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Running title: Stress and depression risk according to *5-HTTLPR*

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Abstract

Variability in the serotonin transporter (*5-HTTLPR*) gene can influence the risk of depression associated with adversity, as well as cortisol stress reactivity, although not consistently. No study has examined the impact of both a stressful environment and corticotropic-axis dysfunction on depression, as a function of *5-HTTLPR*. This population-based study included 334 subjects aged 65 and older. Depression was measured at both diagnostic (major depression according to DSM-IV) and symptomatic (subthreshold depression) levels of caseness, in addition to *5-HTTLPR* and *rs25531* genotyping and diurnal cortisol measures. For participants with the *SS* genotype, higher morning cortisol levels were associated with a 4-fold increased risk of depression. Among *LL* participants, both evening cortisol levels and recent stressful events increased depression risk, although only the latter remained significant after multivariable adjustment. Conversely, *SL* individuals appeared somewhat resilient to depression in terms of cortisol and recent stress. These findings indicate that *5-HTTLPR* genetic variability appears to influence the association between stress-related factors and late-life depression, although the gene-environment interactions failed to reach statistical significance levels. Participants homozygous for the short allele appeared to have a cortisol-related neuroendocrine vulnerability to depression, while long allele homozygotes were more reactive to stressful events in terms of depression risk.

Key words: Cortisol; depression; elderly; hypothalamic-pituitary-adrenal axis; serotonin transporter-linked promoter region; stress.

1. Introduction

In the past, clinicians have made the distinction between 'endogenous' and 'reactive' depression in accordance with the empirical observation that depressive episodes may be triggered in some patients by environmental stressors, but not in others, where it was attributable to genetic vulnerability. The most recent meta-analysis confirmed a robust link between the short (*S*) form of the serotonin transporter gene (*5-HTTLPR*), experiencing stress, and resulting depression (Sharpley et al., 2014). Around 35% of these studies however, failed to show any significant association or found opposite results, with carriers of the long (*L*) form being at a higher risk of depression following life stress. The studies reporting opposing findings did not appear to be flawed by reduced power or methodological weaknesses, and the results were independent of study design, depression measure and type of stressful event (Sharpley et al., 2014). Age has been frequently evoked as a potential source of inconsistent findings for depression (Uher and McGuffin, 2008) and post-traumatic stress disorder (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). This may explain why there is no generally accepted gene-dose model. Another important source of heterogeneity pointed by Sharpley et al. and potential limitation of previous studies is the method used to assess stress, *i.e.* whether extrinsic stress was self-reported or objectively recognized (Sharpley et al., 2014). They also raised the possibility of different neurobiological underpinnings and pathways for depression but, so far, intrinsic biological stress measures have not been examined.

The hypothalamic-pituitary-adrenal (HPA) axis is one of the principal stress signaling pathways, and results in releases of cortisol (Kudielka et al., 2012). Cortisol secretion is not only a good indicator of HPA responsivity and differential biological response to short-term versus long-term effect of stress (Miller et al., 2007; Morris et al., 2012) but it also constitutes one important neurobiological characteristic of depression (Stetler and Miller, 2011). Heightened diurnal cortisol levels have been frequently reported in depressed individuals (Belvederi Murri et al., 2014), and remain high after recovery from major depression (Beluche et al., 2009), suggesting it may constitute a trait marker for vulnerability to depression. Conversely, exposure to traumatic events has been associated with lower evening cortisol (Chaudieu et al., 2008; Morris et al., 2012). There is also a link between serotonergic signaling and HPA

axis functioning (Vazquez et al., 2012), and the *5-HTTLPR* gene has been shown to influence cortisol reactivity in young adults exposed to acute psychosocial stress, whereas the unique study in elderly reported an inverse association (Miller et al., 2013). So far, no study has examined the impact of both an adverse environment and corticotropic axis dysfunctioning, which could represent different aspects of stress with distinct biological effects (Grabe et al., 2011) and consequences for depression risk. Whether this could differ as a function of *5-HTTLPR* genotype, yielding contradictory findings has also not been investigated.

This study evaluated whether both recent stressful events and cortisol levels are associated with late-life depression and if this differs according to *5-HTTLPR* genotype, while taking into account sociodemographic and stress-related factors, as well as past major depression.

2. Subjects and Methods

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 (Ritchie et al., 2009). The standardized interview in the Esprit study included information on socio-demographic characteristics, physical health, and medical history of the participants. This study was based on a random sample of 344 non-demented participants who underwent depression assessment, responded to all questions concerning their experience of recent stressful events, had complete diurnal cortisol samples, and agreed to provide blood samples for *5-HTTLPR* genotyping. Repeated diurnal salivary cortisol samples and measures were taken, as published previously (**see Supplemental Methods** for details). Genotyping of *5-HTTLPR* and the A/G polymorphism (*rs25531*) within the promoter region were performed as described previously (Ritchie et al., 2009), which was validated with independent genotyping of matched buccal DNA samples. Major depression and anxiety disorder were diagnosed according to DSM-IV criteria using the Mini International Neuropsychiatric Interview (MINI, French version 5.00), a standardized psychiatric examination validated in the general population (Sheehan et al., 1998). Case-level late-life depression was defined as a MINI diagnosis of current major depression or high levels of depressive symptomatology (score ≥16) on the Center for Epidemiologic Studies-Depression Scale

(Radloff, 1977). Exposure to stressful events during the past year was assessed using the validated Gospel Oak questionnaire (Harwood et al., 1998). The national ethics committee approved the study and all participants provided written informed consent.

The association between the *5-HTTLPR* allelic frequency and depression was examined by logistic regression analysis, using the conventional biallelic and the triallelic model further considering the *rs25531* polymorphism within the promoter. Previous association studies have suggested dominant, codominant, and recessive models of the *5-HTTLPR S* (or *S'*) allele without a clear consensus (Uher and McGuffin, 2008). To overcome this problem, we compared both possible allele groups separately by aggregating samples according to an *S* (or *S'*) recessive model (having two alleles), and an *S* (or *S'*) dominant model (having at least one allele). We also examined whether there was a statistical significant interaction between genotype (*5-HTTLPR*) and stress-related variables on the risk of depression. In keeping with the original aim of our study, we then stratified analysis by *5-HTTLPR*, and generated multivariable models to determine the association between stress-related variables (morning and evening cortisol levels, recent stressful events), and depression. SAS (v9.4, SAS Institute, NC, USA) was used for the statistical analyses with a significance level of $p < 0.05$.

3. Results

Participant characteristics are summarized in **Table 1** and 4.5% currently used antidepressants. The mean (SD) age of first onset depression was 48.3 (16.6) years and 43% of the participants with past depression had recurrent episodes. There were no significant differences in socio-demographic or clinical characteristics across genotype groups. In logistic regression models adjusted for age and sex, experiencing a recent stressful event and a history of major depression were both associated with a >2-fold increased risk of clinical depression in the whole sample, but cortisol levels were not associated with depression risk (**Table 2A**). There was some evidence that *5-HTTLPR* (+/- *rs25531*) genotype modified the association between stress-related factors and depression risk, in particular for recent stressful events, although the multiplicative interaction term failed to reach statistical significance ($p<0.10$ in both the dominant and recessive models) (**Tables S1 and S2**).

After stratification by genotype, the associations of recent stressful events and depression with depression more than doubled in strength for *L* homozygous individuals, but were clearly not significant for individuals homozygous for the *S* allele (**Table 2B**). A distinct pattern was found when examining cortisol levels. Higher morning cortisol levels were associated with a 4-fold increased risk of depression in the *SS* individuals only. Conversely, a negative association between evening cortisol and depression risk was found specifically for *LL* participants. In multivariable models combining the significant stress-related risk factors, evening cortisol levels were no longer significantly associated with depression in *LL* participants, after accounting for recent stress (evening cortisol: OR=0.57, 95% CI=0.29-1.11, p=0.10; recent stressful events: OR=5.16, 95% CI=1.35-19.78, p=0.017). These findings remained similar after inclusion of past major depression **(Table S3A**) and were not modified when taking into account recurrent episodes or current antidepressant use. Additional variables shown in Table 1 were not significantly associated with depression (all $p \geq 0.15$). The associations remained consistent when examining the triallelic model, and in addition recent stressful events were associated with depression among *S'L'* heterozygotes (**Table S3B**).

4. Discussion

To our knowledge, this is the first study to investigate the impact of stress on depression, using both extrinsic (self-report of stressful events) and intrinsic (cortisol secretion) stress indicators. Our findings show differential stress-related susceptibility to late-life depression, with some indication that this might also vary depending on *5-HTTLPR* genotype, although the GxE interaction term failed to reach the 5% significance level. In stratified analysis, the risk of depression was significantly associated with higher morning cortisol levels specifically in the *SS* participants and this was independent of stressful events. In the *LL* participants however, stressful events and past major depression were significantly associated with increased depression risk. These significant associations were independent of socio-demographic characteristics and comorbidity. No stress-related factors were significantly associated with depression in the *SL* individuals despite double the number of participants in this group compared with the *SS* or *LL* participants. This may explain why in this study and others, there is no clear conclusion

regarding the inheritance model of the *5-HTTLPR* polymorphism (morning cortisol suggests a recessive model but past major depression a dominant one).

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).

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 or recurrent depressive episodes (Bremmer et al., 2007). In the elderly, late-life depressive symptoms have been found to be associated with both hyperactivity and hypoactivity of the HPA axis, with distinct characteristics, *e.g.* low cortisol levels being specifically associated with recurrent depression (Bremmer et al., 2007). However these studies were limited by only one cortisol sample and they did not take into account stressful life events. This could be particularly critical considering that elderly people are more likely to accumulate stressful experiences with potential influences on cortisol secretion, and that with increasing age the HPA axis could be more vulnerable to dysregulation (Belvederi Murri et al., 2014).

Our findings in the general elderly population concur with these previous reports; cortisol hypersecretion reflecting current psychological load, whereas hyposecretion possibly reflecting exposure history and chronic stress. Furthermore, we present some preliminary evidence that this might depend on *5-HTTLPR* genotype, which would also be supported by some prior research findings. Lowered evening cortisol in *LL* depressed participants could actually reflect or be a consequence of adversity, as the former was not significant after multivariable adjustment. This is consistent with our previous findings in this Esprit population of a significant association between exposure to traumas (Chaudieu et al., 2008) and

lower evening cortisol levels. It may be that *LL* participants are more vulnerable to intrinsically stress related environmental factors which could reflect 'reactive' depression, however this hypothesis could not be directly tested in our study. The specific association of past depression, as well as early life events (Ritchie et al., 2009) with current depression in the *LL* participants, also supports this hypothesis. Conversely, heightened morning cortisol could represent a biological correlate or even a causal factor for late-life depression in *SS* individuals and might be a marker of 'endogenous' depression. In contrast, *SL* individuals appeared resilient to both intrinsically and extrinsically stress-related factors in terms of depression risk. A schematic model was drawn to illustrate these findings (**Figure S1**). Equivalent results were found for both *S'S'* and *L'L'* homozygous. For the *S'L'* heterozygotes, recent stressful events were associated with depression, but to a much weaker extent than for *L'L'* participants. A number of allelic variants have been described in the *5-HTTLPR* polymorphisms in humans and it is currently not clear whether the magnitude of any association may be affected by this variability and if *rs25531* has any impact on the functionality of the short *5-HTTLPR* allele (Uher and McGuffin, 2008). Caution however must also be taken in the interpretation of these findings, given that the interaction terms did not reach statistical significance and the effect sizes were modest. It would be interesting to now try and replicate these findings in a very large independent cohort.

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 would also support this and further suggests that increased morning cortisol in the *SS* depressed participants may be under genetic control. The set-point of the HPA axis could be influenced by genes involved in corticosteroid signaling. 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).

Although the size of our study was relatively large for a study of this kind (with data both on diurnal

cortisol secretion and *5-HTTLPR* genotyping), the stratified analysis included groups of less than 100 individuals, thus potentially limiting the overall power of the study. Data related to recent stressful events were retrospective, but participants diagnosed with possible dementia were excluded to minimize recall bias. Strengths of our study are that it was population-based and involved 344 elderly people with *5-HTTLPR* genotyping data (including information on the *rs25531* variant) as well as complete diurnal cortisol secretion and validated measures of recent stressful events and depression. We were not able to consider atypical depression subtypes but we controlled for key stress-related covariates thus minimizing any confounding as well as for genotyping accuracy through duplicate samples collected at different times and with independent genotyping, to ensure the integrity of this data. Finally, since multiple analyses have been performed and we stratified analysis by *5-HTTLPR* genotype, we cannot exclude that some associations were due to chance. Further studies are needed to replicate our findings.

Our findings demonstrate differential stress-related susceptibility to late-life depression and the potential that these effects might vary depending on *5-HTTLPR* genotype. This would suggest individual neuroendocrine heterogeneity of depression which may require different clinical management.

Disclosure statement

The authors declare no conflict of interest to disclose.

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References

Anacker, C., Zunszain, P.A., Carvalho, L.A., Pariante, C.M., 2011. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36, 415-25.

Beluche, I., Chaudieu, I., Norton, J., Carriere, I., Boulenger, J.P., Ritchie, K., Ancelin, M.L., 2009. Persistence of abnormal cortisol levels in elderly persons after recovery from major depression. J Psychiatr Res. 43, 777-83.

Belvederi Murri, M., Pariante, C., Mondelli, V., Masotti, M., Atti, A.R., Mellacqua, Z., Antonioli, M., Ghio, L., Menchetti, M., Zanetidou, S., Innamorati, M., Amore, M., 2014. HPA axis and aging in depression: systematic review and meta-analysis. Psychoneuroendocrinology 41, 46-62.

Bremmer, M.A., Deeg, D.J., Beekman, A.T., Penninx, B.W., Lips, P., Hoogendijk, W.J., 2007. Major depression in late life is associated with both hypo- and hypercortisolemia. Biol Psychiatry 62, 479-86.

Chaudieu, I., Beluche, I., Norton, J., Boulenger, J.P., Ritchie, K., Ancelin, M.L., 2008. Abnormal reactions to environmental stress in elderly persons with anxiety disorders: evidence from a population study of diurnal cortisol changes. J Affect Disord 106, 307-13.

Franz, C.E., York, T.P., Eaves, L.J., Mendoza, S.P., Hauger, R.L., Hellhammer, D.H., Jacobson, K.C., Levine, S., Lupien, S.J., Lyons, M.J., Prom-Wormley, E., Xian, H., Kremen, W.S., 2010. Genetic and environmental influences on cortisol regulation across days and contexts in middle-aged men. Behav Genet 40, 467-79.

Grabe, H.J., Schwahn, C., Appel, K., Mahler, J., Schulz, A., Spitzer, C., Barnow, S., John, U., Freyberger, H.J., Rosskopf, D., Volzke, H., 2011. Update on the 2005 paper: moderation of mental and physical distress by polymorphisms in the 5-HT transporter gene by interacting with social stressors and chronic disease burden. Mol Psychiatry 16, 354-6.

Harwood, R.H., Prince, M.J., Mann, A.H., Ebrahim, S., 1998. The prevalence of diagnoses, impairments, disabilities and handicaps in a population of elderly people living in a defined geographical area: the Gospel Oak project. Age Ageing 27, 707-14.

Kudielka, B.M., Gierens, A., Hellhammer, D.H., Wust, S., Schlotz, W., 2012. Salivary cortisol in ambulatory assessment--some dos, some don'ts, and some open questions. Psychosom Med 74, 418-31.

Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull 133, 25-45.

Miller, R., Wankerl, M., Stalder, T., Kirschbaum, C., Alexander, N., 2013. The serotonin transporter gene-linked polymorphic region (5-HTTLPR) and cortisol stress reactivity: a meta-analysis. Mol Psychiatry 18, 1018-24.

Morris, M.C., Compas, B.E., Garber, J., 2012. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. Clin Psychol Rev 32, 301-15.

Navarro-Mateu, F., Escamez, T., Koenen, K.C., Alonso, J., Sanchez-Meca, J., 2013. Meta-analyses of the 5-HTTLPR polymorphisms and post-traumatic stress disorder. PLoS One 8, e66227.

Radloff, L., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Measurement 1, 385-401.

Ritchie, K., Jaussent, I., Stewart, R., Dupuy, A.M., Courtet, P., Ancelin, M.L., Malafosse, A., 2009. Association of adverse childhood environment and 5-HTTLPR genotype with late-life depression. J Clin Psychiatry 70, 1281-8.

Sharpley, C.F., Palanisamy, S.K., Glyde, N.S., Dillingham, P.W., Agnew, L.L., 2014. An update on the interaction between the serotonin transporter promoter variant (5-HTTLPR), stress and depression, plus an exploration of non-confirming findings. Behav Brain Res 273, 89-105.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59, 22-33.

Stetler, C., Miller, G.E., 2011. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom Med 73, 114-26.

Uher, R., McGuffin, P., 2008. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Mol Psychiatry 13, 131-46.

Vazquez, D.M., Neal, C.R., Jr., Patel, P.D., Kaciroti, N., Lopez, J.F., 2012. Regulation of corticoid and serotonin receptor brain system following early life exposure of glucocorticoids: long term implications for the neurobiology of mood. Psychoneuroendocrinology 37, 421-37.

Table 1
Characteristics of the participants according to their *5-HTTLPR* genotype[a]

5-HTTLPR	Whole sample (N=334[b])	*SS* (N=82)	*SL* (N=164)	*LL* (N=88)	Comparison *SS/SL/LL* (global test *p*-value)
	Mean (SD)				***t*-test**
Age (years)	76.5 (6.3)	76.3 (6.3)	76.2 (6.3)	77.2 (6.3)	0.53
Body mass index (kg/m^2)	25.3 (3.5)	25.5 (3.7)	25.4 (3.5)	24.8 (3.5)	0.42
	% (n)				***Chi*^{2}-test**
Sex (female)	49.4% (165)	48.8% (40)	47.6% (78)	53.4% (47)	0.67
< 12 years of education	51.5% (171)	54.3% (44)	51.2% (84)	49.4% (43)	0.81
At least one recent stressful event[c]	39.5% (132)	35.4% (29)	43.2% (71)	36.4% (32)	0.38
Clinical level of depression[d]	14.4% (48)	14.6% (12)	11.6% (19)	19.3% (17)	0.25
Past major depression[d]	26.4% (81)	28.4% (21)	22.7% (34)	31.3% (26)	0.32
Current anxiety disorders[e]	7.8% (25)	4.9% (4)	9.0% (14)	8.4% (7)	0.53
Cardiovascular ischemic pathologies[f]	8.4% (28)	6.1% (5)	7.9% (13)	11.4% (7)	0.44
Cognitive impairment (MMSE <26)	8.5% (28)	9.9% (8)	8.5% (14)	7.0% (6)	0.80

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression Scale; MINI, Mini International Neuropsychiatric Interview; MMSE, Mini-Mental State Examination; *5-HTTLPR*, serotonin transporter gene-linked polymorphic region.

[a] The *5-HTTLPR* genotype frequency did not significantly deviate from Hardy-Weinberg equilibrium (χ^2=0.10, df=1, *p*=0.75) and reflected frequencies seen in white Europeans (Miller et al., 2013).

[b] Except for body mass index and education (n=332), MMSE (n=331), current anxiety disorder (n=320), and past major depression (n=307).

[c] Number of recent stressful events during the past year assessed using the validated Gospel Oak questionnaire (Harwood et al., 1998).

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

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

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

Table 2

Risk of depression[a] associated with stress-related factors[b]

A. In the whole sample (N=334[c])

	Odds Ratio [95% Confidence Interval] (*p*)
Recent stressful event[d]	2.28 [1.18-4.40] (0.014)
Past major depression[e]	2.03 [1.01-4.09] (0.047)
Morning cortisol[f]	1.45 [0.84-2.50] (0.18)
Evening cortisol[f]	0.80 [0.56-1.14] (0.21)
5-HTTLPR:	
SS vs. SL	1.25 [0.56-2.81] (0.58)
LL vs. SL	1.87 [0.90-3.88] (0.09)
SS vs. LL	0.65 [0.27-1.55] (0.33)
L allele vs. SS	1.03 [0.49-2.14] (0.95)
S allele vs LL	0.57 [0.29-1.13] (0.11)

B. According to *5-HTTLPR* genotype (biallelic model)

		Odds Ratio [95% Confidence Interval] (*p*)						
5-HTTLPR	***p*[g]**	***SS (N=82)***	***SL (N=164)***	***LL (N=88)***	***p*[g]**	***S allele (N=246)***	***p*[g]**	***L allele (N=252)***
Recent stressful event[d]	0.13	0.80 [0.19-3.41]	2.26 [0.80-6.34]	6.45 [1.82-22.9]	0.09	1.48 [0.67-3.28]	0.09	3.39 [1.54-7.46]
Past major depression[e]	0.19	1.01 [0.43-4.54]	1.41 [0.43-4.62]	4.47 [1.34-14.9]	0.07	1.27 [0.51-3.17]	0.35	2.51 [1.12-5.60]
Morning cortisol[f]	0.20	4.09 [1.03-16.2]	0.89 [0.41-1.92]	1.72 [0.57-5.21]	0.76	1.44 [0.76-2.72]	0.12	1.44 [0.76-2.72]
Evening cortisol[f]	0.23	1.00 [0.45-2.22]	1.01 [0.58-1.73]	0.46 [0.24-0.87]	0.08	1.05 [0.68-1.62]	0.62	0.74 [0.50-1.11]

Abbreviations: CES-D, Center for Epidemiologic Studies-Depression Scale; CI, confidence interval; MINI, Mini International Neuropsychiatric Interview; OR, odds ratio; *5-HTTLPR*, serotonin transporter gene-linked polymorphic region.

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

[b] Logistic regression model adjusted for age and sex.

[c] Except for past major depression; n=307 (74 *SS*, 150 *SL*, 83 *LL*).

[d] At least one recent stressful event during the past year assessed using the validated Gospel Oak questionnaire (Harwood et al., 1998).

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

[f] Morning and evening basal salivary cortisol were calculated at fixed times (8.00am for morning and 10.00pm for evening cortisol) from the regression of the four-cortisol values on the sampling times, for each participant (see Supplemental Methods).

[g] p-value for the interaction term between stress-related variable and genotype in the multivariable regression model.

SUPPLEMENTARY MATERIALS

Supplemental Methods

Cortisol measurement

Cortisol measures were taken under naturalistic conditions with participants having the choice of 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). Saliva samples were not collected from participants with dementia. Participants with missing time points, inadequate saliva volume or atypical cortisol baseline profiles (flat pattern or abnormal time peak) were excluded from the study sample. The compliance rates were excellent with the systematic return of saliva samples by all the subjects. Hence, the study was initially based on a random sample of 360 non-demented participants who had complete salivary cortisol samples, with a typical eucortisolemic pattern, and were not being treated with medications likely to modify cortisol levels (*e.g.* glucocorticoids, benzodiazepines, and hormonal treatment for women), as described previously (Ancelin et al., 2013; Beluche et al., 2009; Chaudieu et al., 2008). Of these, 19 participants were missing *5-HTTLPR* genotype, 1 did not have a depression assessment, and 6 failed to provide information on recent stressful events. This left 334 participants in the analyses. 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 ($p<0.003$), but did not differ regarding other characteristics, including recent stressful events ($p=0.14$).

Participants were instructed not to drink, eat or smoke for at least 30 mins before saliva collection and to start the protocol at least 1h after awakening. Subsequent samples were collected at 3, 7, and 14 h after the first morning sampling and exact times were recorded. Participants carried on their normal daily activities with limited physical exertion in order to maximize ecological validity. They did not report any additional stressors on the day of sampling. and the basal levels were similar to previous studies in the elderly (Ice et al., 2004). Salivary cortisol levels were determined by direct radioimmunoassay (Diagnostic Systems

Laboratories-Webster, Texas) (Hellhammer et al., 1987). Intra-assay and inter-assay coefficients of variation averaged 5%.

Since the distribution of raw cortisol is typically skewed, and the normal diurnal profile can be approximated by an exponential curve, cortisol values were log-transformed. Cortisol levels were calculated at fixed times (8.00am for morning and 10.00pm for evening cortisol) from the regression of the four-cortisol values on the sampling times, for each participant as published previously (Beluche et al., 2009; Chaudieu et al., 2008). These times constitute very common HPA axis indicators and were chosen as the most contrasting conditions of the diurnal cycle (at which cortisol levels reached their 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).

References for Supplementary Methods

Ancelin, M.L., Carriere, I., Scali, J., Ritchie, K., Chaudieu, I., Ryan, J., 2013. Angiotensin-converting enzyme gene variants are associated with both cortisol secretion and late-life depression. Transl Psychiatry 3, e322.

Beluche, I., Chaudieu, I., Norton, J., Carriere, I., Boulenger, J.P., Ritchie, K., Ancelin, M.L., 2009. Persistence of abnormal cortisol levels in elderly persons after recovery from major depression. J Psychiatr Res. 43, 777-83.

Belvederi Murri, M., Pariante, C., Mondelli, V., Masotti, M., Atti, A.R., Mellacqua, Z., Antonioli, M., Ghio, L., Menchetti, M., Zanetidou, S., Innamorati, M., Amore, M., 2014. HPA axis and aging in depression: systematic review and meta-analysis. Psychoneuroendocrinology 41, 46-62.

Chaudieu, I., Beluche, I., Norton, J., Boulenger, J.P., Ritchie, K., Ancelin, M.L., 2008. Abnormal reactions to environmental stress in elderly persons with anxiety disorders: evidence from a population study of diurnal cortisol changes. J Affect Disord 106, 307-13.

Franz, C.E., York, T.P., Eaves, L.J., Mendoza, S.P., Hauger, R.L., Hellhammer, D.H., Jacobson, K.C., Levine, S., Lupien, S.J., Lyons, M.J., Prom-Wormley, E., Xian, H., Kremen, W.S., 2010. Genetic and environmental influences on cortisol regulation across days and contexts in middle-aged men. Behav Genet 40, 467-79.

Harwood, R.H., Prince, M.J., Mann, A.H., Ebrahim, S., 1998. The prevalence of diagnoses, impairments, disabilities and handicaps in a population of elderly people living in a defined geographical area: the Gospel Oak project. Age Ageing 27, 707-14.

Hellhammer, D.H., Kirschbaum, C., Belkien, L. 1987. Measurement of salivary cortisol under psychological stimulation. in: Hingtgen, J.N., Hellhammer, D.H., Huppman, G. (Eds.). Adavanced Methods in Psychobiology. Hogrefe, Toronto, pp 281-9.

Ice, G.H., Katz-Stein, A., Himes, J., Kane, R.L., 2004. Diurnal cycles of salivary cortisol in older adults. Psychoneuroendocrinology 29, 355-70.

Jacobs, N., Nicolson, N.A., Derom, C., Delespaul, P., van Os, J., Myin-Germeys, I., 2005. Electronic monitoring of salivary cortisol sampling compliance in daily life. Life Sci 76, 2431-43.

Kraemer, H.C., Giese-Davis, J., Yutsis, M., O'Hara, R., Neri, E., Gallagher-Thompson, D., Taylor, C.B., Spiegel, D., 2006. Design decisions to optimize reliability of daytime cortisol slopes in an older population. Am J Geriatr Psychiatry 14, 325-33.

Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull 133, 25-45.

Morris, M.C., Compas, B.E., Garber, J., 2012. Relations among posttraumatic stress disorder, comorbid major depression, and HPA function: a systematic review and meta-analysis. Clin Psychol Rev 32, 301-15.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59, 22-33.

Table S1. Logistic regression models[a] for the association between stress-related variables[b] and depression[c], considering effect modification by *5-HTTLPR* (biallelic model)

	***S* recessive (*L allele vs. SS*)**			***S* dominant (*S allele vs. LL*)**			**3 genotype group (*LL, SL, SS*)**	
	β coefficient (SE)	*p*		*β coefficient (SE)*	*p*		*β coefficient (SE)*[d]	*Global p-value*
≥ 1 stressful event (E)	-0.16 (0.68)	0.81		1.67 (0.62)	0.007		0.86 (0.52)	0.10
Genotype (G)	-0.65 (0.50)	0.19		0.09 (0.55)	0.87		1: 0.23 (0.62) 2: 0.74 (0.56)	0.41
Interaction: G x E	1.33 (0.79)	0.09		-1.22 (0.73)	0.09		1: 0.82 (0.80) 2: -1.03 (0.85)	0.13
Past major depression (E)	0.14 (0.71)	0.84		1.55 (0.60)	0.01		0.22 (0.59)	0.71
Genotype (G)	-0.18 (0.46)	0.70		0.007 (0.48)	0.99		1: 0.06 (0.51) 2: 0.20 (0.49)	0.92
Interaction: G x E	0.76 (0.80)	0.35		-1.35 (0.74)	0.07		1: 1.33 (0.83) 2: -0.08 (0.91)	0.19
Morning cortisol (E)	1.29 (0.66)	0.05		0.55 (0.57)	0.34		-0.05 (0.39)	0.90
Genotype (G)	6.60 (4.22)	0.12		0.51 (3.67)	0.89		1: -2.65 (3.83) 2: -7.47 (4.41)	0.23
Interaction: G x E	-1.14 (0.73)	0.12		-1.96 (0.65)	0.76		1: 0.60 (0.69) 2: 1.35 (0.77)	0.20
Evening cortisol (E)	-0.07 (0.36)	0.85		-0.63 (0.30)	0.03		0.01 (0.27)	0.97
Genotype (G)	0.67 (1.44)	0.64		-2.61 (1.23)	0.03		1: 2.73 (1.34) 2: 0.47 (1.58)	0.11
Interaction: G x E	-0.20 (0.41)	0.62		0.63 (0.37)	0.08		1: -0.65 (0.40) 2: -0.07 (0.45)	0.23

[a] Adjusted for age and sex.
[b] Full definitions of stress-related variables are provided in Table 1.
[c] Having a MINI diagnosis of current major depression or high levels of depressive symptomatology (CES-D score ≥ 16).
[d] 1*: LL vs. SL*; 2: *SS vs. SL.*

Table S2. Logistic regression models[a] for the association between stress-related variables[b] and depression[c], considering effect modification by *5-HTTLPR* (triallelic model)

	***S* recessive (*L' allele vs. S'S'*)**			***S* dominant (*S' allele vs. L'L'*)**			**3 genotype group (*L'L', S'L', S'S'*)**	
	β coefficient (SE)	*p*		*β coefficient (SE)*	*p*		*β coefficient (SE)*[d]	*global p-value*
≥ 1 stressful event (E)	-0.23 (0.67)	0.73		1.74 (0.69)	0.01		1.75 (0.69)	0.01
Genotype (G)	-0.29 (0.50)	0.56		0.17 (0.60)	0.78		1: 0.05 (0.65) 2: 0.33 (0.66)	0.84
Interaction : G x E	1.48 (0.78)	0.058		-1.18 (0.78)	0.13		1: -0.76 (0.84) 2: -1.99 (0.96)	0.11
Past major depression (E)	0.21 (0.69)	0.77		1.53 (0.66)	0.02		1.53 (0.66)	0.02
Genotype (G)	0.26 (0.46)	0.57		0.01 (0.51)	0.98		1: 0.13 (0.54) 2: -0.17 (0.60)	0.82
Interaction : G x E	0.70 (0.79)	0.38		-1.18 (0.78)	0.13		1: -1.06 (0.85) 2: -1.32 (0.95)	0.32
Morning cortisol (E)	1.28 (0.62)	0.04		0.93 (0.66)	0.16		0.93 (0.66)	0.16
Genotype (G)	6.85 (4.02)	0.09		3.17 (4.11)	0.44		1: 5.43 (4.25) 2: -2.85 (5.18)	0.10
Interaction : G x E	-1.11 (0.69)	0.11		-0.66 (0.73)	0.37		1: -1.04 (0.76) 2: 0.35 (0.90)	0.10
Evening cortisol (E)	-0.09 (0.35)	0.79		-0.45 (0.32)	0.16		-0.45 (0.32)	0.16
Genotype (G)	0.86 (1.40)	0.54		-1.58 (1.26)	0.21		1: -1.53 (1.36) 2: -1.84 (1.63)	0.43
Interaction : G x E	-0.13 (0.40)	0.73		0.34 (0.37)	0.36		1: 0.36 (0.41) 2: 0.36 (0.47)	0.63

[a] Adjusted for age and sex
[b] Full definitions of stress-related variables are provided in Table 1.
[c] Having a MINI diagnosis of current major depression or high levels of depressive symptomatology (CES-D score ≥ 16).
[d] 1*: L'L' vs. S'L'*; 2: *S'S' vs. S'L'*.

Table S3. Multivariable logistic regression models for the risk of depression[a] adjusted for age, sex, and relevant stress-related factors, according to *5-HTTLPR* genotype

	Odds Ratio [95% Confidence Interval] (*p*)				
A. Biallelic model	***All***	***SS (N=82)***	***SL (N=164)***	***LL (N=83)***	***L allele***
Recent stressful event[b]	2.58 [1.30-5.10] (0.007)	n.a.[e]	n.a.	4.96 [1.16-21.26] (0.031)	3.15 [1.37-7.23] (0.007)
Past major depression[c]	2.11 [1.03-4.32] (0.041)	n.a.	n.a.	5.91 [1.46-23.92] (0.013)	2.64 [1.15-6.08] (0.022)
Morning cortisol (continuous)[d]	n.a.	4.09 [1.03-16.2] (0.045)	n.a.	n.a.	n.a.
Evening cortisol (continuous)[d]	n.a.	n.a.	n.a.	0.57 [0.29-1.11] (0.10)	0.83 [0.54-1.29] (0.41)
B. Triallelic model	***All***	***S'S' (N=99)***	***S'L' (N=158)***	***L'L' (N=65)***	***L' allele***
Recent stressful event[b]	2.64 [1.34-5.20] (0.005)	n.a.	2.79 [1.05-7.44] (0.040)	7.02 [1.30-37.85] (0.024)	4.10 [1.78-9.42] (0.0009)
Past major depression[c]	2.20 [1.08-4.51] (0.030)	n.a.	n.a.	5.73 [1.23-26.77] (0.027)	2.69 [1.16-6.27] (0.022)
Morning cortisol (continuous)[d]	n.a.	4.09 [1.14-14.7] (0.031)	n.a.	n.a.	n.a.
Evening cortisol (continuous)[d]	n.a.	n.a.	n.a.	0.68 [0.31-1.48] (0.33)	n.a.

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

[b] At least one recent stressful event during the past year assessed using the validated Gospel Oak questionnaire (Harwood et al., 1998).

[c] Diagnosis of past major depression according to DSM-IV criteria and using the MINI (Sheehan et al., 1998).

[d] Morning and evening basal salivary cortisol were calculated at fixed times (8.00am for morning and 10.00pm for evening cortisol) from the regression of the four-cortisol values on the sampling times, for each participant (see Supplemental Methods).

[e] n.a. = not applicable; not included in the final multivariable model if $p > 0.10$ in model adjusted for age and sex.

Figure S1. Hypothetical model for the differential stress-related susceptibility to late-life depression according to *5-HTTLPR* genotype.

Green arrows indicate associations found in this study (light green is not significant after adjustment) and the purple arrow indicates potential association inferred here. MDD: major depressive disorder; RSE: recent stressful event.

LL participants appeared more vulnerable to stress-related environmental factors (reactive depression), morning cortisol could represent a biological correlate or a causal factor for depression in *SS* individuals (endogenous depression), whereas *SL* individuals appeared resilient to both intrinsically and extrinsically stress-related factors.