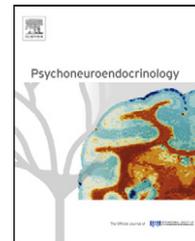




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The cortisol awakening response in amyotrophic lateral sclerosis is blunted and correlates with clinical status and depressive mood

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Manual muscle test;
Beck depression inventory;
Hamilton depression rating scale

Summary Considerable evidence indicates that amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease of the motor system, has an enormous impact on the patient's emotional and physical well-being. As previous findings indicated that particularly the rise in cortisol levels immediately after awakening, i.e., the cortisol awakening response (CAR), is associated with indices of physical and emotional well-being, we compared the CAR of 29 admitted ALS patients with that of 12 age-matched caregiver controls. Saliva samples for cortisol measurement were collected immediately, 15, 30 and 45 min after awakening. The severity of ALS progression was quantified using the ALS functional rating scale (ALSFRS) and manual muscle test (MMT). Depressive mood status in ALS patients was determined with the Beck Depression Inventory (BDI) and Hamilton Depression Rating Scale (HDRS). Salivary cortisol levels of ALS patients did not differ from those of caregiver controls at awakening, 15 min or 45 min after awakening, but were significantly lower at 30 min after awakening. Area under the curve analysis confirmed that the CAR was significantly smaller in ALS patients than in caregiver controls. A smaller CAR in ALS patients was significantly correlated to poorer clinical status, as assessed with both the ALSFRS and MMT rating instruments. Further, a smaller CAR significantly correlated with a more severe depressive mood status. No correlations were observed between total cortisol output during the first 45 min post-awakening and clinical or depressive status. In conclusion, our findings indicate that ALS patients show a blunted CAR, correlated with disease and depression severity.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder of unknown etiology, characterized by weakness, muscle wasting, fasciculation and increased

reflexes. It affects the upper and lower motor neurons in cerebral cortex, brainstem and spinal cord (Mitchell and Borasio, 2007). Although it is well established that ALS has an enormous impact on the patient's physical and emotional well-being (Mitchell and Borasio, 2007), little is known concerning possible changes in hypothalamic–pituitary–adrenal (HPA) axis activity or other stress–response systems in ALS. In one study, Patacchioli et al. (2003) reported a loss of circadian rhythmicity in cortisol secretion in ALS patients. However, this study did not examine a possible association between adrenal activity and the status of physical dysfunction, such as dysphagia, dysarthria or difficulty with activities of daily living, or with the patients' mood status. A wealth of studies has shown that the cortisol awakening response (CAR), defined as the period of cortisol secretory activity in the first 30–60 min post-awakening, is regulated distinctly from the diurnal pattern of HPA-axis activity and, rather, reflects specific secretory activity associated with awakening (Wilhelm et al., 2007). The CAR has a relatively high intra-individual stability (Hucklebridge et al., 2005; Wüst et al., 2000) and is considered a reliable measure of HPA-axis activity in the morning (for review see Fries et al., 2009). For several years, the magnitude of the CAR has been associated with a wide range of physiological and psychological parameters (for review see Clow et al., 2010). A recent meta-analysis (Chida and Steptoe, 2009) indicated that chronic stress is associated with a more pronounced CAR. In contrast, burnout, fatigue and post-traumatic stress disorder are typically associated with a blunted CAR. The present study investigated whether the CAR of patients with ALS differed from that of a carefully selected group of age-matched caregiver control subjects. Furthermore, we examined whether the magnitude of the CAR in ALS patients correlated with their clinical and/or depressive mood status.

2. Method

2.1. Subjects

Participants were recruited from the ALS clinic of the Wonkwang Gwangju Medical Center. Forty patients with definitive ALS were originally selected. ALS was diagnosed on the basis of the revised El Escorial criteria (Brooks et al., 2000). Additionally, we obtained records of neurological and electromyogram examination, imaging of brain or spinal cord and blood testing on all subjects. Four of these 40 patients had to be excluded because they were taking antidepressants, steroids or hypnotic drugs. Seven additional patients were excluded because of non-adherence to the sampling method. Thus, the final study population consisted of 29 administered ALS patients (19 males, 10 females; mean age: 51.5 ± 1.8 y). All female patients were postmenopausal. The time from symptom onset to saliva collection ranged from 15 to 64 months (mean: 29.3 ± 12.5 months) and the time elapsed since admission into our ALS clinic ranged from 12 to 164 days (mean: 58.5 ± 65.6 days). Eighteen patients were taking riluzole, which has been proven to slow down ALS progression (Lacom-

blez et al., 1996); 12 patients were taking vitamins, and four patients were taking non-steroidal anti-inflammatory drugs. According to Sofuoglu et al. (2008), riluzole administration, in a dose comparable to that taken by our patients, does not affect plasma cortisol levels when assessed 60, 120, or 180 min later. To date, no effect of any of the other medications taken by our patients (i.e., vitamins or non-steroidal anti-inflammatory drugs) on cortisol secretion has been investigated.

Eighteen age-matched ALS patients' caregivers were initially recruited as control subjects from the same medical center. Three of these caregivers had to be excluded either because of non-adherence to the sampling method (i.e., eating food and brushing teeth during sampling) or because saliva samples were reddish and contaminated with sputum. Three additional control subjects provided too little saliva or withdrew from the experiment before completion of saliva sampling. Thus, the final control population consisted of 12 caregivers (4 males, 8 females; mean age: 54.1 ± 3.7 y). Seven of the female control subjects were postmenopausal. One female control subject was in the perimenopausal status with a delayed and irregular menstrual cycle. All caregivers lived together with their patients in our hospital. Our hospital provides space and instruments for caregivers to care for their patients closely. Therefore, caregivers had the same schedule of daily activities as had ALS patients, including sleep-awakening time, mealtime, and exercise. Caregiver controls were free of medication and did not have any neurological or psychiatric disorder at the time of testing. All participants gave informed consent. The study was approved by the Institutional Review Board of the Wonkwang Gwangju Medical Center.

2.2. Measures

2.2.1. Salivary cortisol collection and assay

Since all ALS patients were hospitalized in our clinic, they had very similar daily schedules for care, including the duration of rehabilitation, mealtime and sleep-awakening schedule. Patients were asked to go to bed before midnight and wake up at 07:00 h. If a patient was not awake at 07:00 h on the sampling day, he or she was awakened by their physician or caregiver and saliva was collected according to a fixed sampling protocol (Wüst et al., 2000). Each patient provided four saliva samples: the first immediately after awakening, the second 15 min, the third 30 min and the fourth 45 min after awakening. Patients were asked to stay in bed for the duration of saliva samplings and refrain from eating and drinking.

Caregiver controls were instructed to keep the same sampling procedures as those described above for the ALS patients. Caregiver controls performed the saliva sampling in the same clinic, and under supervision. They were also instructed to keep the same sleep-awakening schedule (wake up at 07:00 h) for several days before the sampling day and to stay in bed and refrain from eating and drinking until sampling was completed. All ALS patients and caregiver controls recorded the time of going to bed, wake up and collection of the samples. Samples were collected by the research staff the same day. For both patients and controls, saliva sampling was always performed on a weekday.

For each sample, a minimum volume of 2 ml of saliva was collected. Samples were frozen at -80 °C until assay. Free cortisol in saliva samples was determined using a modified radioimmunoassay as previously described (Ahn et al., 2007).

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The intra- and inter-assay variability were below 7% and 8%, respectively. The analytical sensitivity for cortisol was 10 pg/mL.

2.2.2. Evaluation of clinical disability

The clinical status of ALS was evaluated with the Korean version of the ALS Functional Rating Scale-Revised (ALSFRS) (Kim et al., 2007) and the Manual Muscle Test (MMT) by experienced study neurologists. The ALSFRS is a validated rating instrument for monitoring the progression of disability. The ALSFRS, which has the addition of items to assess respiratory symptoms, is an assessment determining the degree of impairment in ALS patients' abilities to function independently in activities of everyday living. It consists of 12 items to evaluate bulbar function, motor function and respiratory function and each item is scored from 0 (unable) to 4 (normal) (Kim et al., 2007). Thus, a higher score reflects a better physical functioning.

Muscle strength is a clinically relevant measure of disease progression in ALS. MMT is the most simple and commonly used procedure for evaluating the strength and function of an individual muscle or muscle group in which the patient voluntarily contracts the muscle against gravity load or manual resistance. It is scored from 0 (none) to 5 (normal) (Cudkovic et al., 2004). We evaluated MMT of the shoulder, elbow, wrist, hip, knee and ankle joints on both sides and the summed MMT score of each patient was used as a measure of impairment in independent mobility. Thus, higher MMT scores reflect better clinical status.

MMT and ALSFRS are assessed periodically in our ALS clinic. Values used for the purpose of this study were assessed 2–3 days before saliva sampling.

2.2.3. Evaluation of depression

The Korean version of the Beck Depression Inventory II (BDI) is a 21-item self-report instrument, one of the most widely used instruments for measuring the severity of depression. When presented with the BDI, subjects were asked to consider each statement as it relates to the way they have felt for the past two weeks. There is a four-point scale for each item, ranging

from 0 to 3 (Sung et al., 2008). Thus, a higher total score reflects a more severe depressive mood status.

The Korean version of the Hamilton Depression Rating Scale (HDRS) is a clinician-administered 17-item multiple-choice questionnaire to rate the severity of depression. The questionnaire is currently one of the most commonly used scales for rating depression in medical research. Nine of the items are scored on a five-point scale, ranging from 0 to 4. The other eight items are scored on a three-point scale, from 0 to 2. A score of zero represents the absence of depressive symptoms (Yi et al., 2005). Thus, a higher total score reflects a more severe depressive mood status.

BDI and HDRS were assessed on the same day as saliva sampling took place.

2.3. Data analysis

Cortisol levels were analyzed with repeated-measures ANOVA with Group as between-subject factor and Time as within-subject factor. Additional analysis used Duncan's multiplier comparison tests to determine the source of the detected significances. In order to obtain indices for the CAR, the global area under the curve (AUC_G) for total cortisol output and the area under the response (or increase) curve (AUC_I) for the responsivity of the system were computed (Pruessner et al., 2003). Pearson correlations were used to examine whether MMT and ALSFRS scores or BDI and HDRS correlated with AUC_G and AUC_I measures of the CAR, respectively. In order to assess the contribution of depression to AUC, we conducted partial correlation analyses with control for disease severity. A $p < 0.05$ was considered statistically significant for all comparisons.

3. Results

3.1. Sleep-awakening schedule and demographics

Wake-up time, i.e., the time of the first saliva sampling, of ALS patients did not differ from that of caregiver controls at

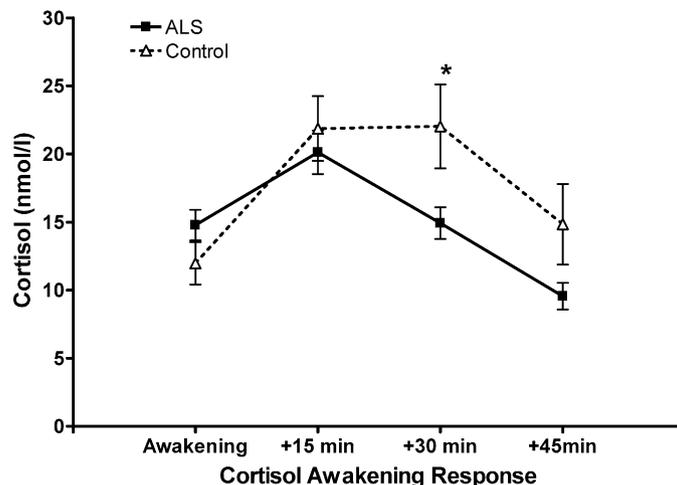


Figure 1 Cortisol awakening response (CAR) of 29 amyotrophic lateral sclerosis (ALS) patients and 12 age-matched caregiver controls. Data represent mean \pm SEM. * $p < 0.05$ indicates a significant difference between ALS patients and caregiver controls at 30 min after awakening.

Table 1 Cortisol awakening response, disease severity and depression scores in male and female ALS patients and caregiver controls.

	ALS patients			Caregiver controls		
	Male (<i>n</i> = 19)	Female (<i>n</i> = 10)	<i>p</i> value	Male (<i>n</i> = 4)	Female (<i>n</i> = 8)	<i>p</i> value
AUCi	158.3 ± 21.2 ^a	173.8 ± 43.1	0.75	303.8 ± 167.3	333.1 ± 63.7	0.88
AUCg	728.9 ± 65.2	646.0 ± 57.2	0.35	847.9 ± 190.5	797.5 ± 106.6	0.83
ALSFRS	28.3 ± 1.3	35.0 ± 2.5	0.03			
MMT	30.2 ± 1.4	32.6 ± 1.9	0.32			
BDI	23.2 ± 2.2	20.7 ± 3.3	0.54			
HDRS	18.9 ± 2.2	17.1 ± 3.0	0.60			

^a Values are presented as mean ± SEM.

the sampling day (ALS patients: 6.5 ± 0.1 h, caregiver controls: 6.6 ± 0.1 h; *p* = 0.42). Sleep duration of the two groups during the night before saliva sampling also did not differ (ALS patients: 7.4 ± 0.2 h, caregiver controls: 7.3 ± 0.1 h; *p* = 0.46). Further, ALS patients and caregiver controls did not differ with respect to gender (Fisher's exact test, *p* = 0.09) or age (ALS patients: 51.5 ± 1.8 y, caregiver controls: 54.1 ± 3.7 y; *p* = 0.49).

3.2. ALS patients show a blunted cortisol awakening response

Fig. 1 shows salivary cortisol levels of ALS patients and caregiver controls after awakening. A repeated-measures ANOVA revealed a significant main effect of Time ($F_{3,37} = 19.89$, *p* < 0.0001), indicative of cortisol rhythmicity, and a significant Time × Group interaction ($F_{3,37} = 4.79$, *p* < 0.0001). At awakening, 15 min and 45 min after awakening, cortisol levels of ALS patients did not differ from those of caregiver controls. At 15 min after awakening, cortisol levels of both groups were significantly higher than those at awakening (*p* < 0.001). However, at 30 min after awakening, cortisol levels of ALS patients were down to awakening levels whereas cortisol levels of caregiver controls were still elevated (*p* < 0.05 vs. awakening). Moreover, cortisol levels of ALS patients were significantly lower than those of caregiver

controls (*p* < 0.05), indicative of a blunted CAR in ALS patients. At 45 min after awakening, cortisol levels of both groups decreased as compared with those at 30 min after awakening (ALS patients: *p* < 0.0001; caregiver controls: *p* < 0.05). However, cortisol levels of ALS patients at 45 min were significantly lower than those at awakening (*p* < 0.0001), whereas cortisol levels of caregiver controls at 45 min did not differ from those at awakening.

Accordingly, the AUCi, reflecting the increase in cortisol levels after awakening, of ALS patients was significantly smaller than that of the caregiver controls (ALS patients: 163.6 ± 19.9; caregiver controls: 323.3 ± 65.5; $t(39) = -2.33$, *p* < 0.05). In contrast, the AUCg for total cortisol output during the first 45 min after awakening did not differ significantly between patients and caregiver controls (ALS patients: 700.3 ± 47.0; caregiver controls: 814.3 ± 90.4; $t(39) = -1.22$, *p* = 0.23). We further analyzed the data by adding gender as a factor in the analysis. Gender alone or the interaction between Gender and Group did not influence either the AUCi or AUCg (see Table 1).

3.3. The cortisol awakening response in ALS patients correlates with clinical status

As is shown in Fig. 2, the AUCi for the cortisol response after awakening correlated significantly with both the MMT and ALSFRS measures of disease severity (MMT: *r* = 0.525;

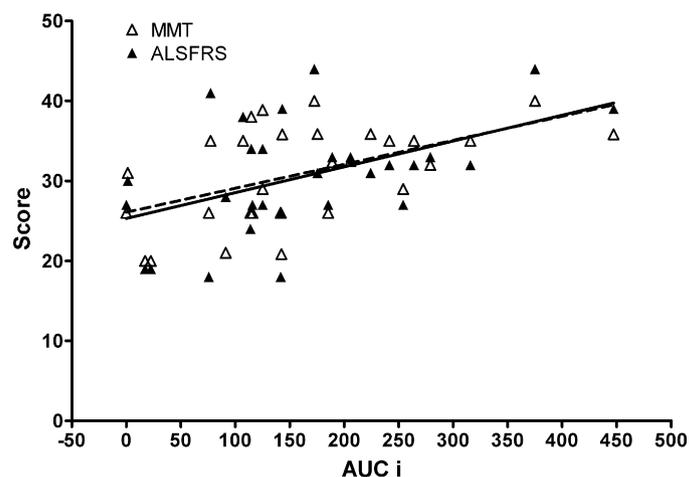


Figure 2 Scatter plot on the association between the area under the response (or increase) curve (AUCi) and Manual Muscle Test (MMT) or Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) in 29 patients affected by amyotrophic lateral sclerosis (MMT; *r* = 0.525; *p* = 0.003, ALSFRS; *r* = 0.484; *p* = 0.007).

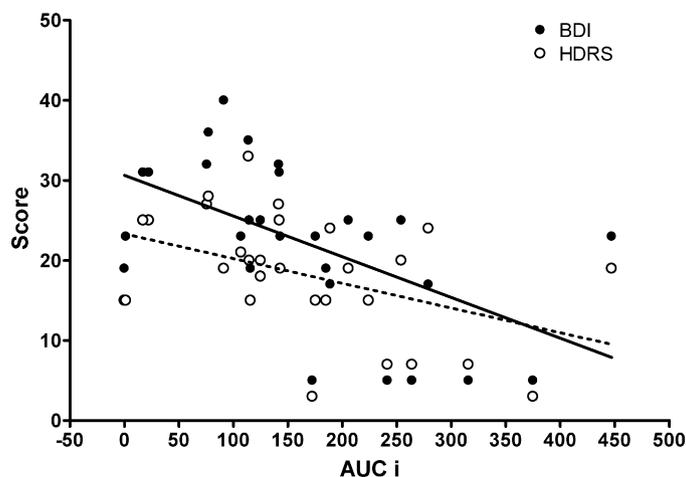


Figure 3 Scatter plot on the association between the area under the response (or increase) curve (AUC_i) and Beck Depression Inventory (BDI) or Hamilton Depression Rating Scale (HDRS) in 29 patients affected by amyotrophic lateral sclerosis (BDI; $r = -0.555$; $p = 0.002$, HDRS; $r = -0.439$; $p = 0.02$).

$p = 0.003$, ALSFRS: $r = 0.484$; $p = 0.007$), indicating that lower MMT or ALSFRS scores (i.e., higher disease severity) were associated with smaller AUC_i values. Addition of the factor age as a co-variate into the analysis did not significantly alter the results. The AUC_g, reflecting total cortisol output during the first 45 min after awakening, did not correlate significantly with either MMT or ALSFRS measures of disease severity (MMT: $r = 0.233$; $p = 0.22$, ALSFRS: $r = -0.099$; $p = 0.61$) (data not shown). MMT scores did not differ between male and female ALS patients, but the mean ALSFRS score of male patients was slightly, but significantly, lower (i.e., poorer clinical status) than that of female patients ($p = 0.03$) (Table 1).

3.4. The cortisol awakening response in ALS patients correlates with depressive status

As is shown in Fig. 3, the AUC_i for the cortisol response after awakening also correlated significantly with both the BDI and HDRS measures of depressive mood status (BDI: $r = -0.555$; $p = 0.002$, HDRS: $r = -0.439$; $p = 0.02$), indicating that smaller AUC_i values were associated with higher BDI or HDRS scores (i.e., higher depressive status). After controlling for disease severity, BDI scores remained significantly associated with the magnitude of AUC_i, whereas HDRS scores did not (Table 2). Addition of the factor age as a co-variate into the analysis did not significantly alter the results. The AUC_g did not significantly correlate with either the BDI or HDRS measures of depressive mood status (BDI: $r = -0.318$; $p = 0.09$,

HDRS: $r = -0.252$; $p = 0.19$) (data not shown). BDI and HDRS scores did not differ between male and female ALS patients (Table 1).

4. Discussion

The main finding of this study is that ALS patients show a blunted CAR as compared to a carefully selected group of caregiver controls living in the same hospital environment. Moreover, a smaller CAR in ALS patients was associated with poorer clinical status (as assessed with both the ALSFRS and MMT rating instruments) as well as a more severe depressive mood status (as assessed with the BDI and HDRS rating instruments). In contrast, overall cortisol output during the first 45 min after awakening did not correlate with clinical or depressive mood status, nor differed between patients and controls. Although the ALSFRS score of male ALS patients was slightly, but significantly, lower than that of female patients, no significant differences were found in the magnitude of the CAR, total cortisol output or depression scores between male and female patients.

Thus, these findings suggest a reduced responsiveness of the HPA-axis in ALS. Only one prior study investigated adrenal activity in ALS (Patacchioli et al., 2003). This study analyzed morning (08:00 h) and evening cortisol levels (20:00 h) from ALS patients and controls. The authors reported a loss of circadian rhythm of cortisol levels in ALS due to a reduced decline of cortisol levels in the evening, but did not find any differences in morning cortisol levels between the two groups. It is possible that differences in sampling method could, at least in part, explain the different findings. As these authors did not provide any information on sleep-awakening schedule or the duration from awakening to sampling time, morning cortisol levels measured in that study could represent any of the samples during the morning cortisol response.

The CAR is considered to be a reliable indicator of HPA-axis function and has been studied extensively, not only in healthy populations, but also in relation to many disorders (for review see Wüst et al., 2000). For example, a blunted CAR has been found in chronically ill patients (Kudielka and Kirschbaum,

Table 2 Correlation between cortisol awakening response and depression after controlling for disease severity.

	Predicting AUC _i	
	BDI	HDRS
Control for MMT	$r = -0.358$ $p = 0.06$	$r = -0.216$ $p = 0.27$
Control for ALSFRS	$r = -0.415$ $p = 0.03$	$r = -0.254$ $p = 0.19$

2003), patients with severe global amnesia (Wolf et al., 2005), patients with post-traumatic stress disorder (Wessa et al., 2006) or those with chronic fatigue syndrome (Roberts et al., 2004). However, other studies described an elevated CAR in patients with upper respiratory illness (Edwards et al., 2003) or in women with borderline personality disorder (Lieb et al., 2004). Thus, although there appears to be some discrepancy in the findings, generally the findings suggest that a blunted CAR in ALS patients may be a consequence of the emotional or physical distress and associated symptoms of chronic fatigue and depression. ALS patients suffer from enormous emotional challenges with progressive erosion of physical ability, profound changes in social and professional roles and basic functions such as eating and conversing, and often financial insecurity. Although ALS is most often associated with relentless physical deterioration, and providing relief for this is important, emotional suffering, especially depression, is also strongly associated with ALS and psychological adjustment appears to be an important prognostic factor for ALS (for review see McLeod and Clarke, 2007). In the present study, we showed that after controlling for disease disability, i.e., MMT and ALSFRS, depressive mood status remained significantly associated with AUCi. Thus, our findings are consistent with the view that the blunted CAR in ALS patients was associated with both disease disability and depressive mood status.

In addition to the emotional consequences of ALS, the physical changes associated with ALS might have contributed to the altered CAR. ALS is characterized by alterations in energy metabolism (Desport et al., 2005; Vaisman et al., 2009), a decrease in muscle mass without prominent changes in the viscera (Vaisman et al., 2009), and limited ambulation due to impairments in motor function. Overall, these changes suggest reduced energy demands in ALS patients. As some evidence suggests that increasing and decreasing energy demands of the brain can induce corresponding changes in early morning rises in HPA-axis activity (Benedict et al., 2009), one might argue that a reduced energy metabolism in ALS patients could be one of the factors contributing to a blunted CAR response.

Although the pathogenesis of ALS is incompletely understood, some explanations have been suggested, including mitochondrial dysfunction (Curti et al., 1996), structural defects in the superoxide dismutase (Deng et al., 1993), selective loss in neuronal glutamate transport (Rothstein et al., 1995), autoimmunity (Appel et al., 1993), involvement of the immune system in spinal cord and motor cortex (Engelhardt and Appel, 1990) and alteration in energy metabolism (Zhang et al., 2005). In addition, several studies have suggested non-motor system-related symptoms such as cognitive impairment with behavioral changes (for review see Phukan et al., 2007). As cortisol is widely related to energy metabolism, immune function, cognition and neuronal degeneration, the present findings of a reduced responsivity of the HPA-axis in ALS might provide interesting and novel information toward a better understanding of these various pathological and symptomatic characteristics. Some studies suggest that HPA-axis dysfunction might directly regulate neurodegenerative disease progress, including ALS. Due to neuronal damage, a proliferation of glial fibrillary acidic protein (GFAP)-positive astrocytes could occur, leading to the formation of a glial scar in the central nervous system (De

Nicola et al., 1998). Experimental findings in an animal model of neurodegenerative disease, characterized by severe motor neuron degeneration, indicated that glucocorticoids suppress GFAP expression in spinal cord (De Nicola et al., 1998). However, at present it is not known whether a blunted CAR alone (i.e., without affecting total 24-h cortisol production), is sufficient to influence ALS disease progression.

We selected caregivers as a control group in order to ensure that sleep-awakening schedule and daily activities are closely matched to that of the ALS patients. Several studies that investigated demographic and health-related influences on the CAR indicated that sleep-related factors, including waking time, sleep duration, and sleep quality can potentially alter the CAR (for review see Fries et al., 2009). Therefore, with a control group not recruited from hospitalization, it might be very difficult to interpret the effects observed. Some studies investigating the CAR in patient populations have used control groups consisting of patients with another, but related disorder (Huber et al., 2006). However, for our ALS study it would be difficult to find such an appropriate neurodegenerative disease control group.

Limitations of this study are its cross-sectional nature and the small number of caregiver controls. Another limitation, despite its reported high intra-individual stability (Hucklebridge et al., 2005), is the assessment of the CAR based on a single day of salivary cortisol collections. In addition, although possible effects of the vitamins and non-steroidal anti-inflammatory drugs on cortisol levels have not been investigated, their influence, if any, on the CAR would be minor, as patients took the low dose of vitamin E or acetylsalicylic acid after breakfast.

In sum, the findings of the present study show a blunted CAR in ALS patients. The magnitude of the CAR was significantly associated with clinical severity and depressive mood. Future studies are needed to investigate the neurological or psychological mechanisms underlying this alteration in HPA responsivity in ALS patients.

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Conflict of interest

There are no conflicts of interest.

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