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FACULDADE DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

MÁRCIO DA SILVEIRA CORRÊA

Análise dos Efeitos do Estresse Crônico e do Envelhecimento sobre a Cognição de Cuidadores Familiares de Pacientes com Doença de Alzheimer e sua Relação com os Níveis de Cortisol, DHEA e BDNF.

Porto Alegre

2015

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Tese apresentada como requisito para a obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora:

Prof. Dra. Elke Bromberg

PORTO ALEGRE

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2015

À minha filha, Luiza Fagundes Corrêa, que tenhas o espírito da curiosidade e da investigação aos porquês da vida.

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RESUMO

O crescimento da população idosa é um fenômeno mundial e vem acompanhado do aumento da incidência e prevalência de demências, principalmente a Doença de Alzheimer (DA). À medida que a DA progride cresce a demanda por cuidados especiais, tarefa desempenhada em grande parte por cuidadores familiares, os quais muitas vezes são os cônjuges dos pacientes, ou seja, também são idosos. Estes cuidadores sofrem constantemente de estresse crônico, o qual é capaz de promover prejuízos à saúde num amplo espectro de disfunções, entre as quais as alterações cognitivas. A literatura evidencia: (I) relações entre estresse crônico, envelhecimento e alterações cognitivas; (II) relações entre estresse crônico e alteração dos níveis de hormônios, como o cortisol e a dehidroepiandrosterona (DHEA), e neurotrofinas, como o fator neurotrófico derivado de cérebro (BDNF); (III) o potencial do cortisol, DHEA e BDNF modularem mecanismos subjacentes a processos cognitivos. No presente trabalho estas evidências foram avaliadas de forma conjunta, de maneira a auxiliar na caracterização dos mecanismos fisiopatológicos envolvidos na associação entre estresse crônico, envelhecimento e déficits cognitivos.

Os efeitos do estresse crônico sobre as funções cognitivas dependentes dos lobos frontais e temporais foram investigadas em cuidadores familiares de pacientes com DA (n= 17; 32 a 84 anos de idade). Foi analisado também o impacto do estresse crônico nos níveis de Cortisol, DHEA e BDNF, bem como a relação destes parâmetros fisiológicos com o desempenho em testes neuropsicológicos. Os achados iniciais indicaram que o estresse crônico é capaz de prejudicar o desempenho em tarefas que avaliam atenção, funções executivas e a memória declarativa bem como elevar a razão cortisol/DHEA e diminuir os níveis séricos de BDNF.

Em um segundo momento, analisamos o efeito da idade sobre a relação entre estresse crônico, cognição e níveis de cortisol, DHEA e BDNF, uma vez que cuidadores idosos teoricamente seriam mais suscetíveis aos efeitos do estresse crônico que cuidadores de meia idade. Os resultados obtidos comprovaram esta hipótese, indicando alterações hormonais e cognitivas mais importantes nos cuidadores idosos do que nos mais jovens. Todavia, os cuidadores de meia-idade também apresentaram um prejuízo cognitivo importante e mostraram-se mais sensíveis aos efeitos do estresse crônico sobre os níveis de BDNF do que os cuidadores idosos.

Os resultados apresentados nesta tese indicam que as alterações cognitivas relacionadas ao estresse crônico são resultantes, pelo menos parcialmente, de alterações nos níveis de cortisol, DHEA e BDNF. Entretanto, o grau em que os aspectos cognitivos são afetados e sua relação com os hormônios e a neurotrofina avaliados dependem da faixa etária do cuidador.

Palavras-chave: Estresse Crônico, Envelhecimento, Cuidadores de Alzheimer, Déficits Cognitivos, Cortisol/DHEA, BDNF

ABSTRACT

The growth of elder population is a global occurrence and is followed by an increased prevalence and incidence of dementia, especially Alzheimer disease (AD). As the disease progresses, there is a larger demand for special care, task mainly performed by their family, who mostly are also elderly spouses of the patients. These caregivers are being constantly afflicted by chronic stress, which in turn can become harmful to their health in a number of disorders, such as cognitive impairment. Current literature highlights: (I) Relation between chronic stress, aging and cognitive impairment; (II) Relation between chronic stress and changes on hormone levels, such as cortisol and dehydroepiandrosterone (DHEA), and neurotrophic factors, i.e. Brain-Derived Neurotrophic Factor (BDNF); (III) BDNF, DHEA and cortisol potentially modulate cognitive-related mechanisms. In the present study, the evidences were assessed conjointly to better characterize physiological and pathological mechanisms involved in the association between chronic stress, aging and cognitive deficits.

Chronic stress effects on frontal and temporal lobes-dependent cognitive functions were investigated in familial caregivers of AD patients (n=17; 32 to 84 years old). The impact of chronic stress on cortisol, DHEA and BDNF levels was also assessed, and the relation of these physiological features on cognitive performance. Initial findings point that chronic stress is capable of impairing attention, executive functions and declarative memory, also increasing cortisol/DHEA ratio and reducing serum BDNF levels.

Then, we assessed age effects on the relation of chronic stress, cognition; cortisol, DHEA, and BDNF, as older caregivers are, theoretically, more prone to chronic stress effects than its younger counterparts are. Our results supports this hypothesis, indicating that hormone and cognitive changes are of greater importance on older caregivers when compared with young caregivers. However, middle-aged caregivers also presented a significant cognitive impairment and more suceptibility to chronic stress effects on BDNF levels than older caregivers.

The results presented in this thesis highlights that chronic stress-related cognitive alterations are resulting from, at least partly, cortisol, DHEA and BDNF level changes. However, the rate in which these cognitive features are affected, and its relation with the assessed hormones and neurotrophin are dependent on the caregivers' age.

Keywords: Chronic Stress, Aging, Caregivers, Alzheimer, Cognitive Deficits, Cortisol/DHEA, BDNF

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CAPÍTULO I

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1 INTRODUÇÃO

2 JUSTIFICATIVA

3 OBJETIVOS

3.1 OBJETIVO GERAL

3.2 OBJETIVOS ESPECÍFICOS

1 INTRODUÇÃO

1.1 ESTRESSE

Atualmente, a palavra estresse tem algumas definições na literatura que geram ambigüidades de interpretações. Ora, serve para definir um evento (estressor) ou uma resposta (resposta ao estresse). Normalmente, é utilizado em um sentido negativo, sendo empregado para atribuir um estado de aflição, bem como pode ser interpretado como um estado crônico de desequiíbrio na resposta ao estresse (McEwen, 2008). No entanto, um ponto fundamental é que os estudos, nessa área, entendem que o estresse é uma ameaça, real ou implícita, para homeostase, na qual se refere à manutenção de uma gama estreita de parâmetros fisiológicos vitais necessários para a sobrevivência (McEwen, 2000).

A reação perante o agente estressor tem por finalidade fundamental a preservação da vida. Desde o nascimento temos a condição básica de lutar ou fugir frente ao perigo, o que vai ocorrer através da reação do estresse. Portanto, estresse nem sempre é um fator de desgaste emocional e físico, e sim, é um mecanismo natural de defesa do organismo.

Outro ponto a ser levado em conta é que a forma como reagimos diante de um estressor vai depender da nossa capacidade de enfrentar um agente estressor e a habilidade de superá-lo através de estratégias de enfrentamento adotadas (Antoniazzi et al., 1998; Dunn and Conley, 2015; Razurel et al.,

2013). Desse modo, torna-se importante observar que a interpretação ao estresse, dependerá da avaliação interna frente a situação estressora (Li et al., 2012; Margis et al., 2003; Razurel et al., 2013). Dependendo da forma como o indivíduo reage a ele, poderá gerar alterações psicológicas e fisiológicas negativas (Bremner, 1999; Dedovic et al., 2009; Favassa, Armiliato & Kalinine 2005) que são extremamente prejudiciais ao organismo. O efeito de estresse crônico pode acarretar danos físicos (aparecimento de afecções como doenças gástricas e cardiovasculares) quanto psíquicos (depressão e ansiedade) (Kozlov and Kozlova, 2014; Lucassen et al., 2014; Sandrá, Legal & Jablonski 2004).

Diante do exposto acima, diversos estudos têm demostrado os efeitos do estresse agudo no organismo (Dedovic et al., 2009; Murakami et al., 2005; Vedhara et al., 2000), bem como o estresse crônico (McEwen, 2012; Shi et., 2010; Caswell et al., 2003) em modelos humanos e animais.

Atualmente, estudos com cuidadores de pacientes com demência vêm ganhando espaço no cenário científico devido ao considerável estresse crônico, no qual frequentemente são expostos (Gallagher-Thompson et al., 2007; Jeckel et al., 2010; Palma et al., 2007). Apesar desses trabalhos não serem robustos, eles demonstram importantes e negativos efeitos sistêmicos do estresse crônico nesses indivíduos (Richardson et al., 2013; Vitaliano et al., 2005; 2010, 2011).

1.2 ESTRESSE EM CUIDADORES DE DOENTES DE ALZHEIMER

É notório o aumento do número de idosos na população mundial e no Brasil esse crescimento não é diferente (Brasil, 2006). Estima-se que, em 2025, o Brasil ocupará o sexto lugar quanto ao contingente de idosos, alcançando cerca de 32 milhões de pessoas com 60 anos ou mais de idade (Brasil, 2010).

Esse envelhecimento populacional gera um iminente aumento da incidência e prevalência de demências, principalmente a doença de Alzheimer (DA), síndrome crônica neurodegenerativa caracterizada por uma progressiva perda de memória e diminuição da função intelectual (Lopes & Bottino, 2002; Thies & Bleiler, 2013; Vitaliano, Zhang & Scanlan, 2003).

À medida que essa doença progride surge a demanda por cuidados especiais, função importante desempenhada pelos cuidadores (Gaioli, Furegato & Santos, 2012; Inouye, Pedrazzani & Pavarini, 2010) que prestam toda a assistência necessária para o doente, devido à perda da autonomia observada com o avanço da doença (Abreu, Forlença & Barros, 2005). Entende-se por cuidador o indivíduo, da família ou não, que presta cuidados a alguém que apresenta dependência e suas tarefas envolvem o acompanhamento nas atividades diárias, auxiliando na qualidade de vida dessa pessoa (Brasil, 2006).

A literatura aponta que os cuidadores de pacientes com DA são, principalmente, cônjuges do sexo feminino e que vivem com o paciente (Caswall, 2003; Vitaliano et al., 2010). Outra característica importante é que o tempo do cuidado é integral, geralmente levando esse cuidador a dedicar-se ao

familiar 24 horas por dia e, consequentemente, sobrecarregando-o (Paulson & Lichtenberg, 2011; Lemos, Gazzola & Ramos, 2006). O Guia do Cuidador, Brasil (2008), afirma que os cuidadores ficam sobrecarregados, pois são responsáveis pela assistência integral ao paciente. Adicionalmente, esses indivíduos acompanham diariamente a piora do prognóstico do familiar. Desta forma, é comum o cuidador desenvolver problemas físicos e psicológicos (Richardson et al., 2013).

As perdas físicas e mentais que ocorrem no idoso portador da DA provocam efeitos adversos sobre a saúde do cuidador (Nunnemann et al., 2012; Vugt et al. 2006; Lee et al., 2004). Em uma revisão sobre o impacto da DA no cuidador, Cruz & Hamdan (2008) mostram que os cuidadores de doentes de Alzheimer possuem maiores chances de apresentar sintomas psiquiátricos e de saúde, além de estarem sujeitos a maior freqüência de conflitos familiares, problemas no trabalho e pior julgamento sobre a própria saúde, sentindo-se mais estressados se comparados a cuidadores de outros tipos de demências ou a pessoas da mesma idade que não exercem tal função. Nessa mesma revisão, eles afirmam que esses cuidadores consultam 46% mais médicos e utilizam mais medicamentos psicotrópicos - como antidepressivos e antipsicóticos - do que cuidadores de pacientes com outras doenças.

Outro fator importante é o alto índice de ansiedade e depressão evidenciado nessa população, o qual pode atingir até 50% dos cuidadores, representando risco duas vezes maior de desenvolver essas doenças que o restante da população (Pinto et al., 2009; Ferrara et al., 2008; Mahoney et al., 2005). Em média três quartos do dia do cuidador são destinados ao paciente,

proporção essa que cresce com o progresso da doença, pois quanto mais prejudicado cognitivamente, meno independente ele é, acarretando assim menos tempo livre e mais ansiedade para o cuidador (Toson, 2009; Cooper, Balamurali, Livingston, 2007; Vitaliano, Zhang & Scanlan, 2003).

A tensão emocional na vida de um cuidador pode interferir no cuidado que este presta ao seu familiar, sendo inclusive fator preditor de maior número de hospitalizações, institucionalizações do paciente e maior mortalidade entre os cuidadores (Brasil, 2010; Toson 2009).

Nem todos os cuidadores desenvolvem doenças ou se tornam insatisfeitos com a tarefa de cuidar. Isso pode ser explicado pela utilização de diferentes estratégias individuais para lidar com as situações consideradas desgastantes (Gaioli, Furegato & Santos, 2012; Inouye, Pedrazzani & Pavarini, 2010). Melhores estratégias de gerenciamento de problemas influenciam no ajustamento emocional do cuidador, refletindo-se em uma melhor assistência ao paciente (Paulson & Lichtenberg, 2011; Selwood et al., 2007).

No entanto, apesar do aumento do número de trabalhos com cuidadores ter ganhado espaço no cenário científico, ainda são escassos no Brasil (Karsch, 2003). Cruz & Hamdan (2008) afirmam que são necessários mais estudos com essa população, principalmente no que diz respeito ao impacto da sobrecarga sobre a cognição, uma das funções mais sensíveis aos efeitos do estresse crônico (Lupien et al., 2009; Sandi, 2004).

1.3 ESTRESSE E ASPECTOS COGNITIVOS

O estresse tem um potencial efeito modulador em diversos sistemas do organismo. No cérebro, o estresse inicia uma cascata de eventos, estimulando suas reações adaptativas a essa nova situação. Dessa forma, durante o estresse agudo as alterações fisiológicas são positivas para o organismo, pois o permitem reagir nas respostas de luta ou fuga (McEwen, 2000). No entanto, quando o estresse é mantido por longos períodos de tempo, células-alvo do sitema nerovoso central (SNC) são afetadas, alterando diversas funções cognitivas (Sandi, 2004).

O efeito do estresse sobre as funções cognitivas, bem como seus correlatos neuronais e neuromoleculares, é tema de muitos estudos, (Hanson et al., 2011; Nooshinfar et al., 2011; Sandi, 2004; McEwen, 2000; Bremner, 1999). A maioria destes trabalhos tem mostrado que ele pode provocar diversas alterações em importantes estruturas do cérebro, como por exemplo, o hipocampo e o córtex pré-frontal, regiões envolvidas em aspectos cognitivos (Kozisek, Middlemas & Bylund, 2008; Yamada & Nabeshima, 2003).

Dentre os componentes das funções cognitivas que parecem estar afetados em indivíduos em estado de estresse crônico estão: a memória de trabalho, a atenção, resposta inibitória, velocidade de processamento (Holmes & Wellman, 2009; Lindauer et al., 2005; Sandi, 2004; Bremner, 1999).

As funções executivas, responsáveis pela seleção e processamento das informações, são mediadas pelo córtex pré-frontal (Holmes & Wellmanb, 2009). Existem evidências em modelos animais, principalmente em ratos, que a

indução de estresse por períodos longos pode gerar lesões e atrofia em neurônios pré-frontais (Sandi, 2004). A compreensão dos mecanismos que provocam alterações morfofisiológicas no cortex pré-frontal podem fornecer informações importantes sobre a fisiopatologia da disfunção executiva no estresse crônico e promover melhores estratégias para o manejo e tratamento dos pacientes (Holmes & Wellmanb, 2009).

Em um estudo que analisou as funções executivas em cuidadores de pacientes com Alzheimer, Caswell e seus colaboradores (2003) mostraram que os cuidadores tinham uma pior performance, quando comparados a sujeitos que não exerciam tal função. Em outro trabalho, os cuidadores também apresentaram piores escores em testes que mediam algumas funções executivas, como memória de trabalho, controle inibitório e atenção (Oken, Fonareva & Wahbeh, 2011). Apesar do número reduzido de pesquisas nessa área, esses trabalhos parecem sugerir que essas funções cognitivas não estão sendo preservados nessa população.

No que diz respeito aos diferentes tipos de mémória, são poucos os estudos que investigam esses aspectos em cuidadores de pacientes demenciados. Os achados sugerem prejuízo na memória declarativa (Vugt et al. 2006; Lee et al., 2004; Boucher et al., 1996), função dependente do hipocampo e do córtex pré-frontal (Joëls & Krugers, 2007; Artola et al., 2006; Buwalda et al., 2005; Huang, Yang & Hsu, 2005; Braver et al, 2001; Glisky et al, 2001).

Estudos mostram redução do volume do lobo temporal de ratos submetidos a estresse (Conrad, 2008) e sugerem dano na potenciação de

longa duração (LTP), um dos principais mecanismos subjacentes à formação e armazenamento de memória no cérebro (Datson et al.,2008). Há também trabalhos de neuroimagem em humanos mostrando uma redução do volume do hipocampo (Lindauer et al., 2005 e 2004; Villarreal et al., 2002; Bremner 1999) e do córtex pré-frontal (Karl et al., 2006; Carrion et al., 2001) em casos de estresse crônico. McEwen (2000) sugere que o estresse prolongado pode levar a supressão da neurogênese e morte neuronal, ocasionando diversos prejuízos cognitivos ao indivíduo.

Verifica-se também a existência de estudos que sugerem o comprometimento das capacidades cognitivas em patologias relacionadas com o estrese crônico, como é o caso de pacientes com Transtorno de Estresse Pós-Traumático (PTSD) (Brewin, 2011; Lindauer et al., 2005). Todavia, o funcionamento cognitivo dos cuidadores de pacientes demenciados, os quais também estão sob uma sobrecarga crônica de estresse, é pouco explorado. Segundo Lupien e seus colaboradores (2009) o prejuízo cognitivo presente nesses indivíduos está relacionado com a exposição crônica a hormônios que podem apresentar efeitos neurotóxicos quando liberados em excesso e por tempo prolongado.

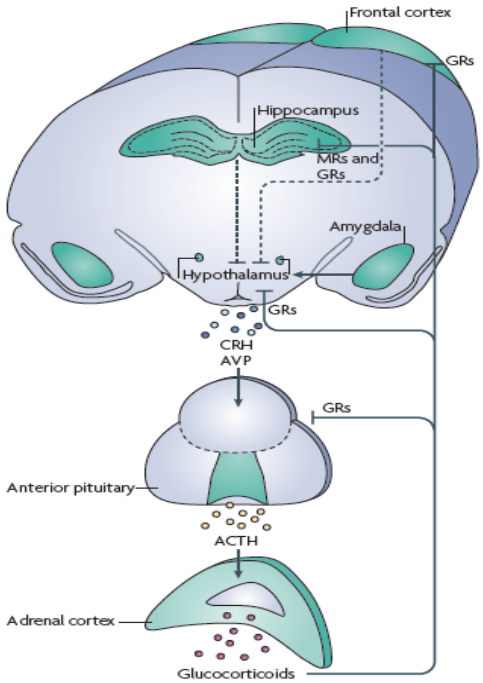
1.4 ESTRESSE E ASPECTOS HORMONAIS

A principal hipótese para as disfunções cognitivas em indivíduos com estresse crônico é a alteração do eixo hipotálamo-hipófise-adrenal (HPA) que levaria a um aumento nos níveis circulantes de cortisol o qual acabaria

afetando aspectos anatomicos e funcionais de estruturas cerebrais relacionadas ao desempenho cognitivo (Conrad, 2008; McEven, 2001).

O Cortisol é um hormônio produzido na zona fasciculata da região cortical das glândulas supra-renais (Kozlov and Kozlova, 2014), sendo secretado em resposta a estímulos estressores. Neurônios hipotalâmicos liberam um neuropeptídeo, o hormônio liberador de corticotropina (CRH), o qual estimula a hipófise anterior a secretar o hormônio adrenocorticotrópico (ACTH) para a corrente sanguínea, o qual por sua vez estimula as adrenais liberarem cortisol (Figura 1) (Lupien, 2009).

Figura.1 Mecanismo neurofisiológico da resposta ao estresse



Fonte: Lupien et al. *Effects of stress throughout the lifespan on the brain, behaviour and cognition*. *Nature Reviews Neuroscience* 10, 434-445, 2009.

O cortisol é extremamente importante para o controle de diversas funções fisiológicas do organismo, como por exemplo, a regulação dos níveis glicêmicos, do tônus vascular e das respostas imunes e antiinflamatórias, além da modulação de diversas funções do sistema nervoso central e da participação na regulação da resposta do organismo a agentes estressores (Lucassen et al., 2014).

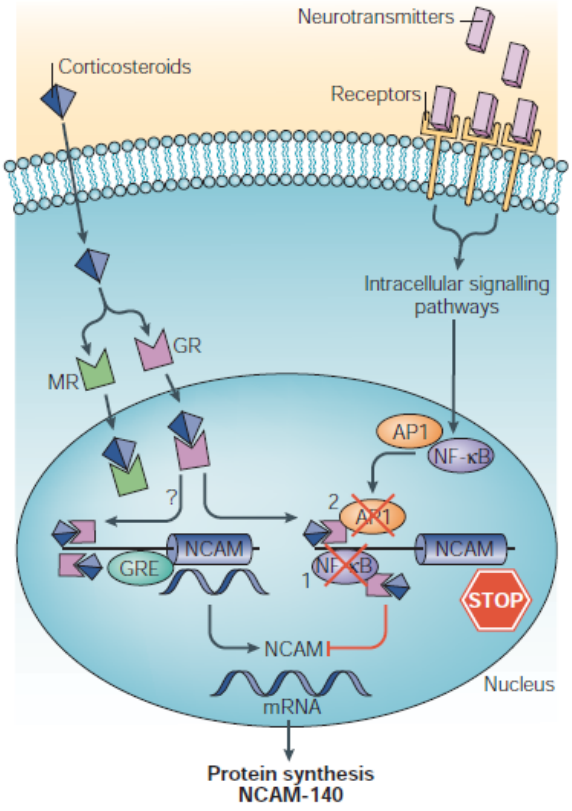
No sistema nervoso, esse hormônio atravessa a barreira hematoencefálica se liga a receptores glicorticóides (GR) e mineralocorticóides (MR) (Datson et al. 2008). A literatura demonstra importantes diferenças entre esses receptores, tais como a afinidade pelo cortisol, modulação do eixo HPA e a sua distribuição nas estruturas do SNC. Os glicocorticóides têm uma superior afinidade aos receptores MR quando comparados aos GR. Na regulação do eixo HPA, os receptores MR tem uma atividade de manuntenção basal do eixo, e os GR mantêm uma resposta *feedback* negativo do sistema HPA, controlando as concentrações de glicocorticóides em respostas ao estresse (Kloet, 2004a; 2004b).

Em situações de estresse crônico, como em cuidadores de pacientes demenciados (Cruz & Handam, 2008), pode ocorrer uma superestimulação do eixo hipotálamo-hipófise-adrenal (HPA), gerando uma liberação excessiva de cortisol. Esse esteróide em excesso no SNC torna-se neurotóxico, alterando anatômo-fisiologicamente as estruturas cerebrais onde atua (Datson et al., 2008). De acordo com Sandi (2004), a hipercotisolemia provoca diminuição da arborização dendrítica em neurônios hipocampais (estrutura com expressiva quantidade de receptores GR e MR), especificamente na área CA3, bem como alteração na ramificação dos neurônios da amígdala, região importante nos

processos cognitivos e com moderada quantidade de receptores GR. Outra área altamente sensível a alta concentração de cortisol é o córtex pré-frontal, altamente carregada de receptores GR, já foram demonstrados diversos efeitos deletérios sobre neurônios dessa região, como redução no comprimento dendrítico, alteração axonal, bem como morte neuronal (Shansky & Morrison, 2009)

Há estudos demonstrando que o excesso dos níveis circulantes de cortisol provoca um aumento na ativação dos receptores GR e MR, inibindo a potenciação de longa duração (LTP), na região CA1 do hipocampo (Artola et al., 2006; Kim, Song & Kosten 2006; Kim and Diamond, 2002), mecanismo extremamente importante na aquisição de memória no sistema nervoso central (Kumar, 2011; Joëls & Krugers, 2007; Buwalda et al., 2005; Huang, Yang & Hsu, 2005). A ação prolongada desse glicocorticóide também gera a supressão da neurogênese e morte neuronal, promovendo disfunções cognitivas (McEwen, 2000), bem como altera a modulação da atividade de diferentes sistemas de neurotransmissores, afetando a plasticidade sináptica e a memória (Henckens et al., 2011; Datson et al., 2008). De acordo com Sandi (2004), essas alterações ocorrem, principalmente, via a modulação do cortisol na supressão de fatores de transcrição de neurotransmissores (Figura. 2).

Figura. 2 Mecanismo de ação dos glicocorticódes na alteração de fatores de transcrição durante situações de estresse crônico.



Fonte: Sandi. *Stress,Cognitive Impairment and Cell Adhesion Molecules. Nature*. 5 (12): 917-30, 2004.

Existem estudos que sugerem uma relação entre o potencial neurotóxico do cortisol (Lupien et al., 2004; Haller, Mikics e Makara, 2008) na modulação do sistema glutamatérgico (por promover a liberação de glutamato) e gabaérgico (por atuar especificamente na expressão RNAm para subunidades especificas dos receptores GABAa) (McEwen, 2008) e diminuição da disponibilidade de neurotrofinas que promovem a plasticidade sináptica, como o fator neurotrófico derivado do cérebro (BDNF) (Duman & Monteggia, 2006; Murakami et al., 2005).

No entanto, nessas situações de estresse elevado ocorre a liberação também de outro esteróide que promove ações contrárias a esse glicocorticóide (Young, Gallagher e Porter, 2002). A dehidroepiandrosterona, (DHEA) como é chamado, é um hormônio sintetizado pela zona reticular na porção medular das adrenais (Stárka et al., 2015) e também secretado no sistema nervoso central, razão pela qual esse anti-glicocorticóide é chamado neuroesteróide (Wolf et al. 1997).

Em modelos animais, como por exemplo, em hamisters, somente 50% são oriundos das supra-adrenais. Já em humanos, a maior parte dos níveis séricos do DHEA tem origem nas adrenais, o restante é produzido pelas gônodas, por tecido adiposo e outra considerável parcela é produzida no SNC, principalmente em astrócitos, porém não em oligodendrócitos. Estudos demonstram que o citocromo P450c17 tem uma importante contribuição na produção do DHEA dentro desses tecidos (Stárka et al., 2015).

A literatura demonstra que a ação do DHEA como antiglicocorticóide, reparadora dos efeitos neurotóxicos do cortisol, age via modulação dos receptores: receptor ácido-N-metil-D-aspártico (NMDA-R), receptor ácido y-aminobutírico (GABA-R) e receptor sigma (Sigma-1). No entanto, até o presente momento, nenhum receptor específico foi identificado para o DHEA no SNC (Lazaridis et al., 2011; Logonge et al., 2011; Stárka et al., 2015)

O DHEA tem ações antioxidantes, sendo considerado um hormônio antienvelhecimento, além de promover reparo tecidual e controlar os efeitos da hipercotisolemia gerada pelo estresse crônico. (Longone et al., 2011). Recentes estudos sobre esse neuroesteróide demonstram que ele exerce

efeitos neurotróficos por interagir diretamente com os receptores de membrana neuronal TrkA e p75NTR, receptores do fator de crescimento do nervo (NGF). Esta ativação impede a perda apoptótica induzida por NGF (Lazaridis et al., 2011).

Alguns estudos sugerem que a DHEA reduz os níveis de receptores glicocorticóides (Gallagher et al. 2007), inibe os neurotransmissores excitatórios (como os glutamatérgicos e colinérgicos) (Webb et al., 2006), promove o aumento de neurotrofinas que aumentam a sobrevivência e o reparo neuronal (Hassani et al., 2011).

Desta forma, a relação cortisol/DHEA tem sido considerada como mais informativa da ação relativa dos dois esteróides no cérebro do que os valores isolados dos mesmos (Maninger et al., 2009; Kaminska et al., 2000; Hechter, Grossman & Chatterton, 1997).

Nessa mesma linha, a literatura aponta que alterações na razão cortisol/DHEA tem um potencial negativo na expressão de fatores protetores do sistema nervoso central, como por exemplo as neurotrofinas (Pluchino et al., 2013; Wolkowitz et al., 2010)

1.5 ESTRESSE E FATORES NEUROTRÓFICOS

Alguns estudos mostraram que durante o estresse crônico é reduzida a expressão de neurotrofinas envolvidas na plasticidade sináptica, sobrevivência e reparo neuronal, como por exemplo, o fator neurotrófico derivado do cérebro

(BDNF) (Issa et al., 2010; Vinberg et al., 2009). O BDNF é uma pequena proteína pertencente a família das neurotrofinas, estruturalmente relacionada ao fator de crescimento neuronal (NGF), neutrotrofina 3 (NT3) e neutrofina 4 (NT4), as quais são abundantemente expressadas no cérebro dos mamíferos, especialmente no hipocampo, córtex e amígdala, ligando-se com grande afinidade ao seu receptor Tirosina Quinase (TrkB) e também ao receptor p75 (Murer, Yan & Raisman-Vozari, 2001). Estudos com animais demonstraram que o BDNF pode atravessar a barreira hematoencefálica (Pan et al., 1998; Pan, Banks, Kastin, 1998) desta forma, os níveis periféricos refletem os níveis centrais (Klein et al., 2011; Kozisek et al., 2008; Sartorius et al., 2009; Yulug *et al*., 2009).

A maioria das pesquisas sobre expressão e modulação de BDNF é feita em ratos (Nooshinfar et al., 2011; Issa et al., 2010; Valenzuela et al, 2007; Milgram et al., 2006), enquanto outras em menor número são realizadas em humanos (Chan et al., 2008; Mattson *et al*., 2004). Dentre os neurotransmissores e hormônios que afetam a expressão de BDNF estão a acetilcolina, serotonina, óxido nítrico, glicocorticóides, mineralocorticóides e esteróides sexuais, bem como os agonistas dos receptores de glutamato que induzem, enquanto os agonistas dos receptores GABAa que inibem a expressão dessa neurotrofina (Murer, Yan & Raisman-Vozari, 2001)

Sertoz e seus colaboradores (2008) sugerem que o nível de BDNF pode ser aceito como um marcador biológico do estresse, pois alguns estudos sobre essa neurotrofina encontraram uma redução dos seus níveis em situação de estresse (Issa et al., 2010), psicopatologias como depressão e em doenças neurodegenerativas como Alzheimer, Parkinson e Huntington (Kozisek et al.,

2008). Essa diminuição dos níveis de BDNF pode contribuir para a atrofia do hipocampo e do cortex prefrontal (Duman et al., 2006).

Apesar desses achados direcionarem essa neurotrofina como biomarcador da cronicidade do estresse, pouco se sabe da sua característica de produção e secreção ao longo da vida. Estudos com modelos animais, apontam que há um pico em seus níveis no ápice do desenvolvimento cerebral, permanecendo constante ao longo do processo de envelhecimento (Del arco et al 2011; Perovic et al., 2013). Em humanos, há trabalhos que demonstram um declínio durante a terceira idade (Golden et al., 2010; Gunstad et al., 2008) ou também a manuntenção dos níveis centrais (Webster, 2006).

Evidências recentes sugerem que o BDNF está associado com o aprendizado e a memória (Li et al., 2009). Ernfors & Bramham (2003) afirmam que ele atua na modulação da potenciação de longa duração (LTP), processo envolvido na formação de memória. Outros estudos, indicam que a redução dos níveis dessa neurotrofina, além de estar associada com neurodegeneração e alteração na plasticidade sináptica, está relacionada com déficits cognitivos, sugerindo que ela possa ser importante na manutenção da performance cognitiva (Yoshii & Constantine-Paton, 2010; Yu et al., 2008), apesar de existirem outros estudos que não acharam essa relação (Nacmias et al., 2004; Strauss et al., 2004).

Outras linhas de estudos apontam que os níveis dessa neurotrofina são modulados pelo envelhecimento (Calabrese et al., 2013; Laing et al., 2012). Silhol e seus colaboradores (2005) demostraram uma redução nos níveis de BDNF no hipocampo, região da qual dependem os processos cognitivos, em

ratos. Já Erickson e seus colaboradores (2010) acharam uma relação significativa entre a redução do volume do hipocampo, baixos níveis de BDNF e déficits cognitivo.

Como exposto acima, a literatura indica a relação do estresse e baixos níveis de BDNF em importantes estruturas do cérebro relacionadas aos aspectos cognitivos (Shou- Sem- Shi, et al., 2010). No entanto, até o presente momento, não há estudos com cuidadores familiares de pacientes com Alzheimer como para avaliar os efeitos de estresse crônico e envelhecimento nessa relação.

1.6 ENVELHECIMENTO, ESTRESSE E COGNIÇÃO

Estima-se que exista, atualmente, cerca de 20 milhões de idosos em nosso país, o que representa pelo menos 10% da população brasileira (Brasil, 2010). Estudos apontam que a população idosa mundial em 2050 será de aproximadamente 2 bilhões de pessoas e que esse envelhecimento populacional é uma resposta à mudança de três indicadores de saúde: a queda da taxa de fecundidade, a redução da mortalidade e o aumento da expectativa de vida (Vaupel, 2010).

Segundo a Organização Pan-Americana de Saúde (OPAS) o envelhecimento pode ser entendido como “um processo seqüencial, individual, acumulativo, irreversível, universal, não patológico, de deterioração de um organismo maduro com menos capacidade de fazer frente ao estresse do

meio-ambiente, desta forma, aumentando sua possibilidade de morte" (Brasil, 2006). Nessa mesma linha, Ferreira e seus colaboradores (2010) afirmam que o envelhecimento pode ser conceituado como um conjunto de modificações morfológicas, fisiológicas, bioquímicas e psicológicas, que determinam a perda progressiva da capacidade de adaptação do indivíduo ao meio ambiente, sendo considerado um processo dinâmico e progressivo.

O envelhecimento é um processo natural, no qual pode ocorrer diminuição progressiva da reserva funcional dos indivíduos (Brasil, 2006). No entanto, em alguns casos, esse processo é agravado, dependendo de fatores ambientais, emocionais e cognitivos, gerando um estado patológico que influencia na diferenciação desse sistema, ou seja, o envelhecimento não acontece da mesma forma e ao mesmo tempo para todos os indivíduos (Lampert, 2009).

Dentre algumas mudanças que podem ocorrer no envelhecimento estão às alterações relacionadas à cognição. Ao envelhecer, a maioria das pessoas se queixa de esquecimentos cotidianos. Esses déficits cognitivos relacionados à idade são bastante salientes em tarefas que envolvem memória declarativa e em tarefas que dependem de memória de trabalho (Grady & Craik, 2000). Outros estudos afirmam que durante o envelhecimento o lobo frontal, relacionado com esses tipos de memória, está comprometido, o que poderia explicar prejuízos nesses tipos de memória (Braver et al, 2001; Glisky et al, 2001).

Segundo os estudos de neuroimagem, outra região que também apresenta alterações durante o envelhecimento, é o lobo temporal (Raz et al.,

2010; Driscoll et al., 2009), estrutura importante nos processos de memória (Joëls & Krugers, 2007; Artola et al., 2006; Buwalda et al., 2005; Huang, Yang & Hsu, 2005). Ta e seus colaboradores (2011) sugerem que durante o envelhecimento pode ocorrer perda neuronal, redução da potenciação de longa duração e diminuição do crescimento dendrítico no hipocampo.

Desta forma, a associação entre estresse e envelhecimento pode promover prejuízos importantes às funções neuronais de diferentes estruturas cerebrais, exacerbando os déficits cognitivos decorrrentes outros fatores (Datson et al., 2008; Artola et al., 2006; Kim, Song & Kosten 2006; Braver et al, 2001).

Portanto, torna-se fundamental analisar os efeitos do estresse sobre o padrão cognitivo, neuro-hormonais e neurotrófica em cuidadores familiares de diferentes faixas etárias, a fim de correlacionar a sobrecarga emocional com o envelhecimento nessa população.

2 JUSTIFICATIVA

O número de idosos com demência está crescendo rapidamente no Brasil, especialmente no que diz respeito à doença de Alzheimer. Entretanto, há carência de dados empíricos sobre o impacto do estresse na saúde dos cuidadores familiares desses pacientes (Garrido & Menezes, 2004).

Acompanhar a progressão de um familiar com DA gera, em quem cuida, doenças como depressão e ansiedade, ou seja, uma sobrecarga emocional (Freitas et al., 2008), tornando os cuidadores mais vulneráveis ao estresse e, consequentemente, às enfermidades geradas por esse problema (Fleury, Keleer & Murdaugh, 2000; Norton et al., 2010; Vitaliano, 2011).

É interessante compreender que a maioria desses cuidadores familiares são cônjuges dos pacientes, portanto indivíduos que já estariam mais predispostos as alterações do processo de envelhecimento natural em estruturas cerebrais relacionadas com os aspectos cognitivos (McEwen et al., 2012; Vitaliano, 2011, 2010; Lupien et al., 2009; Sandi, 2004). Dessa forma, o presente trabalho torna-se relevante do ponto de vista clínico e social, na medida em que pretende avaliar vários parâmetros neuropsicológicos, neurohormonais e neurotrófico destes cuidadores, nos quais a literatura indica alterações em situações de estresse e sensibilidade aos efeitos do envelhecimento (Datson et al., 2008; Lupien et al., 2009; Murakami et al., 2005).

Pretendemos contribuir para o esclarecimento de possíveis déficits cognitivos oriundos do estresse crônico vivido pelo cuidador familiar, bem como

avaliar a relação entre estresse e envelhecimento na predisposição a alterações cognitivas, tais como, a memória, função executiva e atenção, bem como fisiológicas, como os níveis de Cortisol, DHEA e BDNF. Acreditamos que nossos resultados possam fornecer subsídios para o estabelecimento de medidas preventivas que minimizem os impactos do estresse nos cuidadores, melhorando a qualidade de vida destes indivíduos e implicando positivamente na assistência ao paciente com DA.

3 OBJETIVOS

3.1 GERAL

Analisar os efeitos do estresse crônico e do envelhecimento em diferentes funções cognitivas de cuidadores familiares de pacientes com Doença de Alzheimer, relacionando-os com os níveis plasmáticos do fator neurotrófico derivado de cérebro (BDNF), cortisol e deidroepiandrosterona (DHEA).

3.2 ESPECÍFICOS

- Avaliar os efeitos do estresse crônico sobre testes que medem as funções cognitivas dos lobos frontal e temporal;

- Caracterizar os efeitos do estresse crônico sobre:

  - Os níveis de cortisol e DHEA;
  - Os níveis de BDNF;

- Verificar a relação entre estresse crônico e envelhecimento sobre:

  - As funções cognitivas
  - O padrão de secreção de cortisol, DHEA e BDNF;
  - O grau de correlação entre os aspectos cognitivos e parâmetros fisiológicos (cortisol, DHEA e BDNF).

CAPÍTULO II

“Psychophysiological Correlates of Cognitive Deficits in Family Caregivers of patients with Alzheimer Disease”

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PSYCHOPHYSIOLOGICAL CORRELATES OF COGNITIVE DEFICITS IN FAMILY CAREGIVERS OF PATIENTS WITH ALZHEIMER DISEASE

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Abstract—***Background:*** **The progressive loss of memory and autonomy of Alzheimer's Disease (AD) patients, together with their characteristic behavioral and psychological symptoms, subjects their family caregivers to chronic stress. Several studies indicate that these caregivers are predisposed to cognitive impairments, but the physiological correlates of these alterations remain to be elucidated.**

Objective: **Analyze the effects of chronic stress of family caregivers of AD patients on cognition, cortisol/DHEA ratios and BDNF levels and investigate the relation between these variables.**

Experimental procedure: **Seventeen family caregivers (64.83 ± 3.64 years) of patients with AD and eighteen non-caregivers (58.29 ± 3.16 years) completed stress, depression and anxiety inventories. Exclusion criteria were current neurological disorders, major unstable medical illnesses, use of medications that could interfere with cognitive or HPA axis function and dementia. Attention, working memory and executive function were assessed with Digit Span and Trail Making tests, and declarative memory was analyzed with the Logical Memory test. Saliva was collected at 8 AM and 10 PM and its cortisol and DHEA levels determined by radioimmunoassay. Serum BDNF levels were measured by sandwich-ELISA. Results were analyzed with independent samples *t* test, covariance analysis and linear regressions. The statistical significance was set at $p < 0.05$ and all *p* values were adjusted with Holm's Method.**

Results: **Caregivers showed more stress, depression and anxiety symptoms than non-caregivers, as well as significantly worse performances on attention, working memory and executive function tests. Caregivers also had higher cortisol/DHEA ratios and lower BDNF levels than non-caregivers. Cortisol/DHEA ratios, especially at 10 PM, were negatively related with all cognitive tasks in which caregivers showed impaired performance. On the other hand, the only cognitive task that related with the BDNF level was digit span.**

Conclusions: **This study showed that caregivers' cognitive impairment is related with alterations on cortisol/DHEA ratios, and that chronic stress experienced by these subjects has the potential to alter their BDNF levels.**

Key words: caregivers, chronic stress, cognitive deficits, cortisol, DHEA, BDNF.

INTRODUCTION

The world population has been experiencing significant aging—the process that results in rising proportions of older persons in the total population—since the mid-twentieth century (DESA United Nations, 2002) and, consequently, a greater incidence and prevalence of aging-related dementias, like Alzheimer Disease (AD) (review in Thies and Bleiler, 2013). AD is characterized by a progressive decline in cognitive and functional abilities, demanding a growing need of care as the patient's clinical condition worsens (Hazzan et al., 2014). This caregiving task is mostly done by the patients' relatives, especially their spouses or children (Ferrara et al., 2008). Several studies argue that these caregivers suffer from chronic stress (Vitaliano, 2010; Vitaliano et al., 2011).

Although acute stress has physiological benefits to the organism, chronic stress may promote severe and broad health dysfunctions (McEwen, 2000, 2004; Lupien et al., 2009). The effect of chronic stress on cognition, as well as its cellular and molecular correlates, has been the

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Abbreviations: AD, Alzheimer's Disease; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CAR, cortisol awakening response; CNS, central nervous system; DHEA, dehydroepiandrosterone; GRs, glucocorticoid receptors; HPA, Hypothalamus–Pituitary–Adrenal; ISSL, Lipp Stress Symptoms Inventory for Adults; MAOI, monoamine oxidase inhibitor; MMSE, Mini Mental Status Examination; SSRI, selective serotonin reuptake inhibitor; WAIS III, Wechsler Adult Intelligence Scale.

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subject of several studies (Bremner, 1999; McEwen, 2000; Sandi, 2004; Hanson et al., 2011; Nooshinfar et al., 2011). Most of these researches indicate that chronic stress may imply a number of changes on important brain structures responsible for cognitive aspects, such as the prefrontal cortex and hippocampus (Yamada and Nabeshima, 2003; Kozisek et al., 2008). Executive function, attention, working and declarative memories are among the most affected cognitive domains (Bremner, 1999; Sandi, 2004; Lindauer et al., 2006; Holmes and Wellman, 2009). Attention, working memory and executive function, responsible for information assortment and processing, are mediated by the prefrontal cortex (Holmes and Wellman, 2009) and deficits on these cognitive domains were already verified in caregivers of dementia patients (Vitaliano et al., 2005; Oken et al., 2011). Hippocampus-dependent declarative memory impairment (Braver et al., 2001; Glisky et al., 2001; Buwalda et al., 2005; Huang et al., 2005; Artola et al., 2006; Joëls and Krugers, 2007) is also reported in studies of caregivers with chronic physical and emotional burden (de Vugt et al., 2006; Palma et al., 2011).

The main hypothesis for the cognitive impairment of chronically stressed individuals is the Hypothalamus–Pituitary–Adrenal (HPA) axis dysfunction, which results in increased cortisol levels. This hypercortisolemia has adverse effects on morphological and physiological aspects of structures related with cognitive functions (Conrad, 2008). These effects seem to be largely mediated by the lower affinity glucocorticoid receptors (GRs), which become heavily occupied with corticosteroids in response to stress (Kim and Diamond, 2002). One consequence of such deleterious effects is the impairment of long-term potentiation induction (Kim and Diamond, 2002; Artola et al., 2006), a central nervous system (CNS) mechanism for memory maintenance (Buwalda et al., 2005; Huang et al., 2005; Joëls and Krugers, 2007; Kumar, 2011). Moreover, the extended exposure to high levels of glucocorticoids is considered neurotoxic, since these hormones can disturb different neurotransmitter systems, synaptic plasticity, neurogenesis and lead to neuronal death, dysfunctions that were already related with cognitive impairment (McEwen, 2000; Datson et al., 2008; Henckens et al., 2012).

Previous studies with caregivers show that they have high cortisol levels and/or disturbances on the circadian secretion pattern of this glucocorticoid (Oken et al., 2011; Palma et al., 2011). In healthy subjects, besides the elevation of the cortisol levels during stress, there is also the release of dehydroepiandrosterone (DHEA), an antiglucocorticoid (Young et al., 2002; Dong and Zheng, 2012) able to reduce GR levels (Gallagher et al., 2007) and promote neuronal survival and repair by stimulating an increase in neurotrophin levels (Shoae-Hassani et al., 2011). However, in caregivers of dementia patients, there is a decrease in DHEA levels (Jeckel et al., 2010). Thus, the ratio between cortisol and DHEA would be a more reliable evaluation of the effects of stress on CNS than the individual analysis of one or another hormone (Kaminska et al., 2000; Maninger et al., 2009). Until the present time, only one study examined the cortisol/DHEA ratio in family caregivers of AD patients (Jeckel et al., 2010) and none analyzed the relation between their cortisol/DHEA ratios and cognitive parameters.

Besides alterations of cortisol and DHEA levels, some studies showed that the expression of neurotrophins involved with synaptic plasticity and neuronal survival and repair, such as the brain-derived neurotrophic factor (BDNF), is greatly reduced during chronic stress (Vinberg et al., 2009; Issa et al., 2010). This decrease in BDNF levels might be related with the glucocorticoid increase (Kawashima et al., 2010; Jeanneteau and Chao, 2013; Pluchino et al., 2013; Suri and Vaidya, 2013). Moreover, a large body of evidence established a link between BDNF reduction and impaired neuronal plasticity and survival (Calabrese et al., 2009, 2013). Studies with patients of neurodegenerative diseases imply that BDNF can be important for the maintenance of a normal cognitive function (Diniz and Teixeira, 2011; Laske et al., 2011; Carlino et al., 2013). However, to date, no study has examined levels of this neurotrophin in caregivers, despite the existing evidences of their cognitive impairment (de Vugt et al., 2006; Oken et al., 2011) and physiologic alterations that predispose to BDNF decline (Pluchino et al., 2013; Suri and Vaidya, 2013).

This study aims to contribute to the understanding of the neurophysiological correlates of the cognitive impairments of familial caregivers of AD patients. Therefore, we tested the following hypotheses (I) that the cognitive performance of caregivers would be susceptible to the effects of chronic stress; (II) that cortisol/DHEA ratios would be higher and the BDNF levels would be lower in caregivers; (III) that cognitive results would be negatively related with the cortisol/DHEA ratios and positively related with BDNF levels.

EXPERIMENTAL PROCEDURES

Participants

Seventeen family caregivers (64.83 ± 3.64 years old; 13 women) of patients with AD were recruited from the Brazilian Alzheimer Association – Porto Alegre, RS, Brazil. To be included, caregivers had to be providing care for 8 h/day, for at least a year, at the time of the study. A control group, composed by eighteen non-caregivers (58.29 ± 3.16 years old, 14 women) recruited in the community, was also included in the study. Exclusion criteria comprised previous or current neurological disorders, major unstable medical illnesses, use of medications that could interfere with cognitive or HPA axis function, hormone replacement therapy, previous or current use of illegal psychoactive drugs and scores on Mini Mental Status Examination (MMSE) (Folstein et al., 1975) indicative of dementia. Cutoff values for the Brazilian version of MMSE were <18 for middle educational level (4–8 years of education) and <26 for high educational level (more than 8 years of education) (Bertolucci et al., 1994). Symptoms of depression and anxiety were assessed for all participants with the Brazilian adapted and validated version (Cunha, 2001) of Beck Depression Inventory (BDI) and Beck Anxiety Inventory

(BAI). Chronic stress symptoms were assessed by Lipp Stress Symptoms Inventory for Adults (ISSL) (Lipp and Guevara, 1994). The body mass index (BMI) of subjects was also controlled, since it can relate with levels of cortisol, DHEA and BDNF (Svec and Shawar, 1997; Mazza et al., 1999; Ukkola et al., 2001; Monteleone et al., 2004; Gunstad et al., 2006). All participants were required to abstain from any alcohol use 24 h before testing and none of them were smokers. The ethical approval for the study was obtained from the Research Ethics Committee of Pontifical Catholic University, Porto Alegre, RS, Brazil and all participants gave their informed consent.

Neuropsychological measures

Neuropsychological tests were administered to evaluate frontal and temporal lobe functions. The Forward and Backward Digit Span Tests, subtests of the Wechsler Adult Intelligence Scale (WAIS III) (Wechsler, 1997) adapted for the Brazilian population (Nascimento, 2004), were used to evaluate attention and working memory. In the forward version, the participant hears a sequence of numbers and then is asked to repeat them in the same order. In the backward version, the participant hears a sequence of digits and must repeat them in reverse order. The test begins with sequences of two to three numbers, increasing until the participant does two consecutive errors. Each correct sequence equals one point. The score is the sum of correctly repeated sequences. The Trail Making A and B Tests (Strauss et al., 2006) were administered for attention and executive function assessment, as well as processing speed. In version A the participant is instructed to link randomly distributed numbers in a crescent order (connect the dots). In version B the participant must link numbers and letters, alternating between them, in a crescent and alphabetical order. The time (seconds) taken to complete each version of the task is used to measure the participants' performance.

The Logical Memory Tests I and II, subtests of the Wechsler Memory Scale III (Wechsler, 1987), were used to analyze immediate and delayed recall. Two short stories are read once to the participant and must be repeated by him immediately after the reading (Logical Memory I) and after 30 min (Logical Memory II). Each story incorporates 25 specific points (story elements), each of which must be recalled to obtain credit. The immediate and delayed scores are the sum of the number of story elements remembered by the subject during immediate and delayed recall, respectively. Performance on the two stories is summed up, yielding a maximum score of 50 points in each version (immediate or delayed recall) of the task.

Salivary cortisol and DHEA measurement

Participants were asked to collect saliva samples at 8 AM and 10 PM on the day of the experiment. The samples were stored between 0 °C and 4 °C by the subjects and delivered to the laboratory within 3 days, where they were frozen at −80 °C until further analysis. After thawing, each sample was divided for cortisol and DHEA assessment. Samples for cortisol analysis were centrifuged at 1500 rpm for 3 min and then analyzed by radioimmunoassay (Beckman Coulter kit – Immunotech) using a gamma counter. The assay sensitivity was estimated at 0.09 nmol/L. Samples for DHEA analysis were centrifuged for 3 min at 2500 rpm and then measured by radioimmunoassay (Beckman Coulter kit – Immunotech). The sensitivity of the DHEA assay was estimated at 0.06 nmol/L. All samples for both cortisol and DHEA were analyzed in duplicate, and results from each of the sampling times were expressed in nmol/L (Corrêa et al., 2012).

Serum BDNF measurement

For BDNF analysis, five milliliters of blood were collected from each subject by venopuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at 4000×*g* for 10 min, and serum was frozen at −80 °C until further analysis. BDNF serum levels were measured by sandwich- ELISA, using a commercial kit according to the manufacturer's instructions (Milipore, USA). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 h at 4 °C with the samples diluted 1:100 in sample diluent and standard curve ranging from 7.8 to 500 ng/ml of BNDF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BNDF monoclonal antibody (diluted 1:1000 in sample diluent), which was incubated for 3 h at room temperature. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted 1:1000) for 1 h at room temperature was carried out. After the addition of substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density and BDNF concentration (Cunha et al., 2006).

Statistical analysis

Results were expressed as mean ± standard error. Samples of all variables were analyzed with the Shapiro–Wilk Test of Normality and no data transformations were needed to meet the assumptions of parametric analysis. Differences between groups on demographic and clinical characteristics were analyzed with independent samples *t* tests and chi-squared statistics. Measures of cognitive performance and cortisol, DHEA, cortisol/DHEA and BDNF levels were initially analyzed with ANOVAs (one-way between groups, one-way repeated measures and two-way repeated measures, whenever appropriate). Thereafter these measures were analyzed with ANCOVAs (one-way between groups, one-way repeated measures and two-way repeated measures, whenever appropriate) and statistical results presented after adjustment for the possible confounding factors (age, scores on BDI, BAI and medication). These possible confounding factors were introduced stepwise in the ANCOVAs. Thus, for each of the cognitive and physiologic measures, four ANCOVAs were run to adjust for: (1) age; (2) age and

BDI scores; (3) age, BDI and BAI scores; (4) age, scores on BDI, BAI and medication. Rationale for the inclusion of these covariates is described in the Results section. The relation between the results obtained in the cognitive tasks and the hormonal and BDNF levels were analyzed with linear regressions, which included the data of all subjects (caregivers and non-caregivers). Effect size [eta squared (η2ρ) or Rsquare (R^2)] was reported for statistical analysis of all neuropsychological, hormonal and BDNF results. Power of all statistical analysis was greater than 80%, unless differently stated in the Results section. All p values were adjusted for multiple testing with Holm's Method considering the main hypothesis of this study. The statistical significance was set at $p < 0.05$.

RESULTS

Demographic and clinical characteristics

Table 1 shows the demographic and psychiatric characteristics of caregivers and non-caregivers. Groups did not differ in age [$t = 1.348$, df = 33, $p = 0.187$], gender [Pearson Chi-Square = 0.509, $p = 0.476$], years of education [$t = -0.343$, df = 33, $p = 0.734$], MMSE [$t = 0.250$, df = 33, $p = 0.804$] and BMI [$t = -0.19$, df = 33, $p = 0.985$]. However, caregivers showed significantly higher scores on depression [BDI, $t = 6.674$, df = 33, $p < 0.001$], anxiety [BAI, $t = 3.700$, df = 33, $p = 0.001$] and stress [ISSL, $t = 14.316$, df = 33, $p < 0.001$] symptoms than non-caregivers.

Some of the caregivers were on antidepressant and/or anxiolytic medication as follows: five volunteers were taking only antidepressants (selective serotonin reuptake inhibitor (SSRI), $n = 3$; monoamine oxidase inhibitor (MAOI), $n = 1$; tricyclic plus SSRI, $n = 1$), one used antidepressant in combination with anxiolytic medication (SSRI plus bromazepan, $n = 1$) and another used only anxiolytic medication (bupropion, $n = 1$).

In order to strengthen the internal validity and generalizability of our results, different covariates were introduced in the statistical analysis of neuropsychological and physiological data. Even without significant between group differences ($p > 0.05$), age was entered as a possible confounding factor since controls and caregivers were not perfectly matched for this variable (as shown by the group means) and that the age range was large (32–84 years for caregivers and 40–84 years in controls). Moreover, literature has plenty of suggestions that age might have significant effects on the outcome of neuropsychological (Harada et al., 2013; Samson and Barnes, 2013), cortisol/DHEA (Yen and Laughlin, 1998; Ferrari and Magri, 2008) and BDNF data (Erickson et al., 2012; Leuner and Shors, 2013). Results of screening tools for depressive and anxiety symptoms, as well as medication, were also used as covariates due to the evident group differences seen for these variables, as well as their reported effects on cognitive functions (Pringle et al., 2011; Hartley and Phelps, 2012; Beaudreau et al., 2013; Snyder, 2013; Trivedi and Greer, 2014), cortisol/DHEA (Herbert, 1998; Markopoulou et al., 2009; Dubrovsky, 2005; Staufenbiel et al., 2013) and BDNF levels (Balu et al., 2008; Autry and Monteggia, 2012; Kimpton, 2012). To enter medication as a covariate we created a single dummy variable of whether or not a subject was taking antidepressant or anxiolytic drugs.

Table 1. Mean ± standard error of the mean of demographic and psychiatric measures of caregivers and controls

	Caregivers	Controls
Age (years)	64.83 ± 3.64	58.29 ± 3.16
Sex (female/male)	13/5	14/3
Education (years)	12.83 ± 0.67	13.17 ± 0.74
MMSE	28.00 ± 0.35	27.88 ± 0.30
BDI	15.88 ± 1.27*	5.82 ± 0.80
BAI	9.00 ± 1.19 **	3.47 ± 0.87
BMI	24.93 ± 0.86	24.95 ± 0.63
Assisted time (Weekly Hours)	125.22 ± 12.98	
Caregiving time (Years)	5.22 ± 0.81	
Phase of stress (ISSL) n (%)		
Alert	0 (0)	0 (0)
Resistance	7 (39)	0 (0)
Near-exhaustion	5 (28)	0 (0)
Exhaustion	6 (33)	0 (0)
Symptoms of stress (ISSL) n (%)		
Physical	7 (39)	0 (0)
Psychological	11 (61)	0 (0)

Abbreviations: MMSE, Mini Mental Status Examination; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory and BMI, Body Mass Index.
* $p < 0.001$ in relation to controls.
** $p < 0.01$ in relation to controls.

Neuropsychological data

Initial results indicated that caregivers had a significantly worse performance than controls at all neuropsychological tests: Forward [$F(1,33) = 58.539$, $\eta 2\rho = 0.639$, $p < 0.001$] and Backward [$F(1,33) = 77.103$, $\eta 2\rho = 0.700$, $p < 0.001$] Digit Span, Trail Making A [$F(1,33) = 9.417$, $\eta 2\rho = 0.222$, $p = 0.004$] and B [$F(1,33) = 31.112$, $\eta 2\rho = 0.485$, $p < 0.001$] and Logical Memory I [$F(1,33) = 33.085$, $\eta 2\rho = 0.506$, $p < 0.001$] and II [$F(1,33) = 34.663$, $\eta 2\rho = 0.512$, $p < 0.001$].

Table 2 shows results of the experimental groups in the cognitive tasks after the covariate analysis. Significant group differences were maintained even after

Table 2. Mean ± standard error of mean of neuropsychological measures of caregivers and controls. Analysis adjusted for age, depression symptoms, anxiety symptoms and medication

	Caregivers	Controls
Digit Span (Forward)	4.52 ± 0.29*	6.56 ± 0.30
Digit Span (Backward)	2.78 ± 0.34*	5.70 ± 0.35
Trail Making A (s)	42.08 ± 2.62	40.49 ± 2.73
Trail Making B (s)	106.46 ± 5.79**	77.04 ± 6.03
Logical Memory I	21.92 ± 1.26	26.43 ± 1.32
Logical Memory II	16.13 ± 1.31	21.35 ± 1.36

* Between group differences: $p < 0.001$.
** Between group differences: $p < 0.05$.

additional adjustment for age, BDI, BAI and medication for Forward [$F(1,29) = 16.147$, $\eta 2\rho = 0.358$, $p < 0.001$] and Backward Digit Span [$F(1,29) = 24.368$, $\eta 2\rho = 0.457$, $p < 0.001$], as well as for Trail Making B [$F(1,29) = 8.733$, $\eta 2\rho = 0.231$, $p = 0.024$]. However, the outcomes for Trail Making A and Logical Memory Tests were altered with the insertion of covariates. More specifically, between group differences in Trail Making A were maintained with the introduction of age [$F(1,32) = 7.166$, $\eta 2\rho = 0.186$, $p = 0.012$] as a covariate, but vanished as soon as BDI scores were added to the model [$F(1,31) = 0.588$, $\eta 2\rho = 0.019$, $p = 0.499$]. Between group differences in Logical Memory I [$F(1,29) = 4.294$, $\eta 2\rho = 0.129$, $p = 0.081$] and II [$F(1,29) = 5.424$, $\eta 2\rho = 0.158$, $p = 0.094$] faded with the introduction of medication in the ANCOVA analysis.

Cortisol and DHEA levels

The first figure shows the salivary cortisol (1a) and DHEA (1b) levels which gave raise to one of our main variables, i.e., cortisol/DHEA ratio. Two-way ANOVAs were run to analyze hormonal levels (cortisol or DHEA) of controls and caregivers (between-subjects effects) at different sampling times (8 AM and 10 PM, within-subjects factors), followed by one-way ANOVAs and ANCOVAs to further explore the results.

Cortisol levels. Only time proved to have significant effects on cortisol levels [$F(1,33) = 179.550$, $\eta 2\rho = 0.845$, $p < 0.001$], but adjustment for age indicated that there was also a significant group effect [$F(1,32) = 7.816$, $\eta 2\rho = 0.196$, $p = 0.013$]. Additional adjustment for scores on BDI, BAI and medication did not change the significant effects of time [$F(1,29) = 73.709$, $\eta 2\rho = 0.718$, $p < 0.001$] and group [$F(1,29) = 8.005$, $\eta 2\rho = 0.216$, $p = 0.016$] on cortisol levels.

Further exploration of cortisol data indicated that the levels of this hormone were higher at 8 AM than at 10 PM, both for caregivers [$F(1,17) = 62.624$, $\eta 2\rho = 0.789$, $p < 0.001$] and controls [$F(1,16) = 65.560$, $\eta 2\rho = 0.835$, $p < 0.001$]. These significant time effects persisted even after additional adjustment for age, scores on BDI, BAI and medication, as can be seen for the ANCOVA results of caregivers [$F(1,13) = 65.560$, $\eta 2\rho = 0.835$, $p < 0.001$] and controls [$F(1,13) = 16.277$, $\eta 2\rho = 0.556$, $p < 0.001$]. Between group comparisons showed that caregivers had higher cortisol levels at 10 PM [$F(1,33) = 26.589$, $\eta 2\rho = 0.371$, $p < 0.001$] when compared with controls. Adjustment for age, BDI and BAI did not change the outcome of the between group comparisons, but insertion of medication in the ANCOVA led the differences in cortisol levels at 8 AM to reach statistical significance [$F(1,29) = 5.118$, $\eta 2\rho = 0.150$, $p = 0.031$], while the outcome for group differences at 10 PM was maintained [$F(1,29) = 6.688$, $\eta 2\rho = 0.187$, $p = 0.045$].

DHEA levels. DHEA levels showed significant effects of time [$F(1,33) = 103.772$, $\eta 2\rho = 0.759$, $p < 0.001$], group [$F(1,33) = 4.415$, $\eta 2\rho = 0.188$, $p = 0.043$], as well as an interaction of these variables [$F(1,33) = 4.570$, $\eta 2\rho = 0.122$, $p = 0.004$]. However, introduction of age as a covariate eliminated the effect of group [$F(1,32) = 2.413$, $\eta 2\rho = 0.070$, $p = 0.130$], as well as the time and group interaction [$F(1,32) = 2.588$, $\eta 2\rho = 0.074$, $p = 0.120$]. Stepwise inclusion of the other covariates (scores on BAI, BDI and medication) did not change these outcomes. Thus, after all adjustments of DHEA levels, only time continued to have a significant effect [$F(1,29) = 32.207$, $\eta 2\rho = 0.526$, $p < 0.001$]. Time effects on DHEA were related to higher levels at 8 AM than at 10 PM, both for caregivers [$F(1,17) = 37.290$, $\eta 2\rho = 0.687$, $p < 0.001$] and controls [$F(1,16) = 66.381$, $\eta 2\rho = 0.806$, $p < 0.001$]. The differences between DHEA levels at 8 AM and 10 PM were maintained after adjusting for age, scores on BDI, BAI and medication, as indicated by the ANCOVAs results of caregivers [$F(1,13) = 15.121$, $\eta 2\rho = 0.538$, $p = 0.004$] and controls [$F(1,13) = 15.606$, $\eta 2\rho = 0.546$, $p = 0.002$].

To summarize, cortisol and DHEA levels of controls and caregivers showed the characteristic circadian pattern of higher levels in the morning and lower levels in the evening. Significant group effects were seen only for cortisol levels, which were higher for caregivers than controls at 8 AM and 10 PM.

Cortisol/DHEA ratios

The two-way ANOVA of cortisol/DHEA ratios indicated significant effects of group [$F(1,33) = 23.584$, $\eta 2\rho = 0.417$, $p < 0.001$] and time [$F(1,33) = 226.326$, $\eta 2\rho = 0.873$, $p < 0.001$], but no interaction between these variables [$F(1,33) = 0.003$, $\eta 2\rho = 0.000$, $p = 0.958$]. These outcomes were maintained after adjustment for age, scores on BDI, BAI and medication [$F(1,29) = 10.860$, $\eta 2\rho = 0.272$, $p = 0.006$ for group; $F(1,29) = 33.389$, $\eta 2\rho = 0.576$, $p < 0.001$ for time; $F(1,29) = 2.295$, $\eta 2\rho = 0.073$, $p = 0.141$ for interaction]. As can be seen in Fig. 1c, cortisol/DHEA ratios were higher at 8 AM than at 10 PM for controls [$F(1,16) = 177.328$, $\eta 2\rho = 0.917$, $p < 0.001$] and caregivers [$F(1,17) = 86.173$, $\eta 2\rho = 0.835$, $p < 0.001$]. Moreover, caregivers had higher cortisol/DHEA ratios than non-caregivers at 8 AM [$F(1,33) = 8.059$, $\eta 2\rho = 0.196$, $p = 0.008$] and 10 PM [$F(1,33) = 24.800$, $\eta 2\rho = 0.429$, $p < 0.001$]. Adjustment for the covariates did not change the outcome for the circadian pattern of cortisol/DHEA ratios of controls [$F(1,16) = 7.254$, $\eta 2\rho = 0.358$, $p = 0.018$] and caregivers [$F(1,17) = 33.909$, $\eta 2\rho = 0.723$, $p < 0.001$), nor the significant group differences seen at 8 AM [$F(1,33) = 7.614$, $\eta 2\rho = 0.208$, $p = 0.020$] and 10 PM [$F(1,33) = 6.554$, $\eta 2\rho = 0.184$, $p = 0.016$].

BDNF levels

Between group differences for BDNF were first tested with a one-way ANOVA. As can be seen in Fig. 2, results indicated significantly lower levels of BDNF in caregivers compared with controls [$F(1,33) = 5.222$, $\eta 2\rho = 0.137$, $p = 0.029$, power = 0.604]. This significant difference between groups was maintained

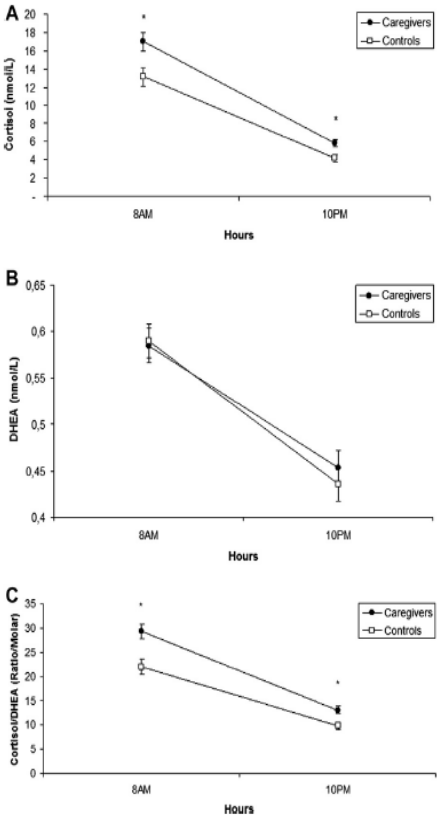

Fig. 1. Levels (mean ± standard error of mean) of cortisol (A), DHEA (B) and cortisol/DHEA ratios (C) in saliva samples of caregivers and controls at 8 AM and 10 PM. Analysis adjusted for age, depression symptoms, anxiety symptoms and medication. Within group analysis showed that both caregivers and non-caregivers had higher cortisol, DHEA and cortisol/DHEA ratios at 8 AM than at 10 PM (all $p < 0.001$). *$p < 0.05$, between-group differences.

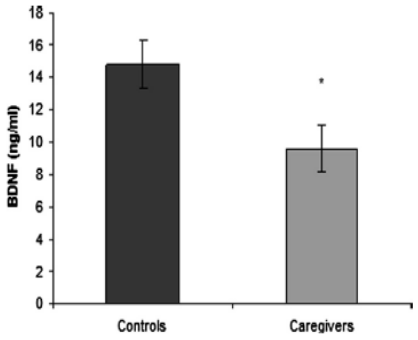

Fig. 2. Levels of BDNF (mean ± standard error of mean) of caregivers and controls adjusted for age. *$p < 0.05$, between-group differences.

after adjustment for age [$F(1,32) = 6.203$, $\eta 2\rho = 0.163$, $p = 0.018$, power = 0.676], but vanished as soon as BDI was added to the ANCOVA [$F(1,31) = 0.075$, $\eta 2\rho = 0.064$, $p = 0.156$, power = 0.291], remaining without further changes after adjustment for scores on BAI and medication [$F(1,29) = 2.278$, $\eta 2\rho = 0.073$, $p = 0.142$, power = 0.309].

Relations among cortisol/DHEA ratios and cognition

Caregivers showed significant cognitive impairments ($p < 0.05$) and alterations on cortisol/DHEA ratios ($p < 0.05$) that persisted after adjustment for the confounding variables (age, scores on BDI, BAI and medication) and multiple comparisons. Thus, linear regressions were carried out to further explore the relations among these parameters. No covariates were inserted in the regression models, since we analyzed only the performance on cognitive tasks that did not show significant alterations in the between groups statistical analysis outcome after the introduction of the confounding factors (Digit Span Forward and Backward, Trail Making B). Linear regressions indicated that the cortisol/DHEA ratios at 8 AM were significantly and negatively related only with the performance in the Backward Digit Span [$R^2 = 0.182$, $B = -0.155$, $p = 0.033$]. On the other hand, cortisol/DHEA ratios at 10 AM showed negative relations with the scores in Forward [$R^2 = 0.387$, $B = -0.290$, $p < 0.001$] and Backward [$R^2 = 0.433$, $B = -0.360$, $p < 0.001$] Digit Span, as well as in Trail Making B [$R^2 = 0.624$, $B = 7.547$, $p < 0.001$].

Relations among BDNF, Hormones and Cognition

As discussed earlier, between group differences of BDNF did not withstand the introduction of BDI in the ANCOVA. However, the results also indicated an important loss of statistical power with the introduction of BDI in the model (from 0.604 to 0.291). Thus, since these results for BDNF should not be considered conclusive, we also checked if there was any relation among BNDF levels and 10 PM cortisol or cortisol/DHEA levels, since literature mentions that higher levels of cortisol (or stress) could be related with lower BDNF levels (Murakami et al., 2005; Shi et al., 2010). However, no significant results were found for the linear regressions of these variables, even with the adjustment for BDI, BAI and medication (all $p > 0.05$). Relations of BDNF levels with cognitive parameters (Forward and Backward Digit Span, Trail Making B) were also analyzed. However, BDNF showed significant relations only to one of the cognitive tests, namely the Backward Digit Span [$R^2 = 0.168$, $B = 0.128$, $p = 0.045$].

DISCUSSION

This study evaluated the cognitive performance and the levels of cortisol, DHEA and BDNF in family caregivers of AD patients. Results showed that caregivers had deficits on attention, working memory and executive function as compared with a control group. Caregivers

also exhibited significantly higher cortisol/DHEA ratios (8 AM and 10 PM) and lower BDNF levels. Moreover, linear regression analysis suggests a significant relation between the alterations seen in these physiologic parameters, especially for cortisol/DHEA ratios, and the cognitive deficits found in caregivers.

Sample characterization

Caregivers that participated in this study proved to be highly committed to their relatives with AD, as can be seen by the long assistance period (several months or years) and high weekly workload dedicated to patients. This assistance profile, in which the partners or close-related relatives are responsible for most patient care, is common (Vitaliano et al., 2005; Palma et al., 2011) and associated with physical and emotional burden for the caregiver (Mahoney et al., 2005; Collins and Swartz, 2011). Caregivers of the present study were clearly distressed with relation to the control group. Moreover, psychological stress symptoms prevailed and the majority of caregivers (61%) had already progressed to the near-exhaustion and exhaustion phases, which indicate a breakdown on resistance and loss of adjustment capacity (Lipp, 2003). Among the changes that can be observed during these phases are the depression and anxiety symptoms (Lipp, 2003; Ramiro et al., 2014), which are very common among dementia caregivers (Mahoney et al., 2005; Ferrara et al., 2008; Vitaliano et al., 2009) and likely to affect cognitive functions (Vitaliano et al., 2009; Oken et al., 2011). Our caregivers had higher anxiety and depression scores than the control group, although the symptoms of most caregivers were below the cutoff scores for moderate depression and anxiety (Cunha, 2001). However, it must be taken into account that an important number of individuals in the caregiver group was already being treated with anxiolytics and/or antidepressants, which very likely contributed to maintain psychiatric manifestations at minimal to moderate levels, reducing the impact of these psychiatric conditions on cognition (Pringle et al., 2011).

Cognitive performance

Caregivers of the current study exhibited a lower performance than controls on some cognitive tasks, indicating impaired attention, executive function and working memory. These results are consistent with previous studies that evaluated familial caregivers of patients with dementia (Caswell et al., 2003; MacKenzie et al., 2009; Vitaliano, 2010; Oken et al., 2011; Vitaliano et al., 2011). Moreover, caregivers' impairments on Forward and Backward Digit Span (measures of attention and working memory) and Trail Making B (measure of attention and executive function) cannot be attributed to their greater depression and anxiety levels, to the anxiolytics and/or antidepressants that they were taking or the age composition of the group, since covariance analysis showed that none of these variables could eliminate the significant between group differences seen for these cognitive tasks. Considering that attention, executive function and working memory are mainly dependent on the prefrontal cortex (Holmes and Wellman, 2009; Rossi et al., 2009; Benchenane et al., 2011; Shansky and Lipps, 2013), we suggest that the functions of this brain structure are impaired in caregivers.

Stress and physiological changes

Many studies provide evidences, both on animal and human models, of the negative effects that chronic stress can cause on the prefrontal cortex. The structural changes can be seen from the macroscopic level, such as the volume reduction of the prefrontal cortex (McEwen, 2012), to the microscopic alterations on neurons and neural circuitry (Shansky and Morrison, 2009; Leuner and Shors, 2013). These stress-dependent morphological changes in the prefrontal cortex may result in dysfunctions of excitability, plasticity and neuronal survival (Leuner and Shors, 2013; Lucassen et al., 2014).

Several studies suggest that the morphological, physiological and cognitive changes due to chronic stress may be related with alterations of glucocorticoid levels (McEwen, 2000; Lupien et al., 2009). As previously outlined in the introduction, the most common effect of chronic stress is the upregulation of the HPA axis and the resulting increase of cortisol levels (Hellhammer et al., 2009). The high glucocorticoid receptor density at the frontal lobes make these regions particularly vulnerable to the hypercortisolemia effects (Lupien et al., 2009), which seem to increase the risk of cognitive impairment (Karlamangla et al., 2005).

The distinct rise in cortisol levels upon awakening (also known as the cortisol awakening response or CAR), is considered a trait measure for HPA axis activity (Pruessner et al., 1997), and thus could give important information about the effects of chronic stress suffered by caregivers (Wahbeh et al., 2008). However, we choose to measure cortisol levels only at 8 AM and 10 PM. This decision was done because the saliva samples should be collected by caregivers at home, which could compromise the strict standardization and timing necessary for CAR (Wilhelm et al., 2007), especially in the case of our caregivers, since they informed that the care for the patients started as soon as they wake up. Thus, saliva collection for CAR could turn into an extra stress for these caregivers.

Caregivers of the current study showed the characteristic circadian rhythm previously described for cortisol of healthy subjects, i.e. higher levels in the morning and lower levels in the evening (Törnhage, 2009; Evans et al., 2011). After statistical adjustment for confounding variables (age, depression and anxiety symptoms, and medication), results showed that caregivers had higher cortisol levels than controls at 8 AM and 10 PM. These results are in agreement with most studies of cortisol alterations in caregivers (Gallagher-Thompson et al., 2006; Oken et al., 2011) and in contrast with some others, which reported no significant changes (Mills et al., 1997) or lower levels of this hormone (Vedhara et al., 2002). Besides long-term alterations in HPA axis function related with chronic stress, these alterations in cortisol levels could also be related with the circadian pattern of patients' behavior. This is especially true for the 10 PM

elevation of cortisol levels seen in this and another study of our research group (Palma et al., 2011). Thus, the higher cortisol levels of caregivers at 10 PM could also be modulated by the Sundowning Syndrome of the AD patients, which refers to a group of neuropsychiatric symptoms as restlessness, confusion, anxiety and aggressive behaviors that emerge, or increase in intensity, at late afternoon and early night (Khachiyants et al., 2011). In this context, it is worth mentioning that the relation between the sundowning syndrome and the caregivers' stress levels was already described two decades ago (Gallagher-Thompson et al., 1992). However, only studies designed to evaluate patient's sundowning symptoms and cortisol levels of their caregivers can establish if there really is a relation among these variables.

The analysis of chronic stress effects on cognition shall also consider, besides neurotoxic substances such as cortisol, the possible alterations on neuroprotective factors, such as DHEA and BDNF. In the end, the final effect of stress on cognition should depend on the balance between factors that impair and that protect neuronal function and survival.

DHEA is important for neurogenesis and neuronal differentiation, survival and plasticity. Besides, this hormone shows anti-glucocorticoid activity. This anti-glucocorticoid effect seems to be related to DHEA's capacity to modulate the metabolism of cortisol and the availability of its nuclear receptors (see Maninger et al., 2009 for a review). Based on these assumptions and a previous study that observed higher levels of DHEA in caregivers in relation to their controls (Jeckel et al., 2010), we expected to find significant differences between caregivers and controls in DHEA levels in this study. Although we observed higher DHEA levels in caregivers at 8 AM, this significant difference did not withstand the introduction of medication as a covariate.

Cortisol/DHEA ratio and cognition

As stated earlier, the cortisol/DHEA ratio seems to be a more reliable marker for cognitive changes than cortisol or DHEA alone (Maninger et al., 2009). This is not surprising since both are important allostatic overload predictors (McEwen, 2004). Our caregivers had a higher cortisol/DHEA ratio than controls at the sampled times (8 AM and 10 PM). The internal validity and generalizability of these findings are strengthened by the fact that the group differences of cortisol/DHEA ratios remained, even with the introduction of age, BDI and BAI scores, and medication in the statistical analysis. Previous literature suggests that an imbalance between neurotoxic and neuroprotector factors could be related to cognitive impairments. Relations observed in our study between cortisol/DHEA ratios and the performance on cognitive tasks are in accordance with this hypothesis. Although linear regressions showed negative relations of cortisol/DHEA ratios at 8 AM and 10 PM on cognitive performance, the broader and deeper associations were observed for the cortisol/DHEA ratios at 10 PM. Regressions with the cortisol/DHEA ratios at 8 AM suggested only minor effects ($R^2 \leqslant 0.18$) on attention and working memory. On the other hand, regressions of cortisol/DHEA ratios at 10 PM showed significant and more important effects ($R^2 = 0.4–0.6$) on most investigated cognitive domains (attention, executive function, working memory), suggesting that the hormonal imbalance of this time point has deeper effects on the frontal lobes. These findings increase considerably the scarce knowledge of the psychophysiological correlates of cognitive alterations in dementia caregivers.

BDNF levels

We also evaluated the effects of caregivers' physical and emotional burden on BDNF levels. Previous literature on animal models and psychiatric patients suggest that some increase in cortisol may elicit BDNF expression, while an additional rise or chronic exposition to this hormone could suppress the expression of this neurotrophin (Gray et al., 2013; Numakawa et al., 2013). In this study we show, for the first time, that caregivers of AD patients can also have, besides higher cortisol/DHEA ratios, lower BDNF levels. Therefore, the pattern of BDNF alterations seen in caregivers is in accordance with the expected effects of chronic stress (Shi et al., 2010; Pluchino et al., 2013) and hypercortisolemia (Kawashima et al., 2010; Jeanneteau and Chao, 2013; Pluchino et al., 2013; Suri and Vaidya, 2013) on this neurotrophin. However, it is important to consider that the between group differences seen for BDNF levels were not large (eta square = 0.137), and that statistical analysis could not rule out the potential interference of depression and anxiety symptoms, as well as the effects of medication, on BDNF (see Study limitations section).

Regression analyzes showed no significant relation between hormonal alterations and BDNF levels. Moreover, the only cognitive domain which exhibited a small association with BDNF was working memory. Lack of a relation between BDNF levels and the other cognitive domains could be related to the small sample size and small differences of BDNF levels between caregivers and controls. Researches that indicated significant correlations between BDNF levels and cognitive performance usually addressed severe psychiatric disorders (e.g. depression, schizophrenia, bipolar disorder), with greater BDNF differences between case and control groups than those observed in the current study (Dias et al., 2009; Carlino et al., 2011, 2013; Oral et al., 2012).

Study limitations

According to the discussion above, it is clear that one of the limitations regarding the current study is sample size. Although our results for cognitive performance and hormonal levels were in agreement with our hypothesis and previous literature, a larger sample may have been more accommodating for drawing stronger conclusions, especially with regard to BDNF.

It was not possible to rule out either potential effects of the greater depression and anxiety levels of caregivers, or the effects of the medications used on BDNF levels. The power of the statistical analysis dropped below reliable values when BAI, BDI and medication were introduced

in ANCOVAs. Literature indicates that depression and anxiety symptoms tend to decrease BDNF levels (Shimizu et al., 2003). However, five caregivers (28% of the sample) were under treatment with antidepressant medication that can increase BDNF levels [SSRI (Mattson et al., 2004), MAOI (Altar et al., 2003) and Tricyclic antidepressants (Nibuya et al., 1995; Nestler et al., 2002)] and only two (11% of the sample) were medicated with anxiolytic drugs that could decrease BDNF levels [benzodiazepines (Ventriglia et al., 2013)]. Thus, it is possible that the use of SSRI, MAOI and Tricyclic antidepressants contributed to attenuate the fall of BDNF levels of caregivers. In other words, significant differences between caregivers and controls for BDNF could be expected to be greater without the interference of antidepressant medication. Even so, it must be reminded that besides pharmacological composition, characteristics of medication use (such as the treatment duration, dose and the combination of different drugs) can also modulate BDNF levels (Balu et al., 2008; Calabrese et al., 2009; Matrisciano et al., 2009; Ventriglia et al., 2013). Therefore, the results obtained for BDNF in this study should not be taken as conclusive.

Besides affecting BDNF levels, anxiolytic and antidepressant drugs could also interfere with the evaluation of other parameters, such as cortisol levels (Calabrese et al., 2009) and cognitive performance (Levkovitz et al., 2002; Herrera-Guzmán et al., 2009; Murrough et al., 2011). However, as shown in the Results section, medication by itself was not able to eliminate significant group differences in these variables.

It is also important to note that caregivers have a higher risk of dementia (Norton et al., 2010; Vitaliano, 2010) and that the MMSE, used in our study as an exclusion criterion for dementia, cannot rule out the possibility that at least some of the caregivers of this study could have mild cognitive impairment or even mild dementia.

Despite these limitations, we believe that the results of this study are important to shed some light on the neurobiological bases of cognitive deficits in caregivers and motivate more investigations about the psychophysiological correlates of their cognitive decline.

CONCLUSIONS

The results obtained in this study showed that caregivers' cognitive impairment is related with alterations on cortisol/DHEA ratios, and that the chronic stress experienced by these subjects also has the potential to alter their BDNF levels. Future studies should investigate if these effects of chronic stress are the same for middle age and older adults, since aged caregivers are subjected to the normal effects of aging (Ferrari and Magri, 2008; Suhr et al., 2008; Laing et al., 2012; Shimada et al., 2014) besides the caregiving stress. The clarification of these aspects would be of utmost importance to the establishment of proper managing and rehabilitation techniques designed for caregivers, assuring a better quality of life for them and their relatives with dementia.

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CAPÍTULO III

“Age Effects on Cognitive and Physiological Parameters in Family Caregivers of Alzheimer's Disease Patients”

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Age effects on Cognitive and Physiological Parameters in Familial Caregivers of Alzheimer's Disease Patients

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Abstract

Older familial caregivers of Alzheimer's disease patients are subjected to stress related cognitive and psychophysiological dysfunctions that may affect their quality of life and ability to provide care. Younger caregivers were never properly evaluated. We hypothesized that they would show qualitatively similar alterations to the older ones. Cognitive measures of 17 younger (31-58 years) and 18 older (63 -84 years) caregivers, and 17 younger (37-57 years) and 18 older non-caregiver controls (62-84 years), were evaluated together with their cortisol and dehydroepiandrosterone (DHEA) in saliva by radioimmunoassay and brain-derived neurotrophic factor in serum (BDNF) by Elisa. Although younger caregivers had milder impairments on memory and executive functions than older caregivers, their performance fell to the same or lower levels than that of healthy older controls. Decreases in DHEA and BDNF levels correlated with the cognitive dysfunctions seen in older and younger caregivers, respectively. Cortisol at 10PM increased in both caregiver groups. In conclusion, younger caregivers were prone to cognitive impairment like older ones, although the degree and the neuropsychological correlates of the cognitive dysfunctions were somewhat different between them. This work has implications for caregiver and care-recipient health and for research on the neurobiology of stress related cognitive dysfunctions.

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Key Words: BDNF, Cortisol, DHEA, Memory, Executive Functions

1 Introduction

In the next decades a huge increase in dementia cases is expected as a consequence of global population aging (1). Among dementias, Alzheimer's disease (AD) is the most prevalent (2). Assistance of AD patients is provided mostly by familial caregivers, mainly spouses and daughters (3,4), who are faced with an overwhelming and challenging task that can last over a long period of time. Due to the high dependence level of their demented relatives, caregivers are often subjected to an assistance-related physical and emotional burden (5).

Until now, studies on familial caregivers have focused mainly on the effects of emotional distress on older subjects, mostly patients' spouses (6). The chronic stress suffered by these caregivers, in association with their advanced age, predisposes them to psychological, behavioral and physiological risk factors for cognitive decline and dementia (3,7,8). Different studies indicate impairments of executive functions (working memory, attention and processing speed) and declarative memory in older caregivers. These cognitive domains depend heavily on two of the most sensible brain structures to stress, the prefrontal cortex (PFC) and hippocampus (9), which are particularly dense on cortisol receptors (10), a hormone that is expected to be altered in acute as well as in chronic stress (11).

Most studies of cortisol levels (measured in blood or saliva samples) in dementia caregivers suggest an up regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis and, as a consequence, hypercortisolemia (12,13). Moreover, aging can also predispose to elevation of cortisol levels (14,15). The negative effects of hypercortisolemia on neuronal plasticity, survival and neurogenesis are well documented (16,17) and could be related, at least partly, to the cognitive impairment seen in caregivers (18,19). However, cortisol is not the only steroid that has its levels altered in response to stress. As shown in former studies (20), stressful events also increase dehydroepiandrosterone (DHEA) levels, which have anti-glucocorticoid effects and lessen the negative effects of cortisol on central nervous system (21). Thus, it is suggested that cortisol/DHEA ratio is a more reliable marker for cognitive changes than cortisol or DHEA

alone (22). In fact, a previous study has shown that increased cortisol/DHEA ratios are related to the cognitive decline seen in dementia caregivers (19).

The evidence discussed above also suggests that cortisol and DHEA alterations are not the only responsible for the cognitive impairments seen in caregivers. Recently our research group showed a decrease in brain-derived neurotrophic factor (BDNF) levels in familial caregivers (19). It is normally assumed, based on animal (23) and human (24) studies, that peripheral and central BDNF are correlated. This neurotrophin modulates synaptic plasticity, neurogenesis and neuronal survival (25), is reduced in chronic stress situations (26) and expected to decrease with age (27), although controversy exists in relation to this issue (28,29). There is also evidence that serum BDNF levesl correlate with cognitive performance in different physiological and clinical conditions (24,25,30). However, its participation in cognitive impairments of dementia caregivers has yet to be proven.

As pointed out before, until now there are no studies that specifically evaluate the cognitive performance in younger caregivers, like patients' children. However, this is an important issue, because as caregivers they are also prone to have mood alterations (depression, anxiety), poorer sleep quality, social isolation and less time to take care of themselves, among other negative effects related to their caregiver activity (8,31). Thus, younger caregivers are exposed to almost the same risk factors as the older ones (with the evident exception of age) to cognitive decline. Therefore, the aim of this study was to compare the effects of chronic stress related to the caregiving activity on younger and older caregivers' cognition, as well as investigate physiological parameters that could be modulated by stress and related to their cognitive performance. Our hypothesis were that (I) cognitive impairment would be seen in both age groups, being more pronounced in the older caregivers; (II) hormonal and BDNF levels would be altered in caregivers, with more important stress effects on older caregivers and (III) hormonal and BDNF levels would show significant relations with cognitive performance in caregivers.

2 Material and Methods

2.1 Participants

Seventeen younger (48.82 ± 2.07 years; 15 women) and seventeen older (74.16 ± 1.82 years; 15 women) family caregivers of AD patients were recruited from the Brazilian Alzheimer Association (Porto Alegre, Brazil). To be eligible caregivers had to be providing care for 8 hours/day, for at least a year, at the time of the study. Additionally, seventeen younger (46.23 ± 1.37 years, 14 women) and eighteen older (68.22 ± 1.51 years, 13 women) control (non-caregiver) subjects were recruited from the community. Exclusion criteria were visual and hearing impairment, use of medications that could interfere with HPA axis or cognition, past or current use of psychoactive drugs, unstable medical conditions, neurological trauma or diseases, scores on Mini Mental State Exam (MMSE) (32) compatible with dementia (cut off < 26) and scores on Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) indicative of severe depressive (cut off > 30) or anxiety (cut off > 30) symptoms (33). All participants were evaluated for chronic stress, with the Lipp Stress Symptoms Inventory for Adults (ISSL) (34), and body mass index (BMI), since some studies have related this parameter with steroid and BDNF levels (35,36). None of the subjects was a smoker and all participants were asked to refrain from any use of alcohol 24 hours prior the beginning of the study. This study was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (Porto Alegre, Brazil) and have therefore been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All participants gave their informed consent.

2.2 Neuropsychological measures

Frontal lobe functions were assessed with neuropsychological tests that measured different components of executive function. More specifically, we employed the Digit-span tests of the Wechsler Adult Intelligence Scale (WAIS-III) (37) adapted for the Brazilian population (38), to asses working memory. Trail Making A and B test (39) and Stroop test (39), versions word (I) and color (II), were used to evaluate attention and processing speed. Finally, we used the Stroop test, version word/color (III) to evaluate the inhibitory response capacity (39).

Temporal lobe functions were assessed by the Logical Memory Test, a subtest of the Wechsler Memory Scale (40). This task evaluates immediate and delayed recall of declarative memory and is heavily dependent on the hippocampal formation.

All procedures related to the neuropsychological assessment followed the recommended guidelines for each specific task and were briefly described elsewhere (19).

2.3 Cortisol and DHEA analysis

As previously described (19), participants were asked to collect saliva samples at home, at 8 AM and 10 PM, on the day of the neuropsychological assessment. The samples were stored between 0ºC and 4◦C by the subjects and delivered to the laboratory within 3 days, where they were frozen at −80 ◦C until further analysis. Samples for cortisol and DHEA were analyzed by radioimmunoassay (Beckman Coulter kit - Immunotech) using a gamma counter (41) and showed sensitivities of 0.09 nmol/L and 0.06 nmol/L, respectively.

2.4 BDNF

A nursing professional collected 5ml of peripheral blood of each volunteer by venipuncture into an anticoagulant-free vacuum tube. The clotted blood samples were then centrifuged at 4000 rpm for 10 minutes, and serum was kept frozen at -80°C until further analysis. Serum BDNF analysis was performed by an ELISA kit following the manufacturer instructions (Milipore/USA) as previously described (42). A short description of the analysis can be found in the supplemental file.

2.5 Statistical Analysis

Differences between groups on demographic and clinical characteristics were analyzed with chi-squared statistics and one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test whenever necessary. Measures of cognitive performance and cortisol, DHEA, cortisol/DHEA and BDNF levels were initially analyzed with a mixed design analysis of variance (MANOVA), followed by ANOVAs and Bonferroni's post hoc tests to evaluate specific group differences. As clinical data indicated significant between groups differences on anxiety (BAI) and depressive (BDI) symptoms, we completed the statistical analysis with one-way analysis of

covariance (ANCOVA), to evaluate the effect of BAI and BDI as covariates on neuropsychological, hormonal and BDNF analysis. Linear regressions were run between results of neuropsychological tests and hormonal and BDNF levels. Results were expressed as mean ± standard error. The statistical significance was set at $P < 0.05$, power of all statistical analysis was greater than 80 % and effect sizes [eta squared ($\eta 2p$) or Rsquare (R2)] were reported for all statistical significant results.

3 Results

3.1 Demographic and clinical characteristics

Demographic and psychiatric features of the four experimental groups are shown in Table 1. Significant age differences [$\eta 2p = 0.749$, $p<0.001$] were seen between younger and older subjects ($p<0.001$), but not between the two younger ($p=1.00$) or the two older groups ($p=0.094$). There were also no significant differences between groups in gender [Pearson Chi-Square = 1.582, $p = 0.663$], years of education [$p = 0.232$], MMSE [$p=0.5$] and BMI [$p = 0.852$]. On the other hand, scores on depressive [$\eta 2p = 0.613$, $p<0.001$], anxiety [$\eta 2p = 0.441$, $p<0.001$] and stress [$\eta 2p = 0.842$, $p<0.001$] screening tests were significantly different between groups. As shown by Bonferroni's post hoc test, scores of younger and older caregivers on BDI, BAI and ISSL were similar (all $p>0.05$) and higher than that of the control groups (all $p< 0.01$).

Insert table 1

Some caregivers were taking antidepressant and/or anxiolytic medications: six volunteers were taking only antidepressants [selective serotonin reuptake inhibitor (SSRI), n=4; monoamine oxidase inhibitor (MAOI), n= 1; tricyclic plus SSRI, n= 1]; two used antidepressant in combination with anxiolytic medication [SSRI plus benzodiazepinic, n= 2] and another used only anxiolytic medication [bupropion, n= 1].

3.2 Neuropsychological data

The MANOVAs indicated significant age effects on working memory [η2*p*=0.188, p<0.001 for Forward Digit span; η2*p*=0.080, p=0.019 for Backward Digit span], attention and processing speed [η2*p*=0.253, p<0.001 for Trail Making A, η2*p*=0.247, p<0.001 for Trail Making B, η2*p*=0.340, p<0.001 for Stroop I and η2*p*=0.458, p<0.001 for Stroop II], as well as on inhibitory response capacity [η2*p*=0.376, p<0.001]. Further investigation of these results with ANOVAs [η2*p*=0.397 to 0.645, all p<0.05] and Bonferroni's post hoc tests showed that older controls had a lower performance in relation to younger controls in all these tasks (all p<0.01), with exception of Trail Making A in which an interaction between age and stress was observed [η2*p*=0.125, p=0.003], thus limiting the age effects to older caregivers. Moreover, older caregivers also showed significantly worse performances than their younger counterparts in all tasks cited above [p<0.001], with exception of the Backward Digit span [p=0.754] (Table 2).

Insert Table 2

Statistical analysis also showed significant effects of chronic stress on all cognitive functions investigated, as can be seen for the MANOVA results of working memory [η2*p*=0.562, p<0.001 for Forward Digit span; η2*p*=0.634, p< 0.001 for Backward Digit span], attention and processing speed [η2*p*=0.246, p<0.001 for Trail Making A; η2*p*=0.246, p<0.001 for Trail Making B; η2*p*=0.284, p<0.001 for Stroop I and η2*p*=0.349, p<0.001 for Stroop II], inhibitory response capacity [η2*p*=325, p<0.001] and declarative memory [η2p=0.554, p<0.001 for Logical Memory I; η2*p*=0.525, p<0.001 for Logical Memory II]. Further analysis of these results with ANOVAs [η2*p*=0.397 to 0.645, all p<0.05] and Bonferroni's post hoc tests confirmed that younger and older caregivers had significantly lower scores thant their age matched controls on all neuropsychological tasks [all p<0.05]. The only exception was in Trail making A. As explained before, this task showed and interaction between age and stress [η2*p*=0.125, p=0.003], limiting the stress effects to older caregivers. It is also important to draw attention to the fact that the performance of younger caregivers was significantly lower that that of older controls in Forward

and Backward Digit Span, as well as in Logical Memory I and II (all $p<0.05$). On the other cognitive tasks (Trail Making A and B, and Stroop I, II and III) no significant differences were found between younger caregivers and older healthy controls (Table 2)

To summarize, our results indicate that the chronic stress due to the caregiving activities: (I) usually promote greater deficits on older than younger caregivers (working memory, processing speed and inhibitory control); (II) impair younger caregivers in such manner that their performance fell to the same (attention, processing speed, inhibitory control) or even lower (working and declarative memory) levels than that of older controls. Covariance analyses showed that BDI and BAI scores had no significant effects as covariates (all $p>0.05$) on these results and, consequently, were unable to change the neuropsychological outcomes described above.

3.3 Hormonal levels

Figure 1 represents the levels of cortisol (a) and DHEA (b), as well as the ratio of these hormones (c).

Insert Figure 1(a), 1(b) and 1(c)

3.3.1 Cortisol levels

The mixed ANOVA indicated a significant effect of time on cortisol levels [$\eta 2p=0.902$, $p<0.001$], as well as an interaction between time, age and stress [$\eta 2p=0.084$, $p=0.016$]. The significant time effect can be explained by higher cortisol levels at 8 AM than at 10PM in all experimental groups (all $p<0.001$). The interaction between time, age and stress can be better appreciated by the analysis of the group differences at 8AM [$\eta 2p=0.137$, $p=0.021$] and 10PM [$\eta 2p=0.293$, $p<0.001$]. At 8AM an age effect can be seen among stressed subjects, so that cortisol levels were higher for younger than older caregivers ($p=0.003$). No significant

differences were seen for the 8AM levels of this steroid between controls and caregivers ($p>0.05$), nor between younger and older controls ($p>0.05$). At 10PM a different pattern of results emerged: cortisol levels of younger and older caregivers were similar ($p>0.05$) and higher than that of their respective age controls (all $p<0.05$). The significant group differences seen for cortisol levels at 8 AM and 10PM remained even with the introduction of BDI and BAI scores in the statistical model, since they had no significant effects as covariates (all $p>0.05$) on cortisol analysis. In short, chronic stress effects on cortisol levels of younger and older caregivers were seen only at 10 PM and were not affected by depressive and anxiety symptoms.

3.3.2 DHEA levels

The statistical analysis of DHEA results indicated a significant effect of time [$\eta 2p=0.824$, $p<0.001$] and age [$\eta 2p=0.345$, $p<0.001$], but no effect of stress was detected [$\eta 2p=0.032$, $p=0.145$]. However, there was also a significant interaction between time and stress [$\eta 2p=0.285$, $p=0.001$]. Further analysis of these results indicated significant between group differences at 8AM [$\eta 2p=0.450$, $p<0.001$] and 10PM [$\eta 2p=0.198$, $p=0.002$]. Bonferroni's post hoc tests indicated a significant decline of DHEA levels with age for younger and older controls ($p<0.001$) and for younger and older caregivers ($p<0.001$) at 8AM. Older caregivers had the lowest DHEA levels of all groups at this sampling time (all $p<0.05$), whereas younger caregivers showed similar levels of this hormone to their respective age control group ($p=0.490$). At 10PM no significant age differences were seen between younger and older controls ($p>0.05$). However, younger caregivers showed higher DHEA levels than older controls ($p=0.005$) and caregivers ($p=0.005$). Covariance analysis indicated that BAI and BDI scores had no significant effects (all $p>0.05$) as covariates and, consequently, were unable to change the significant group differences described above for DHEA levels at 8AM and 10PM. To summarize, a clear stress effect on DHEA levels were seen only at 8AM and only for older caregivers. This outcome was not affected by depressive or anxiety symptoms.

3.3.3 Cortisol/DHEA ratios

The results obtained with the mixed ANOVA for cortisol/DHEA ratios indicated a significant effect of time [η2p=0.842, p<0.001] and age [η2p=0.116, p=0.004], no effect of stress [η2p=0.040, p=0.102] and a significant interaction between time, age and stress [η2p=0.061, p=0.043]. The significant time effects can be explained by higher cortisol/DHEA ratios at 8AM than at 10PM in all experimental groups (all p<0.05). The one way ANOVAs indicated significant group differences only at 10 PM [η2p=0.298, p<0.001]. At this sampling time we can see a clear age effect, with younger controls and caregivers showing lower cortisol/DHEA ratios then their respective older counterparts (all p<0.05). The time, age and stress interaction can be understood when we realize that older caregivers had the highest levels (p<0.01 in relation to younger subjects) at 10PM. These significant differences seen for cortisol/DHEA ratios at 10PM remained even with the introduction of BDI and BAI scores in the statistical model, since they had no significant effects as covariates (all p>0.05) on the analysis. In short, the cortisol/ DHEA ratios suggest interactions between age and stress only at 10PM, so that older caregivers seem to be the most affected by these variables.

3.4 BDNF levels

The BDNF results showed a clear effect of stress [η2p=0.097, p=0.010] and an interaction between stress and age [η2p=0.079, p=0.021] on the levels of this neurotrophin. However, age had no effect on BDNF levels by itself [η2p=0.011, p=0.390]. Post-hoc tests indicated that the significant group differences [η2p=0.165, p=0.007] seen for BDNF are among younger caregivers and their corresponding age controls (p=0.005), as shown in figure 2. Thus, stress and age interacted to lower the levels of this neurotrophin in younger caregivers. This outcome remained unchanged even after the introduction of BDI and BAI in the covariance analysis [η2p=0.145, p=0.018], since they had no significant effects as covariates.

Insert Figure 2

3.5 Relations among Cognition and hormonal or BDNF levels

The evident effects of age and chronic stress on cognition, hormonal and BDNF levels described above led us to further investigate a possible relation between these variables. Thus, separate linear regressions were run for younger and older subjects, in order to search for relations between cognitive performance (dependent variable) and physiologic parameters altered by stress (hormonal and BDNF levels, independent variables) within the different age groups.

The hormonal parameters analyzed were cortisol levels at 10PM (for younger and older subjects) and DHEA levels at 8AM (for older subjects), which were the only variables that showed a significant stress effect between caregivers and their respective age controls. The results of these linear regressions indicated that younger subjects had a significant relation between scores on neuropsychological tests and cortisol levels only for the Trail Making B task [$R2=0.316$, $B=10,393$, $p=0.001$]. None of the linear regressions made for older subjects showed any significant relation between cognitive performance and cortisol levels (all $p>0.05$). On the other hand, DHEA levels of older subjects showed significant relations with performance on the Forward and Backward Digit Span, as well as on Trail Making B and on Logical Memory I and II tasks (all $p<0.05$), as can be seen on Table 3.

Insert table 3 about here

Linear regressions for cognitive performance and BDNF levels were run only for younger subjects, which showed significant differences in the levels of this neurotrophin between control and caregivers, as described above. As can be seen in table 3, BDNF levels of younger subjects were significantly related to most of the analyzed cognitive domains, including working memory [Forward ($p<0.001$) and Backward Digit Span ($p<0.001$)], attention [Stroop I

(p=0.004) and II (p=0.030)], inhibitory response [Stroop III (p=0.026)] and memory [Logical Memory I (p=0.013) and II (p<0.001)] regressions.

In general, the results described above revealed that: (I) cortisol levels at 10PM were not related to the cognitive outcomes of younger or older subjects, with the exception of Trail B performance in younger volunteers; (II) decreased levels of DHEA at 8AM are related to a worst cognitive outcome in older subjects; (III) lower BDNF levels were related to a decrease of cognitive performance in younger subjects.

4 Discussion

The aim of this study was to investigate the impact of chronic stress due to caregiving of AD patients on cognition, hormonal and BDNF levels of younger and older familial caregivers. The results indicated cognitive impairments in both caregiver groups, with surprisingly important deficits in the younger one, in which the performance fell to the same or lower levels than that of healthy older controls, suggesting a precocious cognitive aging. Moreover, cortisol levels at 10PM were increased in both caregiver groups, whereas DHEA levels at 8AM fell only in older caregivers. These hormonal alterations were not capable to induce significant differences on cortisol/DHEA ratios between caregivers and their respective age controls. Even so, lower DHEA levels at 8AM proved to be significantly related to a worst cognitive outcome in older subjects. On the other hand, BDNF levels showed a decrease only in younger caregivers, in wich they were related to a poorer cognitive performance.

A significant emotional burden, characterized by the prevalence of psychological stress symptoms on the ISSL scale, was seen among younger and older caregivers. Moreover, a significant portion of the caregivers were on the near-exhaustion and exhaustion stages (43), presumably as a consequence of the long lasting, high weekly load of caregiving activities and the suffering resulting from the close relationship between caregivers and patients (5,44). In accordance with this scenario, we also found more depression and anxiety symptoms among caregivers. Even so, BDI and BAI scores for both caregiver groups were below the cutoff for moderate depression and anxiety symptomatology (33), and this is likely the explanation to the

lack of effect seen for scores of BAI and BDI as significant covariates in neuropsychological, hormonal and BDNF analysis. Probably the depression and anxiety symptoms were maintained at low levels because some caregivers were taking antidepressant and/or anxiolytic medication, as described in the results section. As shown by previous studies, the use of such medication is very common among caregivers (44,45).

The cognitive impairments seen in this study for older caregivers' working and declarative memory, attention, processing speed and inhibitory response capacity are in accordance with many other studies (3,7,8,46–49) . However, our results add two important new informations. First, older caregivers were especially prone to the negative effects of the caregiving activities on prefrontal functions, as suggested by the greater cognitive decline in prefrontal dependent tasks in relation to younger caregivers. Until now this result was only hypothesized by literature (3,49), based on the knowledge that both age and chronic stress are capable to predispose to cognitive decline (50). Second, younger caregivers seem to be predisposed to a precocious cognitive aging, since besides the lower performance than younger controls in practically all neuropsychological tests, their scores on the prefrontal and temporal lobe dependent tasks fell to the same (attention and processing speed, inhibitory control and declarative memory) or even lower (working memory) levels than that of older controls. It is important to highlight that some studies reported increased incidence rates for dementia among spouses of persons with dementia (3,7). However, these studies are focused in caregiving activity as a late-life stressor. What could be expected for the dementia incidence rate among middle-aged familial caregivers, like patients' children, for which this psychosocial stressor initiates earlier? Although there is no response yet, the results of the neuropsychological tests of our younger caregivers clearly point to their risk of cognitive decline and the need of more studies on this age group.

Differently from most other chronic stress studies, we analyzed cortisol secretion only at 8AM and 10PM. Although not common, this method was choosen because it allowed caregivers to collect saliva samples at home (increasing study adherence), without the complications implied in multiple samplings along the day (51) or the risk to compromise the strict standardization and timing required by other techniques, such as cortisol awaking response (12,52). Our experimental paradigm showed that caregivers had a typical rhythm of cortisol

secretion (53,54), whit serum concentrations decreasing form the morning to the night. However, the 10 PM levels of this hormone were clearly influenced by stress, showing higher values for younger and older caregivers than for their respective age controls. Similar results were found in previous studies of our and other research groups (13,19,55) and could be explained by a HPA imbalance (19,56) and/ or behavioral alterations of patients at early night, known as Sundowning Syndrome, which can impose extra difficulties for patients' managing (57).

A clear stress effect on DHEA was seen only at 8AM and only for older caregivers. Similar DHEA alterations were also reported in other studies whit caregivers (56), although controversies exist (19). It is worth noting that our elderly caregivers also had lower mean levels of cortisol than their age controls at 8 AM (although statistical significance was not reached), probably reflecting the expected positive, albeit weak, correlation between cortisol and DHEA levels in response to HPA axis control (58).

As expected, cortisol/DHEA ratios showed a tendency to increase in caregivers, but statistical significance was not reached. Although the cortisol/DHEA ratio seem to be a more reliable marker for cognitive changes than cortisol or DHEA alone (21), only two other studies investigated it in caregivers. Corrêa et al. found increased cortisol/DHEA ratios at 8AM and 10PM, mainly due to increases in cortisol levels. Jeckel and collaborators also found increased cortisol/DHEA ratios in caregivers, however they were the consequence of low DHEA levels, not increasing cortisol values (56).

The discrepancies seen between different studies on caregivers' cortisol, DHEA and cortisol/DHEA levels could be explained by the different age compositions of the experimental samples, as suggested by the age effects seen in this and other studies (10). Moreover, other factors such as caregivers' stress levels and coping strategies (59), as well as the stage of patients disease (60), could contribute with some variability in the data. Thus, at the present state of knowledge, it is important to emphasize the need for more studies to adequately investigate each of these parameters and their contribution to hormonal alterations in caregivers.

Besides cortisol and DHEA levels, we also investigated serum BDNF of caregivers. Previous studies found that chronic stress can influence neurotrophin levels (26). Researchers suggest a possible relation between hypercortisolemia and lower BDNF levels (26,61,62) and recently our laboratory reported, for the first time, a decrease in BDNF levels in familial caregivers (32-84 years old) of AD patients (19). In the present study we also found decreased levels of BDNF in caregivers, but only for the younger ones. Thus, our results point to an interaction of age and chronic stress on BDNF levels in such a manner that younger caregivers were more affected than the older ones.

Assuming that peripheral cortisol, DHEA and BDNF are related to their levels in brain (63,64) and knowing that they are involved in cellular and molecular mechanisms of cognition (26), we investigated if the effects of chronic stress on these physiologic parameters could be related to the cognitive impairment seen in caregivers. The linear regressions between these variables revealed that cortisol levels at 10PM were not related to the cognitive outcomes of younger or older subjects. However, decreased levels of DHEA at 8 AM were related to a worst cognitive outcome in older subjects, while lower BDNF levels were related to a decrease of cognitive performance in younger subjects. More specifically, decreased DHEA levels were related to poorer outcomes on working memory, processing speed and declarative memory (R^2 ranging from 0.12 – 0.27), suggesting that DHEA could be one of the mediators of chronic stress effects on cognitive impairments in older caregivers (21). In turn, BDNF levels correlated with all cognitive domains assessed in young subjects (R^2 ranging from 0.13 – 0.47), such as working memory, attention, inhibitory response capacity, processing speed and declarative memory.

According to the discussion above, it is clear that one of the limitations regarding the current study is sample size. Although our results for cognitive performance were in agreement with our hypothesis and previous literature, a larger sample may have been more accommodating for drawing stronger conclusions on hormonal levels, cortisol/DHEA ratios and BDNF levels, as well as the relation of these parameters with cognition. Furthermore, some of our caregivers were using medication for depression and anxiety, and the effects of these medications on the variables analyzed in this study could not be ruled out. Even so, it seems to us that these medications could hardly affect our results. First, the number of caregivers in each

age group making use of them was low. Second, the different reported medicaments can have opposing effects on cognition, hormonal and BDNF levels, depending on their dosage, usage time and combination (65–67). Thus, it seems very unlikely that these medications could deviate the obtained results in a specific direction and alter our conclusions. Nevertheless, it would be wise to design future studies to investigate the potential of anxiolytic and antidepressant medication on caregiver's outcomes.

5 Conclusions

Despite the limitations discussed above, the present study showed that younger caregivers had important cognitive dysfunctions and that older caregivers are even more compromised. Hormonal and BDNF levels were affected by caregiver's chronic stress and partly related to their cognitive impairments. We are confident that our results are important to expand the knowledge in this area and hope that our results draw the attention of policymakers and clinicians to the fact that the mental health of midle-aged caregivers deserves as much attention as that of older ones. Even minor cognitive problems in caregivers may affect their quality of life and ability to provide adequate care, whit major implications for formal health systems at a time when demand is increasing. Thus, the development of inteventions aimed to help families whit AD patients to manege the stressfull effects of caregiving activity is urgent.

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Disclosure statement

The authors have no conflicts of interest to disclose.

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Table 1 Demographic and psychiatric measures (mean ± standard error) of the different age groups of controls and caregivers.

	Younger Controls	Younger Caregivers	Older Controls	Older Caregivers
Age (years)	46.23 ± 1.37	48.82 ± 2.07	68.22 ± 1.51 *	74.16 ± 1.82 *
Sex (F/M)	14/3	15/2	13/5	15/3
Education (years)	13.52 ± 0.53	13.58 ± 0.52	11.88 ± 0.84	12.94 ± 0.62
MMSE	28.05 ± 0.29	28.35 ± 0.34	27.38 ± 0.43	27.05 ± 0.33
BDI	5.70 ± 0.78	15.70 ± 1.30 **	4.27 ± 0.88	16.61 ± 1.31 **
BAI	3.05 ± 0.58	7.29 ± 0.89 **	2.94 ± 0.80	10.61 ± 1.16 **
BMI	24.80 ± 1.16	24.96 ± 1.03	25.93 ± 0.97	24.70 ± 0.97
Assisted Time (Weekly Hours)		99.76 ± 14.82		136.88 ± 12.63
Caregiving (years)		3.52 ± 0.44		5.27 ± 0.87
Phase of Stress (ISSL) n (%)				
Alert	0 (0)	0 (0)	0 (0)	0 (0)
Resistance	0 (0)	7 (41)	0 (0)	11 (61)
Near Exhaustion	0 (0)	7 (41)	0 (0)	1 (6)
Exhaustion	0 (0)	3 (18)	0 (0)	6 (33)
Symptoms of Stress (ISSL) n (%)				
Physical	0 (0)	5 (29)	0 (0)	4 (22)
Psychological	0 (0)	12 (71)	0 (0)	14 (78)

Abbreviations: MMSE, Mini Mental Status Examination; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory and BMI, Body Mass Index; ISSL, Lipp Stress Symptoms Inventory for Adults.
* $p<0.05$ in relation to younger controls and caregivers
** $p<0.01$ in relation to controls

Table 2 Performance (mean ± standard error) of younger and older controls and caregivers on neuropsychological tests.

	Younger Controls	Younger Caregivers	Older Controls	Older Caregivers
Digit Span (Forward)	7,17 ± 0.26	5.11 ± 0.22 #	6.44 ± 0.33	3.88 ± 0.13 *
Digit Span (Backward)	5.88 ± 0.18	2.88 ± 0.26 #	5.16 ± 0.39	2.27 ± 0.17 #
Trail Making A	34.88 ± 1.97	39.94 ± 2.77	40.22 ± 2.68	65.11 ± 4.66 *
Trail Making B	67.05 ± 3.29	102.17 ± 6.75 ¤	102.27 ± 13.80 ¤	144.44 ± 4.53 *
Stropp I	93.17 ± 2.14	77.29 ± 2.68 ¤	75.27 ± 3.87 ¤	62.22 ± 2.15 *
Stropp II	73.64 ± 2.09	59.47 ± 1.94 ¤	56.00 ± 2.73 ¤	43.11 ± 2.19 *
Stropp III	54.70 ± 2.10	37.05 ± 2.14 ¤	35.38 ± 3.40 ¤	24.83 ± 2.00 *
Logic Memory I	27.70 ± 0.63	18.52 ± 1.35 #	26.50 ± 1.02	17.50 ± 0.87 #
Logic Memory II	23.52 ± 1.00	13.70 ± 0.99 #	20.61 ± 1.11	13,33 ± 0.86 #

* $p<0.05$ in relation to all groups
# $p<0.05$ in relation to control groups
¤ $p<0.05$ in relation to younger controls group

Table 3 Results of linear regressions for cognitive performance and physiological parameters (DHEA or BDNF) in younger and older subjects.

	Older subjects DHEA at 8AM		
	R2	B	*p*
Span Forward	0.187	6,319	0.008
Span Reverse	0.265	8,712	0.001
Trail Making B	0.150	-161,086	0.020
Logical Memory	0.151	20,419	0.019
Logical Memory	0.123	16,953	0.036
	Younger subjects BDNF		
	R2	B	*p*
Span Forward	0.377	0.080	<0.001
Span Reverse	0.475	0.113	<0.001
Stroop I	0.233	0.561	0.004
Stroop II	0.139	0.371	0.030
Stroop III	0.145	0.432	0.0026
Logical Memory I	0.180	0.245	0.013
Logical Memory II	0.327	0.335	<0.001

Fig. 1

Levels (mean ± standard error) of cortisol (a), DHEA (b) and Cortisol/DHEA ratios (c) in saliva samples at 8AM and 10PM between groups. * $p < 0.05$

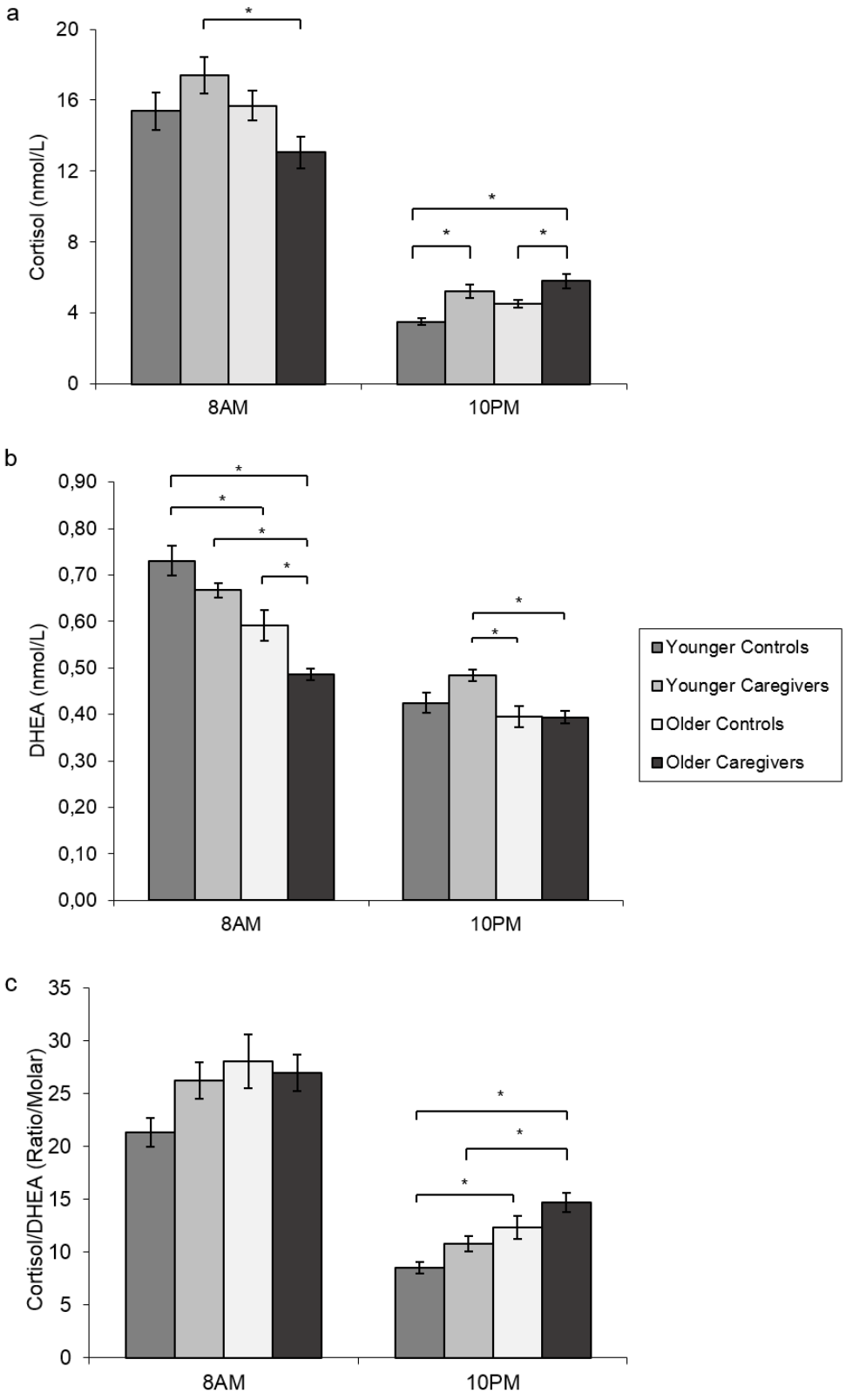
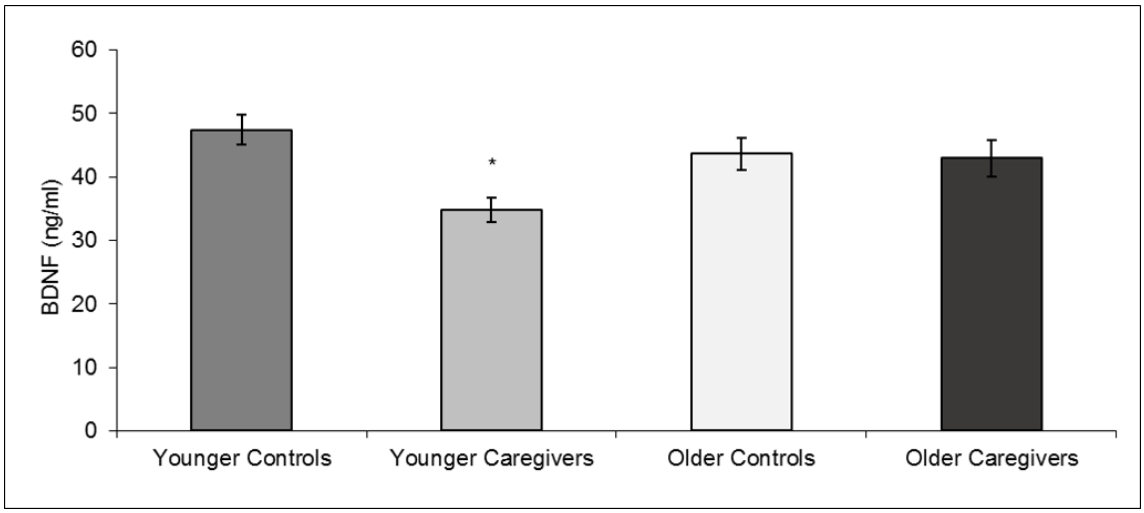


Fig. 2

BDNF levels (mean ± standard error) of younger and older controls and caregivers



* $p < 0.05$ in relation to younger controls.

CAPÍTULO IV

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4 CONSIDERAÇÕES FINAIS

5 PERSPECTIVAS

4 CONSIDERAÇÕES FINAIS

Cuidadores familiares de pacientes com Alzheimer são considerados um modelo para estudos sobre os efeitos do estresse crônico no organismo. A alta sobrecarga emocional, a qual são submetidos diariamente, torna estes indivíduos suscetíveis aos efeitos negativos do estresse, os quais comprometem diferentes mecanismos e funções fisiológicas. Um dos mecanismos mais alterados é a produção e secreção dos glicocorticóides, sendo o hormônio cortisol um dos biomarcadores mais estudados.

Diante do exposto, nossa hipótese inicial, apresentada no Capítulo II, foi analisar os efeitos do estresse crônico sobre parâmetros neurofisiológicos (cortisol, DHEA e BDNF) e cognitivos (atenção, função executiva e memória declarativa) nos cuidadores. Os resultados de nosso primeiro experimento demosntraram que esses indivíduos apresentam elevação dos níveis de cortisol no período noturno, confirmando achados de estudos com modelos de estresse, bem como trabalhos com cuidadores familiares de pacientes com outros tipos de demências, que mostram hipersecreção desse hormônio nesse horário (Gallagher-Thompson et al., 2006; Palma et al., 2011).

A literatura mostra que os glicocorticoides são capazes de modular aspectos cognitivos. Mais especificamente, níveis cronicamente elevados de cortisol podem prejudicar os mecanismos subjacentes a diferentes processos cognitivos. Nossos resultados estão de acordo com estes achados. Os cuidadores avaliados demonstram alterações de memória declarativa. Este tipo de memória dependente do hipocampo, estrutura do lobo temporal que

apresenta elevada densidade de receptores para gicocorticóides. Adicionalmente, a atenção e função executiva, ambas dependentes do córtex pré-frontal (o qual também apresenta elevada densidade de receptores para cortisol), também se mostraram prejudicadas nos cuidadores.

Outro aspecto investigado em nosso primeiro experimento diz respeito a avaliação do efeito do estresse crônico sobre os níveis de cortisol, DHEA e mais especificamente, na razão entre ambos. Os achados não demonstraram alterações nos níveis circadianos do DHEA, como hipotetizado para verificar sua ação antiglicocorticóide. No entanto, o efeito mais pronunciado foi na proporção entre esses hormônios. A razão cortisol/DHEA monstrou-se sensível ao estresse, especialmente as 10PM, quando foi relacionada negativamente com todas as tarefas cognitivas analisadas, ou seja, tanto com lobo frontal quanto com temporal. Dessa forma, demonstramos que essa alteração hormonal poderia explicar parcialmente as alterações cognitivas encontradas.

Adicionalmente, nós tentamos esclarecer o comportamento dos níveis de BDNF perante a sobrecarga emocional. As análises demonstraram baixos níveis dessa neurotrofina nos cuidadores, no entanto somente relacionada com um teste que media a função do córtex pré-frontal.

Esse desfecho do estresse sobre essa neurotrofina em cuidadores foi o primeiro publicado na literatura, até o presente momento. Todavia, a partir desse achado surgiu a necessidade de avaliar como a idade poderia estar relacionada aos efeitos negativos do estresse nessa população. Então, como apresentado no Capítulo III, nós analisamos esses mesmos parâmentros

cognitivos, homonais e neurotrófico em diferentes grupos etários de cuidadores familiares e sujeitos que não exerciam tal atividade.

Como observado no primeiro trabalho, escores moderados de sintomatologia depressiva e ansiosa foram evidenciados em cuidadores, provavelmente resultante do longo e intenso comprometimento dos cuidadores com seus pacientes demenciados. A maioria dos voluntários estava na fase de resistência do estresse. Contudo, considerável parcela de cuidadores encontrava-se nas fases de quase exaustão e exaustão da escala de Lipp (Lipp & Guevara, 1994), quando o organismo já não consegue lidar adequadamente com o estresse, comprometendo a homeostasia do organismo. Os sintomas de estresse mais observados foram os psicológicos, demonstrando uma clara sobrecarga emocional, se comparada aos sintomas físicos.

Nos resultados cognitivos, foi evidente o prejuízo dos cuidadores idosos, grupo que apresentou os piores desempenhos nos testes de memória declarativa e função executiva. Outra constatação relevante dos resultados cognitivos foi que os cuidadores de meia-idade monstraram um desempenho semelhante aos controles idosos, sugerindo um envelhecimento cognitivo precoce. Como a maioria dos estudos com cuidadores é realizada com os cônjuges dos pacientes, mais estudos com cuidadores mais jovens são necessários para corroborar esse achado promissor.

Os cuidadores idosos também apontam alterações importantes a respeito dos níveis hormonais. Esses indivíduos apresentaram os níveis mais altos de cortisol e da razão cortisol/DHEA no período noturno. Para os níveis de DHEA, os resultados mais evidentes foram também para os cuidadores

idosos, nos quais mostraram uma hiposecreção pela manhã. Análises estatísticas mais detalhadas demonstraram que os resultados cognitivos, que avaliavam a função frontal e temporal, foram potencialmente relacionados aos baixos níveis de DHEA 8AM em idosos.

Por fim, o nível de BDNF mostrou-se significativamente inferior em cuidadores de meia-idade e negativamente relacionado aos déficits cognitivos, para as tarefas que mediam a função do córtex pré-frontal e hipocampo. Esse achado é o primeiro na literatura em cuidadores e poderia explicar em parte as alterações cognitivas evidenciadas em grade parte dos estudos com essa população.

Por conseguinte, tanto nos Capitulos II e III, notamos um prejuízo cognitivo e alterações homonais e neurotrófica em cuidadores familiares de pacientes com Alzheimer como hipotetizado. No entanto, é importante resaltar que nossos resultados esclarecem parcialmente o impacto do estresse crônico e da idade nos aspectos avaliados nos estudos e, portanto, não podem ser generalizados. São necessários muitos estudos ainda avaliando outros parâmetros fisiológicos, bem como testes de neuroimagem, a fim de fortalecer esses resultados preliminares.

Futuros estudos devem verificar alguns fatores que podem obscurecer os resultados homonais e neurotróficos, como os efeitos das medicações antidepressivas e ansiolíticas, nas quais essas populações obrigatoramente fazem uso, devido aos consideráveis níveis de depressão e ansiedade que são vivenciados. Nos dois trabalhos realizados, não foi possível verificar se as medicações estavam de alguma forma modulando tais aspectos avaliados.

Outra preocupação importante nos estudos apresentados são o tamanho das amostras, que apesar do poder significativo, é outro fator revelante para estudos com modelos humanos. Todavia, como já explorado nos estudos, os cuidadores são responsáveis pela totalidade dos cuidados diários dos pacientes, tornando-se difícil a disponibilização dos mesmos em ensaios clínicos. Um ponto que deve ser discutido também é que estudos transversais são difíceis de diagnosticar um comportamento exato dos efeitos crônicos do estresse e do envelhecimento para essa população. Por fim, uma limitação importante dos estudos apresentados foi a falta da avaliação das estratégias de enfrentamento desses indivíduos. Boa parte de estudos com modelos de estresse crônico avaliam a resposta do *Coping*, a fim de verificar como voluntários lidam com o estresse experimentado. Acreditamos que esses resultados poderiam melhor esclarecer o desfecho dos nossos estudos.

Diante do exposto, acreditamos que esses achados são de extrema importância para compreensão do comportamento cognitivo e neurofisiológico dos efeitos do estresse crônico e da idade em cuidadores. Esses trabalhos trazem entendimento para o estabelecimento das técnicas de gestão e de reabilitação adequadas para os cuidadores, garantindo uma melhor qualidade de vida para eles e seus familiares com a doença de Alzheimer.

5 PERSPECTIVAS

- Avaliar as estratégias de enfrentamento (*Coping*) dos cuidadores jovens e idosos e relacionar com o desempenho cognitivo, hormonal e neurotrófico, bem como com a sintomatologia depressiva e ansiosa desses indivíduos;

- Analisar o padrão de secreção hormonal em outros horários do ritmo circadiano, tais como, a resposta do cortisol ao acordar (*CAR*) e pela tarde;

- Verificar o comportamento de outras neurotrofinas nos diferentes grupos experimentais, tais como, Neurotrofina 3 (NT3), Neurotrofina (NT4), Fator de Crescimento Neural (NGF), a fim de identificar uma possível alteração e relacionar com o desempenho cognitivo e os níveis hormonais;

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ANEXOS

ANEXO A - Artigo publicado “The role of encoding strategies in contextual memory deficits in patients with bipolar disorder”, Neuropsychological Rehabilitation, 2014.

ANEXO B – Resumo publicado “Emotional Burden Effects on Attention and Executive Function in Family Caregivers of Alzheimer Patients”, Frontiers in Human Neuroscience, 2015.

ANEXO A

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The role of encoding strategies in contextual memory deficits in patients with bipolar disorder

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Contextual memory is important for the encoding and retrieval of episodic memory, which is often impaired in euthymic patients with bipolar disorder (BD). The objective was to investigate the effect of low and high cognitive support on encoding in an incidental contextual memory task in euthymic patients with BD. Twenty-three patients with a BD type I diagnosis (aged

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ANEXO B

frontiers in Human Neuroscience

Emotional Burden Effects on Attention and Executive Function in Family Caregivers of Alzheimer Patients.

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INTRODUCTION: Family caregivers of patients with Alzheimer's disease have a significant emotional burden. Chronic stress affects cognitive functions such as attention and executive function. OBJECTIVE: Assess the chronic stress effects on attention and executive function in family caregivers of Alzheimer patients. METHODS: Participants were caregivers (n=18, age 64.83 ± 3.64 years old, 13 women) and controls (n=17, age 58.29 ± 3.16 years old, 14 women). Participants performed Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Lipp's Stress Symptoms Inventory (ISSL). All volunteers were subjected to Digit Span (forward and backward), Trail Making (A and B) and Stroop (words, colors and colors/words) tests to measure attention and different components of executive function. T-test and linear regression were used for statistics analysis and P <0.05 was considered statistically significant. RESULTS: The caregivers presented higher scores of depressive (p=0.001) and anxiety symptoms (p<0.001) than controls. In the ISSL, only caregivers presented stress (33% resistance, 27% near exhaustion, 39% exhaustion). In all cognitive tests, the caregivers had poorer performance than the controls. There was significant difference between groups in Digit Span (forward and backward) p<0.001, Trail Making A (p=0.004) and B (p<0.001) and Stroop to words (p=0.004), colors (p=0.003) and colors/words (p=0.038) tests. We found a positive relation between the cognitive task performance and the results of BAI (p=0.008 – R2=0.477), BDI (p=0.001 – R2=0.561) and ISSL (p<0.001 – R2=0.693). CONCLUSION: Caregivers had impaired performance in attention and executive tasks. Chronic stress, as well as anxiety and depression symptoms, seem to have an important role on these cognitive impairments. Thus, measures aimed for emotional burden reduction may be important to remediate attention and executive function of caregivers.

Keywords: Anxiety, Attention, Depression, Executive Function, stress

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