Original Article

PORTO BIOMEDICAL JOURNAL
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# Assessing response to stressful emotions: a controlled crossover study using pupillometry

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## Abstract

**Background:** Fear and horror induce autonomic protective responses, acting as "survival intelligence." Pupillometry is an innovative method that captures real-time autonomic nervous system reactions to stress.

**Objective:** To evaluate the feasibility of pupillometry to assess the acute response to a passive real-life stressor—viewing a truthful war scene.

**Methods:** Thirteen medical students (10 women) with an average age of 20.4 years were enrolled in a nonrandomized controlled crossover trial. Selected clips from two different audiovisual stimuli (M1: *Saving Private Ryan* as a fear and horror inducer and M2: *Life Is Beautiful* as a control) were watched for 15 minutes, separated by a washout period of 48–72 hours. The differences in pupillometry parameters between the exposure movie and the assessment time (T0 and T1 for M1 and T0 and T1 for M2) were evaluated using a Wilcoxon test. The Wilcoxon test was also used to assess the difference between M1 and M2 within each assessment time point (T0 and T1).

**Results:** A significant difference in response to acute fear and horror-induced stress was observed in pupillometry parameters {baseline [6.90 (5.95; 7.40) vs. 6.60 (5.55; 7.10), $P = 0.030$] and final pupil diameter [4.50 (3.90; 5.20) vs. 4.10 (3.50; 4.60), $P = 0.012$]} between M1 and M2 in T1, suggesting the acute increase in sympathetic parameters. Although not significant, there was also a difference in pupillometry parameters (final pupil diameter [$P = 0.060$], average constriction velocity [$P = 0.059$]) after watching M1 compared with T0.

**Conclusion:** Our proof-of-concept study suggests that pupillometry may be used to evaluate changes in the activity of the autonomic nervous system induced by an acute passive stress stimulus.

**Keywords:** pupillometry, medical students, stress, emotions, autonomic nervous system

## Introduction

Fear and horror are protective mechanisms that may act as "survival intelligence." These emotions are rooted in the brain's defense system, particularly the amygdala, and are designed to protect individuals from dangerous and threatening situations.[1] Horror movies, for example, tap into these mechanisms by creating simulated fear experiences that engage the brain's automatic fear responses.[2] However, the amygdala, recognized as the principal instigator of fear in humans, is believed to function based on the principle of prioritizing caution. It often responds excessively to unclear stimuli, treating them as potential threats and consequently inducing fear, even when there may be no actual harm.[3] The prefrontal cortex plays a role in mitigating this exaggerated response by independently evaluating potential threats and, if deemed unjustified, suppressing the fear triggered by the amygdala.[3]

The physiological responses essential for surviving are achieved through the coordinated activation and control of neuroendocrine and autonomic stress systems, mediated by overlapping circuits in the limbic forebrain, hypothalamus, and brainstem, also known as the central autonomic network (CAN).[4,5] There is growing evidence for the role of the autonomic nervous system (ANS) in maintaining survival mechanisms by responding to internal and external challenges, including blood pressure maintenance, thermoregulation, and stress response.[4,6] However,

The authors declare no conflicts of interest.

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There was no funding for this study.



Porto Biomed. J. (2025) 10:1(e279)

Received: 2 April 2024 / Received in final form: 29 November 2024 / Accepted: 3 December 2024

http://dx.doi.org/10.1097/j.pbj.0000000000000279

besides this critical role, the ANS is also involved in emotional response.[7,8] Fear or anxiety, in particular, is characterized by sympathetic activation and vagal deactivation, two branches of the ANS that have an opposing activity to maintain balance and to prepare the individual to meet the survival challenges by adjusting several functions such as cardiovascular, respiratory, gastrointestinal, skeletomuscular, and endocrine, as well as regulating emotional, cognitive, and behavior processes.[8]

Measuring the autonomic nervous system response to a passive stressor stimulus is crucial for understanding the body's physiological reactions to stress. In clinical settings, autonomic testing is used to assess the role of the ANS, using different methods such as heart rate variability (HRV) or blood pressure response.[9] However, several studies have shown that pupillary response using pupillometry can also be used as a physiological indicator to assess alterations in the activity of the ANS and a valuable tool in the clinical assessment of neurologically impaired patients.[10] Furthermore, pupillometry can detect sympathetic or parasympathetic deficits in patients with autonomic disorders, providing a convenient and straightforward method for evaluating autonomic function.[11] Although previous studies have used pupillometry together with other physiological measures to comprehensively understand ANS activity as a complementary tool to HRV,[12] pupillometry allows for an independent assessment of sympathetic and parasympathetic nerve activity.[13]

The pupillary response is controlled by both branches of the ANS (parasympathetic and sympathetic nervous system): Constriction is controlled by the parasympathetic nervous system while pupillary dilation is a sympathetically driven response beginning in the hypothalamus and following a three-neuron pathway to end up with the contraction of the dilator pupillae muscle.[14] When individuals are exposed to any physical or emotionally stressful stimulus or situation, their pupils dilate in response to the activation of the sympathetic nervous system.[14,15] Pupil diameter can vary from 1.5 to 9 mm, and from exposure to a stressor, with increased cognitive or emotional arousal, the pupil dilates within 200 ms and returns to baseline after the event within a few seconds.[16,17] However, previous studies have shown that the pupil's response to stressors is complex and may involve a combination of cognitive arousal (increased cognitive arousal corresponds to a prolonged recovery period); dark adaptation, which is proposed to be slowed proportionally to the amount of stress that an individual has experienced; and other physiological responses.[17,18] Besides, pupil dilation and constriction occur without conscious control and consequently, the physiological response of the pupil to stressors cannot be intentionally managed or inhibited by the individual.[16]

Therefore, pupillometry has been reported as an effective method to assess stress and pupil dilation, which is considered in research to be a reliable indicator of emotional arousal and autonomic nervous system activation.[15,16] Pupillometry is a clinically validated method used to evaluate pupillary changes, measuring pupillary responses in a noninvasive and relatively inexpensive manner and allowing the capture of the activity of the ANS in real time.[17] It can provide insights into both parasympathetic and sympathetic functions, with specific indices such as baseline pupil diameter, maximum constriction velocity, and absolute constriction amplitude being particularly informative.[19]

Considering that pupillometry is a validated form of measuring ANS activity and stress, in this proof-of-concept study, we aimed to assess the feasibility of pupillometry to assess the acute response to a passive real-life stressor—viewing a truthful war scene.

## Methods

### Participants and study design

Thirteen participants were enrolled in a nonrandomized controlled crossover trial at the Faculty of Medicine of the University of Porto (FMUP) facilities. The participants consisted of medical students (11 women), aged 19–23 years, from Portugal (5 participants, all women) and Turkey (8 participants, 5 women) participating in a Research Twinning Project, an initiative of the European Medical Students' Association (EMSA) organized in collaboration between the Students' Association of the Faculty of Medicine of University of Porto (EMSA AEFMUP) and Students' Association of the Istanbul Faculty of Medicine Çapa-Fatih (EMSA Istanbul). A participant with multiple sclerosis was excluded from the study, as multiple sclerosis is a chronic progressive demyelinating disease of the central nervous system, which may also affect the ANS.[20] All the participants provided written informed consent. This study was conducted following the Declaration of Helsinki.

### Questionnaire

The evaluation included a self-reported questionnaire before the first evaluation and a simplified version of the same questionnaire in the second evaluation. The questionnaires aimed to evaluate sociodemographic data and health-related information. The following variables were asked: age, gender, presence of any known active disease previously diagnosed, current medication, practice of physical exercise, and smoking status 30 minutes before the audiovisual stimulus. The characteristics of the participants are presented in Table 1.

### Pupillometry

Clinical examinations of each participant with pupillometry were conducted on two different days separated by 48–72 hours, during mornings from November 07, 2023, to November 10, 2023. Pupillary measurements were performed using a portable infrared PLR-200 Pupillometer (NeurOptics Inc, CA). The participants were asked to spend 15 minutes in a semidark and quiet room to allow their eyes to adjust to the low lighting levels before measurement. Two pairs of infrared-emitting diodes of a 180-nm wavelength illuminated the right eye, and the measure was obtained 3 seconds after. At the end of the measurement cycle, a pupillary light response curve for the right eye was recorded. In case of blinking, the measurement was repeated.

The following parameters were obtained for each participant: diameter (millimeters) of the pupil before the light stimulus (initial) and at the constriction peak (minimal), relative constriction amplitude (%), maximum constriction velocity, average constriction and dilation velocities (mm/s), and total time (seconds) taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction (T75). Pupillometry is a noninvasive technique that can provide valuable data on the functioning of the two branches of the ANS since pupil constriction is controlled by the parasympathetic nervous system and pupil dilation reflects the sympathetic nervous system activity.

Participants were randomized into two groups ($n = 7$ and $n = 6$). Pupillometry was performed before (T0) and after (T1) participants were exposed to two different audiovisual stimuli consisting of 15-minute clips separated by a washout period of 48–72 hours. Intervention stimulus was part of Movie 1 "Saving

**Table 1**

**Sociodemographic and health-related characteristics of participants.**

| Characteristics | n | | | |
|---|---|---|---|---|
| | Total (n = 13) | Portugal (n = 5) | Turkey (n = 8) | P |
| Age (y)* | 20.0 (19.0; 23.0) | 20.0 (19.0; 23.0) | 20.5 (19.0; 22.0) | |
| Female | 10 (76.9) | 5 (100) | 5 (62.5) | .134 |
| Any disease† | 2 (15.4) | 1 (20.0) | 1 (12.5) | .726 |
| Medication‡ | 1 (7.7) | 0 | 1 (12.5) | .429 |
| Physical exercise | | | | .225 |
| 0 min/wk | 8 (61.5) | 2 (40.0) | 6 (75.0) | |
| ≤150 min/wk | 4 (30.8) | 2 (40.0) | 2 (25.0) | |
| >150 min/wk | 1 (7.7) | 1 (20.0) | 0 | |
| Smoke before the audiovisual stimulus | 4 (30.8) | 0 | 4 (50.0) | .068 |

* Median (min–max).
† Polycystic ovary syndrome, food allergy.
‡ Oral contraceptive.

Private Ryan" (Spielberg, S., 1998) chosen to induce acute fear and horror-induced stress (02h20 to 02h35), and the control was part of Movie 2 "Life Is Beautiful" (Benigni, R., 1997) used to elicit the emotions of affection, amusement, and/or happiness (00h01 to 00h16) (Fig. 1).

### *Statistical analyses*

Continuous variables were described by the median (25th–75th percentile). Categorical variables were expressed as absolute and percentage frequency. Since our data followed a non-normal distribution, we used nonparametric tests for the analysis. The Mann–Whitney U test was used to evaluate the differences in the characteristics between Portuguese and Turkish medical students such as gender, presence of any disease, medication, physical exercise, and smoking status. The differences between the time of assessment (T0 and T1 for audiovisual stimulus 1 [M1] and T0 and T1 for audiovisual stimulus 2 [M2]) were evaluated using a Wilcoxon test. This test was also used to assess the difference between M1 and M2 within each time point of assessment (T0 and T1). Significant differences were reported with an α-value of less than 5% ($P$-value < 0.05). Statistical analyses were performed using SPSS statistical package software v27.0 (NeurOptics Inc., IBM, New York).

## Results

A significant difference in response to acute fear and horror-induced stress was observed in pupillometry parameters {baseline [median (25th–75th percentile), 6.90 (5.95; 7.40) vs. 6.60 (5.55; 7.10), $P$ = 0.030], and final pupil diameter [4.50 (3.90; 5.20) vs. 4.10 (3.50; 4.60), $P$ = 0.012]} between M1 and M2 in T1, suggesting the acute increase in sympathetic parameters (Table 2). Although not significant, there was also a difference in the constriction amplitude between M1 and M2 in T0 (35.0 [29.0; 39.0] vs. 35.0 [31.5; 41.5], $P$ = 0.061), and T1 (33.0 [29.5; 35.0] vs. 33.0 [32.5; 39.0], $P$ = 0.052) (Table 2). There was also a difference in pupillometry parameters {final pupil diameter [4.30 (3.75; 5.10) vs. 4.50 (3.90; 5.20), $P$ = 0.060], and average constriction velocity [3.99 (3.42; 4.45) vs. 3.67 (3.30; 3.98), $P$ = 0.059]} after watching M1 compared with T0 (Table 2).

## Discussion

The purpose of this study was to evaluate the feasibility of pupillometry to assess the acute response to a passive real-life stressor, viewing a truthful war scene, chosen to induce acute fear and horror-induced stress. Our proof-of-concept study showed that there was a significant acute increase in sympathetic parameters after being exposed to an acute passive stressor

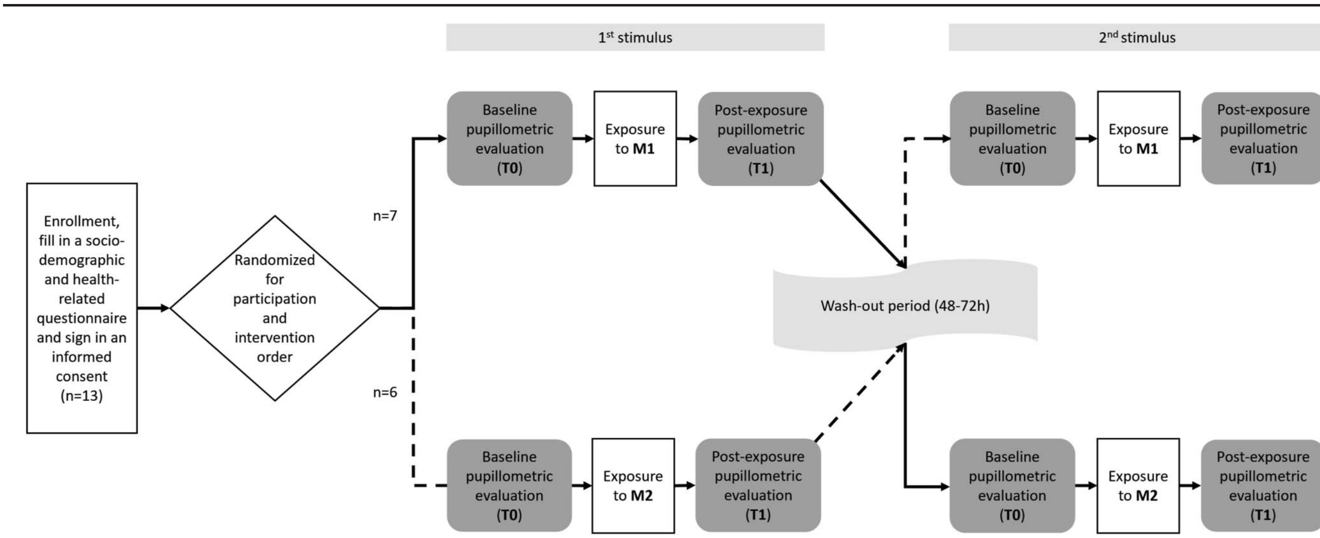


Figure 1. Flowchart of study participants.

**Table 2**

**Median (25th–75th percentile) values of pupillometry parameters by time of assessment (T0 and T1) and by stimulus (Movies 1 and 2).**

| Pupillometry parameters | | Time of assessment [median (25th–75th percentile)] | | P |
|---|---|---|---|---|
| | | Before (T0) | After (T1) | |
| Baseline pupil diameter | Movie 1 | 6.70 (5.75; 7.35) | 6.90 (5.95; 7.40) | .114 |
| | Movie 2 | 6.70 (5.80; 7.15) | 6.60 (5.55; 7.10) | .292 |
| | P | 0.622 | **0.030** | |
| Final pupil diameter | Movie 1 | 4.30 (3.75; 5.10) | 4.50 (3.90; 5.20) | .060 |
| | Movie 2 | 4.30 (3.60; 4.90) | 4.10 (3.50; 4.60) | .751 |
| | P | 0.128 | **0.012** | — |
| ACV | Movie 1 | 3.99 (3.42; 4.45) | 3.67 (3.30; 3.98) | .059 |
| | Movie 2 | 3.89 (3.51; 4.19) | 3.93 (3.59; 4.43) | .480 |
| | P | 0.279 | 0.142 | — |
| ADV | Movie 1 | 0.95 (0.78; 1.09) | 0.85 (0.76; 1.01) | .421 |
| | Movie 2 | 0.99 (0.86; 1.15) | 0.97 (0.86; 1.08) | .625 |
| | P | 0.382 | 0.234 | — |
| Constriction amplitude | Movie 1 | 35.0 (29.0; 39.0) | 33.0 (29.5; 35.0) | .073 |
| | Movie 2 | 35.0 (31.5; 41.5) | 33.0 (32.5; 39.0) | .379 |
| | P | 0.061 | 0.052 | — |
| MCV | Movie 1 | 5.51 (4.59; 6.23) | 4.96 (4.66; 5.53) | .101 |
| | Movie 2 | 5.29 (4.75; 5.66) | 5.35 (4.76; 5.95) | .944 |
| | P | 0.196 | 0.345 | — |
| T75 | Movie 1 | 3.46 (3.09; 3.89) | 3.20 (2.75; 3.30) | .139 |
| | Movie 2 | 2.80 (2.58; 3.39) | 3.76 (2.81; 3.92) | .086 |
| | P | 0.123 | 0.314 | — |

Movie 1: Saving Private Ryan. Movie 2: Life is beautiful. Baseline pupil diameter: initial diameter of the pupil before. Final pupil diameter: diameter of the pupil at constriction peak. Values in bold denote significant differences.
ACV, average constriction velocity; ADV, average dilation velocity; relative constriction amplitude; MCV, maximum constriction velocity; T75, total time taken by the pupil to recover to 75% of the initial resting pupil size after reaching peak of constriction.

(intervention stimulus), compared with a stimulus used to elicit the emotions of affection, amusement, and/or happiness. Therefore, pupillometry may be used to evaluate the autonomic nervous system changes induced by an acute passive stress stimulus.

This study has a few limitations. In this study, only fourteen individuals actively participated, a small sample size that has reduced statistical power and that may not be representative of medical students. However, although a higher number of participants could enhance the overall robustness and generalizability of our results, with the small number of included participants, we observed significant differences. Only pupillometry was used in this study to assess the response of the ANS to emotional stress. Although some previous studies have considered the combination with other stress biomarkers such as blood pressure or skin temperature,[21] research has also shown that pupil diameter can be used as a reliable indicator of ANS activity and that the measurement of pupil diameter can be a convenient and reliable approach for assessing mental stress.[22] Our eyes, specifically pupil dilation, controlled by the autonomic nervous system provide significant insights into emotional responses and cognitive processing involved in emotion regulation.[23,24]

Our study has important strengths. It is a controlled crossover study, meaning that each participant serves as their control, reducing interparticipant variability. Consequently, reducing individual differences can enhance study precision, improve internal validity, and reduce the required sample size for equivalent statistical significance.[25,26]

Previous studies on pupillary changes during emotional picture viewing also supported that the pupil's response to emotional arousal, characterized by larger changes in the presence of emotionally arousing stimuli, is mediated by sympathetic activity.[15] This fact highlights the link between emotional arousal and autonomic activation. Therefore, pupillometry has emerged as a valuable tool for assessing emotional responses. In the past years, there has been a growing interest and research in stress recognition systems based on computing methods using pupillary parameters.[27,28] Pupil dilation and other eye-related metrics have been explored as comfortable and noninvasive potential indicators of stress, cognitive load, and emotional states. The outcomes derived from the analysis of pupil parameters were contrasted with other physiological signals, namely electrocardiogram and electrodermal activity, and this highlighted the superior ability of pupil diameter to distinguish between stress and relaxation when compared with the other physiological signals.[27,29]

The use of pupillometry in clinical practice as a noninvasive biomarker of the ANS response to emotional stress is, therefore, an innovative method that carries several advantages, making it a powerful tool in the study of emotional stress responses, particularly appealing for real-life implementations.[28] Stress typically causes pupil dilation, and the extent, speed, and recovery of this dilation offer insights into how individuals are processing and reacting to the stimulus. Ginton et al.[30] also used pupillometry to assess the role of emotional regulation in the association between trauma exposure and post-traumatic stress disease (PTSD) symptomatology. The results by Ginton et al.[30] suggested that increased pupillary dilation in PTSD may reflect impaired parasympathetic nervous system processes. Recent studies have shown that social support, such as spousal handholding, can significantly reduce pupil reactivity to stress when measured with pupillometry, indicating a buffering effect on the autonomic nervous system.[31] In addition, pupillometry provides valuable information on the neurobiological basis of stress resilience since pupil responses, linked to the locus coeruleus–noradrenergic system, have been associated with changes in anxiety and depression in response to prolonged real-life stress.[32]

Among various psychophysiological indicators of stress, which include cardiovascular, electrodermal, or respiratory activity, the study of pupillary diameter is preferred because of its nonintrusiveness, real-time assessment, and possibility to continuous monitoring and observation of dynamic changes during various emotional states. Besides, pupillometry provides objective and quantifiable data, minimizing reliance on self-reporting or subjective interpretations. It also has a wide applicability in different individuals and settings such as psychology, neuroscience, human–computer interaction, and physiological computing.[28]

Given that our study population consisted of medical students, we can explore the implications that our findings may have in real life, namely in stress recognition and developing measures to overcome that stress. Our results suggested that the exposure to a stress-induced passive stimulus may change the activity of the ANS among medical students, namely a change in the parameters that characterize the activity of the sympathetic nervous system (SNS). However, caution is needed when interpreting the results, because of the small number of participants included. Several studies have shown that medical school brings significant psychological distress with a high load of adverse effects on students' mental health, such as depression and anxiety, which negatively influence students' academic performance.[33-35] The most common stressors include worries about the future, financial difficulties, and academic pressures, and they often use active coping strategies, such as seeking emotional support and engaging in recreational activities, to manage this stress.[36,37] Emotional stress may play an important role in activating the SNS, as suggested in our proof-of-concept study, which may be triggered by various stressors, from severe threats to more subtle discrepancies in expectations.[38] On the contrary, it is also described that increased endogenous sympathetic activity decreases awareness and reactivity.[39] These findings may also contribute to the development of effective stress management strategies in medical education, including identifying stressors on campus, engaging in discussions with students, and offering appropriate support for coping with individuals.[36] Several studies have found that green spaces in university environments can help reduce stress in students by having a restorative effect on the ANS and leading to greater relaxation, recovery, and increased parasympathetic activity.[39-41] By contrast, the presence of or exposure to built environments has been associated with increased sympathetic activity.[40,41]

For future research, applying a questionnaire to measure the sense of coherence would be interesting. The sense of coherence scale has been used in clinical research, with a focus on its relationship with emotional and psychosocial well-being and quality of life.

## Conclusion

The pupil's response during exposure to a passive stressor stimulus reflects that emotional arousal may be related to a change in sympathetic activity. Our proof-of-concept study suggests that pupillometry may be used to evaluate the autonomic nervous system changes induced by an acute passive stress stimulus.

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