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Title: Global stress response during a social stress test: impact of alexithymia and its subfactors

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Highlights

- We study the association between alexithymia and its subfactors with HPA and SAM activity.
- Stress was induced experimentally using a public-speaking paradigm.
- The increased HPA activity was related to only one alexithymia subfactor, DIFF.

Global stress response during a social stress test: impact of alexithymia and its subfactors

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The corresponding author certifies that all the authors approved the entirety of the submitted material and contributed actively to the study.

Running title: Impact of Alexithymia subfactors on physiological responses to a social stress test

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Summary:

Objectives: Alexithymia is a personality trait characterized by difficulties in identifying, describing and communicating one's own emotions. Recent studies have associated specific effects of this trait and its subfactors with hypothalamo-pituitary-adrenal (HPA) axis markers during stress. The aim of this study was to analyze the association between alexithymia and its subfactors with HPA and sympatho-adrenal medullar (SAM) activity. Stress was induced experimentally using a public-speaking paradigm. Salivary cortisol, Alpha-amylase (AA), chromogranin A (CgA) and heart rate (HR) were collected during the defined periods of baseline, stress, and recovery in 19 males and 24 female healthy university students.

Results: Subjects reacted to the stressor with a significant cortisol and SAM response. Subjects scoring high on alexithymia reacted significantly more intensely than low scorers in basal anticipatory as well as peak cortisol and area under the curve. Regression analyses revealed that the increased HPA activity was related to only one alexithymia subfactor, the difficulty in differentiating feelings and distinguishing them from bodily sensations and emotion arousal.

Conclusion: Alexithymia and its subfactors were specifically related to cortisol responses. This research should be replicated with more subjects and should take into account more parameters reflecting sympathetic and/or parasympathetic activation, as well as HPA axis. Factors such as coping strategies and the perception of the situation as a challenge have also to be explored.

KEYWORDS: Psychosocial stress; Alexithymia; Saliva, Cortisol; Chromogranin A; Alpha-amylase, Heart rate;

1. Introduction

Alexithymia, a common personality trait, is normally distributed in the general population (Franz et al. 2008). It is characterized by a difficulty in identifying, describing, and expressing one's emotions (Sifneos, 1973). It has been related to certain stress-related psychological disorders such as pain syndromes (Huber et al. 2009) or eating disorders (Keating et al. 2013). Besides a global alexithymia score, the Toronto Alexithymia Scale (Bagby et al. 1994), measures three subfactors: namely, difficulties in identifying feelings (DIFF), difficulties in describing feelings (DDF), and externally oriented thinking or a preoccupation with the details of external events (EOT). Alexithymia can be considered as a stress vulnerability factor, possibly by altering stress responses, indeed, impaired psychosomatic processing attributed to alexithymia induces alterations in physiological parameters (de Timary et al. 2008). On the one hand, some authors have suggested that the influence of alexithymia on the expression of stress-related pathological states might involve poor resistance to stress. This idea was described as the "alexithymia-stress hypothesis" (Martin and Pihl, 1985). On the other hand, Papciak et al. (1985) proposed the "decoupling hypothesis", which states a mismatch between physiological arousal and emotional awareness in alexithymic individuals under stressful situation.

Job interviews are commonly reported as stressful experiences, mainly psychosocial in nature (Campisi et al. 2012). It has been shown that the use of a potent laboratory stress protocol reproducing job interview (i.e. the Trier Social Stress Test, TSST, Kirschbaum et al. 1993) activates the sympathetic-adrenomedullary (SAM) system, and the hypothalamic-pituitary-adrenocortical (HPA) axis (Nater et al. 2005). Both systems interact in managing the adaptive response to stressful events, and biomarkers of these systems can be evaluated non-invasively in saliva, this method allows repeated samples in a short time (Lac, 2001; Filaire et al. 2009;

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Tanaka et al. 2012). Activation of the HPA axis induces the secretion of cortisol, which stimulates the mobilization of the energy needed to overcome the stressor. Therefore, cortisol is considered as the main biomarker in stress research (Hellhammer et al. 2009).

Besides the endocrine secretions of cortisol, the physiological response to psychological stressors implies the activation of the SAM, with a sympathetic activation resulting in the release of noradrenaline from sympathetic nerve terminals and adrenaline and noradrenaline from the adrenal medulla, which result in a range of rapid physiological and behavioral responses such as increases in heart rate (HR) and blood pressure and heightened vigilance (Goldstein, 1987; Obayashi, 2013). Direct measurements of salivary adrenaline and noradrenaline seem not to reflect SAM activity (Schwab et al. 1992). However, salivary alpha-amylase (sAA) appears as a promising marker, as non-invasive and easily obtainable (Nater et al. 2005; Granger et al. 2007) since Ehlert et al. (2006) provided evidence through pharmacological manipulation of the SAM system. In fact, these authors showed that yohimbine administration activates not only autonomic parameters but also sAA via adrenergic mechanisms, suggesting that sAA might be an indirect indicator of the central sympathetic system. Alpha-amylase, one of the principal salivary proteins appearing as a number of isoenzymes, is produced by the serous acinar cells of the parotid and submandibular glands. Amylase accounts for 10–20% of the total salivary gland-produced protein content and is mostly synthesized by the parotid gland (Zakowski and Bruns, 1985). The secretion of AA by acinar cells of the salivary glands is regulated by the autonomic neuronal pathways. Psychosocial stress increases sAA secretion (Filaire et al. 2009; Filaire et al. 2010; Tanaka et al. 2012).

Another interesting protein is the Chromogranin A (CgA), a soluble protein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve

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endings (Dimsdale et al. 1992); thus, it is considered to be a valuable indicator of sympatho-adrenal activity (Taupenot et al. 2003). Recently, salivary CgA was shown to be produced by the human submandibular gland and secreted into saliva (Saruta et al. 2005), making it a sensitive and reliable index of psychological stress. Some authors reported a rapid and sensitive elevation of salivary CgA in response to psychosomatic stressors such as public speaking (Nakane et al. 1998). Salivary CgA has gained attention as a novel stress marker. Whereas cortisol has long been assayed as a stress marker that reflects both mental and physical stress, concentrations of salivary CgA correlate only with mental stress (Nakane et al. 1998). Thus, CgA and alpha-amylase appear as potential non-invasive tools for evaluating the SAM following psychological stress.

There were few publications on alexithymia and HPA system until now. Lindholm et al. (1990) have reported an association between alexithymia and a positive dexamethasone suppression test. Conversely, McCaslin et al. (2006) found no association between alexithymia and cortisol reactivity to a video stress challenge. De Timary et al. (2008) noted that an increased cortisol level before being exposed to social stressor was associated with high scores in the DDF scale (difficulties in describing feelings). These authors suggested that alexithymia modulates cortisol concentration, possibly by affecting the anticipatory cognitive appraisal of situations. In the same study, these authors also observed that DIFF subfactor (difficulties in identifying feelings) was negatively correlated to cortisol. Recently, Härtwig et al. (2013) noted that alexithymic individuals have a lower cortisol awakening response (CAR), this parameter is a valid measure of basal HPA-system activity and considered as an anticipatory reaction to the challenges of the oncoming day (Clow et al. 2010; Kudielka and Wüst, 2010). According to McIntosh et al. (2014), alexithymia also appears to be linked to an increased noradrenergic activity; as well as to

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an elevated Norepinephrine/Cortisol ratio. These authors reported that the DIFF score was the strongest predictor of a greater Norepinephrine/Cortisol ratio. This tonic sympathetic hyperarousal in persons with alexithymia has also been reported by previous authors (Papciak et al. 1985; Stone and Nielson, 2001). According to the decoupling hypothesis, it seems that increased norepinephrine reflects greater efforts to meet the demands of a stressful experience while lower cortisol indicates the absence of behavioral consequences.

Thus, it appears that alexithymia enhances HPA reactivity and alters the SAM responses, but this point is still in debate. Moreover, studies have shown a differential association of specific alexithymia subfactors in diseases (Luminet et al. 2006), suggesting the alexithymia construct is not homogenous in its effects. However, to our knowledge, few studies have been conducted on the subfactors of alexithymia, and the question remains open as to whether these subfactors differentially interact with the SAM and HPA systems (Berthoz et al. 2002; Pollatos et al. 2011). Moreover, only a few studies to date have investigated the impact of alexithymia on HPA and SAM responses during an acute stress, among graduate students, none of them tested it during a job interview, which is a pertinent paradigm given the fact that candidates to a job perceive the interviews as stressful (Hiramoto et al. 2009).

Therefore, the aim of this study was to evaluate how alexithymia and its subfactors affect HPA and SAM systems during a simulated job interview among psychologically and physically healthy students. The stress responsive HPA system was assessed through salivary cortisol concentrations and the SAM system was evaluated using sAA and CgA. Heart rate was also measured to take into account neuro-vegetative reactivity to stress, since ineffective processing of somatic and affective perturbations attributed to alexithymia also shape autonomic responses (Martin and Pihl, 1985; Stone and Nielson, 2001). Because alexithymia primarily entails an

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inability to identify and describe emotional states (McInstosh et al. 2014), we hypothesized that participants with elevated scores on the TAS would exhibit, greater HPA and SAM dysfunction, i.e., increased CgA and sAA and decreased cortisol levels, and that subfactors of alexithymia modulate these responses.

2. Method

2.1. Participants

Taking into account the exclusion criteria, the recruitment procedure consisted of health status and psychological screening questionnaires. Forty-three university students, including 19 healthy males (mean age 23.9 years ± 4.4 (mean ± SD); body weight: 76.3 kg ± 10.1; height: 179.6 cm ± 10.1; body mass index: $23.6 ± 3.0$ kg.m^{-2}) and 24 females (mean age was 28.2 years ± 9.9 (body weight: 63.0 kg ± 10.4; height: 166.9 cm ± 8.6; body mass index: $22.5 ± 2.9$ kg.m^{-2}) participated in this study.

None of the participants reported any drug intake; alcohol within a normal consumption range was admitted. None of the subjects were currently under medication (except for contraceptives). Exclusion criteria were using medications that might alter HPA activity such as corticoids, or SAM axis such as sympathomimetics or beta-blockers, or suffering from known psychological disorders. Participants were only included if they did not have a history of any Axis disorders, particularly in anxiety disorders, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV, American Psychiatric Association, 1994). The population was screened for health status using a detailed anamnestic questionnaire, the French version of the Big-Five Inventory (Plaisant et al. 2010), and the Trait Anxiety Inventory (Bruchon-Schweitzer

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and Paulhan, 1993). All subjects were non-smokers and none of the subjects reported dietary supplements that affect HPA activity.

This study was conducted according to the Helsinki Declaration. All participants gave written informed consent before entering the study.

2.2. Procedure

Alexithymia questionnaire (TAS-20; Bagby et al. 1994), and consent form providing information about the study, confidentiality and contact information was sent by email one week before the experiment

We operationally defined High and Low Alexithymia according to the median score. In the low alexithymia group, (n = 20), total TAS score was 38.8 ± 4.6 (mean $\pm$ SD), as compared to the high alexithymia group (n =23), which had a total TAS score of 54.13 ± 5.3.

Public-speaking paradigm

We used a modified public-speaking anxiety paradigm based on the Trier Social Stress Test (Kirschbaum et al. 1993) with some changes to induce an emotional real-life situation.

After arrival at the laboratory, the participants seated in a quiet sound attenuated room for 60 minutes. Then, the first saliva sample (baseline) was obtained. Room lighting was kept constant throughout the experimental session. Fig.1 presents the schedule of the experimentation.

Here: Figure 1

Ten minutes before the test, participants were instructed that they were going to give a five-minute self-introduction speech in front of an audience (2 assessors: male and female) as if they were applying for a job. The instructions were as follow: "You are applying for a job that is particularly important to you. You will have five minutes to present yourself in front of a camera

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and describe your personal characteristics (e.g. personality, skills, and experiences) that you view as strengths and that would allow you to be considered for this job. You must also indicate those characteristics that might be perceived as weaknesses and how you have or are willing to improve them. Be as sincere as possible. This is an important prerequisite for this position." To increase anticipatory stress, the participants were told that their speech would be videotaped.

Next, they entered the experimental room and were asked to stand on an "X" marked on the floor to deliver their speech with the video camera set to record mode and a LCD monitor. The participant faced the two assessors who each had a clipboard and took notes while the speech was delivered.

As instructed, speech was interrupted after five minutes and the assessors gave feedback and asked questions about the participant's performance.

Assessors were completely blind as to the psychological characteristics of the subjects. The assessors' responses were scripted and presented in the same order for each subject, but they were also adapted to the content of each speech for more credibility.

After the task each subject had to report their perceived task difficulty and perceived stress through a self-report including two questions rated on a 5-point Likert scale. The two items were:

1. How much stress did you feel during the task of public speaking?

2. Do you think that the task of public speaking was difficult?

Before leaving the experimental room, participants provided the third saliva sample (S3) and then were thanked for their participation. After leaving the experiment room, the subjects

remained in the laboratory for another 45 min. and provided three other saliva samples (S4, S5, S6), after which they were debriefed and allowed to leave.

The stress situation lasted a total of 20 min. During the experiment, a researcher supervised distribution of the scales (immediately before and after the task), and verified the subjective emotional states of participants at each given time point.

2.3. Psychological measures

Alexithymia: The French version of the Toronto Alexithymia Scale (TAS-20) was used as a measure of alexithymia (Bagby et al. 1994). This French version was validated by Loas et al. (1996) and used in several studies by the same authors (Loas et al. 1997; Loas et al. 2001). This questionnaire consists of 20 items answered on a 5-point scale, targeting three specific dimensions: DIFF (e.g., "When I am upset, I do not know if I am sad, frightened or angry"), DDF (e.g., "I find it hard to describe how I feel about people"), and EOT (e.g., "I prefer talking to people about daily activities rather than their feelings"). Two dimensions of the construct were used: Difficulty Identifying Feelings (DIFF) and Difficulty Describing Feelings (DDF). In fact, the literature on the psychometric properties of the TAS-20 (see review, Kooiman et al. 2002) reports that the third dimension, Externally Oriented Thinking (EOT), is unreliable; therefore, it was not analyzed. In the present study, the Cronbach's alpha was 0.80 for DIFF and 0.83for DDF.

2.4. Heart rate measurement

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Heart rates were assessed continuously using a Polar 800 CX (Polar, Electro GmbH, Buttelborn, Germany). Heart rate data collection started 60 min prior to the interview and ended 45 min. after interview participation.

2.5. Saliva measurements

Time of day was controlled because cortisol levels are known for small spontaneous fluctuations in the late afternoon; all tests were done between 16.00 h and 19.30 h Eating, and drinking beverages containing alcohol, caffeine, or fruit juices were not allowed for 60 min. before sampling. The subjects were told not to undergo intensive physical activity for the 48h prior to the experiment and to refrain from any sporting activities at all 24h before the study. Besides these restrictions, participants were free to follow their normal daily routines on the sampling days.

All samples were collected using Salivette collection tubes (Sarstedt Co., Nümbrecht, Germany). The cotton roll was omitted and the salivette container was used for saliva sampling. Participants donated whole unstimulated saliva using the passive drool technique for 3 min. They were instructed to swallow to empty the mouth before the unstimulated whole saliva sample was collected. The collection of whole saliva by passive drool is the most reliable option, as cotton or polyester-based materials tend to increase acidity and provide false concentrations of saliva components (Papacosta and Nassis, 2011).

Saliva samples were scheduled at 60 and 0 minutes pre-stress and at 3, 15, 30, and 45 minutes post-stress (see Figure 1).

Saliva samples were stored at -45°C until biochemical analysis. Tubes were centrifuged for 10 min at 3000 rpm to obtain clear saliva. Saliva volume was estimated by weighing to the nearest milligram and the saliva density was assumed to be 1.0 g.ml^{-1} (Cole and Eastoe, 1988).

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Saliva flow rate (ml.min^{-1}) was determined by dividing the volume of saliva by the collection time. The flow rate of saliva of valid samples should not be < 0.1 ml.min^{-1}. Salivary CgA, cortisol, and alpha-amylase were assayed using kits (YK070 Human CgA EIA kit; Yanaihara Institute, Shizuoka, Japan; cortisol EIA kit and alpha-amylase assay kit; Salimetrics inc., State College, PA, USA, respectively). Intra-assay maximal coefficients of variation were 8.15% for CgA, 6.7% for alpha-amylase, and 3.65% for cortisol. Inter-assay maximal coefficients of variation were 12.42% for CgA, 5.8% for alpha-amylase, and 6.41% for cortisol. Total protein concentration in saliva was determined using BioRad protein assay kit with human serum albumin as a standard.

Cortisol concentration was expressed as nmol.l^{-1}. sCgA and sAA concentrations were expressed as pmol.ml^{-1} and U.ml^{-1}, respectively. All samples were processed in duplicate during the same assay section.

2.6. Statistical analyses

SPSS for Windows Version 16.0 was used to analyze the data. Prior to analysis, the normality of the data distribution of each physiological variable was established using Kolmogorov-Smirnov test. Anthropometric and psychological characteristics are expressed as means and standard deviations (SD). All the other results are expressed as means and standard errors (SEM). Statistical significance was set at $p < .05$.

Area under the curve was calculated according to the formula described by Pruessner et al. (2003). Area under the curve with respect to the ground (AUC_G) was calculated for samples 2 to 6 for all parameters in order to obtain information about the total amount of a given substance excreted in a specific time period (Pruessner et al. 2003).

For the calculation of $AUCG$, this formula was applied:

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$$AUCG = \sum_{i=1}^{n-1} (S(i+1) + Si).ti/2)$$

In order to examine changes over time, the area under the curve with respect to increase (AUC_I) was also calculated for samples 2-6, using the formula devised by Pruessner et al. (2003).

$$AUCI = \sum_{i=1}^{n-1} (S(i+1) + Si).ti/2) - (Si \sum_{i=1}^{n-1} ti)$$

Possible effects of sex gender, alexithymia, and time on cortisol, sAA, CgA, and HR were tested with a Mixed Multivariate analyses of variance (MANOVA) for repeated measures. When a significant main effect or interaction was detected MANOVA was completed with ANOVA on single variables and Tukey's post-hoc tests were used when appropriate. The effect of time (sampling) was also tested through Trend Analysis.

3. Results

3.1. Psychological measures

The means scores for subjective feelings of stress and perceived task difficulty were 3.44 ±1.05, and 3.30 ±1.14, respectively. No significant differences between high/low alexithymia were noted either on subjective stress or task difficulty.

The MANOVA did not show any Sex or Alexithymia effects or any interaction implying these factors. However, there is a strong effect of time on the physiological variables globally tested (Table 1).

Here Table 1

3.2. Physiological parameters

Figure 2 illustrates the effect of time for each variable, as expected there is a significant quadratic trend (Wilks = .035, F(5,26) = 142, p <.000). Since the main multivariate effect of time

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is significant, univariate ANOVA were performed on each variable. Table 2 summarizes these ANOVA on the Time factor; all variables were significantly affected by the sample Time factor.

Here Figure 2

3.2.1. Heart rate

As revealed by post-hoc tests, mean heart rates at each time sample are significantly different except between times 2 and 3, times 4 and 5, and times 5 and 6. It is worth noting that heart rate increased immediately following TSST compared to baseline values, and did not return to resting values (S1) within 45 min. of the stressor termination. However, no significant differences between high/low alexithymia subjects were noted in heart rates.

3.2.2. Salivary parameters

Salivary flow rates did not change significantly over time, (between 0.53 ± 1.7 and 0.54 ± 0.5 ml.min^{-1}).

Total protein concentration in saliva did not change significantly during all the experimentation.

Salivary cortisol showed similar pattern to the heart rate through time (Figure 2) with a peak at 15 minutes after the end of the experimentation (S4). The concentrations 45 min. after the interview (S6) was significantly higher than those compared at S2 (just before the interview). The only insignificant differences in cortisol were between S2 and S6, and S3 and S5.

Alpha-amylase activity increased significantly after the interview (S3). The concentrations at S6 (45 min. after the interview) was significantly lower than those compared at S2 (just before the interview; $p<.001$). No significant differences between high/low alexithymia subjects were noted

The CgA concentrations were significantly different from each other with the exception of S2 and S6 which are similar. Again, the largest increases occurred after the interview and the

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concentrations dropped more slowly afterwards. The decrement of sCgA from peak point was significant and gradual until the 45th recovery minute (S6); at the end of the recovery period the value of sCgA was similar to S2. No significant differences between high/low alexithymia subjects were noted

Area under the curve with respect to the ground, a global stress response indicators of the whole situation, revealed a significant effect of Alexithymia as tested with a 2 (Sex) X 2 (Alexithymia) X 4 (variables) MANOVA (Wilks = .739, F(4,36) = 3.17 ; p = .024). Univariate analysis performed on each variable showed that the effect is present on cortisol only (F (1,39) = 9.19; p = .004). That is, the subjects who scored high on the alexithymia scale showed a significantly higher cortisol secretion than those who scored low.

There was an effect of alexithymia on the AUCg (β= .36, t = 2.5, p < .01). To evaluate the weight of alexithymia subfactors we performed four multiple regression analyses with AUCg, S2, S3 and S4 as dependent variables. The independent variables = DIFF and DDF were entered simultaneously into the model. As shown in Table 3, only the DIFF factor was a significant predictor of cortisol response (note that it significantly predicted AUCg and cortisol concentrations after the interview (S3 and S4).

Area under the curve with respect to increase (AUCI) of stress response indicators revealed no significant interaction effect of sex and alexithymia as tested with a 2 (Sex) X 2 (Alexithymia) X 4 (variables) MANOVA. There was no effect of alexithymia on the AUCI.

Here Table 3

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4. Discussion

Our study was original in that it is - to our knowledge - the first study to consider the link between alexithymia subfactors on HPA and SAM response to a controlled social stress among psychologically and physically healthy students. It showed that, among all tested stress indicators, the "area under the curve with respect to the ground" of cortisol was the only variable positively related to alexithymia; this relation being mainly attributed to DIFF subfactor. Therefore, alexithymia was associated with significantly increased cortisol before, during and after the stress exposure (Table 3). No link was noted between this personality trait and SAM axis as evaluated through sAA and sCgA.

Few studies have tested the relationship between alexithymia and cortisol responses. Mc Caslin et al. (2006) showed no link between alexithymia and cortisol reactivity to a video stress challenge. De Timary et al. (2008) reported that alexithymia, and DDF factor, modulates the anticipation to the stressor rather than the response to the stressor itself and probably the cognitive processing of emotions. They also reported higher salivary cortisol levels in high-alexithymia individuals, who were about to be involved in an imminent socially challenging situation. Moreover, DIFF was negatively correlated to cortisol, partially explaining the observations of Henry et al. (1992) of an apparently negative correlation between alexithymia and cortisol. Pedrosa Gil et al. (2008) reported no significant correlations between high-alexithymia patients and cortisol levels, measured by the area under the curve-ground (AUC-G), area under the curve-increase (AUC-I). Thus, data in the literature are still not consistent. Our data showed that alexithymia was associated with significantly increased cortisol before, during and after the stress exposure (Table 3), suggesting that alexithymia and its DIFF subfactor modulate the anticipation and the recovery to the stressor. Therefore, one can hypothesize that

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the cognitive processing of emotion was altered, as suggested by Timary et al. (2008) who stated that alexithymia modifies the anticipatory cognitive appraisal of situations. However, it should be noted that, in our study, no significant differences between high/low alexithymia were observed either on subjective stress or on task difficulty. The relatively small number of subjects may also explain the lack of statistically significant effect of alexithymia on subjective stress. Usually, studies dealing with these psychological variables involve much more subjects. Nevertheless, as indicated by our data, whether or not this effect exists, it is probably weak. Confounding effect of sex can be ruled out, no sex effect being observed in this study. Recently, Härtwig et al. (2013) noted that psychologically and physically healthy high-alexithymia individuals showed a HPA system hypoactivity as measured by the CAR, the CAR being a valid measure of basal HPA-system activity (Kudielka and Wüst, 2010). Alexithymia has been hypothesized to be associated with chronic stress. It seems that chronic stress may initially result in an increased activity of the HPA axis, but if the stress persists over a substantially long time, it may lead to HPA hypoactivity (Fries et al. 2005). Thus, an explanation for the lower CAR in Härtwig's (2013) high-alexithymia cohort could be the older age insomuch as older adults in that study likely have experienced more cumulative life stress in comparison to our younger cohort (Härtwig et al. 2013).

In a recent study, McIntosh et al. (2014) noted that alexithymic participants living with HIV had significantly higher levels of norepinephrine (NE) as well as an elevated NE/Cortisol ratio. Their model suggested that the DIFF factor score was the strongest predictor of a greater NE/Cortisol ratio. Clearly, the literature regarding alexithymia and autonomic responses to stress or negative affects is equivocal. There are two separate approaches to explain the patterns of autonomic nervous system activity in relation to alexithymia. Linden et al. (1996) and Bermond et al. (2010)

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highlighted the hypoarousal theory, stating that alexithymic persons are less physiologically aroused. In contrast, several authors have provided evidence of tonic or exaggerated sympathetic hyperarousal in persons with alexithymia, (Papciak et al. 1985; Stone et Nielson, 2001), likely the result of a decoupling of subjective stress-appraisals and autonomic responses (Martin and Pihl, 1985). According to the decoupling hypothesis increased norepinephrine reflects greater efforts to meet the demands of a stressful experience while lower cortisol indicates the absence of behavioral consequences (Henry et al. 1992). This pattern is not apparent in our study as there are no higher levels of alexithymia predicting greater skew in the proportion of salivary sAA and CgA as compared to cortisol levels. Salivary α-amylase (sAA) has been suggested to be an index of SAM activity, because the sympathetic and parasympathetic branches of the autonomic nervous system innervate salivary glands. Sympathetic stimulation increases salivary protein secretion, whereas parasympathetic stimulation increases salivary flow rate (Baum, 1993). SAA activity is positively correlated with the acute sympathetic nervous system (SNS) stress response in children and adults (Nater et al. 2006). As chromogranin A (CgA) is a reliable marker of SAM system activity, CgA could be used along with sAA (Gallina et al. 2011). CgA is considered to be a reliable index for evaluating psychological stress and a quantitative index of the activity of the sympathetic nervous system (SNS) innervating glands (Takatsuji et al. 2008). Our study only confirms that a threatening, negative, or unexpected experience evokes an activation of the stress response inducing a chain of neuroendocrine and other nervous system reactions. However, no evidence of tonic or exaggerated sympathetic hyper arousal in relation with alexithymia was observed. This does not corroborate some studies having reported that alexithymia would be associated with both an increased noradrenergic activity and a decreased cortisol release; a finding which has been reported in alexithymic males with and without depression (Spitzer et al.

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2005). In a recent study, Pollatos et al. (2011) reported no significant effect of alexithymia on heart rate reactivity and skin conductance; both variables are considered reliable indices of sympathetic nervous activity. These results were also observed in our study concerning HR, even if the job interview induced an increase of the neuro-vegetative reactivity (Figure 2). Heart rate is influenced by both sympathetic and vagal activity. Hence, as suggested by Pollatos et al. (2011), differential effects on vagal and/or sympathetic activity produced by the social stressor might occur and explain why alexithymia does not interact with HR in our social stress task. Referring to SAM and SNS axis, we can hypothesize that other factors such as coping strategies and the perception of the situation as a challenge or a threat may alter the relationship between alexithymia and these axes.

In summary, the results of the current study suggest that difficulty in differentiating feelings and distinguishing them from bodily sensations and emotion arousal is associated with a hyper-responsive HPA system. Those links were not present with the SAM axis. Because the relevant literature on this topic presents conflicting results, more research is clearly necessary. This research should be replicated with more subjects and should take into account more parameters reflecting sympathetic and/or parasympathetic activation, as well as HPA axis. Factors such as coping strategies and the perception of the situation as a challenge have also to be explored.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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Contributors

Authors Le Scanff, Filaire, Martin, Devillers designed the study and wrote the protocol. Author Hua managed the literature searches. Authors Jacques Larue and Hua performed the statistical analysis. Authors Filaire and Ferreira analyzed saliva samples. Authors Filaire and Le Scanff wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Table 1   Multivariate Analysis of Variance with repeated measures on the last factor

2 (Sex) X 2 (Alexithymia) X 6 (Time)

EFFECT	Wilks	F	Df (effect)	df (error)	*p*
SEX	0.86	0.89	5	29	.49
ALEXITHYMIA	0.69	2.52	5	29	.05
SEX*ALEXITHYMIA	0.86	0.89	5	29	.49
TIME	0.00	48.15	25	9	.00
TIME*SEX	0.24	1.09	25	9	.46
TIME*ALEXITHYMIA	0.33	0.72	25	9	.75
TIME*SEX*ALEXITHYMIA	0.14	2.15	25	9	.11

Table2 Univariate ANOVA on Time factor with repeated measures

VARIABLES	dl effect	dl error	F	p <
Heart rate	5	180	54.64	.001
Cortisol	5	210	65.64	.001
Alpha Amylase	5	210	274	.000
ChromograninA	5	210	141	.000

Table 3 Multiple regression analyses predicting the cortisol responses by alexithymia subfactors.

	AUCg	S2	S3	S4
	Area under the curve			
	$F_{(2,40)} = 5.5$ ***	$F_{(2,40)} = 2.82$*	$F_{(2,40)} = 5.03$ **	$F_{(2,40)} = 4.0$ *
	$R^2 = .21$	$R^2 = .0.35$	$R^2 = .20$	$R^2 = .16$
DIFF	Beta = .53 $t = 3.04$ ***	Beta = .30 $t = 1.6$*	Beta = .56 $t = 3.17$***	Beta = .51 $t = 2.8$**
DDF	Beta = -1.43 $t = - .8$	Beta = .07 $t = .38$	Beta = -.32 $t = -1.8$	Beta = -.29 $t = -1.6$

Note. S2, just before the interview; S3, immediately after the interview; S4, 15 minutes after the end of the interview; DIF, Difficulty in Identifying Feelings; DDF, Difficulty in Describing Feelings. AUCg: Area under the Curve. It was calculated using the formula of Pruessner et al., (2003).
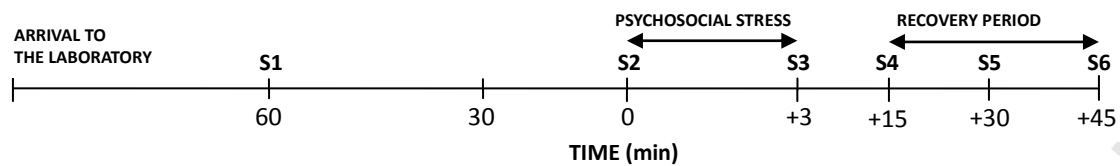
* $p < .05$; ** $p < .01$;*** $p < .001$

Figure 1: Schedule of the experimentation: S1: Saliva Sample 60 min after arrival at the laboratory; S2: Saliva Sample 3 min pre-test; S3: Saliva Sample 3 min post-test; S4: Saliva Sample 15 min post-test; S5: Saliva Sample 30 min post-test; S6: Saliva Sample 45 min post-test.
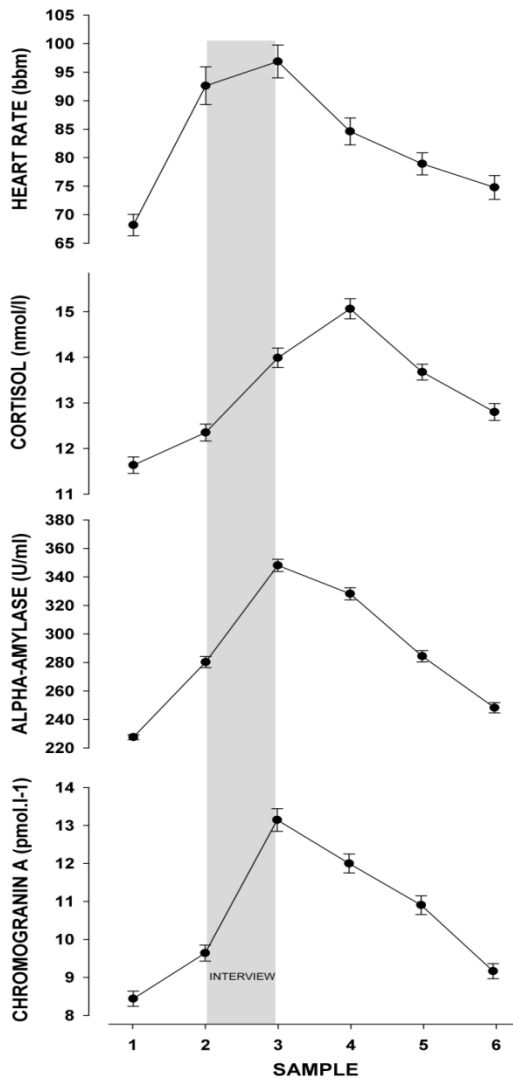
Figure 2: Heart rate**,** Salivary Cortisol (nmol.l^{-1}), Alpha-amylase (U.ml^{-1}), and Chromogranin A (CgA) (pmol.l^{-1}) (mean and SEM) of participants exposed to the interview. Saliva samples were obtained at baseline (S1), just before (S2), immediately after (S3), 15 min, 30 min, and 45 min following exposure (recovery) to the interview.