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Stress and Cognitive Reserve as independent factors of neuropsychological performance in healthy elderly

Estresse e Reserva Cognitiva como determinantes independentes para o desempenho neuropsicológico de idosos saudáveis

João Carlos Centurion Cabral [1]
Gessyka Wanglon Veleda [2]
Martina Mazzoleni [3]
Elton Pinto Colares [4]
Lucas Neiva-Silva [3]
Vera Torres das Neves [3]

Abstract *Exposure to high levels of cortisol and self-reported stress, as well as cognitive reserve, have been linked to Alzheimer's disease pathology. However, there are no studies on the interaction of these variables. The present study aims to assess the associations of measures of cortisol, self-reported stress, and cognitive reserve with neuropsychological performance in healthy elderly people; besides, to test the interactions between these variables. Cross-sectional analyzes were conducted using data on stress, cognitive reserve and clinical conditions in 145 healthy elderly adults. A neuropsychological battery was used to assess executive functions, verbal memory and processing speed. Measurement of salivary cortisol at the circadian nadir was taken. A negative association between different stress measures and performance on tasks of memory, executive functions and processing speed was observed. Elderly people with higher cognitive reserve showed superior performance on all neuropsychological measures. No significant interaction between stress and cognitive reserve to neuropsychological performance was observed. These results indicate that older adults with high levels of stress and reduced cognitive reserve may be more susceptible to cognitive impairment.*

Key words *Salivary cortisol, Glucocorticoid, Stress, Cognitive reserve*

Resumo *A exposição a níveis elevados de cortisol e de estresse psicológico, assim como à reserva cognitiva, têm sido relacionadas a sintomas da Doença de Alzheimer. Contudo, não há estudos sobre a interação dessas variáveis. Objetivamos examinar as associações de medidas de cortisol e estresse psicológico e de reserva cognitiva com o desempenho neuropsicológico de idosos saudáveis, além de analisar a existência de interações entre essas variáveis. Análises transversais foram conduzidas usando dados sobre estresse, reserva cognitiva e condições clínicas em 145 idosos saudáveis. Usamos uma bateria neuropsicológica para medir as funções executivas, memória verbal e velocidade de processamento. Utilizamos uma medida de cortisol salivar para o nadir circadiano. Encontramos uma associação negativa entre diferentes medidas de estresse e o desempenho em tarefas de memória, funções executivas e velocidade de processamento. Idosos com elevada reserva cognitiva apresentaram um desempenho superior em todas as medidas neuropsicológicas. Não houve interação significativa entre estresse e Reserva Cognitiva para o desempenho neuropsicológico. Estes resultados sugerem que idosos com níveis elevados de estresse e reduzida reserva cognitiva podem ser mais suscetíveis ao comprometimento cognitivo.*

Palavras-chave *Cortisol salivar, Glicocorticoides, Estresse, Reserva cognitiva*

[1] Instituto de Psicologia, Universidade Federal do Rio Grande do Sul (UFRGS). Av. Paulo Gama 110, Farroupilha. 90040-060 Porto Alegre RS Brasil. centurion.cabral@ufrgs.br
[2] Hospital Universitário Prof. Polydoro Ernani de São Thiago, Universidade Federal de Santa Catarina. Florianópolis SC Brasil.
[3] Instituto de Ciências Humanas e da Informação, UFRGS. Porto Alegre RS Brasil.
[4] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande. Rio Grande RS Brasil.

Introduction

Social and environmental variables can have a significant effect on neuropsychological functioning, increasing vulnerability to cognitive impairment and dementia in the elderly. Alzheimer's disease (AD), the most common type of dementia, is characterized: (a) clinically, by cognitive decline, especially of episodic memory; (b) morphologically, by brain atrophy, being initially affected the hippocampal formation and entorhinal cortex; and (c) histologically, by reduction of synaptic density, presence of neurofibrillary tangles of Tau protein and aggregates of amyloid-β peptide (Aß)[1,2]. The accumulation of soluble Aß oligomers is considered a critical event in the pathogenesis of AD[3], producing cellular changes that result in dendritic atrophy, synaptic loss, and neuronal death[4,5]. Late-onset AD is heterogeneous and multifactorial, triggered by an interaction between genetic and environmental factors, as well as clinical phenotypes[6]. Even in healthy people, when these interactions disrupt the functioning of nervous system, there may be a reduction of brain reserves, leading to a higher susceptibility to cognitive impairment[7]. Due to the wide variability in cognitive abilities of older people, the factors associated with these differences remain to be elucidated.

Cognitive reserve (CR) refers to individual differences in brain or cognitive processing capacity to deal with injuries to the nervous system[8]. This construct, often linked to educational level and intellectual experiences, has been proposed to try to explain the discrepancy between the severity of disease markers and clinical manifestations in neurological disorders[7]. In a classic post-mortem study, levels of biomarkers for AD in individuals who did not have clinically significant symptoms were evident, conversely these subjects had a higher amount of neurons and brain weight than those patients with the clinical manifestation of disease[9]. Complementing these findings, it was observed that subjects with a high level of education are more resistant to clinical manifestation of AD[10]. In addition, an in vivo study showed that education, occupation and intellectual activity are associated with measures of volume and pattern of brain activity[11]. Although the functional correlate of CR is not completely understood, the amount and interaction of specific presynaptic proteins may be some of CR components that reduce the risk of dementia[12]. However, little is known about the interaction between the CR and other variables that are considered risk factors for cognitive decline in the elderly.

Prolonged exposure to glucocorticoids (GC), especially cortisol in humans, and to psychological stress play a significant role in pathological cognitive impairment in elderly[13-15]. An increasing amount of evidence, including experimental studies in humans, demonstrated that cortisol may impair the formation of declarative memory[16] and may predict cognitive decline in healthy older adults[17]. Also, measures of executive function and processing speed were negatively associated with concentrations of GCs[18]. Chronic stress and increased GC levels were also related to hippocampal neuronal loss, dendritic atrophy and reduced hippocampal volume[15,19-22]. However, these findings are still controversial because several studies have found no such associations, nor with neuropsychological performance[17,23], nor with hippocampal measures[24-26]. Otherwise, different lines of evidence support the notion that chronic stress and increased GCs play a role in the risk for development of AD[24]. Green et al.[27] found, in animal models, that GCs mediate an increase in production and reduce degradation of Aß, enhance their neuronal toxicity and enable the formation of amyloid plaques, additionally increasing the accumulation of tau protein. GCs have also been associated with other pathophysiological features of AD, such as increased release of excitatory amino acids and increased expression of N-methyl-D-Aspartate (NMDA) glutamate receptor, as well as changes in calcium influx[13]. Furthermore, older people may be particularly susceptible to the effects of stress, with reduced capacity to resist damage in neurons after prolonged exposure to GCs[14]. These data are still preliminary and are under intense investigation.

With the establishment of risk factors for the development of AD, the interactions between factors that may increase the likelihood of cognitive impairment in healthy elderly have been increasingly studied[28]. Therefore, the present study aimed to examine the associations of subjective and physiological stress and CR with neuropsychological performance in healthy elderly people. Also, it was verified whether there were interactions between the effects of stress and CR on neuropsychological performance in this population. It was hypothesized that a high level of stress would be associated with reduced neuropsychological performance and high CR would be associated with better performance. Furthermore, it was hypothesized that a high CR would reduce the impact of stress on neuropsychological performance in elderly adults.

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Method

Participants

One hundred and forty-five cognitively healthy and socially active participants, aged above or equal to 60 years, having at least 4 years of schooling, residents of southern Brazil, were recruited through an approach based on sampling units of time and space. For this cross-sectional study, we established units in neighborhoods of Rio Grande city, RS, Brazil, by identifying specific locations and times at which the target population was likely to be found and constructed a sampling frame of these locations. The identified time-space units were randomly visited, allowing the target population to be systematically recruited in accordance with the protocol of Muhib et al.[29]. Potential participants who had a history or evidence of dementia, disabling medical conditions or use of medications that affect cognitive functioning or cortisol levels (e.g., corticosteroids) were excluded. Participants received instructions and signed a term of informed consent before entering the study, which was approved by the Ethics Committee of Health Research of Federal University of Rio Grande.

Procedure

After previous scheduling, data collection was performed by trained research assistants, at the participant's residence. This procedure aimed to reduce the anxiety caused by testing in an atypical environment. Data collection was conducted between 10:00 a.m. and 5:00 p.m., in order to avoid the circadian variation of cortisol secretion, and was performed during one season (i.e., winter). Data were collected in the following order: interview about socio-demographic and health characteristics, and intellectual activity, measures of stress, neuropsychological testing, clinical measures, and lastly, instructions for saliva collection procedure were provided.

Psychological and Clinical Assessments

A questionnaire was elaborated to assess health and sociodemographic characteristics, including health habits (e.g., drinking, smoking, lack of exercise), past medical history (e.g., diabetes mellitus, cardiovascular disease, neurological disease) and social activities (e.g., traveling, entertaining, and attending social gatherings). Moreover, to assess the mental status workup we used the Clinical Dementia Rating Scale (CDR; for more details see below) as a cognitive screening instrument.

To assess stress, three scales were used and one to assess depression symptoms. The Perceived Stress Scale (PSS-14), which measures the perception of stress for the last month, has adequate psychometric qualities in its version for the Brazilian population, with internal consistency and construct validity similar to the original version[30]. The Social Readjustment Rating Scale (SRRS) presents a list of 43 stressful events, and the respondent should indicate, among them, those which occurred during last year[31]. The Hassles and Uplifts scale (HUS), in its version of 53 items, measures daily sensitivity to adverse events (only the part related to negative evaluation of events was used)[32]. Finally, the Geriatric Depression Scale (GDS) in the version with 15 items was used for screening depressive symptoms. This scale showed good accuracy, sensitivity, specificity and reliability for the Brazilian population[33].

Measures of intellectual activity and education were used for the establishment of a proxy measure for CR. Data on years of formal education; school failure; weekly frequency of reading books, newspapers, magazines and other materials (e.g., puzzle exercise and internet); number and fluency of foreign languages spoken (comprehension, speaking, reading and writing) were used to quantify the proxy measures for cognitive reserve. Principal Component Analysis was used to combine these variables and to obtain for CR, as described in "Statistical Analysis" section. Measures of intelligence (e.g., verbal IQ) were not used for the proxy for CR, since such measures are intrinsically associated with neuropsychological performance.

The Clinical Dementia Rating Scale (CDR), also validated for Brazilian population, is a scale used to quantify the severity of symptoms of dementia, assessing global cognition and behavior in six clinical areas: memory, orientation, judgment and problem solving, community affairs, home and leisure activities and personal care. This scale has a sensitivity of 91.2% and specificity of 100%[34]. In the present study, this scale was used as a criterion for exclusion (i.e., six participants were excluded for being classified as equal to or greater than one on CDR score, from an initial sample of 151).

Neuropsychological Assessment

The application of the neuropsychological battery lasted about 90 minutes. The instructions for each task were read aloud by a research assistant and made available in writing to participants before each test. The mention of the term "skills testing" was avoided to minimize anxiety related to performance of the participants. The neuropsychological battery was assembled in order to measure the performance of executive functions, verbal memory and processing speed.

Measures of executive functions included a Verbal Fluency Test, Trail Making Test, Stroop Test, and a Digit Span task. In the Semantic Verbal Fluency Test, the semantic version was used, in which the participant must say as many words as possible, from a given category, during one minute. This test is highly sensitive to prefrontal cortex (PFC) dysfunction[35]. In order to control other sources of variations on people's fluency and categorization (variables measured by test) two applications were carried out, one with the category "animals" and the other with the category "fruit". The score for fluency was calculated by averaging the results of applications. The Trail Making Test B measures cognitive flexibility and attention. After demonstration, the participant must draw lines to connect the randomly spread numbers and letters in an ascending and alternating alphanumeric sequence, in the shortest possible time. Stroop test measures inhibitory control and attention under incongruent stimuli conditions (i.e., words printed in an ink color differing from the color name of the word). The Digit Span task measures working memory, a component of executive function that activates the Dorsolateral PFC cortex[36]. Only the direct order version was used.

The Word List Memory Task (WLMT)[37] assesses the ability to recall ten unrelated words of everyday use. In the learning trials (immediate recall), the same words are displayed in different order in each trial. Each of the three trials has maximum score of ten. Ten minutes after the learning phase, free recall was measured through the Word List Recall Task, in which the ten words shown in the learning trials should be mentioned, without stimulus presentation. Then, the Word List Recognition Task was applied, when the participant had to identify the ten words shown in previous trials, to distinguish them from other ten words of everyday use. In this task, the 20 words were randomly ordered. Each trial allowed 90 seconds for responses. After the learning trials, an abstract task, unrelated to the WLMT (i.e., not using words in its execution) was executed, to avoid interference in the memory encoding process. In this case, a Digit Symbol Substitution Task was used.

Digit Symbol Substitution Task was used to measure processing speed. In this task, a sequence of digit-symbol pairs is used to match and complete a list of unpaired symbols. Participants were asked to make the largest number of pairings possible within 120 seconds. Moreover, Trail Making Test A (25 sequential numbers are linked with a pencil in the shortest possible time) and reading time without interference, from the Stroop Test, were used to measurement processing speed.

Salivary Cortisol Measurement

Salivary cortisol is a biomarker used in stress research to evaluate the activity of the hypothalamic-pituitary-adrenal (HPA) axis, providing a reliable measure of the biologically active (unbound) fraction of this hormone and has good correlation with serum measurements of cortisol[38]. The method of saliva collection is simple, non-invasive, and can be done in various environments, making unnecessary the presence of specialized personnel. Thus, saliva was collected by the participants in their homes, between 9:00 p.m. and 10:00 p.m. one to two days after the neuropsychological assessment, when they received saliva collection instructions. For this purpose, cotton swab of the Salivette sampling devices (Sarstedt, Rommeldorf, Germany) were inserted into the oral cavity. Participants were instructed to make smooth masticatory movements, with mild pressure to avoid damage to the material, until it became saturated with saliva (about 3 minutes). The samples were refrigerated until delivered to research assistant, during the next day. Then, the samples were centrifuged (1800 x g) for 20 minutes and stored at - 20°C until the biochemical analysis. Nocturnal cortisol sampling was chosen to determine the nadir cortisol levels and, also, to avoid spontaneous fluctuations in cortisol secretion pattern, and its high amplitude, which are significantly lower at night[17].

Eighty-one samples were randomly selected from the total pool, and underwent duplicate analysis using an enzyme immunoassay based on the principle of competitive binding (Cortisol Saliva ELISA Kit - Diagnostics Biochem Canada Inc, Ontario, Canada). After thawing, 50 µL of the supernatant fraction was used for each assay,

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as specified by the manufacturer's instructions. An ELISA plate reader with a filter set at 450nm was used. The sensitivity of the kit was 1 ng/ml.

Statistical Analysis

Descriptive and exploratory analysis were performed. Tests for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test) for total sample and each group were conducted. Spearman correlation ($\alpha = 0.05$) was used to measure the relationship between continuous variables. Principal Component Analysis (PCA) was performed to obtain principal components and standard scores (dimensionless) for the latent variables. PCA were performed using a correlation matrix (since the variables have different units), with a varimax rotation and an extraction criterion of eigenvalue > 1. The variable loadings greater than 0.4 were used for interpretation. PCA were used to deal with the problem of multiple comparisons (Familywise Error Rate - FWER), i.e., increased the probability of type I error, reducing the dependent variables. Each PCA generated a single component and a corresponding standard score. These were later named as Cognitive Reserve (CR); Executive Function (EF); Verbal Memory (VM); and Processing Speed (PS). Perceived Stress score and the standardized score of cognitive reserve were categorized into two groups based on median values: High Stress (HS) / Low Stress (LS) and High Cognitive Reserve (HCR) / Low Cognitive Reserve (LCR), respectively. T tests were used to compare the means of dependent variables (EF, VM and PS) for these groups. Finally, a two-way ANOVA was performed to determine the interaction between the independent variables (HS / LS X HCR / LCR) on the standardized dependent variables. The Bonferroni correction was used to reduce the probability of Type I error (FWER), yielding a significance level of 0.016.

Results

Socio-demographic and health characteristics for the entire sample and for the groups of high and low level of stress, were summarized in Table 1. The salivary cortisol levels at nadir had a mean of 6.95 ng/mL (standard error (SE) = 0 , 37), and showed no significant associations with age, gender, education and socioeconomic status of the participants, nor were there significant differences between the groups classified as high or low perceived stress and those classified as high or low cognitive reserve. Each factor (for each PCA) explained approximately 60% of variance. The factor extracted for CR had an eigenvalue of 1.89. The eigenvalue for the EF factor was 2.15. While for the memory score, its eigenvalue was 3.26. Finally, the eigenvalue for PS was 2.06. Neuropsychological scores can be seen in Table 2.

Correlations of measures of stress and CR with performances on neuropsychological tests are shown in Table 3. All significant correlations between measures of stress and neuropsychological performance were weak. The perceived stress was consistently associated with a decrease in verbal memory performance. However, this pattern was not observed in measures for adverse events. The increased level of cortisol was sig-

Table 1. Sociodemographic and health variables.

Variables	Participants (n = 145)	Groups	
		Low Stress (n = 73)	High Stress (n = 72)
Age (years)[a,b]	69.9 (6.7)	70.0 (6.6)	69.7 (6.8)
Gender (female)[c]	66.2	60.3	72.2
Education (years)[a,b]	9.8 (4.6)	10.9 (4.5)	8.8 (4.4)
Body mass index[a]	26.4 (3.8)	26.3 (3.6)	26.6 (4.0)
Sleep (hours per night)[a,b]	7.4 (1.6)	7.4 (1.3)	7.4 (1.8)
Regular physical activity[b,c]	53.1	61.6	44.4
Tobacco use[b,c,d]	10.3	11.0	9.7
Diabetes mellitus[b,c]	23.4	19.2	27.8
Cardiovascular disease[b,c]	58.6	56.2	61.1
Clinical Dementia Rating (0,5)[c,e]	17.9	6.8	28.2
Geriatric depression[a,f]	2.9 (2.9)	1.6 (1.5)	4.2 (3.4)

[a] Mean and standard deviation. [b] Self-report data. [c] Percentage. [d] Regular tobacco use in the last year. [e] CDR 0.5 score. [f] Data obtained by the Geriatric Depression Scale.

nificantly correlated with a worse performance in the recognition task. Nevertheless, its association with other memory scores did not meet the significance level, even having a pattern, i.e., negative correlations with all measures. Again, the perceived stress was associated with a worse performance on cognitive flexibility and attention tasks (Trail Making Test B) and working memory (digit span), both measures of executive functions. It was not found for inhibitory control and fluency measures. The two measures of processing speed were also related with perceived stress. On the other hand, the explained variance in the associations between measures of stress and neuropsychological assessment in the elderly was at most 7%. This inconsistency of results is not seen in the association between CR score and neuropsychological performances, in which all correlations were positive and highly significant. These associations reached an explained variance of up to 33%.

The association of high stress with reduction in neuropsychological performance in the elderly was confirmed by the comparison between High Stress and Low Stress groups (Table 4). The EF score was significantly reduced (p = 0.011) in the High Stress group. However, the mean difference (MD) of both groups was 0.41 (standard error of the difference (SED) = 0.16). The difference between stress groups was also significant (p = 0.016) for verbal memory scores, with a mean difference of 0.39 (SED = 0.16). Similar results were seen for the difference between these groups in processing speed score (p = 0.014; MD = -0.41; SED = 0.16). While these data corroborate the hypothesis that high level of stress and reduced

Table 2. Mean (standard deviation) and range for neuropsychological performance in healthy elderly.

Variables	Score	
	Mean (SD)	**Range**
Executive Function		
Verbal fluency test (semantic)	14.38 (3.7)	17.5
Trail making test B[a]	156.11 (82.0)	382.0
Stroop test (incompatible stimuli)	75.34 (21.8)	96.0
Digit span task	7.59 (1.8)	8.0
Verbal Memory		
Word list immediate recall task 1[b]	4.75 (1.7)	9.0
Word list immediate recall task 2[b]	7.03 (1.7)	8.0
Word list immediate recall task 3[b]	7.62 (1.7)	7.0
Word list recall task[b]	5.85 (2.1)	9.0
Word list recognition task[b]	9.21 (1.1)	5.0
Processing Speed		
Trail making test A[a]	57.35 (38.5)	333.0
Stroop test (compatible stimuli)[a]	72.9 (17.6)	74.0
Digit symbol substitution task	38.57 (14.4)	68.0

Abbreviations: SD, standard deviation. [a] Time. [b] Word list memory task.

Table 3. Associations of measures of stress and cognitive reserve with neuropsychological performance[a].

		Stress					CR
	Variables[b]	**Cortisol[c]**	**PSS14**	**SRRS**	**HUS**	**GDS15**	**SCR**
EF	Stroop (IS)[e]	.025 (.413)[d]	-.100 (.116)	-.088 (.147)	-.032 (.352)	-.119 (.076)	.386 (<.001)
	TMT-B[f]	-.083 (.231)	.187 (.012)	.120 (.076)	.106 (.103)	.116 (.082)	-.386 (<.001)
	Verbal Fluency	-.102 (.183)	-.122 (.072)	.002 (.491)	.082 (.164)	-.198 (.008)	.379 (<.001)
	Digit Span	.158 (.080)	-.260 (.001)	.177 (.016)	-.050 (.274)	-.215 (.005)	.188 (.012)
VM	Immediate Recall 1	-.170 (.064)	-.201 (.008)	-.028 (.368)	.070 (.201)	-.090 (.140)	.274 (<.001)
	Immediate Recall 2	-.069 (.271)	-.189 (.012)	-.055 (.257)	.047 (.286)	-.195 (.009)	.256 (.001)
	Immediate Recall 3	-.118 (.147)	-.151 (.035)	-.025 (.385)	.118 (.079)	-.136 (.051)	.209 (.006)
	Recall	-.140 (.107)	-.181 (.015)	-.109 (.095)	.101 (.113)	-.152 (.034)	.242 (.002)
	Recognition	-.208 (.031)	-.244 (.002)	-.043 (.305)	.075 (.184)	-.163 (.025)	.123 (.070)
PS	TMT-Af	-.138 (.110)	.212 (.005)	.078 (.175)	.123 (.071)	.151 (.035)	-.444 (<.001)
	Stroop (CS)[g]	-.145 (.098)	.108 (.098)	.064 (.221)	-.004 (.480)	.158 (.029)	-.574 (<.001)
	DSST	.216 (.027)	-.254 (.001)	-.124 (.068)	-.097 (.122)	-.157 (.030)	.474 (<.001)

Abbreviations: PSS14, Perceived Stress Scale; SRRS, Social Readjustment Rating Scale; HUS, Hassles & Uplifts Scale; GDS15, Geriatric Depression Scale; CR, Cognitive Reserve; SCR, Standard Score for Cognitive Reserve; DSST, Digit Symbol Substitution Task; TMT, Trail Making Task; EF, Executive Function; VM, Verbal Memory; PS, Processing Speed.
[a] Associations by Spearman's correlation coefficient at the 0.05 significance level. [b] Data were obtained from 145 participants, except where noted. [c] Data were obtained from 81 participants. [d] Correlation size effect (p-value). [e] Stroop test (incompatible stimuli). [f] Time to complete the task. [g]Stroop test (compatible stimuli).

neuropsychological performance are related, we realize that the size of the main effect, though existent, is quite limited.

On the other hand, the means differences in standardized neuropsychological scores for the High CR and Low CR groups were highly significant (Table 4). In these groups, were observed p-values of less than 0.001 for all neuropsychological scores, corroborating our second hypothesis. In addition, the mean differences were -0.84 (SED = 0.15); -0.58 (SED = 0.15); and 0.93 (SED = 0.14); for EF, VM and PS scores, respectively.

Finally, the hypothesis of interaction effects between the independent variables was refuted (Table 4). All F-values were below 1 and η^2 were less than 0.001.

Discussion

Our results support the notion that healthy elderly adults with high level of stress have significantly worse scores on cognitive tests. Although this effect size was small, the relationship is consistent for all three measured variables. This consistency can be checked by the negative correlations pattern of perceived stress scores and cortisol levels with different measures of memory. In addition, depression scores can support these findings. Depression is largely associated with memory impairment and hippocampal atrophy and these may be due, at least in part, to hypercortisolemia, which is frequent in this pathology[25]. A similar pattern was seen for PS measures. Conversely, measures of EF have not shown the same consistency, only working memory and cognitive flexibility were impaired by perception of high stress level, both measures are associated with the dorsolateral PFC activity[36]. Absence of correlations between adverse events or daily hassles may reflect the complexity of physiological stress, which can be modulated by several factors, such as differences in expression of GC receptor genes, differences in patterns of cortisol secretion, epigenetic regulation, concentration of other hormones, reactivity to stress and resilience[13,39].

Certainly, brain aging may increase the susceptibility to the effects of stress. The hippocampus and PFC, brain regions responsible for episodic memory consolidation and executive functions respectively, become increasingly susceptible to deleterious effects[25,40,41]. In hippocampal region, impairments can also result in persistently high levels of cortisol, since this region regulates GC levels through an inhibitory feedback loop[42-44]. The human hippocampus has a high density of mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), given that both mediate cortisol activity[45,46]. With high levels of free cortisol, MRs are fully saturated, while a large proportion of GRs are occupied, and this, via the hippocampus, inhibits the secretion of corticotropin releasing hormone (CRH) from the hypothalamus and thus reducing the cascade responsible for cortisol release from the suprarenal glands[45]. Consequently, hippocampal atrophy may reduce the effectiveness of this control mechanism, thereby increasing the susceptibility to the effects of stress. Indeed, patients with AD have a cortisol secretion pattern progressively higher[47]. Typically, higher evening cortisol levels are related to an impairment of the negative feedback of HPA axis[48]. This is consistent with our data which show an association between high evening cortisol levels and poorer performance in the recognition task, seeing as this task is less influenced by

Table 4. Mean differences between stress groups stress and cognitive reserve groups for neuropsychological standard scores[a,b,c].

Variables	Stress				Cognitive Reserve				Interaction CR X Stress ANOVA	
	High n = 72 m (sd)	Low n = 73 m (sd)	t test t[d]	p	High n = 72 m (sd)	Low n = 73 m (sd)	t test t[d]	p	F[e]	p
EF	-.21 (1.01)	.21 (.95)	2.57	.011	.42 (.88)	-.42 (.94)	-5.55	<.001	.02	.901
VM	-.20 (.99)	.19 (.97)	2.43	.016	.29 (.97)	-.29 (.95)	-3.66	<.001	<.01	.986
PS	.20 (1.10)	-.20 (.85)	-2.49	.014	-.47 (.74)	.46 (1.00)	6.36	<.001	.79	.376

Abbreviations. EF, Executive Function; VM, Verbal Memory; PS, Processing Speed; CR, Cognitive Reserve.
[a] T test was used to determine the neuropsychological differences between stress groups and cognitive reserve groups. [b] Two-way ANOVA was used to determine the interaction between independent variables (stress and cognitive reserve). [c] Two-tailed significance level for all tests was set at 0,0167. [d] Degrees of freedom = 143. [e] Degrees of freedom = 1; 141.

other brain structures, besides the hippocampus, than other memory tasks. Our findings on the EF impairment are also supported by neuroanatomical data about the distribution of MRs and GRs. Besides medial temporal structures, the PFC also has a high expression of GCs receptors, which makes this region particularly susceptible to the cortisol action[13,17]. The present study showed an association between stress and impaired cognitive functioning. All effect sizes found for stress were small, however, it is worth noting that the occurrence of summation of various neurophysiological dysfunctions may further impair cognitive performance in the elderly.

On the other hand, those with higher cognitive reserve showed a moderately elevated performance on neuropsychological tests, when compared to those with lower CR. There was a strong consistency of the data supporting the hypothesis of an association between higher CR with better cognitive performance. CR has had a positive correlation with all measures of neuropsychological performance[49,50]. This pattern of relationship occurred even with the absence of verbal intelligence measures (which were avoided because their correlation with neuropsychological measures is expected) for proxy measure of cognitive reserve. In the same line of our results, recent neuroimaging studies have supported the importance of educational level and intellectual activities for the maintenance of normal brain structures in elderly[8,51]. Sollé-Padullés et al.[11] evidenced, through the use of functional magnetic resonance image, that high CR is not only associated with increased brain volume, they also noted an increase in the efficacy of the neural network (as reflected by a reduction in brain activity for the same performance) during the performance of cognitive tasks in healthy elders. However, these authors did not find the same for patients with Mild Cognitive Impairment (MCI) or AD. Those patients with higher CR had a smaller brain volume in both MCI and AD, and there was also a greater brain activity in patients with AD, indicating a possible anatomophysiological compensatory mechanism for cognitive decline[11]. In another cross-sectional study with elderly[52], differences in the use of compensatory strategies, such as external aids, mnemonic strategies and increased effort investment, were also identified for the scores of CR proxies (i.e., verbal intelligence and educational level). Surprisingly, compensatory strategies were used only by older adults with a verbal intelligence level higher than their educational level. These data demonstrate the heterogeneity in cognitive ability of older adults with differences in CR. Consequently, this highlighted the need to better understand the neurobiological and behavioral determinants of CR for both healthy elderly and those with mild cognitive impairment or neurodegenerative diseases as Alzheimer's disease.

Our hypothesis of an interaction between stress and CR was refuted. High CR did not reduce the main effect of stress on neuropsychological performance in healthy elderly people. Elderly with a high level of stress (i.e., deleterious condition) and high CR (i.e., protective condition) had a superior neuropsychological performance than those with low stress level (i.e., protective condition) and low CR (i.e., deleterious condition). Accordingly, the CR had a more powerful relationship with neuropsychological performance in healthy elderly people. Nevertheless, high CR did not affect the magnitude of neuropsychological impairment associated with high levels of stress in the elderly, i.e., the main effect of stress remained independently of the influence of CR. The physiological mechanisms and cognitive changes related to stress and CR are noticeably distinct and, therefore, may be exercising their brain and behavioral effects without interacting each other.

Some limitations of this study should be considered. First, the relatively small sample for cortisol levels, which usually present high variance among the elderly, reduces the possibility of more robust inference of associations with neuropsychological performance. The nature of research design that we used does not allow a causal inference about the relations between variables. In addition, long neuropsychological battery may be tiring for the elderly participant, which could have added more variance to the data. Concerning the stress variable, excluding participants with symptoms of moderate or severe clinical depression and with more intense cognitive impairment (CDR > 0.5), we may have removed the people most vulnerable to the effects of stress. In other words, to exclude participants with health conditions that stress is a major risk factor, may have underestimated the impact of stress on our results, assessing people with a greater ability to deal with stressful events. It is worth noting, however, that our findings support the hypothesis that older adults with a high level of stress exhibit impairment in several cognitive functions. Similarly, they allow us to infer a positive association between CR and performance in a wide range of cognitive domains. Finally, we can conclude that

there is no evidence of a relationship between the modulating effects of CR on the impact of stress on neuropsychological performance in healthy elderly subjects. A more detailed understanding of the effect of these variables on human cognition, which are recognized as risk factors for the development of AD, is important to identify preventive strategies that aim to decrease cognitive decline in the healthy elderly. There have been intense researches to identify groups vulnerable to AD prior to symptom onset[6]. Thus, our data suggest that healthy elderly subjects with high stress and low CR can be especially vulnerable to cognitive impairment in old age.

Collaborations

JCC Cabral contributed to design of study, acquisition, analysis and interpretation of data, and in drafting the manuscript. GW Veleda contributed to design of study, acquisition, analysis and interpretation of data. M Mazzoleni contributed to design of study, acquisition, analysis and interpretation of data. EP Colares contributed to design, analysis and interpretation of hormonal data. L Neiva-Silva contributed to design of study, mainly concerning epidemiological aspects; contributed to analysis and interpretation of data. VT Neves was responsible for overall orientation as well as for the conception of the project, discussion of results, and critical analysis. All authors read and approved the final manuscript.

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