Physiology & Behavior 223 (2020) 113017

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Could academic experience modulate psychophysiological stress response of biomedical sciences students in laboratory?

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ARTICLE INFO

Keywords:
Heart rate variability
Stress response
Simulation
Autonomic modulation
Experience
Education

ABSTRACT

The aim of the present research was to analyse the subjective and objective psychophysiological stress response of experienced and non-experienced Pharmacy and Biotechnology students in laboratory practices. We analysed in 82 Pharmacy and Biotechnology degree students divided into two groups (non-experienced: n: 53; experienced: n: 29) the autonomic stress response by the analysis of heart rate variability (HRV) before, during and after and distress perception before and after a laboratory practice. Results showed how students in both groups presented a large anticipatory anxiety response at the beginning of the practise (low HRV values). During the entire laboratory practice, non-experienced students showed a maintained sympathetic modulation while experienced students presented a higher parasympathetic modulation (high HRV values) consistent with a habituation process. Laboratory practise performed by Pharmacy and Biotechnology students produced an anticipatory anxiety response independently of their experience, but, non-experienced students showed a lower habituation response in both subjective and objective stress records than experienced students at the end of the laboratory practice.

1. INTRODUCTION

Stress is defined as a no specific response performed by an organism after the demand of a stressor, which implies psychological, cognitive and behavioural modifications, regulated by hypothalamus – pituitary – adrenal gland (HPA) axis by a negative feedback [1]. When bloodstream cortisol levels are high, its secretion is inhibited and regulated by the HPA axis, whose main function is the maintenance of organic homoeostasis through metabolic, cardiovascular, immunological and sympathovagal control [2]. When exposed to a perceived stressor stimuli, HPA axis is activated as consequence of a sympathetic activation, which produces increases in the heart rate, blood pressure, glucose levels, bronchodilation as well as over-activation of cognitive and metabolic resources [3–5]. This response has been found either in applied military combat, in where there is a life perceived risk, thus an acute stressful stimuli [6], showing increases in lactate, heart rate, blood oxygen saturation, muscular strength, salivary cortisol and blood glucose levels [7], and in clinical evaluations in undergraduate students [8], clinical stays or prior to their first written exams in the course [9–13].Previous studies showed how acute stress produces a reduction in blood flow in the prefrontal cortex, which means a decrease in oxygen and nutrients, triggering a decrease of the effectiveness of the executive functions associated [3, 14], affecting brain regions as the hippocampus, leading to concentration difficulties and acutely reducing memory capability [15] either working and operative memory [15–18], decision making [16] and contributing to irritability, anxiety, loss of self-confidence and worriedness [19, 20].

The impact and consequences produced by acute stress has been also studied in some bio-sanitary areas, such as physiotherapy, nursing or psychology. These researches showed how students presented an anticipatory anxiety response measured by a high sympathetic modulation previous to clinical stays [9–11, 13]. However, experience plays an important role in stress management, since authors exposed how it could direclty interfiere and modulate the psychophysiological stress response, being novice and non-experienced grealty affected [21, 22]. In addition, previous experience as been higlighted as a key factor to

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https://doi.org/10.1016/j.physbeh.2020.113017
Received 18 April 2020; Received in revised form 27 May 2020; Accepted 16 June 2020
Available online 18 June 2020
0031-9384/

improve the efficacy and the performance of certain activities in real context [23]. In academic field, the experience produced due to habituation process, is related to the chronic adaptation and exposure to the stressor stimuli, which has been reported in psychology students after their clinical stay [10]. This habituation effect due to the acquired experience was also reported in the military field, where inexperiened subjects showed greater increase in cortisol values than experienced parachute jumpers [24, 25].

However, the impact of acute stress had not been studied in Pharmacy and Biotechnology students yet. These degrees include a large amount of laboratory evaluation practices which undergraduate students must successfully achieve, facing irritating and toxic products, and constant observation and evaluation by the professors. In addition, it is interesting from a pedagogic point of view to know if experience have any kind of impact on the learning process in this type of disciplines. In this line, it is also important to evaluate the stress response and its effects in students since they are in a scenario which involves personal risks that could compromise their own safety and their partners one. Thus, the aim of the present study was to analyse the subjective and objective psychophysiological stress response of Pharmacy and Biotechnology experienced and non-experienced students in laboratory practices. The first hypothesis was that non- experienced students would present a larger anticipatory anxiety response in both, subjective and objective stress markers than experienced students. The second hypothesis was that non-experienced students at the end of the laboratory practice would show a lower habituation response in both subjective and objective stress markers than experienced students.

2. MATERIAL and methods

2.1. Participants

We analysed 82 undergraduate Pharmacy and Biotechnology students of first and last academic year. They were between 17 and 27 years old (19.63 ± 2.29), 34.1% male and 65.9% female. Participants were divided in two groups, non-experienced group (NEG, n: 53, 18.26 ± 1.36 years) and experienced group (EG, n: 29, 22.13 ± 1.30 years). All the procedures were conducted following the Helsinki Declaration (as revised in Brazil, 2013), all the participants filled an informed consent form previous start the research and all the procedures were approved by the University Ethic Committee.

2.2. Design and procedure

We analysed students subjective and objective stress response before, during and after a laboratory practise.

For this aim we used a scale of subjective distress units (SUDS) and the autonomous modulation by the heart rate variability (HRV) parameters to analyse subjective and objective stress response following previous methodologies in this educational scenarios [10, 12, 26].

SUDS were analysed before and after the laboratory practise and the autonomous modulation was measured in five moments: during the 10 min before the laboratory practise (basal measure) (M1), the first third of the practise (M2), the third of the practise (M3) and the 5 min after the practise (post measure) (M4). In the laboratory, students had to follow teacher instructions, develop the main activity, calculate results asked by the practise guidelines and share them with partners. Novel students had to make dissolutions with different chemicals, including hydrochloric acid, sulfuric acid, nitric acid, sodium hydroxide and calcium hydroxide. Last year students had to prepare a granulated formulation that included citric acid, sodium bicarbonate, heat and different instrumental techniques.

Students were involved in a scenario that assumed high stress levels due to the handling of toxic products and irritating substances as well as they had to deal with the presence of a teacher who was examining the activity, increasing academic pressure.

2.3. Measures

2.3.1. Physiological measures

Heart Rate (HR) and Heart Rate Variability (HRV) were recorded before, during and after the laboratory practise by a Polar V800 HR monitor (Kempele, Finland) with a sampling frequency of 1000 Hz, with which the RR intervals (time interval between R waves of the electrocardiogram) can be analysed for the analysis of the HRV and the number of beats per minute for the HR analysis. 10 min were taken as basal measure while students remained still in a waiting area, free of noises and distractions, then during the entire clinical stay and immediately after they finished for 10 min in the same waiting area as in previous researches in academic population [10, 11, 13]. Subsequently, the following parameters of the HRV time domain were analysed using the Kubios HRV software program (University of Kuopio, Kuopio, Finland):

Time-Domain (Nonspectral) Analysis. This analysis was based on the assessment of the intervals between normal beats on 24-hour ECG recordings. During the statistical analysis, generally all of the QRS complexes, the duration between consecutive QRS complexes (NN interval), or the instantaneous heart rates during continuous ECG recordings are determined. We recorded the following time-domain indices: pNN50 which is the percentage of successive normal sinus RR intervals exceeding 50 ms (%) and RMSSD (ms): Is the square root of the mean value of the sum of squared differences of all successive R-R intervals.

Frequency-Domain (Spectral Measures) Analysis. Frequency-domain measures give information about how the power is distributed as a function of frequency. This analysis give us smoother spectral components that can be distinguished as independent from preselected frequency bands and easy post-processing of the spectrum with an automatic calculation of low (LF)- and high-frequency (HF) power components and an easy identification of the central frequency of each component, and accurate estimation even on a small number of samples [27] HF and LF (n.u) were measured in order to measure the peaks of parasympathetic, high-frequency component, frequency range: 0.15–0.40 Hz (HF) and sympathetic low-frequency component frequency range: 0.04–0.15 Hz (LF) values. In addition, LF/HF ratio was evaluated.

In addition, SD1 and SD2 were measured to reflect the fluctuations of the HRV throw a Poincaré chart, physiologically, the transverse axis. SD1 reflects parasympathetic activity while SD2 reflect the long-term changes of RR intervals and is considered as an inverse indicator of sympathetic activity.

2.3.2. Psychological measures

A scale of subjective distress units (SUDS) was the tool used to measure students distress before and after the practise by asking “On a subjective scale from 0 to 100, where 0 means nothing and 100 means the maximum level of stress, which is your stress level right now?”, this scale constitute the Subjective perception of Distress, which has been used in previous studies in this field [8].

2.3.3. Data analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 24.0 for Windows (IBM Spain). Descriptive statistics were analysed for each variable and a multivariate analysis of variance was carried out to evaluate the effect of the practise in physiological and psychological parameters analysed. The Tukey test was used for post-hoc comparisons. The significance level was $p \leq 0.05$.

3. RESULTS

We found significant differences in students ages, presenting NEG participants lower age than EG (18.26 ± 1.36 years vs. 22.13 ± 1.30

Table 1
Modification on heart variability parameters between non-experienced group and experienced group in the laboratory practise.

Pre (M1)	HR max (bpm)	HR mean (bpm)	RMSSD (ms)	PNN50 (%)	LF (n.u)	HF (n.u)	LF/HF	SD1 (ms)	SD2 (ms)
NEG	134.18 ± 28.11	97.06 ± 15.89	41.03 ± 23.65	10.47 ± 10.27	75.65 ± 10.22	26.69 ± 19.70	5.28 ± 10.17	29.02 ± 16.74	66.59 ± 29.78
EG	134.50 ± 30.83	94.68 ± 12.33	43.20 ± 32.87	12.47 ± 16.37	77.61 ± 13.03	22.35 ± 12.98	4.98 ± 3.14	30.55 ± 23.27	64.64 ± 33.90
T	−0.046	.698	−0.345	−0.679	−0.752	1.064	.154	−0.344	.270
P	.963	.487	.731	.499	.454	.290	.878	.732	.788
1/3 of the laboratory practise (M2)	HR max (bpm)	HR mean (bpm)	RMSSD (ms)	PNN50 (%)	LF (n.u)	HF (n.u)	LF/HF	SD1 (ms)	SD2 (ms)
NEG	127.38 ± 27.49	94.49 ± 13.39	35.27 ± 18	9.22 ± 8.46	77.66 ± 8.69	21.85 ± 8.96	4.29 ± 2.36	24.90 ± 12.82	61.75 ± 20.07
EG	118.71 ± 17.48	87.33 ± 9.71	39.99 ± 33.06	13.95 ± 15.34	75.53 ± 8.39	24.14 ± 8.32	3.66 ± 1.97	28.28 ± 23.38	64.86 ± 35.33
T	1.508	2.534	−0.838	−1.805	1.074	−1.133	1.222	−0.847	−0.509
P	.136	0.013	.405	.075	.286	.260	.225	.400	.612
3/3 of the laboratory practise (M3)	HR max (bpm)	HR mean (bpm)	RMSSD (ms)	PNN50 (%)	LF (n.u)	HF (n.u)	LF/HF	SD1 (ms)	SD2 (ms)
NEG	125.38 ± 27.02	91.81 ± 13.41	37.73 ± 17.51	10.79 ± 8.94	77.72 ± 8.60	22.17 ± 8.54	4.09 ± 1.73	26.71 ± 12.38	66.86 ± 21.80
EG	127.48 ± 31.98	87.36 ± 19.50	44.31 ± 35.45	14.07 ± 13.87	76.81 ± 11.09	23.11 ± 11.04	4.23 ± 2.54	31.34 ± 25.07	73.93 ± 39.32
T	−0.309	1.218	−1.128	−1.300	.414	−0.425	−0.290	−1.121	−1.043
P	.758	.227	.263	.197	.680	.672	.772	.266	.300
Post (M4)	HR max (bpm)	HR mean (bpm)	RMSSD (ms)	PNN50 (%)	LF (n.u)	HF (n.u)	LF/HF	SD1 (ms)	SD2 (ms)
NEG	133.02 ± 65.38	93.80 ± 16.94	41.97 ± 22.90	13.02 ± 11.36	74.84 ± 13.22	25.11 ± 13.12	4.24 ± 2.74	29.74 ± 16.25	69.46 ± 23.89
EG	129.07 ± 40.55	88.11 ± 12.51	54.05 ± 37.80	18.56 ± 16.92	76.32 ± 13.64	23.55 ± 13.59	5.03 ± 3.95	38.27 ± 26.76	83.34 ± 39.29
T	.295	1.586	−1.804	−1.766	−0.478	.510	−1.075	−1.797	−1.990
P	.769	.117	.075	.081	.634	.611	.286	.076	.050

PNN50: percentage of consecutive RR intervals that differ >50 ms; RMSSD: Square root of the average of the sum of the squared differences of the intervals RR; HR MEAN: Average heart rate; HR MAX: maximum heart rate; LF: low frequency waves; HF: high frequency waves; LF/HF RATIO: rate between the low and high frequency waves; SD1: variability of the short-term HRV; SD2: variability of the long-term HRV; NEG: non-experienced group; EG: experienced group.

years respectively; p:0.000). EG showed significantly higher SD2 values than NEG in M4. By contrary, we found a significantly higher HR mean values in NEG than in EG in M2 (Table 1).

The SUDS increased no significant in NEG after the laboratory practice (33.67 ± 23.41 vs. 40.37 ± 25.69; $p = 0.07$), but in EG SUDS presented a significant increase (29.82 ± 20.76 vs. 38.96 ± 19.01; $p = 0.003$). SD2 values of NEG were significantly higher in M3 than in M2. In EG we found that the pNN50 and SD2 were significantly higher in M4 than M2 and M1. Additionally, the RMSSD in EG presented lower values in M2 than in M4 and LF/HF ratio were significantly higher in EG in M1 than in M2 (Table 2).

Table 2
Differences in moment comparison between non-experienced group and experienced group in the laboratory practice.

	NEG	EG
HR max	–	–
HR mean	M1 > M2; M1 > M3; M2 > M3	M1 > M4
RMSSD	–	M4 > M2
PNN50	–	M4 > M2; M4 > M1
LF	–	–
HF	–	–
LF/HF	–	M1 > M2
SD1	–	M4 > M2
SD2	M3 > M2	M4 > M2; M4 > M1

PNN50: percentage of consecutive RR intervals that differ >50 ms; RMSSD: Square root of the average of the sum of the squared differences of the intervals RR; HR MEAN: Average heart rate; HR MAX: maximum heart rate; LF: low frequency waves; HF: high frequency waves; LF/HF RATIO: rate between the low and high frequency waves; SD1: variability of the short-term HRV; SD2: variability of the long-term HRV; NEG: non-experienced group; EG: experienced group; M: moment of analysis.

4. DISCUSSION

The aim of the present research was to analyse the subjective and objective psychophysiological stress response of experienced and non-experienced Pharmacy and Biotechnology students in laboratory practices. First hypothesis was not full filled since no significant differences were found between experienced and non-experienced students anticipatory anxiety response. Second hypothesis was fulfilled since non-experienced students at the end of the laboratory practice showed a lower habituation response in both subjective and objective stress records than experienced students.

We found high levels of HR mean, LF and low RMSSD and SD2 values at M1 moment analysis. These values are consistent with an anticipatory anxiety response, showing students an increased sympathetic modulation [28, 29] . This organic response was in consonance with previous researches in clinical evaluations of undergraduate students [8] and prior to the first academic test of the year [9–13]. The uncertainty of the evaluation, the exposure to irritating and toxic products, extreme security measures, the use of special equipment, as well as direct and constant evaluation by the professors, activate this preparatory phylogenetic response in the student. However, there were not significant differences between the groups analysed (experienced and non-experienced) in the pre evaluation moment in any of the HRV variables, meaning that independently of the experience both groups presented an anticipatory response. This lack of difference in the two groups analysed could be related with the fact that the psychological profile have a stronger influence than experience independently of the context whereas there is a perceived stressor stimulus [30] and does influence autonomic modulation [31]. Nevertheless, in other high stress context as parachute jumps previous research found how experience has a direct effect on the psychophysiological stress response since novel paratroopers presented a higher psychophysiological response than experienced [21, 32]. This difference respects the present study could be related to the specific demands of the contexts analysed, being more eliciting and dangerous the parachute one, then having higher influence parameters as experience in the psychophysiological stress response.

In the present research EG showed lower HR mean values than NEG at M2. This cardiovascular response showed the autonomical adaption to the stressor, being related with the higher experience of this group in this specific context [7, 18]. This finding was in consonance with previous studies in where lower cardiovascular response was found in the defence of public speaking and mental arithmetic task [33], as well as in more eliciting contexts such as parachute jumps, where experienced jumpers presented lower HR than novel. However, in parachute scenarios even the HR of experimented was lower than novel, the HR reached by both was higher than the evaluated in the present research, fact due to the higher demands, threat and stress of parachute jumping [34]. In academic field, also nursing students showed a significantly decrease in HR values after a four month clinical stay [13]. These previous researches showed how experience acquired by participants produced the psychophysiological habituation process since at psychological level the perception of threat and uncontrollability decrease, having the physiological correlate of decrease in sympathetic nervous system, that produce the decrease in HR that we are discussing [6, 35-37]. Independently, in the present research we did not find differences in the autonomical response of students of two groups analysed in any other moment or parameter except the HR, future studies should continue with this research line for better knowledge of habituation in academic context.

EG showed higher levels of SD1 and SD2 at M4 than at M2, as well as higher SD2 values at M4 than at M1. These results are consistent with an habituation process, since an adaption to the stressor occurred during the practise These autonomic response was in consonance with previous researches conducted in Psychology students, where participants showed an increase of parasympathetic activity at the end of a ECOE recorded in an increase in SD2 values [12]. The habituation response of Psychology students at psychophysiological level could be related to their high experience, since they were last year students, as our EG participants. Furthermore, we found SD2 values significantly higher in EG than in NEG at the end of the laboratory practice, suggesting an adaptation to the stress context improved after several years facing to the eliciting laboratory context and the practical evaluation that is made in this special context. The influence of experience in the autonomic modulation was also found in other more eliciting and higher stressful context such as parachute jumps, aircraft pilots or military operations [21, 32, 36,38]. In these researches, there were a higher psychophysiological stress response in experienced participants than in our experienced group, fact explained by the high specific demands of these contexts, in where life could be compromised and there is a real-life risk.

4.1. Practical applications

The monitorization of HRV could be a useful tool to measure stress response in students. The use of practical simulations, as well as, understanding students stress response and habituation process could improve student's learning development, since teachers could show their strategies to deal with stressor contexts. Then, in their future professional activity they would know how to face that stressing situations, reaching the habituation process faster and developing their knowledge and skills properly, and consequently, decreasing mistakes due to high stress levels. Thus, simulation becomes an effective work tool which gives students the necessary skills to improve their learning process. On the other hand, the measurement of psychophysiological variables could help professors to improve the evaluation process and to know which limitations could have their students due to high stress situations. They could teach biofeedback abilities which help students to control their stress response.

4.2. Limitations of the study

The main limitation of this study was the small sample size, which was due to the difficulties to access students in real laboratory conditions. Secondly, due to resource availability, there were no measurements of stress hormones (as alpha-amylase, cortisol, adrenaline, etc.). Future studies should consider these issues.

5. CONCLUSION

Laboratory practise performed by Pharmacy and Biotechnology students produced an anticipatory anxiety response independently of their experience, but, non-experienced students showed a lower habituation response in both subjective and objective stress records than experienced students at the end of the laboratory practice.

FUNDING

The present research was developed with the David A. Wilson for Excellence in Teaching and Learning Research Award 2017, Project No. XOTRIO1712

ACKNOWLEDGEMENTS

We would like to acknowledge the contribution of participants and professors for collaborating in the study. We would also like to acknowledge the involvement of the predoctoral research assistants.

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