Development of a prototype for the analysis of multiple responses of the autonomic nervous system

Development of a Prototype for the Analysis of Multiple Responses of the Autonomic Nervous System

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Abstract

A modular hardware prototype is developed for the noninvasive acquisition, processing and transmission of biological signals to analyze the autonomic nervous system (ANS) in synchrony with the video recording of the pupil. The implementation includes 1) two noninvasive sensors, a pulse oximeter and an electrodermal activity sensor, 2) a module able to collect the information and send it to the PC via USB and 3) a graphic user interface (GUI) for visualization, synchronization and data saving. A series of experimental tests were performed to investigate the effect of different stimulations: light, dental occlusion, transcutaneous electrical nerve stimulation (TENS) and mental efforts. They indicate the reliability of the system and the importance of the joint detection of more signals for discriminating different states of the ANS. Specifically, heart rate, Galvanic response and pupil size were compared, showing some coherence in their oscillations and different discrimination capability in different conditions. Their joint detection is thus important for discriminating different states of the ANS.

Keywords: Pulse oximetry, Electrodermal Activity (EDA), Galvanic Skin Response (GSR), Pupillogram, Autonomous Nervous System (ANS), Transcutaneous Electrical Nerve Stimulation (TENS), Embedded systems.

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1. Introduction

The autonomic nervous system (ANS) is the portion of the central nervous system that controls unconscious activities, such as visceral functions and homeostasis. It is divided into two main branches, the sympathetic and the parasympathetic, the first promoting the activation of a physiological response and the other inhibiting it. The ANS is profoundly affected by emotions and somatosensory inputs and plays an important role in pain and stress modulation and perception.

Autonomic testing finds application in the clinical assessment of neurological disorders, particularly those affecting predominantly small nerve fibres [1]. Many studies have been devoted to the quantitative assessment of the ANS response, dating back to more than 3 decades [2][3][4]. However, most of the literature takes into account just one of the numerous physiological systems that are affected by the ANS in turn.

Very frequently the works that have dealt with ANS in different disorders related to its dysfunction have focused on cardio-circulatory parameters [5]. However, there are many other potential peripheral effects of ANS, that have been largely overlooked. For example, a physiological system related to the ANS can be investigated measuring skin conductance, reflecting the sweating of the sweat glands [6]. Moreover, pupil is strongly affected by the ANS [7]. The study of mydriasis and myosis (i.e., the dilation and contraction of pupil) is usually done in a different context (vestibular system). However, recent studies have indicated the possibility of characterizing the condition of the ANS in healthy or pathological conditions, by analyzing the nonlinear pupil oscillations [8][9][10][11][12][13]. In particular, the study of pupillary dynamics was considered useful for evaluating the arousal state during mental effort due to cognitive tasks [14][15] and in relation to the involvement of reward systems [16]. Furthermore, it has been suggested that the use of different ANS parameters, including pupil size, may be useful for better characterizing and quantifying the emotional component linked to the autonomous response [17]. Moreover, further evidence was provided that pupil can be used to evaluate the state of emotional arousal as well as the generic activation of the ANS [18].

One of the limitations of the study of various ANS responses is the use of different instruments for the analysis of different signals. However, it was argued that pupillogram could provide useful information for the study of the arousal state and that it would receive a valid contribution from the association with signals already used for this purpose, such as skin conductance and electrocortical activity [19]. These observations would involve an important expansion of the combined and synchronous study of various parameters associated with pupillography to aspects not only of pathology, but also related to the emotional / affective state. Thus, important outcomes could be expected both for clinical patients and for any study involving the psychic assessment of the arousal state.

Only recently, more reactions of the ANS have been investigated simultaneously [20][21]. The signals that have been often analyzed are the cardiac pulse and the variations of skin conductance. In this study, we are interested in the investigation of those signals jointly with the pupil response. A modular hardware prototype is developed for the noninvasive acquisition, processing and transmission of biological signals and is synchronized with a commercial system for pupil investigation. Our present implementation includes:

- two noninvasive sensors, a pulse oximeter and an electrodermal activity sensor (both often used in the study of the responses of ANS [22][23][24]);
- a module able to collect the information and send it to the PC via USB;
- a graphic user interface (GUI) for visualization and data saving.

Pulse oximetry and skin conductivity are acquired in synchrony with the video recording of the patient's dilation and constriction of the pupil (acquired by a commercial system [25]). The system was developed keeping low the production cost and energy consumption. It was tested in experiments from healthy subjects under different stimulations: light, dental occlusion, transcutaneous electrical nerve stimulation (TENS) and computational task.

2. Design and Implementation

The recording system is shown in Figure 1. Two sensors, described below, are developed and are used together with a commercial system for pupil investigation [25].

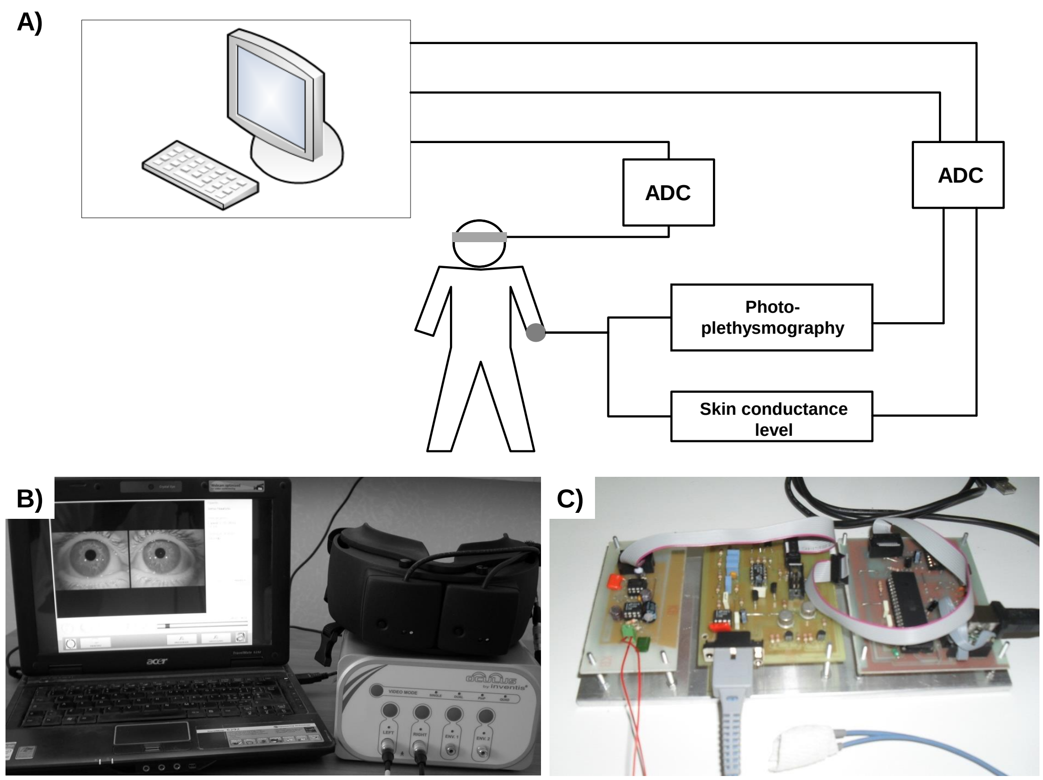

Figure 1: A) General scheme of the instrumentation used. B) Commercial system for pupil investigation [25]. C) Acquisition system recording photoplethysmogram and skin conductivity level.

2.1. Design of the sensors

2.1.1. Pulse oximeter.

The first sensor measures the absorption of red and infrared lights that pass through a patient's finger by light sensors. The light is generated with 2 LEDs that are controlled alternately. A photodiode receives both ambient and modulated light from the LED and generates a current that is related to the oxygen saturation and the cardiac frequency [26][27][28]. The pulse oximetry signal (photoplethysmogram - PPG) has a frequency range between 1 and 10 Hz. The signal is affected by the line interference (50 Hz) and the neon lamps that are usually used in offices and laboratories (giving an interference at 100 Hz), which were removed by notch filters; moreover, a high pass filter attenuated motion artifacts (notice that high performance professional oximeters are stable to ambient light and motion artifacts [29], but our low cost system had to rely on simple solutions). For the actual implementation, the sampling frequency of the PPG is 220 Hz. The circuit design is shown in Figure 2A and B.

2.1.2. Skin conductivity level sensor.

There are two skin resistance responses due to the electrodermal activity (EDA): the tonic and the phasic levels. The tonic level is the absolute level of resistance at a given moment in the absence of a measurable phasic response and is referred to as Skin Conductance Level (SCL). The phasic level or Skin Conductance Response (SCR) is superimposed on the tonic level and corresponds to the response to stimuli. The sum of the tonic and phasic response is the Galvanic Skin Response (GSR).

The skin conductance is obtained by measuring the current flow through the skin in response to a constant applied voltage. The circuit design is shown in Figure 2C. For bipolar recordings, 0.5 V is recommended [30]. For a resolution of 0.01 μS (the minimum variation of the SCR [31][32]), the minimum current to be amplified is of 5 nA. The maximum input current of the amplifier is 15 μA (maximum SCR and SCL), therefore the transimpedance of the circuit must be of 333 kΩ. This circuit has two independent outputs that allow the analysis of each component separately. The sampling frequency is 170 Hz.

2.2. Digital processing of the signal and transmission

The information coming from the analogue sensors was converted into digital form, acquired, pre-processed and transmitted via USB to a PC. The

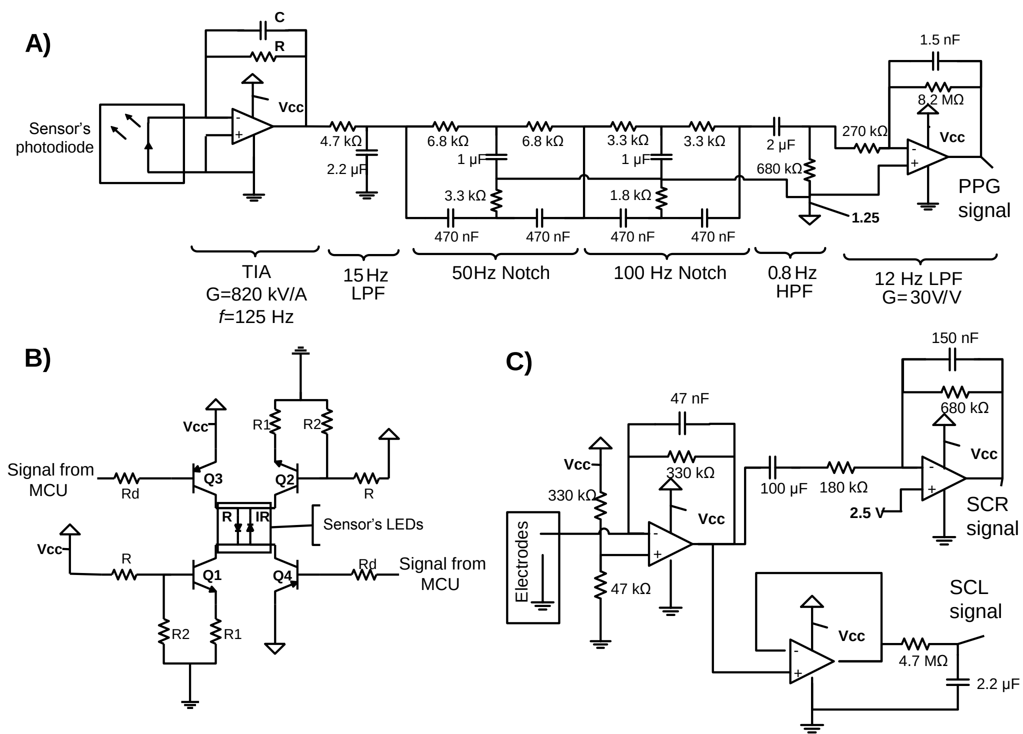

Figure 2: A) Schematic of the photoplethysmograph. B) Schematic of the LEDs driver. The signal from the microcontroller unit (MCU) controls which of the 2 LEDs is ON. C) Schematic of the skin conductance sensor. The 2 outputs are for the Skin Conductance Response (SCR) and the Skin Conductance Level (SCL).

microcontroller Atmel ATmega 16 accomplished these tasks [33][34]. Such a microcontroller was chosen as it is cheap and it has a high speed, a small code size and an analog multiplier (which can be used to implement digital filters). Its most important characteristics are listed in Table 1. The connection of the microcontroller to the system is shown in Figure 3. Notice that the internal 10 bit ADC was used for sampling the skin conductance, whereas an external 16 bits analog-to-digital converter (ADC) was included to sample the PPG (the considered external ADC is AD7715, which is a 16-bit Sigma-Delta ADC that can be interfaced with microcontrollers using the Serial Peripheral Interface, SPI).

The red and infrared lights for the pulse oximetry sensor must be ON in different cycles: the signal period was 1 ms, with a duty cycle of 0.25 and the phase between the two lights was 0.5 ms. The state of the LEDs was controlled using timer interrupt. An average filter (of 5 samples for pulse oximetry and 20 samples for the Galvanic skin response) was implemented in the microcontroller to remove experimental noise from the recorded signals (the sampling frequency was about 5 kHz, so that there was sufficient time to process the acquired data to produce a low pass filtered sample to be acquired). The filtered samples were then transmitted to the PC with the USART, which was interfaced with a USB transceiver.

Parameter	Value
Maximum Speed	16 MHz
Operating voltage	2.7 V - 5.5 V
Power consumption	Active: 1.1 mA
	Idle: 0.35 mA
	Power-down: $< 1\ \mu$A
Flash Memory	16 KB
EEPROM	512 B
Internal SRAM	1 KB
ADC Channels	8
ADC resolution	10 bits
ADC speed (max. resolution)	200 ksps
Timers max. resolution	16 bits
Serial communication	USART
	SPI
	Two-wire Serial

Table 1: General properties of the Atmega16 [33].

To manage the data, a software interface was developed in Visual Basic,

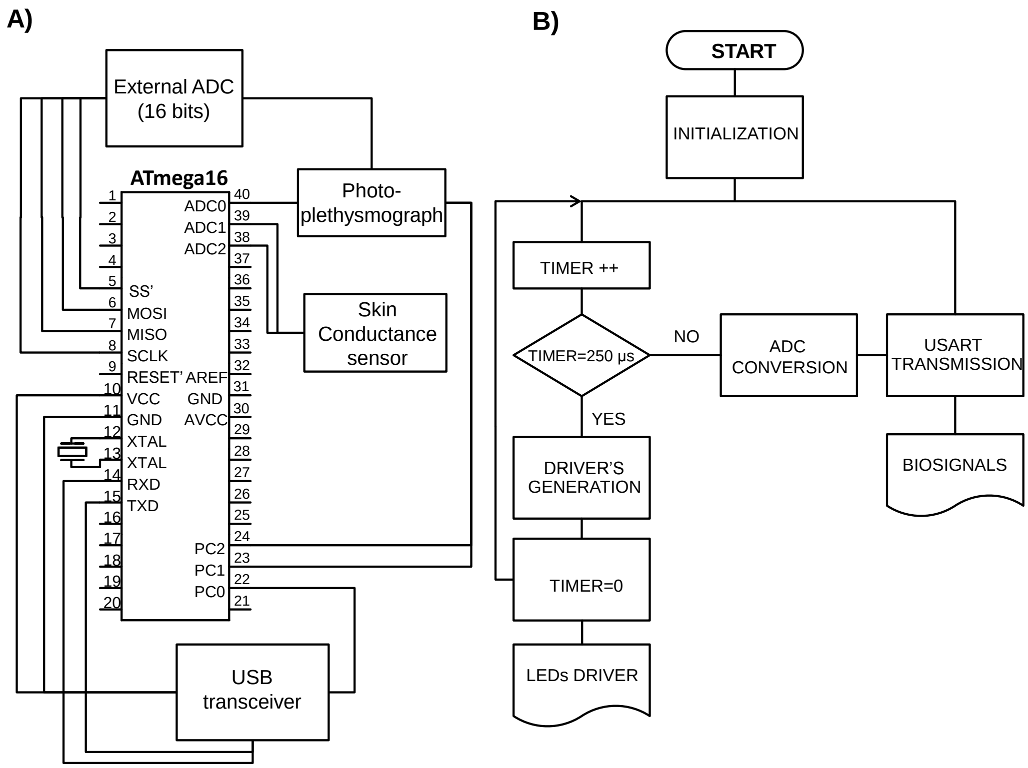

Figure 3: A) General schematic of the digital processing unit. B) Microcontroller general flow diagram.

with the following main functions.

- Registration of patient's information. The user should also be able to import the information from the database of pupillograms.
- Visualization of the signals. The charts update constantly to show the signals while they are being acquired.
- Storage of the signals. The information was stored in a file. This file contains the patient's information, the starting time of the acquisition, the time of each sample and the samples of the signals.

3. Experimental tests

3.1. Signal acquisition

3.1.1. Instrumentation.

Images of the pupils were acquired by the Oculus system (Inventis srl, Padova, Italy), using two infrared CCD cameras (resolution 720x576 pixels, 256 grey levels) mounted on a light helmet (1.5 kg), with sampling frequency of 25 frame/s. The eyes were illuminated with an infrared diode with 880 nm of wave-length; moreover, during experiments on pupil dynamics under constant light conditions, illumination was provided by a yellow-green LED with 740 nm of wave-length.

Pulse oximetry and variations of sweat were monitored with the system described in the previous sections, considering the PPG and the GSR time series.

3.1.2. Synchronization of the signals.

The software of the considered system for pupil investigation [25] does not allow any kind of modifications or access. Therefore, the synchronization of the signals was done by registering the precise moment in which the system started recording. The signals were synchronized with a resolution of 10 ms. Given that the sampling period of the pupillogram was 40 ms, this resolution is enough.

3.1.3. Experimental set up.

The subjects were sitting in a high-back chair. The environment was kept at a constant temperature of 21^{o}C. Visual predominance was determined. The acquisition system was then connected to the patient's non dominant hand. The helmet was applied and was maintained until the end of the recording. This phase took about 4 minutes.

The correct procedure and execution of tests was first explained to the subjects. Then, they were asked for brief tests to make sure that the instructions were well understood. This phase took about 2 minutes.

Two operators worked within the experimental set. The first took care of the subject (pretest and test instructions, helmet handling, check of the correctness of execution), the second controlled hardware and software.

3.1.4. Experiments.

One minute long acquisitions were obtained (after approval by the Internal Review Board of Politecnico di Torino) from 8 young, healthy subjects (age 25.1 ± 1.1 years; 6 females, 2 males) in different stationary conditions (separated by 5 minutes rest), which require a different involvement of the sympathetic and of the parasympathetic control: neutral position of the jaw (rest position: RP) and habitual dental occlusion (HDO)[1], in light or darkness condition; moreover, a test was performed during a computational task, which was assumed to induce a detectable stress of the subject. These 5 conditions (RP in light and darkness, HDO in light and darkness and computational task in darkness) were performed before, during and after the application of low-frequency TENS (5 minutes duration), which was expected to induce relaxation.

3.2. Signal processing and results

3.2.1. Pre-processing of recorded data.

Pupillometric recordings were processed through the algorithm of strongly connected components [35] to measure frame by frame the area of the pupil, expressed as the number of pixels covering it. The area of the pupil was then low pass filtered under 2 Hz (non-causal, zero-phase, Butterworth filter of order 2).

The local maxima in the PPG were used to identify the heartbeats, from which the heart rate (HR) was estimated. The GSR was low pass filtered under 2 Hz.

The mean and standard deviation (STD) were computed for the three following signals: pupillogram, HR and GSR. Moreover, the linear trend was estimated as a basic indicator of the evolution in time of the signals (the trend was defined as the slope of the interpolation line of the data after scaling the time to range between 0 and 1 and normalizing the time series to have zero mean and unit STD). These indexes were used as descriptors of the signals in the different experimental conditions. The two-sided Wilcoxon signed rank test (considering paired data) was then applied to investigate differences of each of the indexes in specific pairs of conditions of interest, after pooling

[1] During dental occlusion, the effect of muscle fatigue and the massive involvement of the autonomic system were excluded by avoiding prolonged teeth clenching. Subjects were asked to swallow and then to contact the teeth lightly without clenching. Attention was paid to check the activity of mimic muscles.

data: RP in darkness compared to the computational task, RP compared to HDO (in darkness), pre-TENS compared to TENS or post-TENS conditions. The significance level was set to $p < 0.05$.

3.2.2. Results.

Examples of recorded signals are shown in Figure 4. The pupil shows an irregular oscillatory behavior, as also the HR. There is a decreasing trend in both HR and GSR, as if the subject was relaxing. The figure shows also the spectral coherence of the pupillogram and the HR at frequencies lower than 1 Hz. The two signals were found to be coherent in subjects under control breathing conditions, where a respiratory component was visible in both the pupillogram and the HR [7]. Here, the considered normal breathing and the short acquisitions resulted in significant coherence (over 0.5) only in a few subjects and conditions.

The significance of the differences of indexes extracted from the signals recorded in different conditions is shown in Table 2. Different indexes have a greater discriminatory value comparing different conditions:

- an index from the HR showed the maximal significance (i.e., minimum p value) in discriminating computational task and RP in darkness (which can be considered as a rest state);
- indexes estimated from the pupillogram had the maximal significance in distinguishing light and darkness, RP and HDO, or pre-TENS and TENS conditions;
- indexes extracted from GSR were the most statistically different in the conditions pre-TENS versus post-TENS and TENS versus post-TENS.

Specifically, HR and mean pupil size increased when comparing computational task with RP in darkness (due to the mental stress induced by the task). The subject started sweating during the computational task, as indicated by the high positive trend of GSR. Pupil was the only system showing significant differences between HDO and RP: pupil size increased as a result of HDO stimulation [8][9][10]. Moreover, it was the only system showing significant differences comparing light and darkness conditions (obviously, increasing the diameter in darkness).

Pupil also indicated the relaxation induced by TENS (there is a significant reduction of pupil size during and after the application of TENS). On the

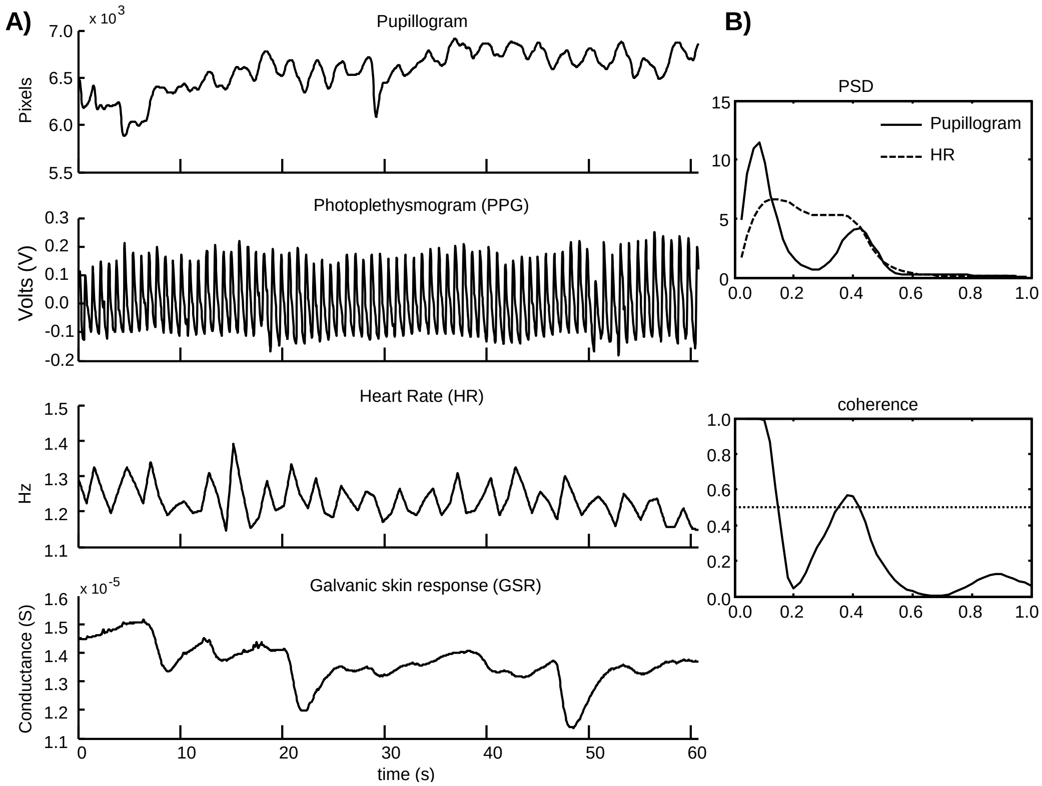

Figure 4: A) Example of data (pupillogram, PPG from which the HR is obtained, GSR), with the subject at rest position of the jaw in darkness. Power spectrum densities (PSD) of pupillogram and heart rate (using Welch's overlapped segment averaging estimator, considering 8 segments with 50% overlap) and magnitude squared coherence.

other hand, GSR increased during and after TENS, with respect to the pre-TENS condition. Possibly, this was due to an accumulation of sweat during the experiment, as the sensors were kept fixed for all its duration.

4. Discussion

The ANS controls many different visceral functions, including heart rate, perspiration, digestion, salivation, respiratory rate, pupillary dynamics, micturition (urination), sexual arousal, breathing and swallowing. The joint acquisition of different autonomic responses could be useful for a deeper insight into ANS physiology and pathology. For example, the study of the autonomic response is important in the following situations [36]:

- sympathetic and parasympathetic lesions after surgical procedures;
- drug's collateral effects;
- diagnosis and follow up of ANS diseases;
- poisoning;
- involuntary reactions of the patient.

The diagnosis in most of these situations is currently vague [37][38][39]. The joint investigation of different autonomic responses could help in clarifying the complex dynamics of the ANS in such conditions. One of the problems when designing an experimental setup is being constrained by the functionalities of commercial systems, which are usually closed and allow only specific protocols. Here, we were interested in investigating synchronously the joint responses of the heart (i.e., the cardiac pulse), the skin (i.e., sweat production) and pupil. Despite the interest in the study of pupillary dynamics, at present there are no tools that are able to simultaneously obtain the acquisition and processing of pupillary dynamics and those of other systems such as the cardiovascular (e.g., through pulse oximetry) and sudorific system (through skin conductivity). Being able to expand the analysis to several systems in a synchronous way would allow a better knowledge of the coupling pathways between somatosensory and affective / emotional needs and responses of the whole of the ANS in physiological and/or pathological conditions, probably indicating the activation of different autonomous circuits in case of different conditions.

In this work, we have designed an experimental setup able to record in synchrony the video of pupil (done with a commercial system [25]) and different responses of the ANS. A modular sensor network was designed and implemented to acquire, process and transmit via USB some biomedical signals reflecting the state of the ANS. Two sensors have been included in the prototype, for pulse oximetry and skin conductivity. However, it is feasible for being extended to include more body sensors, miniaturized and embedded in a portable system. This could be important for future extension of the work, as body sensor networks are finding many applications in the continuous monitoring of sensitive people [40][41][42][43]. Indeed, many different sensors are available to monitor physiological data and could be included: accelerometer, blood glucose sensor, electrodes for bioelectric signals, blood pressure sensor, gyroscope, carbon dioxide gas sensor, etc. [44]. The developed system, even if it is only a prototype, provided robust estimations of the electrodermal activity and of the pulse oximetry, which was then processed to investigate the heart inter-beat interval. Note that the PPG is immune to electrical artifacts, which could be observed on the electrocardiogram during TENS application (however, it is less precise in detecting the HR, as it also depends on the pulse wave velocity). Thus, our system is adequate for the study of the ANS response to TENS reflected in the HR.

Preliminary experimental tests are shown. Even if we considered only short recordings, weak ANS stimulations (dental occlusion, light, TENS and a computational task) in healthy subjects and we extracted simple indexes (mean, STD and trend), we have shown statistically significant variations of at least an index in each pair of conditions. This indicates that the overall information provided by the joint recordings, not just that of each individual signal, should be used for the discrimination of the ANS responses in the different considered conditions. The results are in line with our expectations: HDO and computation elicit the sympathetic response (which should result in HR and GSR increasing and pupil dilation), TENS induces relaxation (determining a decrease of HR, GSR and pupil size). Pupil appeared to be the most sensible system, as it reflected even the small stimuli given by HDO or TENS. HR showed significant variations only in a few conditions (e.g., mean HR was significantly increased only by the computation task). GSR showed an important trend during computation, but it was prone to accumulation effects (with an average increase along the experiment, in spite of the application of TENS).

Other tests and advanced processing techniques could provide specific indications in physiology or pathology, in future joint acquisitions of different responses of the ANS. Additional measures to guarantee reproducibility of the tests need to be developed, such as careful preparation in order to stabilize hemodynamic parameters. Moreover, additional sensors or stimulation signals could also be included in the proposed device, due to its modular architecture.

References

[1] R. Freeman, M. Chapleau, Chapter 7 - testing the autonomic nervous system, Editor(s): Gérard Said, Christian Krarup, Handbook of Clinical Neurology 115 (2013) 115–136.

[2] A. Loewy, M. Spyer, Central regulation of autonomic functions, New York, United States: Oxford University Press (1990).

[3] C. Collet, E. Vernet-Maury, G. Delhomme, A. Dittmar, Autonomic nervous system response patterns to basic emotions, Journal of the Autonomic Nervous System 62 (1997) 45–57.

[4] D. C. Fowles, M. Christie, R. Edelberg, W. Grings, D. Lykken, P. Venables, Publication recommendations for electrodermal measurements, The Society for Psychophysiological Research, Inc. 18 (1981) 232–239.

[5] C. Lombardi, M. Pengo, G. Parati, Obstructive sleep apnea syndrome and autonomic dysfunction, Auton Neurosci. 221 (2019) 102563.

[6] M. Kobayashi, N. Tomioka, Y. Ushiyama, T. Ohhashi, Arithmetic calculation, deep inspiration or handgrip exercise-mediated pre-operational active palmar sweating responses in humans, Auton Neurosci. 104 (1) (2003) 58–65.

[7] G. Calcagnini, F. Censi, S. Lino, S. Cerutti, Spontaneous fluctuations of human pupil reflect central autonomic rhythms, Journal of Methods of Information in Medicine 39 (2000) 142–145.

[8] L. Mesin, A. Monaco, R. Cattaneo, Investigation of nonlinear pupil dynamics by recurrence quantification analysis, BioMed Research International 420509 (2013).

[9] L. Mesin, R. Cattaneo, A. Monaco, E. Pasero, Pupillometric study of the dysregulation of the autonomous nervous system by svm networks, Editors: R.J. Howlett, Lakhmi C. Jain, Smart Innovation, Systems and Technologies, Springer (2013) 107–116.

[10] A. Monaco, R. Cattaneo, L. Mesin, I. Ciarrocchi, F. Sgolastra, Dysregulation of the autonomous nervous system in patients with temporomandibular disorder: a pupillometric study, Plos One 7 (9) (2012) e45424.

[11] A. Monaco, R. Cattaneo, L. Mesin, E. Fiorucci, D. Pietropaoli, Evaluation of autonomic nervous system in sleep apnea patients using pupillometry under occlusal stress: a pilot study, Cranio 32 (2) (2014) 139–147.

[12] F. Onorati, L. Mainardi, F. Sirca, V. Russo, R. Barbieri, Nonlinear analysis of pupillary dynamics, Biomedical Engineering / Biomedizinische Technik 61 (1) (2016) 95–106.

[13] A. Venkata Sivakumar, M. Kalburgi-Narayana, M. Kuppusamy, P. Ramaswamy, S. Bachali, Computerized dynamic pupillometry as a screening tool for evaluation of autonomic activity, Neurophysiologie Clinique 50 (5) (2020) 321–329.

[14] S. Aminihajibashi, T. Hagen, M. Foldal, B. Laeng, E. T., Individual differences in resting-state pupil size: Evidence for association between working memory capacity and pupil size variability, Int J Psychophysiol. 140 (2019) 1–7.

[15] S. Jainta, T. Baccino, Analyzing the pupil response due to increased cognitive demand: an independent component analysis study, Int J Psychophysiol. 77 (1) (2010) 1–7.

[16] K. Fröber, F. Pittino, G. Dreisbach, How sequential changes in reward expectation modulate cognitive control: Pupillometry as a tool to monitor dynamic changes in reward expectation, Int J Psychophysiol. 148 (2020) 35–49.

[17] I. Lee, S. Yoon, S. Lee, H. Lee, H. Park, C. Wallraven, Y. Chae, An amplification of feedback from facial muscles strengthened sympathetic activations to emotional facial cues, Auton Neurosci. 179 (1-2) (2013) 37–42.

[18] M. Bradley, L. Miccoli, M. Escrig, P. Lang, The pupil as a measure of emotional arousal and autonomic activation, Psychophysiology 45 (2008) 602–607.

[19] C. Kelbsch, T. Strasser, Y. Chen, B. Feigl, P. Gamlin, R. Kardon, T. Peters, K. Roecklein, S. Steinhauer, E. Szabadi, A. Zele, H. Wilhelm, B. Wilhelm, Standards in pupillography, Front. Neurol. 10 (2019) 129.

[20] M. Strauss, Handwave: Design and manufacture of a wearable wireless skin conductance sensor and housing, Massachusetts Institute of Technology. Department of Mechanical Engineering (2005).

[21] L. de Jesus, J. Rosana, M. Tristao, H. Storm, A. da Rocha, D. Campos, Heart rate, oxygen saturation, and skin conductance: a comparison study of acute pain in brazilian newborns, 33rd Annual International Conference of the IEEE EMBS. Boston, Massachusetts (2011).

[22] D. Li, H. Zhao, S. Dou, A new signal decomposition to estimate breathing rate and heart rate from photoplethysmography signal, Biomedical Signal Processing and Control 19 (2015) 89 – 95.

[23] S. Ghiasi, A. Greco, R. Barbieri, E. Scilingo, G. Valenza, Assessing autonomic function from electrodermal activity and heart rate variability during cold-pressor test and emotional challenge, Sci Rep. 10 (1) (2020) 5406.

[24] K. Khalfallah, H. Ayoub, J. Calvet, X. Neveu, P. Brunswick, S. Griveau, V. Lair, M. Cassir, F. Bedioui, Noninvasive galvanic skin sensor for early diagnosis of sudomotor disfunction: application to diabetes, IEEE Sensors Journal 12 (3) (2012) 456–463.

[25] S. Amplifon, Oculus: Video-oculoscopio digitale., www.biomedica.amplifon.com (2012).

[26] E. Gil, M. Orini, R. Bailón, J. Vergara, L. Mainardi, P. Laguna, Photoplethysmography pulse rate variability as a surrogate measurement of heart rate variability during non-stationary conditions, Physiol. Meas. 31 (2010) 1271–1290.

[27] M. Nitzany, A. Babchenko, B. Khanokhy, D. Landau, The variability of the photoplethysmographic signal- a potential method for the evaluation of the autonomic nervous system, Physiol. Meas. 19 (1998) 93–102.

[28] E. Rodrigues, R. Godina, C. Cabrita, J. Catalao, Experimental low cost reflective type oximeter for wearable health systems, Biomedical Signal Processing and Control 31 (2017) 419–433.

[29] O. Fathabadi, T. Gale, J. Olivier, P. Dargaville, Automated control of inspired oxygen for preterm infants: What we have and what we need, Biomedical Signal Processing and Control 28 (2016) 9 – 18.

[30] M. Dawson, A. Schell, D. Filion, The electrodermal system, Handbook of Psychophysiology. United States: Cambridge University Press (2000).

[31] T. Reinhardt, C. Schmahl, S. Wust, M. Bohus, Salivary cortisol, heart rate, electrodermal activity and subjective stress responses to the mannheim multicomponent stress test (mmst), Psychiatry Res. 198 (1) (2012) 106–111.

[32] M. Benedek, C. Kaernbach, A continuous measure of phasic electrodermal activity, Journal of Neuroscience Methods 190 (2010) 80–91.

[33] Atmel, 8-bit avr microcontroller with 16k bytes in-system programmable flash: Atmega16, www.atmel.com (2012).

[34] Atmel, Parametric search of avr microcontrollers, www.atmel.com (2012).

[35] R. Tarjan, Depth-first search and linear graph algorithms, Siam J Comput. 1 (1972) 146–160.

[36] T. Chelimsky, D. Robertson, G. Chelimsky, Disorders of the autonomic nervous system, Daroff: Bradley's Neurology in Clinical Practice. Philadelphia, United States: Saunders Elsevier (2012).

[37] H. Chen, A. Nackley, V. Miller, L. Diatchenko, W. Maixner, Multisystem dysregulation in painful temporomandibular disorders, J Pain 14 (2012) 983–996.

[38] W. Maixner, J. Greenspan, R. Dubner, E. Bair, F. Mulkey, V. Miller, C. Knott, G. Slade, R. Ohrbach, L. Diatchenko, F. R.B., Potential autonomic risk factors for chronic tmd: Descriptive data and empirically identified domains from the oppera case-control study, J Pain 12 (11 Suppl) (2011) T75–T91.

[39] A. Zygmunt, J. Stanczyk, Methods of evaluation of autonomic nervous system function, Arch Med Sci. 6 (2010) 11–18.

[40] U. Anliker, J. Ward, P. Lukowicz, G. Troster, F. Dolveck, M. Baer, F. Keita, E. Schenker, F. Catarsi, L. Coluccini, A. Belardinelli, D. Shklarski, M. Alon, E. Hirt, R. Schmid, M. Vuskovic, Amon: a wearable multiparameter medical monitoring and alert system, IEEE Trans Inf Technol Biomed. 8 (2004) 415–427.

[41] S. Lee, S. Jung, C. Lee, K. Jeong, G. Cho, S. Yoo, Wearable ecg monitoring system using conductive fabrics and active electrodes, Lecture Notes in Computer Science 5612 (2009) 778–783.

[42] H. Darwish, Wearable and implantable wireless sensor network solutions for healthcare monitoring, Sensors 12 (2012) 12375–12376.

[43] J. Choi, B. Beena Ahmed, R. Gutierrez-Osuna, Development and evaluation of an ambulatory stress monitor based on wearable sensors, IEEE Transactions On Information Technology In Biomedicine 16 (2) (2012) 279–286.

[44] M. Chen, S. Gonzalez, A. Vasilakos, H. Cao, V. Leung, Body area networks: A survey, Mobile Netw Appl 16 (2011) 171–193.

CONDITIONS	INDEXES with SIGNIFICANT DIFFERENCE
Computation versus **RP (D)**	*Mean HR* (p=0.0002) Comp.: $1.4(1.17, 2.04)$ – RP: 1.08(0.94,1.57) *STD of HR* (p=0.001) Comp.: $0.28(0.17, 0.54)$ – RP: $0.11(0.08, 0.38)$ *STD of GSR* (p=0.0015) Comp.: $1.25(0.54, 2.77)$ – RP: $0.2(0.1, 1.0)$ *Trend of GSR* (p=0.0078) Comp.: $3.03(0.27, 3.28)$ – RP: $-0.12(-1.88, 2.06)$ *Mean pupil area* (p=0.01) Comp.: $6952(6180, 9050)$ – RP: $6565(5839, 8072)$
HDO (D) versus **RP (D)**	*Mean pupil area* (p=0.0029) HDO: $6817(6318, 8636)$ – RP: $6565(5839, 8072)$ *Trend of pupil area* (p=0.012) HDO: $1.05(-0.62, 1.86)$ – RP: $2.10(1.55, 2.63)$ *STD of pupil area* (p=0.024) HDO: $177(142, 279)$ – RP: 267(202,308)
pre-TENS versus **TENS**	*Mean of pupil area* (p=0.009) pre: $6425(3806, 7391)$ – tens: $5818(3134, 7082)$ *STD of GSR* (p=0.031) pre: $0.11(0.05, 0.27)$ – tens: $0.21(0.72, 0.83)$ *STD of HR* (p=0.036) pre: $0.11(0.07, 0.24)$ – tens: $0.12(0.09, 0.57)$
TENS versus **post-TENS**	*Mean of GSR* (p=0.0001) tens: $12.91(9.09, 14.83)$ – post: $13.65(12.86, 16.61)$
pre-TENS versus **post-TENS**	*Mean of GSR* (p=0.006) pre: $12.88(10.88, 13.68)$ – post: $13.65(12.73, 16.61)$ *Mean area of pupil* (p=0.012) pre: $6425(3806, 7392)$ – post: $5823(3151, 6874)$ *STD of HR* (p=0.022) pre: $0.12(0.08, 0.25)$ – post: $0.16(0.08, 0.35)$
Light versus **Darkness**	*Mean area of pupil* (p<<0.001) L: $2987(2545, 3981)$ – D: $7312(6613, 9059)$ *STD of pupil* (p<<0.001) L: $417(333, 539)$ – D: $220(161, 283)$ *Trend of pupil* (p=0.014) L: $0.23(-0.79, 1.29)$ – D: $1.70(0.21, 2.32)$

Table 2: Statistical analysis of the data using Wilcoxon sign rank test. Mean, standard deviation and trend of data were considered (L: light; D: darkness). Median and quartiles of the indexes showing significant differences (p<0.05) are reported (in order of increasing p). Pupil size is indicated in pixels, HR in Hz, GSR in μS; their trends are measured in arbitrary units (they were computed on normalized data and time).