC of the Universidad de Zaragoza. The parameters related to skin impedance, pulse wave and temperature are mostly extracted at the GBIO (biomonitoring group) of the CNM-Universitat Autònoma de Barcelona. Likewise, the LST group at the Universidad Politécnica de Madrid will provide information, details and experience in relation to electromyography.

## Biochemical parameters

Articles referring to both animal and human experimentation report quick variations in blood glucocorticoid levels (cortisol in humans) due to the action of stressors [28]. Usual levels are reached again at varying speeds after the initial peak, even though the cause has not ceased and there are variations superimposed upon the process owing to the circadian rhythm [29]. The free fraction of cortisol spreads through saliva, reflecting the fraction of free cortisol while at the same time avoiding the effects of pulsatile release found in measures in serum. Moreover, it is independent of the salivary flow rate. In short, salivary determination is less invasive, less sensitive to pulsatile variations and facilitates the process of preparing, conserving and analysing samples.

Some studies recently reported in the literature also suggest that it could be useful to analyse cortisol levels in hair. The exact mechanism by which cortisol becomes incorporated within hair remains unknown [30], but trials carried out to date indicate that cortisol is stratified in hair and thus represents a record of its evolution over time. Each centimetre of hair shows the changes in cortisol over one month in the life of the individual.

Alpha-amylase is known to be a marker of the activity of the sympathetic nervous system in response to stress. Levels of α-amylase increase in response to physical and/or psychological stress with a maximum peak at 5-10 minutes' exposure to the stressor; normal values are then quickly re-established. Similarly to cortisol, the secretion of α-amylase has a circadian physiological pattern: its levels diminish in the first 30 minutes after waking and then progressively increase as the day goes on. Again, taking samples in saliva is recommended due to its being non-invasive, simple to extract and more independent from pulsatile secretion.

Copeptin, which is related to stress in an indirect way, will also be appraised, as it is a parameter capable of reflecting the changes in the circulation of vasopressin (AVP), another possible marker of stress, the big advantage being that it is quite stable both in circulation and ex-vivo. Levels of copeptin are sensitive to situations involving physical stress [31], but could also be sensitive to emotional stress [32].

It is a firmly established fact that the secretion of prolactin is strongly affected by stress. An increase in its levels as a response to different types of psychological stressors in humans has been reported and the intensity of the response depends on the intensity of the stress stimulus [33].

Since, as mentioned above, depression, stress, anxiety and fatigue could be part of the clinical expression of a peripheral inflammation, biomarkers of inflammation such as interleukin 6 (IL6) and tumour necrosis factor alpha (TNF-alpha) were included within the set of variables to be taken into account. In fact both biomarkers, IL6 and TNF-alpha, may be elevated in individuals with depressive disorders. Plasma levels of glucose and glycated haemoglobin are also taken into account as metabolic indicators.

Glucose, glycated haemoglobin, prolactin and copeptin are processed in the Biomedical Diagnosis Centre at the Hospital Clínic de Barcelona. Cortisol and α-amylase in saliva are processed in the laboratory of the Endocrinology and Radioimmunoanalysis Service of the Animal Physiology Unit of the Universitat Autònoma de Barcleona, and TNF-alpha and IL6 are processed in the Myeloid Cell Laboratory at the Biological Research Centre.

### Neuroimaging of the brain

Although changes associated with the action of different stressors have been observed in neuroimages of the brain (MRI, PET) [34], neuroimaging is not included in the pilot studies on the same level as the other sources of information due to the added cost and complexity that would be involved.

### Cognitive parameters

Reports have been published in the literature of the appearance of significant changes following exposure to stressors in cognitive or quantitative neurophysiological tests related to, for example, the speed and intensity of certain reflexes, the correct interpretation of cognitive stimuli and also the correct generation of the expected response to them [35, 36]. Some cognitive tests have been included in the protocols participated by healthy students, although they are not expected to represent an important contribution owing to the difficulty in establishing a reference with which to compare them.

### Psychometric tests

Psychometric tests are commonly accepted tools for measuring emotional tension focused on a particular disorder. The PSS, STAI and VASS were selected. All of them are validated, endorsed and very well documented by the medical world and have a tried and tested version in Spanish. They are the reference standard for helping to determine the level of stress reached by the subject in each situation.

The Perceived Stress Scale (PSS) measures the individual's overall degree of stress or the extent to which the subject rates situations in everyday life as stressing [37]. The Stress Visual Analogue Scale (VASS) [38] provides a measure of the subjectively perceived stress at a particular moment when faced with a specific situation. The State-Trait Anxiety Inventory (STAI) evaluates anxiety from two different points of view: STAI-s, the state of the subject in a particular moment, and STAI-t, which is the general tendency to respond to a stressing situation with an increased level of anxiety [39]. Later studies [40,41] show that the STAI is sensitive to the caregivers' stress and can therefore be used as an indicator of stress.

The Psychiatry Research Group of the Hospital Clínico de Zaragoza acted as consultants on the selection of the tests to be applied, as well as the way they should be administered and their later interpretation, and also verified the Spanish version in each case. The same team designed a test, administered in all the trials, which aims to evaluate the subjective effect of the stressor upon the subject. The experimental results obtained to date point towards a promising future for this new 'symptomatic stress scale'.

### Types of stress studied and ad hoc clinical trials

As mentioned above, the study presented in this article attempts to evaluate only psychological stress and/or the psychological consequences resulting from stress. In general, the multivariable biomarker that can be considered the objective of the study aims to provide a quantitative measure of 'how far' a subject is from the situation that is considered both socially and medically 'normal', from the psychological point of view, as regards the subject's baseline state or from the reference that has been taken.

In an attempt to abstract and elaborate the biomarker that allows us to measure different levels resulting from different situations, the study is intended to cover situations involving stress with different types of aetiologies, caused or triggered by different stressors. In general terms, we will distinguish between acute and chronic emotional stress and also psychological stress produced by a physical cause.

In order to induce and evaluate moderate emotional stress, one tool that is widely used and that has been standardised in experimental psychiatry is the Trier Social Stress Test or TSST, cited earlier [42]. To induce stress, the TSST focuses on the alteration produced by memorising a text and then reciting it in public. It is often complemented with an arithmetic test, also to be done in public.

It is important to note that, according to the opinion of experts and the literature consulted, it seems clear we cannot expect to find patterns of variation due to the acute emotional stress produced by a stressor based on 'social phobia' that are analogous to those resulting from the chronic stress suffered by a person acting as caregiver for a chronically ill relative, and for this reason they are considered independently in the two trials. The study was therefore designed as an intermediate case: the stress suffered by the parents of a child who is diagnosed with a condition requiring major surgery in the short term. The parents will be undergoing high-intensity acute emotional stress associated with the diagnosis which will last for some time, will become more intense at certain times and will presumably return to its normal state once the surgical intervention has been successfully completed.

Traumatic stress in the physical sense is represented by the responses of the body to major surgery in children or to a stroke which presented its first symptoms less than 3 hours before collecting the first data. In the first case, children were chosen in order to avoid as far as possible the conscious emotional burden that an adult would undoubtedly have in the same situation. Surgery to correct scoliosis or craniosynostosis was chosen in order to offset as much as possible the direct effects that medication could have on the variables to be measured.

In an attempt to find a bridge between acute emotional stress and physical traumatic stress, a 'heat stress' trial was specified, protocolised and implemented, in which healthy youngsters were put inside an environmental chamber. The cycle consists of one hour of adaptation, one hour ramp time until a temperature of 35 °C and a humidity of 50% are reached, one hour under these conditions, another one hour ramp time and another hour of adaptation. For the sake of comparison, for this trial we selected 50% of the youngsters who also collaborated in the social phobia trial, whereas the other 50% were participating for the first time.

A trial was also designed, protocolised and carried out in patients diagnosed for knee arthroplasty. The surgical protocol that is implemented at the Hospital Clínic de Barcelona requires these patients to participate in an 'empowering' session with the aim of lowering their levels of stress by providing them with detailed information about the possibilities, risks, post-operative functioning, ways to achieve a more effective recovery and anything else that may be worrying the patient. It has been proved that doing so improves clinical outcomes. The trial is conducted during the empowering session and is restricted, that is, the conditions under which the session is carried out and the average type of subjects mean that it is only possible to gather data about a subset of variables that are collected in the other trials, although this is done following exactly the same protocols.

A clinical trial was also designed to evaluate the acute emotional stress that is produced by the diagnosis and the indication of the need for the immediate hospitalisation and isolation of subjects who have previously been diagnosed with bipolar depression. In addition to its contribution to the general aim that is being pursued in the study, this pilot also seeks to document the fact that, as mentioned earlier, the population affected by a depressive disorder is more sensitive to stressors. Two types of reference populations were defined for this pilot study: subjects diagnosed with severe depression who do not need to be hospitalised and another population of healthy individuals with similar characteristics.

**Table I.** Clinical trials.

| | Setting | Institution |
|---|---|---|
| Chronic stress | Caregivers of chronic patients at home | Hospital Clínic de Barcelona |
| | Parents of children with major surgery (scoliosis and craniosynostosis) | Hospital Sant Joan de Déu |
| Acute emotional stress | Healthy students. Social and cognitive stress | UAB, Universidad de Zaragoza, Living lab. (UPM) |
| | Preparation knee arthroplasty | Hospital Clinic de Barcelona |
| | Virtual reality | Living lab. (UPM) |
| | Bipolar depressed patients | Hospital Clínico de Zaragoza |
| Traumatic stress | Children subjected to major surgery (scoliosis and craniosynostosis) | Hospital Sant Joan de Déu |
| | Stroke patients < 3 h | Hospital Clínic de Barcelona/Madrid |
| Heat stress | Healthy youngsters | Environmental chamber UAB |

UAB: Universitat Autònoma de Barcelona; UPM: Universidad Politécnica de Madrid.

The nine trials are shown in the Table I.

In each of the pilots, the variations in the selected parameters are analysed, correlations being established among the electrophysiological, biochemical and psychometric parameters of each subject, for each group and in each of the different states, whenever possible. Secondly, the results are compared with those obtained in the same pilot conducted in another centre. Lastly, attempts will be made to establish correlations among the different types of stress and/or to determine the conditions that allow them to be distinguished. The variables selected by type of trial are shown in Table II.

## Preliminary experimental results

Although the primary aim of this article is to describe the method followed in order to perform a quantitative evaluation of stress and its underlying cause rather than to disseminate the experimental results obtained, at the time of writing we already have some from trials in which the data collection phase has concluded.

In the case of social and cognitive stress in 40 healthy students, a statistically significant increase of nearly 53% was found in the STAI and VASS

**Table II.** List of parameters to be measured according to the type of stress.

| | | | Acute reaction to stress | Chronic reaction to stress | Reaction to traumatic stress |
|---|---|---|---|---|---|
| Physiological parameters | Skin temperature | | × | | × |
| | Skin conductance | | × | | × |
| | Electrocardiogram | | × | × | × |
| | Electromyography | | × | × | × |
| | Pulse wave | | × | × | × |
| | Respiratory rate | | × | × | × |
| | Intra-arterial pressure (catheter) | | | | × |
| Biochemical parameters | In blood | Prolactin | × | | × |
| | | TNF-α | | × | |
| | | Copeptin | × | × | × |
| | | Glucose | × | × | × |
| | In saliva | Free cortisol | × | × | × |
| | | α-amylase | × | × | × |
| | In hair | Cortisol | | × | |
| Psychometric parameters | STAI | | × | × | × |
| | Visual Analogical Scale | | × | × | × |
| | ES3 | | × | × | × |

STAI: State-Trait Anxiety Inventory; TNF-α: tumour necrosis factor alpha.

psychometric tests between the baseline state and that of stress induced by the TSST. A net increase in the concentration of cortisol in saliva was observed in the males of this same population, which together with the tests offers proof of an effective application of the stress stimulus.

In the study conducted on patients programmed for a knee arthroplasty, on the other hand, a mean decrease of 45% was seen in the level of stress after the empowerment session, according to the psychometric tests. The reduction is much greater –around 78%– in the group that pointed to surgery as the motive behind their stress, in contrast to decreases of only 17% in those who claimed that they were not stressed or those who were, but owing to reasons other than the surgical intervention. As a consequence of this differential effect, after the empowerment session no significant differences are observed in the level of stress among the patients who reported being anxious about the operation and those who were not. This proves that the session has a direct effect upon the stress caused by the prospect of undergoing surgery and therefore corroborates the efficacy of the empowerment session that was implemented.

At the time of writing, most of the trials have begun. In some of them the data collection stage has ended, the data thus obtained are now part of the database and work on their analysis has started. This means that, following the method described here, in these pilots the time sequences of data are being filtered, the parameters are being extracted and currently correlations among variables are being analysed two by two.

The next stage consists in comparing the results obtained in the same trials carried out in different centres: healthy students submitted to social and cognitive stress carried out at the Universitat Autònoma de Barcelona, Universidad de Zaragoza and Universidad Politécnica de Madrid. The comparison of results will continue using the same population with different types of stressors; this is the case of the students at the Universitat Autònoma de Barcelona and at the Universidad Politécnica de Madrid who were subjected, on the one hand, to social and cognitive stress and, on the other, to the heat stress trial or one involving virtual reality, respectively.

In the third stage, attempts will be made to accomplish, in each of the pilots, a multivariable classification based on the study of correlations in which more than two variables are involved. Lastly, cross-tab analyses will be performed among the different pilots with the aim of finding, documenting and accounting for similarities and discrepancies among them that may help to gain a better understanding of the relations between the different types of stress and the physiological bases corresponding to their different causations.

## Discussion

The literature that we had access to deals with the two major types of experimentation in animals and in humans. In general, animal experimentation consists in putting the animal in a situation that undoubtedly causes it stress and observing the variations that are produced in some of its vital signs, which are then compared with those of other similar individuals that were not subjected to the stress factors. In most of the literature consulted, the variables studied were biochemical ones taken from blood, but also include some measures of sympathetic activity (above all heart rate, blood pressure and body temperature) obtained by telemetric recordings.

Experimentation in humans, on the other hand, nearly always sets out from the same principle, i.e. a particular stimulus that is accepted as being stressing is considered and the variations that take place in some of the vital signs are studied by comparing them with the individual in his or her baseline state or with paired subjects who are not subjected to the stressor. A wide range of studies have been conducted in human experimentation but nearly all of them opt for focusing the work on a certain type of variable. One of the novelties of the study presented here is the data collection and simultaneous analysis of the variables belonging to all the different types and on the same time base.

In the study of the biochemical variables, the time windows in which the samples are collected are taken very much into account. The electrophysiological variables are also recorded with precision regarding the temporal relationship with the stressing factor, especially when this latter has differentiated phases.

The method followed in this study for taking measurements took special care to maintain both the same time bases for all the electrophysiological variables and the best possible synchronisation between the measurement of the electrophysiological variables, the taking of blood samples and the administration of the psychometric tests. The protocols are also designed to ensure that the same schedule is followed between the subject and the reference for taking blood or saliva samples.

For example, synchronising times to the order of a millisecond between certain stressors and skin temperature has allowed us to observe an extremely close relation between this latter and the changes in the stress situation, thus allowing us to conclude that temperature follows emotional stress exactly. In any case, the preliminary results obtained show that synchronisation is a key factor when it comes to visualising and obtaining high rates of correlation between the data recorded and the psychometric results.

The administration of psychometric tests in parallel to the rest of the measures is also an important aspect presented in this study and which it seems might lead to some interesting conclusions. Furthermore, the preliminary results obtained allow us to establish a statistically significant correlation between the score on the tests and the values of some of the parameters that are measured.

The general method employed and the preliminary results obtained show that we are on the right path towards defining and validating a multivariable marker for the quantification of stress levels. The results to date also suggest that the method can be applied to mental disorders. If the foregoing were confirmed, it would mean defining and validating different multivariable markers that would allow us to perform a quantitative appraisal of the 'state', level of severity or tendencies in the development of mental disorders such as anxiety, dementia, Alzheimer's disease or schizophrenia.

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## Proyecto ES3: intentando la cuantificación y medida del nivel de estrés

**Introducción.** La Organización Mundial de la Salud ha calificado al estrés de 'epidemia mundial', debido a su cada vez mayor incidencia en la salud. El trabajo que se presenta en este artículo representa un intento de cuantificar objetivamente el nivel de estrés.

**Objetivo.** La metodología desarrollada tiene como objetivo medir cuán lejos o cuán cerca se encuentra un sujeto de una situación considerada médica y socialmente como 'normal'.

**Sujetos y métodos.** Se ha realizado un estudio bibliográfico de la fisiopatología del estrés y sus métodos de estudio, en experimentación animal y en humanos. Se han puesto en marcha nueve estudios prospectivos observacionales con distintas tipologías de sujetos y estresores que cubren las diferentes tipologías de estrés.

**Resultados.** Como resultado del estudio bibliográfico, se han identificado las distintas tipologías de estrés, los indicadores que describen resultados significativos, los tests psicométricos y los 'agentes estresantes' bien documentados. Este material ha permitido diseñar la metodología general y el detalle de los nueve ensayos clínicos. Los resultados preliminares obtenidos en algunos de los estudios han servido para validar los indicadores, así como la eficacia de las técnicas utilizadas experimentalmente para disminuir el estrés o para producirlo.

**Conclusiones.** Los resultados preliminares obtenidos en los ensayos experimentales muestran que se está en el camino correcto hacia la definición y validación de marcadores multivariable para la cuantificación de los niveles de estrés, y sugieren que la metodología puede ser aplicada de forma similar al estudio de trastornos mentales.

**Palabras clave.** Biomarcador multivariable. Cuantificación del nivel de estrés. Estrés crónico. Estrés emocional. Estrés traumático. Parámetros electrofisiológicos, bioquímicos y psicométricos.