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ABSTRACT

Background: An adverse psychological environment (e.g. stressful events or depression) has been shown to influence basal cortisol levels and cortisol response to stress. This differs depending on the adverse stimuli, but also varies across individuals and may be influenced by genetic predisposition. An insertion/deletion polymorphism in the serotonin transporter gene (*5-HTTLPR*) is a strong candidate in this regard.

Objective: To investigate how stressful life events and depression are associated with diurnal cortisol levels in community-dwelling elderly and determine whether this varies according to genetic variability in the *5-HTTLPR*.

Methods: This population-based study included 334 subjects aged 65 and older (mean (SD) = 76.5 (6.3)). Diurnal cortisol was measured on two separate days, under quiet (basal) and stressful conditions. The number of recent major stressful events experienced during the past year was assessed from a 12-item validated questionnaire as an index of cumulative recent stressful events. Lifetime trauma was evaluated using the validated Watson's PTSD inventory, which evaluates the most severe traumatic or frightening experience according to DSM criteria. Depression was defined as having a Mini-International Neuropsychiatric Interview (MINI) diagnosis of current major depressive disorder or high levels of depressive symptoms (Center for Epidemiologic Studies-Depression Scale ≥16). *5-HTTLPR* genotyping was performed on blood samples.

Results: Exposure to stressful life events was associated with lower basal evening cortisol levels overall, and in the participants with the *5-HTTLPR L* allele but not the *SS* genotype. The greatest effects (over 50% decrease, $p<0.001$) were observed for the *LL* participants having experienced multiple recent stressful events or severe lifetime traumas. Participants with the *L* allele also had higher evening cortisol stress response. Conversely, depression tended to be associated with a 42% higher basal morning cortisol in the *SS* participants specifically, but did not modify the association between stressful events and cortisol levels.

Conclusion: An adverse psychological environment is associated with basal cortisol levels and cortisol stress response, but this differs according to *5-HTTLPR* genotype.

Keywords: Stress response; adverse events; trauma; depression; hypothalamic-pituitary-adrenal axis; serotonin transporter-linked promoter region.

1. Introduction

One of the most consistent findings in the biology of psychiatric disorders is altered activity of the corticotropic hypothalamic-pituitary-adrenal (HPA) axis, but this varies according to the disorder and across individuals (Lupien et al., 2009). Major depressive disorder has often been associated with enhanced cortisol release in concert with a reduced feedback sensitivity of the HPA axis (Belvederi Murri et al., 2014; Stetler and Miller, 2011), while an opposing picture has been described in post-traumatic stress disorder (PTSD) or trauma-exposed individuals (Morris et al., 2012). Cortisol secretion can be influenced by a number of factors, *e.g.* sampling conditions (time of sampling, basal vs. stress condition, experimental stressor vs. naturalistic protocol), as well as other moderators such as age, sex, comorbidity, and genetic sensitivity to environmental stress (Belvederi Murri et al., 2014; Franz et al., 2010; Kudielka et al., 2012; Miller et al., 2013).

A genetic variant within the promoter region of the serotonin transporter (*5-HTTLPR*) has been associated with differential psychological sensitivity to stressful experiences. The short (*S*) allele of the *5-HTTLPR* is associated with lower expression and decreased activity of the serotonin transporter and could modulate both risk for depression and psychopathological HPA axis signaling. Two recent meta-analyses reported a robust link between the short (*S*) form, reporting lifetime stress experience, and resulting depression (Sharpley et al., 2014), but no significant association between *5-HTTLPR* and PTSD when compared to trauma-exposed controls (Navarro-Mateu et al., 2013). The influences of serotonergic neurotransmission on HPA-axis regulation and the link between serotonergic signaling and *5-HTTLPR* genotype and HPA axis functioning have been largely documented from both human and animal studies (Andrews and Matthews, 2004; Fuller, 1990; Li et al., 1999; Porter et al., 2004; Vazquez et al., 2012). Several experimental studies have investigated the association between *5-HTTLPR* genotype and HPA-axis reactivity to standardized laboratory stress tasks in healthy individuals with mixed results. A recent meta-analysis of 11 studies reported a weak but significant association between the homozygous *SS* genotype and enhanced cortisol reactivity to laboratory-

imposed stressor (Miller et al., 2013), although the unique study in older adults included in this meta-analysis reported an inverse association (Mueller et al., 2011). Studies outside the laboratory are more limited and inconsistent and may depend on population age and moderation by psychopathological environment, *e.g.* life experiences and depressive symptomatology. They have been performed with populations at elevated risk for depression and adversity (self-rated). Two studies did not observe a moderating effect of *5-HTTLPR* genotype on the association between psychosocial adversity and morning cortisol levels in adolescents (Goodyer et al., 2009) or between recent stressful life events and awakening or evening cortisol levels in middle-aged adults (Vinberg et al., 2010). However, when considering cumulative risk exposure, a moderate association was found with cortisol area under the curve (AUC), moderated by *5-HTTLPR* genotype in youth at elevated family risk for depression (Willner et al., 2014). Among *LL* youth, greater cumulative risk was associated with a lower AUC, whereas among those carrying the short allele, there was a trend for an inverse association.

So far, no study has examined whether cortisol levels in response to various adverse psychological environment (real-life stress, trauma or depression), could differ according to *5-HTTLPR* vulnerability. This could be particularly critical in older people, given that they have an accumulation of lifetime stressful events and show specific depression symptoms and etiologic factors as well as age-associated neurobiological changes (Fiske et al., 2009). With increasing age, the HPA axis might also become more vulnerable to dysregulation (Belvederi Murri et al., 2014; Otte et al., 2005). Age has been frequently evoked as a potential source of inconsistent findings for the associations between *5-HTTLPR* and depression (Uher and McGuffin, 2008) and PTSD (Navarro-Mateu et al., 2013). Further, unlike in younger populations where the *S* allele is a risk factor, the *LL* genotype appears a risk factor for mental and physical distress in elderly people highly exposed to chronic disorders and severe stressors (Grabe et al., 2011) as well as increased cortisol reactivity to laboratory stress tasks (Mueller et al., 2011).

The aim of this study was to examine diurnal cortisol levels in community-dwelling elderly under basal and stressful naturalistic conditions, in response to an adverse psychological environment (stressful life events including recent and major life traumas, and late-life depression), and to determine whether this varies according to *5-HTTLPR* genotype.

2. Subjects and Methods

2.1. Participants

The data were derived from a longitudinal study of neuropsychiatric disorders in community-dwelling French elderly, the Esprit study (Ritchie et al., 2004). Eligible participants, who were at least 65 years of age and non-institutionalized, were recruited by random selection from the electoral rolls between 1999 and 2001. Ethics approval for the study was given by the national ethics committee and written informed consent was obtained from all participants. The study was based on a random sample of 360 non-demented participants who had a complete set of salivary cortisol samples, collected at 4 time points, under both quiet and stressful conditions (see below), with a typical eucortisolemic pattern (including decline throughout the day), and were not being treated with medications likely to modify cortisol levels. Of these, 19 participants were missing *5-HTTLPR* genotyping data, 1 did not have a depression assessment, and 6 failed to provide information on recent stressful events (RSE). This left 334 participants in the analyses (mean (SD) age = 76.5 (6.3) years). Compared to the overall Esprit sample (n=1855), the participants included in the present analysis were younger, less frequently women and less likely to have depression, cognitive impairment, and cardiovascular ischemic pathologies ($p<0.003$). They did not differ however regarding other characteristics, particularly recent stressful events (RSE, $p=0.14$) and lifetime traumas ($p=0.27$).

2.2. Cortisol measurement

Salivary cortisol was measured over the course of the day in participants who were not being treated with medication likely to modify cortisol levels (*e.g.* glucocorticoids, benzodiazepines, and hormonal treatment for women), as described previously (Beluche et al., 2009). Saliva samples were not collected from participants with dementia. Participants were instructed not to drink, eat or smoke for at least 30 min before saliva collection. As cortisol levels increase shortly after awakening and to avoid potential interference with sleep-wake transition (Kudielka et al., 2012), participants were asked to collect the first sample in the morning, but that it needed to be 1 hour or more after awakening (morning cortisol). Subsequent samples were then collected 3 (midday cortisol), 7 (afternoon cortisol), and 14 hours (evening cortisol) after the first morning sample (the last sample being collected before midnight to eliminate early cortisol increase occurring during the nocturnal phase). To maximize compliance, the participants were first shown by the lay interviewer at the examination center how to perform salivary collection and were instructed to carefully write down the exact times when collections were made in a diary to be returned with the samples. Furthermore, as in other naturalistic studies, participants were allowed to decide their own wake and sleep times. Participants were encouraged to carry on their normal daily activities with limited physical exertion in order to maximize ecological validity. Samples were taken under two contrasting conditions; at the hospital ("stressful situation"), with the first sampling at their arrival at the examination center, just prior to a lengthy clinical examination which involved various recognized psychosocial stressors (*e.g.* psychiatric examination, cognitive testing, clinical evaluation, and blood collection) and a subsequent quiet day at home with normal daily activities (baseline condition) (Belvederi Murri et al., 2014; Franz et al., 2010). The participants were aware of the duration and global content of the examination. Maximal contrasting conditions were thus obtained, while avoiding novelty or anticipatory effects on the baseline measures. None of the participants reported any additional stressors on the days they performed sampling. The stress versus basal conditions were associated with a highly significant increase in cortisol levels ($p<0.0001$), as published previously (Ancelin et al., 2013a; Beluche et al., 2009; Chaudieu et al., 2008). Cortisol levels were determined from saliva collection (Hellhammer et al., 1987) by direct

radioimmunoassay (Diagnostic Systems Laboratories-Webster, Texas). Intra-assay as well as inter-assay coefficients of variation averaged 5%. Individuals with a typical eucortisolemic pattern are those displaying cortisol decline throughout the day, thus with a negative slope of the regression of the four log-transformed cortisol values on the sampling time. The individuals with missing data and those who did not show a negative slope were excluded as previously reported (Lupien, 2013). They did not differ significantly regarding main characteristics from those retained in the study.

2.3. Adverse psychological environment

Exposure to recent stressful events (RSE) during the past year was assessed using the Gospel Oak questionnaire (Harwood et al., 1998). This 12-item list of major life events included bereavement, rupture of close relationships, severe illness and serious financial or judicial problems. The number of stressful events experienced during the past year was used as an index of cumulative recent stressful events. Severe lifetime traumatic events and symptom severity were assessed using the validated Watson's PTSD Inventory which evaluates the most severe lifetime traumatic event or frightening experience according to DSM criteria (Watson et al., 1991). This questionnaire can provide measures of the severity of the disorder for every symptom and allow the measurement of subsyndromic PTSD as described previously (Chaudieu et al., 2011).

The diagnosis of lifetime major depression and anxiety disorder was made by psychologists and psychiatric nurses according to DSM-IV criteria and using the Mini-International Neuropsychiatric Interview (MINI, French version 5.00), a standardized psychiatric examination validated in the general population (Sheehan et al., 1998) and with high inter-rater reliability (Lecrubier et al., 1997). The interviewers were initially trained for a 3 month period under the supervision of psychiatrists from the Department of Adult Psychiatry at Montpellier University Hospital and interviewer drift was minimized. The positive cases were reviewed by a panel of independent psychiatrists as described previously (Ancelin et al., 2010; Ritchie et al., 2013). The Center for Epidemiologic Studies-

Depression Scale (CES-D), validated in the elderly, was used to evaluate current depressive symptomatology (Radloff, 1977).

In older adults, late-life depression covers a range of mild to severe depressive symptoms which does not always correspond to the DSM criteria for major depression, although it has devastating consequences on physical and social functioning, disability, and mortality (Fiske et al., 2009).To adequately capture this construct, we based our late-life depression assessment on two criteria. Participants with either a MINI diagnosis of current major depression or high levels of depressive symptomatology (CES-D>16) were defined as having a clinical level of late-life depression (Dep), i.e. levels of psychopathology which would warrant clinical intervention (Ancelin et al., 2010).

2.4. 5-HTTLPR genotyping

Blood samples were collected after the baseline clinical interview, enabling DNA extraction and *5-HTTLPR* genotyping as described previously (Ritchie et al., 2009). To verify the accuracy of the data, replicate independent genotyping was also performed using DNA extracted from buccal samples as described previously (Ancelin et al., 2013a).

2.5. Socio-demographic and clinical variables

The standardized interview included information on socio-demographic characteristics, physical health, and medical history of the participants. Weight and height were measured during clinical examination and body mass index (BMI) was calculated and expressed as kg/m^2. Detailed medical questionnaires (with additional information from general practitioners) were used to obtain information on history of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, and arteritis). Global cognitive function was evaluated using the Mini-Mental State Examination (MMSE), a score <26 indicating cognitive impairment (Folstein et al., 1975). Dementia was diagnosed by a neurologist as part of a standardized examination and validated by a

panel of independent neurologists, as described previously (Ancelin et al., 2013b).

2.6. Statistical Analysis

All data were cleaned following standard procedures. Due to the exponential distribution of raw diurnal cortisol data, cortisol values were log-transformed. Given the non-fixed time sampling protocol, cortisol levels were calculated at fixed times from the regression of the four-cortisol values on the sampling times, for each participant and on two different days (basal and stressful situation). Stress response (Δ) was calculated for each participant and for all the four samples across the day as the ratio of (stress cortisol level – basal cortisol level)/ basal cortisol level; and expressed as %, as published previously (Beluche et al., 2009; Chaudieu et al., 2008). Chi-squared tests were used to compare the distribution of *5-HTTLPR* genotypes with those predicted under the Hardy-Weinberg equilibrium.

The primary analyses concerned the average cortisol concentrations at the four times of the day (morning, midday, afternoon and evening) on basal conditions and then under stress condition (expressed as stress response). Subsequent multivariate analyses focused on morning and evening times which constitute very common HPA axis indicators and the most contrasting conditions of the diurnal cycle (at which cortisol levels reached the daily zenith and nadir, respectively), and displaying distinct characteristics. Morning cortisol levels show a much higher heritability whereas evening cortisol has a greater environmental influence and could also be differently influenced by depression and stressful life events (Belvederi Murri et al., 2014; Franz et al., 2010; Miller et al., 2007; Morris et al., 2012). Bivariate associations between cortisol levels and exposure to stressful events (RSE or severe trauma) or Dep were evaluated using ANCOVA adjusted for age and sex. Multivariate models were then generated combining the different adverse environmental factors (stressful events and Dep), to determine the adjusted associations. Further adjustment was made for other covariates known to influence cortisol, based on prior studies, notably BMI (Incollingo Rodriguez et al., 2015), a history of

major depression (Beluche et al., 2009), current phobia and generalized anxiety disorder (Chaudieu et al., 2008). SAS (v9.4, SAS Institute, Inc., North Carolina) was used for the statistical analyses with a significance level of $p<0.05$.

3. Results

3.1. Participant characteristics

Baseline characteristics of the 334 participants are summarized in **Table 1**. Forty percent reported at least one RSE in the last year, 57% experienced severe traumatic events during their life, and 14.4% currently had Dep of whom 4.2% had major depression. One quarter of the participants were homozygous carriers of the *S* allele and 26.3% were *LL*. The 5-*HTTLPR* genotype frequency did not significantly deviate from Hardy-Weinberg equilibrium ($p=0.75$). The socio-demographic and clinical characteristics of *SS*, *SL*, and *LL* participants did not differ significantly ($p\geq0.25$).

3.2. Cortisol levels according to recent stressful events

Fig. 1 shows the diurnal patterns of basal cortisol levels (adjusted for age and sex) as a function of RSE among all participants and according to the *5-HTTLPR* genotype. Overall and for the participants with the *SL* and *LL* genotype, a difference in cortisol levels was observed in the afternoon and evening between the participants reporting RSE and those who did not (see **Supplementary Table S1** for full details). Lower cortisol levels were seen with a higher number of RSE. Conversely, for the *SS* participants, no significant differences were found at any time.

In a similar manner to the basal cortisol levels, RSE were not associated with morning stress response, irrespective of the *5-HTTLPR* genotype whereas the evening stress response ($\Delta_{evening}$) appeared to significantly increase with RSE in *LL* ($p=0.005$) and *SL* ($p=0.007$) participants, but not for those with the *SS* ($p=0.43$) genotype (**Supplementary Fig. S1**). The highest $\Delta_{evening}$ (37.8%) was observed for the *LL* participants having reported at least two RSE, compared with 3.3% in the *LL*

participants having reported no RSE in the last year.

3.3. Cortisol levels according to lifetime trauma and re-experiencing symptoms

Data on lifetime traumatic events were also available for 288 participants of whom 57% reported a trauma according to DSM IV criteria, at a median age of 24 years (ranging from 5–82 years). Of these traumatized participants, 30.5% had reported re-experiencing symptoms, the most common and clinically relevant symptom associated with trauma in PTSD (subclinical PTSD). In a model adjusted for age and sex, significant associations were only found for evening time; cortisol levels were 45.1% lower in the *LL* participants with re-experiencing symptoms compared to non-traumatized *LL* participants (p=0.015) whereas no significant differences were observed for the *SL* and *SS* participants (see Supplementary Table S1). When further adjusting for RSE, the same pattern was observed, evening cortisol levels being 47.8% lower specifically in the *LL* participants with re-experiencing symptoms compared to non-traumatized *LL* participants (p=0.006) (**Supplementary Figure S2**). The evening stress response was also significantly higher for the *LL* participants having reported re-experiencing symptoms ($\Delta_{evening}$=36.9%) compared to 14.6% in non-traumatized participants (p=0.019) whereas no significant differences were found for morning stress response (**Supplementary Fig. S3**).

3.4. Cortisol levels according to Dep

Fig. 2 shows the diurnal pattern of basal cortisol levels (adjusted for age and sex) as a function of Dep in the overall sample and according to 5-*HTTLPR* genotype. Cortisol levels across the day were not significantly different between the depressed and non-depressed participants in the overall sample (p>0.15) and in the *SL* group (p>0.72). For the *SS* participants, morning cortisol levels were 40.6% higher (non-log transformed scale) in the depressed compared with the non-depressed participants

(p=0.059) (see Supplementary Table S1). For the *LL* participants, evening cortisol levels specifically were 46.4% lower (p=0.011) in depressed compared to non-depressed participants. Further adjustment for RSE (**Fig. 3**) had very little effect on the association between Dep and morning cortisol levels in *SS* participants (42.3% higher, p=0.05) as well as further adjusting for past major depression (p=0.047). However, among *LL* participants, there was no longer an association between Dep and evening cortisol levels after inclusion of RSE (p=0.13).

In this model, Dep was not associated with cortisol stress response irrespective of the *5-HTTLPR* genotype (**Supplementary Table S1 and Fig. S4**). Hence, despite higher basal morning cortisol levels, the depressed *SS* participants largely maintained their capacity to further increase cortisol levels under stress conditions, in a similar manner to the non-depressed *SS* participants.

3.5. The influence of RSE and lifetime trauma on cortisol levels, accounting for Dep

We next sought to determine whether accounting for Dep, modified the associations between RSE or lifetime trauma and cortisol. Further adjusting for Dep in the analyses described in § 3.2, did not modify the pattern of associations for cortisol basal levels and stress response regardless of the time (see **Supplementary Table S1**). More particularly, in the *LL* participants, evening basal cortisol levels were 28.1% (non-log transformed scale) and 54.2%, lower for those reporting one and at least two RSE respectively, compared to those who did not (p=0.0097) (**Fig. 4B**). For participants with the *SL* genotype evening cortisol levels were also significantly lower (p=0.0005), by 35.2% and 53.3% respectively, whereas no significant difference was observed for the *SS* participants (p=0.76). The same data were also found in the most complete multivariable model adjusted for age, sex, Dep and lifetime trauma (see **Supplementary Table S1**).

The results with lifetime trauma (cf. § 3.3) were also unchanged after further adjusting for Dep; *LL* participants with re-experiencing compared to those without had 51% lower evening cortisol levels specifically ($p \leq 0.006$), as well as higher evening stress response (p=0.02), irrespective of whether RSE

was in the model (see **Supplementary Table S1**). Further adjusting for BMI, a history of major depression, or anxiety disorder did not change these patterns (data not shown).

4. Discussion

To our knowledge, this is the first study to investigate the impact of different adverse psychological environments (recent stressful events, major life trauma, and Dep) on diurnal cortisol levels as a function of *5-HTTLPR* genotype. These results suggest that stressful events and Dep can independently influence cortisol levels and the cortisol stress response, even when accounting for other factors such as comorbidity, however the associations vary according to genotype. While the *SS* genotype tended to be associated with higher morning cortisol levels in depressed participants, a lower evening cortisol was observed in the participants with *LL* and *SL* genotypes having being exposed to stressful environment, independently of Dep. The strongest associations were found for the homozygous *LL* participants having reported multiple RSE or who experienced severe lifetime trauma. The greatest difference in cortisol levels was seen for elderly individuals reporting both multiple RSE and Dep, compared with non-depressed individuals with no RSE (*LL* had 80% lower evening cortisol (non-log transformed scale), $p<0.0001$; *SS* had 126% higher morning cortisol, $p=0.003$). However the low number of participants in this subgroup precluded drawing definite conclusions.

These data provide further support for the complex interplay between HPA axis functioning and serotonergic signaling with possible modulation according to adverse psychological environment, *e.g.* depression or trauma. In line with this, a large meta-analysis of case-control studies have reported heightened basal cortisol levels throughout the diurnal cycle in depressed patients compared to healthy controls (Belvederi Murri et al., 2014). Conversely, lowered evening cortisol levels, but not morning levels, have consistently been reported in trauma-exposed individuals (see for meta-analysis (Morris et al., 2012)). Hence, in depressed people, morning cortisol levels specifically appeared to be increased,

and independently of trauma, whereas for evening cortisol this may vary according to lifetime traumatic experience (Morris et al., 2012). Our findings thus concur with these meta-analyses and add novel information regarding moderation by *5-HTTLPR* vulnerability. Heightened morning basal cortisol levels being found only in depressed *SS* participants, while evening cortisol levels were specifically lowered, and in a dose (RSE number)-dependent manner, in trauma-exposed participants with the *L* allele. For the *SL* participants the effects were only significant with recent stressful events, whereas for the *LL* homozygous both recent and distant past traumatic events (subclinical PTSD) were associated with lower evening cortisol levels and higher stress response. Our data suggests that the *LL* elderly participants could be vulnerable not only to the short-term but also lasting consequences of stressful events on neuroendocrine function, and possibly to the subsequent development of chronic PTSD symptoms. A recent meta-analysis did not support a direct effect of *5-HTTLPR* polymorphisms on PTSD, but the authors reported that several characteristics, *e.g.* stressor, comorbidity, and population characteristics, especially age and ethnicity could moderate the associations (Navarro-Mateu et al., 2013). Consistently, Grabe et al. reported that in community-dwelling older European/ Caucasian people, the risk of lifetime PTSD increased with the number of *L* alleles as well as the number of traumatic events (Grabe et al., 2009). The same group also reported a switch from the *S* allele to the *LL* genotype for the risk of mental and physical distress in older adults according to the number of chronic diseases (Grabe et al., 2011).

The regulation of the HPA axis is complex and influenced by multiple factors, *e.g.* exposure to different stressors, person-dependent factors, and heritable factors. Twin studies show a much higher heritability of morning cortisol than evening cortisol levels (60% *vs.* 8%), with the latter having a greater environmental influence (Franz et al., 2010). Our data also support a greater effect of stressful environment on evening cortisol levels as well as stress response. Further, they suggest that increased morning cortisol in the *SS* depressed participants may be under genetic control. HPA axis functioning and cortisol levels could be influenced by various genes involved in corticosteroid signaling.

Specifically, the corticosteroid receptors have been involved in a large range of neurobiological correlates that underlie depression, *e.g.* HPA axis hyperactivity, glucocorticoid resistance, and changes in neural plasticity and neurogenesis (Anacker et al., 2011). Antidepressants have been shown to impact all of these mechanisms and to modulate receptor function, providing further support that these receptors may play a pivotal role in the neurobiological disturbances that contribute to depression (Anacker et al., 2011).

Cortisol hypersecretion is thought to characterize the short-term effect of stress with initial activation of the HPA axis, whereas hypo-secretion may develop in the long-term (Miller et al., 2007; Morris et al., 2012) and has been associated with experiencing a high degree of chronic stress (Bremmer et al., 2007). Our findings suggest that in the general elderly population, cortisol hypersecretion may be a reflection of current psychological load (depression), whereas hyposecretion could reflect early as well as recent exposure to stressful events. The exact time when RSE occurred during the past year was not known and we cannot ascertain whether this could reflect short-lasting attenuated HPA axis activity in the context of high cumulative risk or leading to a more persistent stress effect. Furthermore, we demonstrate that this is dependent on *5-HTTLPR* genotype. A moderate association between cumulative risk exposure and cortisol AUC, moderated by *5-HTTLPR* genotype was previously reported in 138 multiethnic youth at elevated family risk for depression (Willner et al., 2014). Among *LL* youth, greater cumulative risk was associated with a lower AUC, whereas among those carrying the short allele, there was a trend for an inverse association. Our study extends this finding to elderly general population and further suggests that early trauma could have a long lasting effect on evening cortisol in *LL* individuals, specifically and independently of Dep.

Limitations to our study include the bias introduced from selecting community-dwelling participants who were in relatively good physical and mental health which may have decreased the overall power of the study, possibly underestimating the associations found. Bias from population

stratification needs to be considered. However, prior genotyping data of these participants indicated that less than 1% were non-Caucasian (Ancelin et al., 2013a) and genotype frequency was similar with that already published in white Europeans (Miller et al., 2013). Although this study in the elderly population was limited to only one day on two conditions but with four salivary cortisol measures, and despite exclusion of the participants with missing data and an atypical pattern, the basal characteristics of cortisol are similar to previous studies in older adults with more frequent sampling (Ice et al., 2004). Data related to life events were retrospective, which may introduce recall bias, but participants diagnosed with probable/possible dementia were excluded. Finally, since multiple analyses have been performed we cannot exclude that some associations were due to chance. However, many of the associations between stressful life events and participants with the *L* allele remained significant even after applying overly conservative multiple testing correction. On the other hand, further studies are needed to replicate our findings with depressive symptomatology which may be underpowered due to the small number of Dep cases.

Strengths are that it was the first population-based study involving 334 elderly people having complete data on *5-HTTLPR* and diurnal cortisol levels under basal and stressful conditions. Cortisol levels were measured under naturalistic conditions and using a non-fixed time-sampling protocol (Belvederi Murri et al., 2014; Franz et al., 2010), known to improve compliance in the elderly (Jacobs et al., 2005; Kraemer et al., 2006), and compliance rates were excellent, with the systematic return of saliva samples by all the participants. Thus cortisol measures most likely reflect their "normal/everyday" levels previously reported in the elderly (Ice et al., 2004). Dep was assessed by trained staff using two distinct measures validated in the general population, including a structured diagnostic interview (Radloff, 1977; Sheehan et al., 1998) according to DSM-IV criteria. RSE and lifetime traumatisms (DSM criteria) were evaluated using validated questionnaires. We controlled for important stress-related covariates thus minimizing any confounding or moderating effect, which contrasts with previous studies which have predominantly presented minimally adjusted analyses.

Finally, we were able to control for accuracy through duplicate samples collected at different time and with independent genotyping.

In conclusion, this study is the first to provide a thorough investigation of the extent to which different adverse psychological environments can influence diurnal cortisol levels and cortisol stress response, and how this varies according to the environmental sensitivity of *5-HTTLPR* polymorphism. We have shown that older adults can display a differential pattern of HPA dysregulation according to their psychopathological characteristics and *5-HTTLPR* genetic vulnerability. Stressful environment was associated with significantly lower basal evening cortisol levels and heightened stress response in *L* allele carriers and Dep tended to be associated with a higher basal morning cortisol in the *SS* individuals. Furthermore, this suggests that there is a difference in the biological effects of acute stress versus chronic stress or trauma and could also complement the current debate on potential mechanisms mediating vulnerability for the development of psychiatric disorders as a function of *5-HTTLPR* genotype.

Disclosure statement

The authors declare no conflict of interest to disclose.

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Table 1

Characteristics of the 334[a] participants

Characteristic	
	Mean (SD)
Age (years)	76.5 (6.3)
Body Mass Index (kg/m^2)	25.3 (3.5)
	% (n)
Sex (female)	49.4% (165)
< 12 years of education (%)	51.5% (332)
Recent stressful events (RSE)[b]	
0	60.5% (202)
1	22.7% (76)
≥ 2	16.8% (56)
Lifetime traumatic events[c]	
No Trauma	43.0% (124)
Trauma without re-experiencing	39.6% (114)
Trauma with reexperiencing	17.4% (50)
Depression[d]	14.4% (48)
Past major depression[e]	26.4% (81)
Current antidepressant use	4.5% (15)
Current anxiety disorders[e]	7.8% (25)
Cardiovascular ischemic pathologies[f]	8.4% (28)
Cognitive impairment (MMSE score <26)	8.5% (28)
5-HTTLPR genotype	
SS	24.6% (82)
SL	49.1% (164)
LL	26.3% (88)

[a] Except for BMI and education (n=332), MMSE (n=331), current anxiety disorder (n=320), past major depression (n=307), and lifetime traumatic events (n=288).

[b] Number of RSE during the past year assessed using the Gospel Oak questionnaire (Harwood et al., 1998).

[c] Severe lifetime traumatic events were assessed with Watson's PTSD Inventory according to DSM criteria (Watson et al., 1991).

[d] Having a MINI diagnosis of current major depression or high levels of depressive symptomatology (CES-D≥16).

[e] Diagnosis of major depression and anxiety disorders (generalized anxiety disorder, phobias) according to DSM-IV criteria and using the MINI (Sheehan et al., 1998).

[f] History of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, arteritis).

Figure captions

Fig. 1. Diurnal basal cortisol secretion as a function of recent traumatic events (RSE) in the whole sample (n=334) and according to the *5-HTTLPR* genotype[a,b].

[a] Time concentrations correspond to the means of Ln of cortisol concentration (expressed as ng/dl) adjusted for age and sex.
[b] The number of participants in each group is indicated between brackets.

Fig. 2. Diurnal basal cortisol secretion as a function of current depression (Dep) in the overall sample (n=334) and according to the *5-HTTLPR* genotype[a,b].

[a] Time concentrations correspond to the means of Ln of cortisol concentration (expressed as ng/dl) adjusted for age and sex.
[b] The number of participants in each group is indicated between brackets.

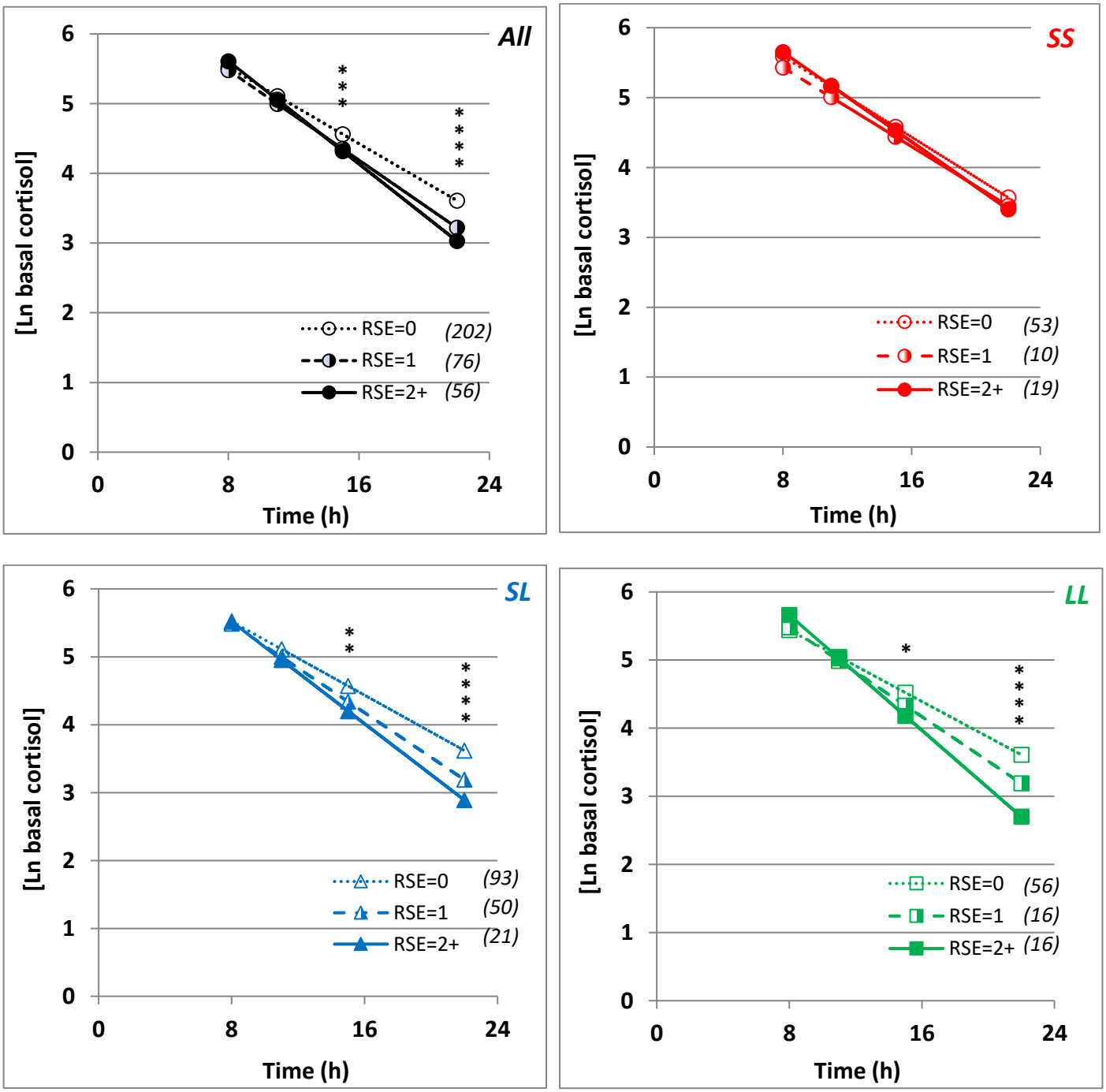
Fig. 3. Morning (A) and evening (B) basal cortisol as a function of current depression (Dep) and according to *5-HTTLPR* genotype (n=334)[a,b].

[a] The number of participants in each group is indicated in Fig. 2.
[b] Time concentrations correspond to the means (SE) of Ln of cortisol concentration (expressed as ng/dl) adjusted for age, sex, and recent stressful events.

Fig. 4. Morning (A) and evening (B) basal cortisol as a function of recent stressful events (RSE) according to *5-HTTLPR* genotype and accounting for depression (n=334)[a,b].

[a] The number of participants in each group is indicated in Fig. 1.
[b] Time concentrations correspond to the means (SE) of Ln of cortisol concentration (expressed as ng/dl) adjusted for age, sex, and depression.

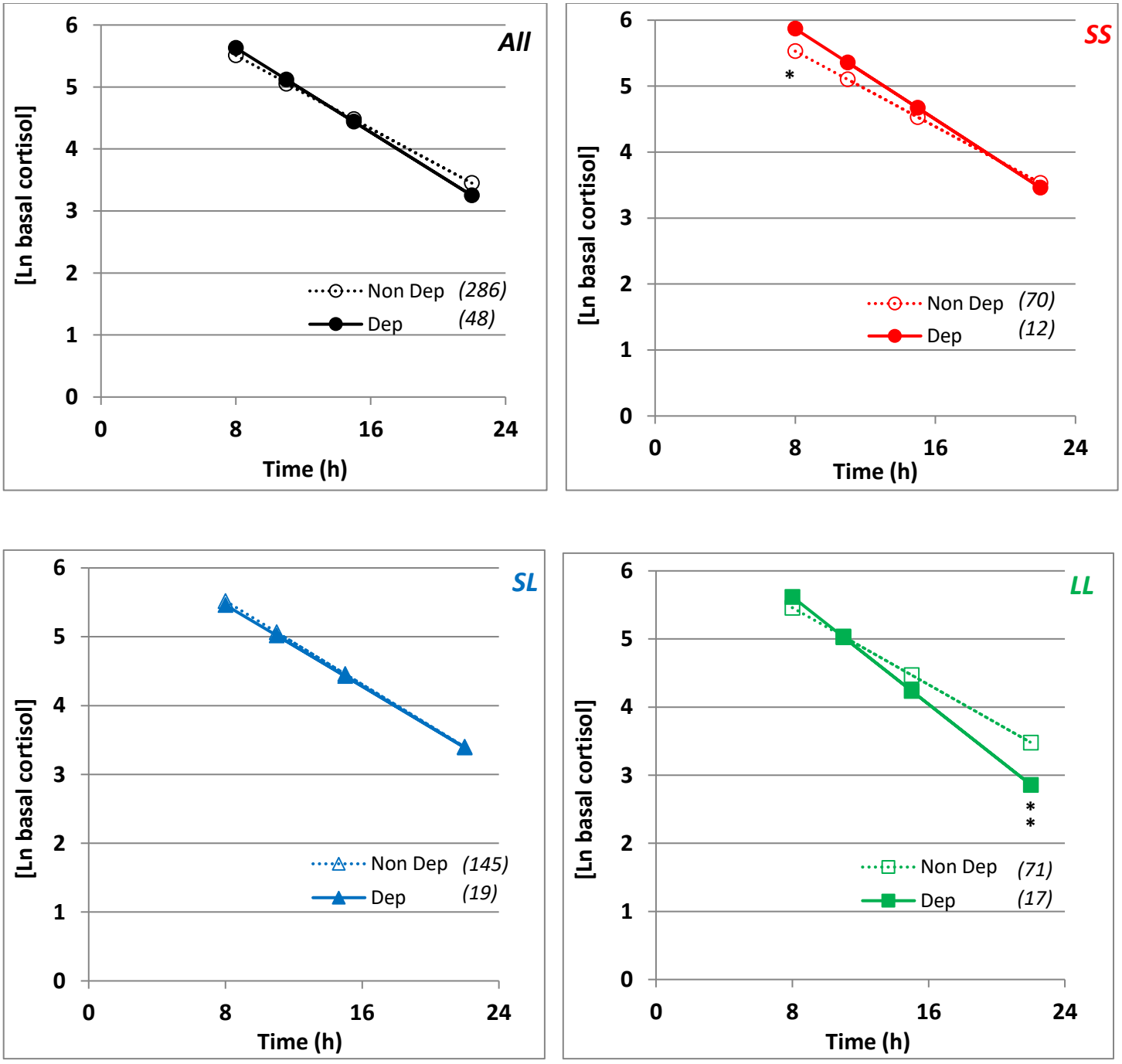
Fig. 1



*p=0.058; **p=0.015; ***p=0.003; ****p≤0.001 (if not indicated p≥0.40).

RSE: Number of recent stressful events

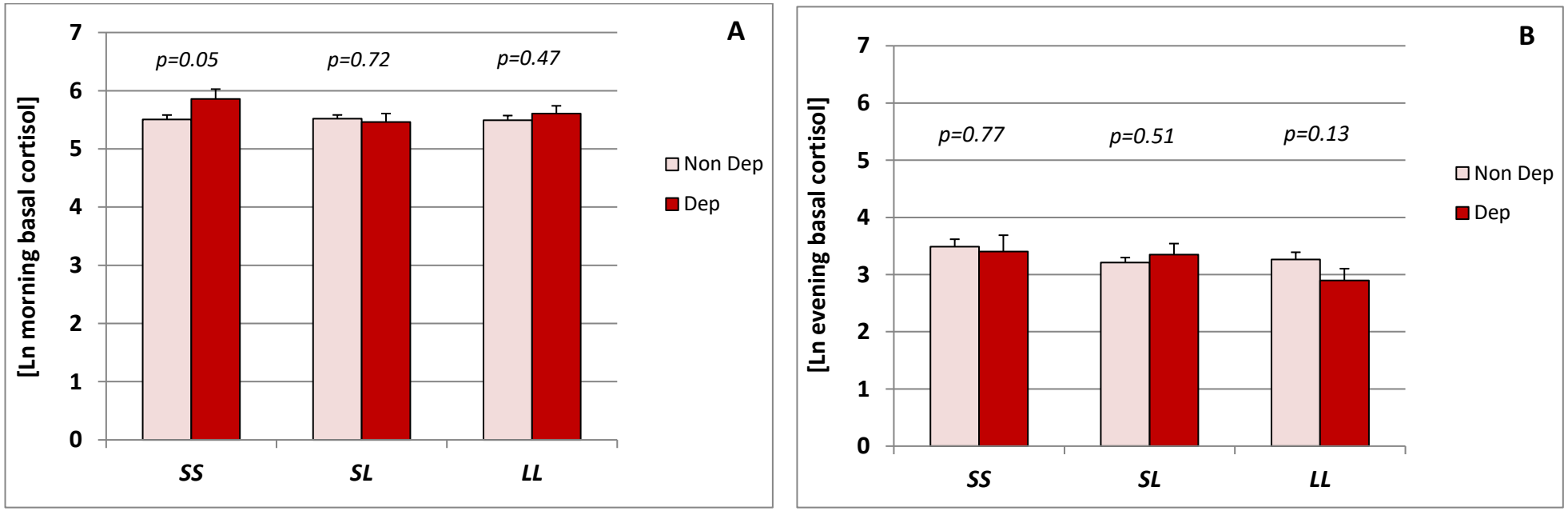
Fig. 2.



*p=0.059; **p=0.011 (if not indicated p>0.11).

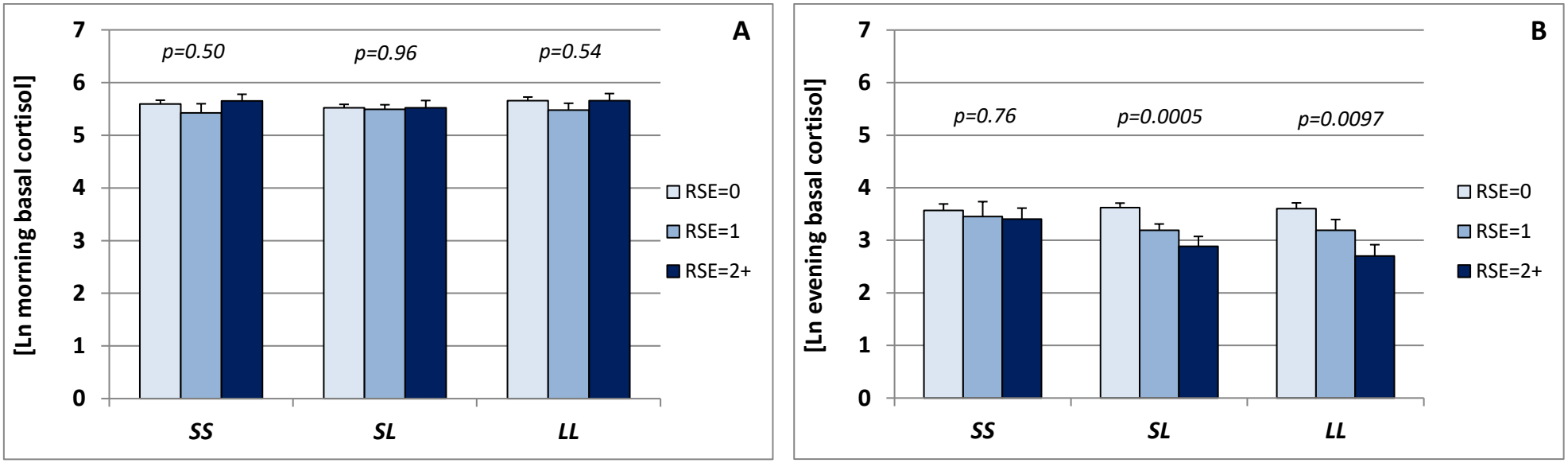
Dep: Depressed

Fig. 3.



Dep: Depressed

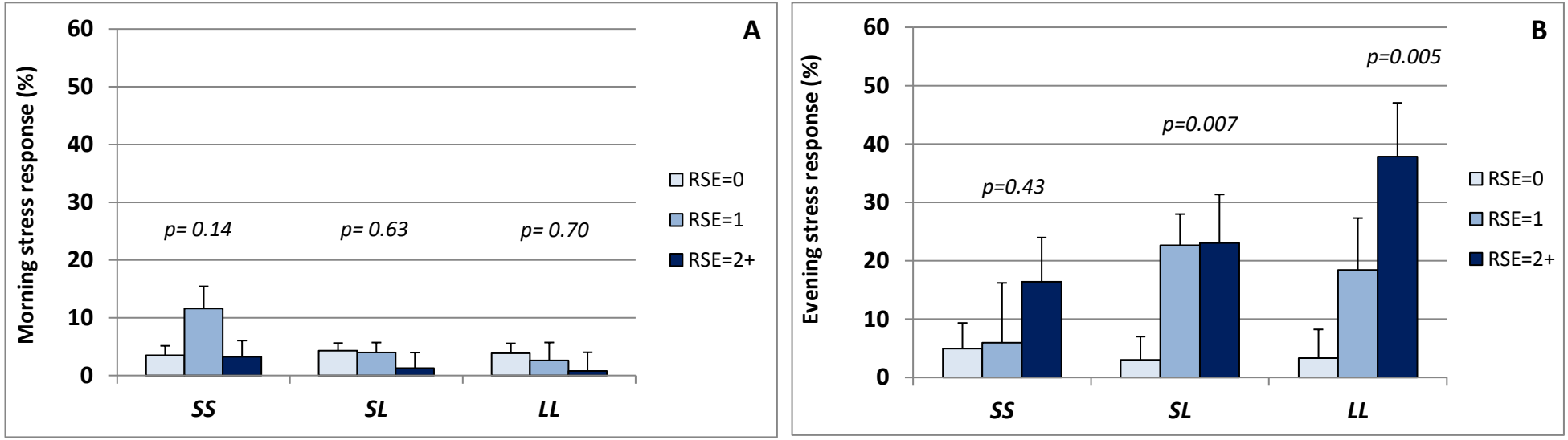
Fig. 4.



RSE: Number of recent stressful events

ANNEX: SUPPLEMENTARY INFORMATION

Supplementary Fig. S1. Morning (A) and evening (B) cortisol stress response as a function of recent stressful events (RSE) and according to *5-HTTLPR* genotype (n=334)[a,b].

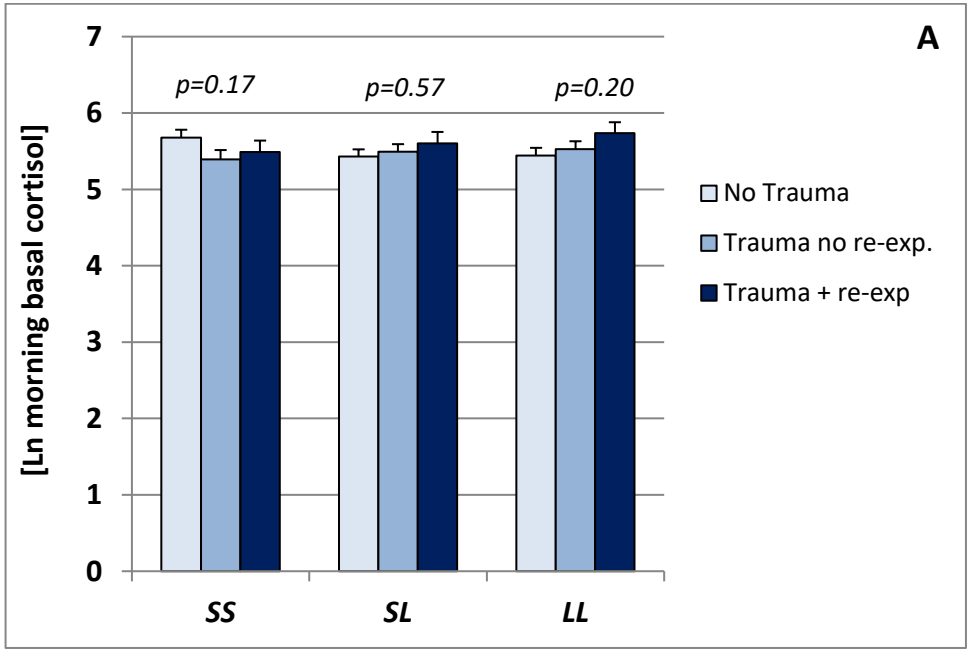


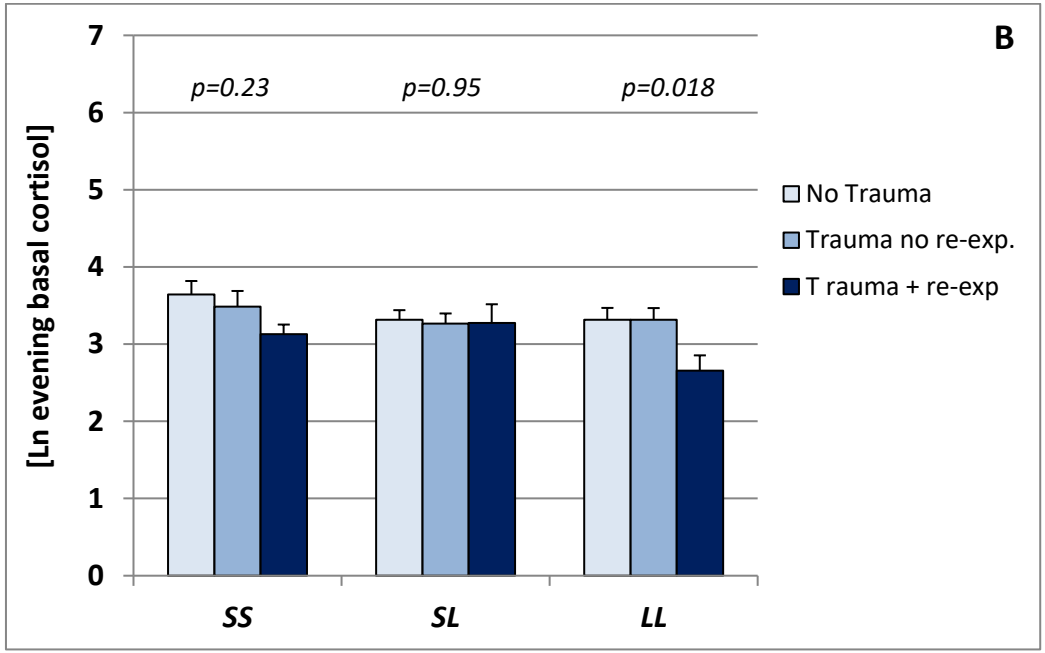
Global p-values are indicated.

[a] The number of participants in each group is indicated in Fig. 1.

[b] Means (SE) adjusted for age and sex of the stress response (expressed as %) and calculated as the ratio of (stress cortisol level – basal cortisol level)/ (basal cortisol level).

Supplementary Fig. S2. Morning (A) and evening (B) cortisol in non-traumatized participants (n=124) as well as in traumatized participants with (n=50) or without (n=114) re-experiencing symptoms and according to *5-HTTLPR* genotype[a]

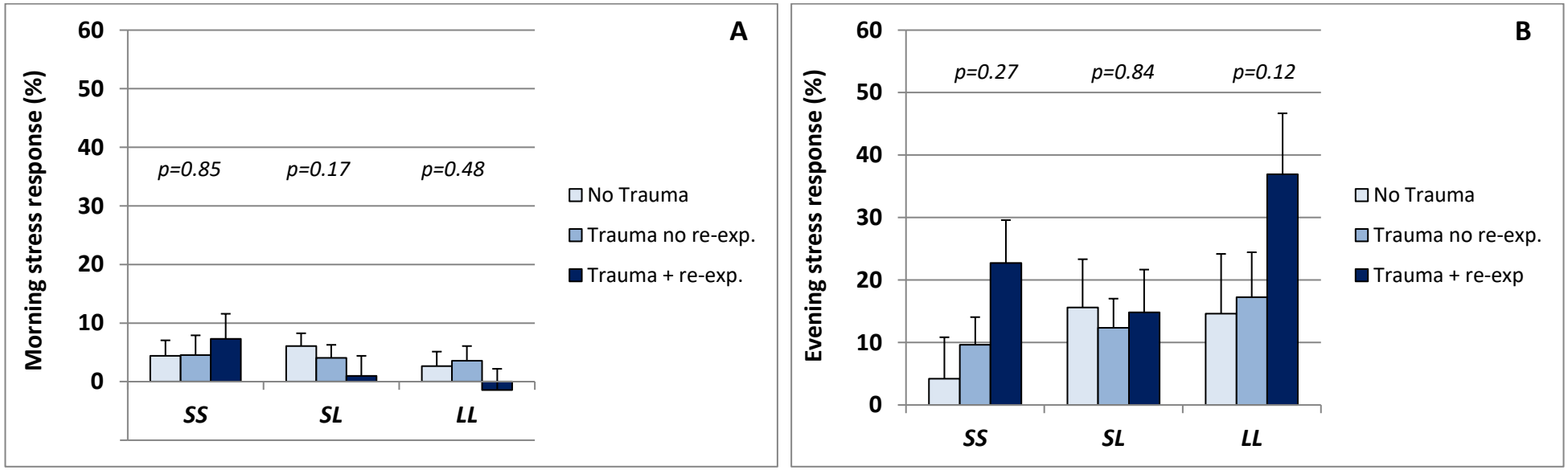




Global p-values are indicated.

[a]Time concentrations correspond to the means (SE) of Ln of cortisol concentration (expressed as ng/dl) adjusted for age, sex, and RSE.

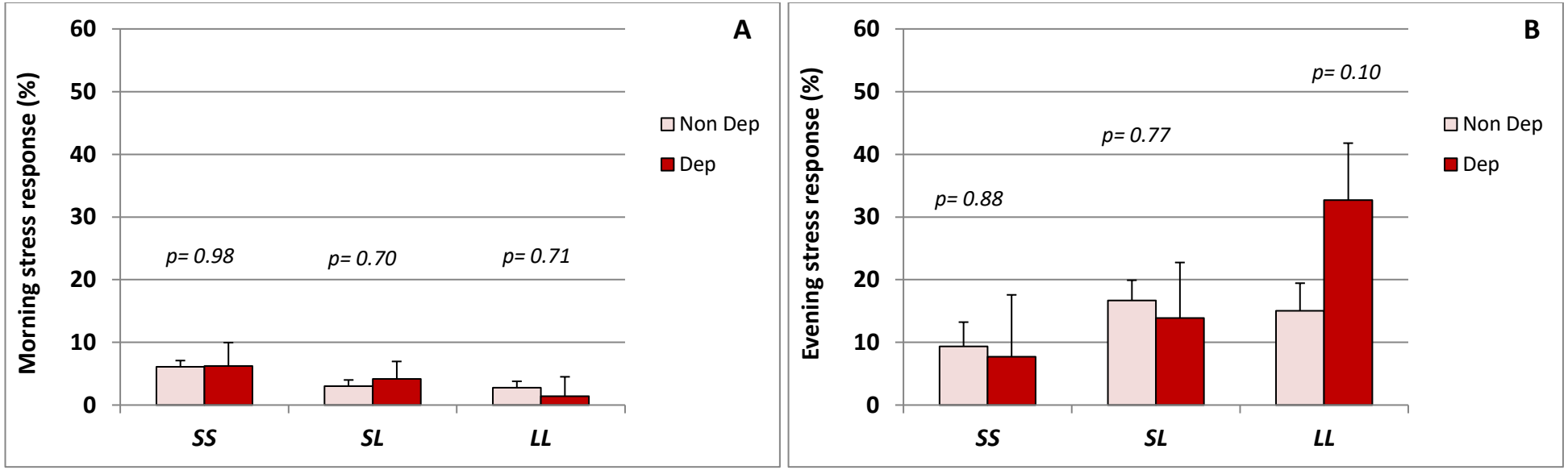
Supplementary Fig. S3. Morning (A) and evening (B) cortisol stress response in non-traumatized participants (n=124) as well as in traumatized participants with (n=50) or without (n=114) re-experiencing symptoms and according to *5-HTTLPR* genotype[a]



Global p-values are indicated.

[a] Means (SE) adjusted for age, sex, and RSE of the stress response (expressed as %) and calculated as the ratio of (stress cortisol level – basal cortisol level)/ (basal cortisol level).

Supplementary Fig. S4. Morning (C) and evening (D) cortisol stress response as a function of current depression (Dep) and according to *5-HTTLPR* genotype[a]



[a] The number of participants in each group is indicated in Fig. 2.

[b] Means (SE) adjusted for age, sex, and RSE of the stress response (expressed as %) and calculated as the ratio of (stress cortisol level – basal cortisol level)/ (basal cortisol level).