Short Communication

Temporal dynamics of cortisol-associated changes in mRNA expression of glucocorticoid responsive genes *FKBP5, GILZ, SDPR, PER1, PER2* and *PER3* in healthy humans

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A R T I C L E   I N F O

A B S T R A C T

Secretion of the stress hormone cortisol follows a circadian rhythm and is stimulated following stress exposure. Cortisol regulates the transcription of several genes, primarily through activation of the glucocorticoid receptor (GR). Previously, we showed an upregulation of *PERIOD* genes *PER1* and *PER3* after pharmacological/glucocorticoid challenge *in vivo* and *in vitro*. The current study aims to investigate the temporal association between unstimulated, diurnal cortisol secretion and the expression of selected *GR*-target genes (*PER1, PER2, PER3, FKBP5, GILZ* and *SDPR*) *in vivo* to determine the timing of the most pronounced coupling between cortisol and mRNA expression.

Unstimulated plasma and saliva cortisol concentrations and gene expression levels in whole blood were measured every 15 min from early morning until 16:00 h in 18 healthy men. Time-lagged correlations of cortisol concentrations with mRNA expression levels were assessed allowing lags between -240 and + 240 min.

Strong positive correlations at non-zero lags between cortisol levels and the expression of *FKBP5* (plasma: $r = 0.74$ (CI = 0.65-0.81), $p < 0.001$, lag + 90 min; saliva: $r = 0.71$ (CI = 0.61-0.78), $p < 0.001$, lag + 75 min), and *GILZ* (plasma: $r = 0.59$ (CI = 0.46-0.69), $p < 0.001$, lag + 30 min; saliva $r = 0.53$ (CI = 0.41-0.63), $p < 0.001$, lag + 15 min) were observed. Expressions of *PERIOD* genes and *SDPR* correlated only weakly with cortisol (all $|r| < 0.25$).

Our findings demonstrate strong correlations between cortisol secretion and gene expression in humans under unstimulated conditions. The observed time-lags can guide future research aiming to characterize glucocorticoid-dependent gene expression in clinical samples with stress-related disorders.

1. Introduction

The glucocorticoid cortisol, regulated by the hypothalamus-pituitary-adrenal (HPA) axis, plays an essential role in both stress-related and baseline homeostasis. In peripheral tissues, it exerts its function mainly through binding to the glucocorticoid receptor (GR), encoded by *NR3C1*. The GR is a ligand-activated transcription factor predominantly located in the cytoplasm in a multiprotein complex consisting of chaperones and co-chaperones (Wochnik et al., 2005). Activation of the GR leads to up- and down-regulation of target gene expression, both through direct interaction between activated GR dimers and glucocorticoid responsive elements (GREs) in gene regulatory regions, and indirectly through interaction with other transcription factors. Cortisol-associated changes of messenger RNA (mRNA) levels can be regarded as indicators for cellular sensitivity towards glucocorticoids, and potential biomarkers in the investigation of stress-related disorders. Chronic or traumatic stress exposure is associated with increased risk for a range of mental or physical disorders. Further, it has been suggested that dysregulations of the HPA axis, which are associated with alterations in the negative feedback sensitivity, might be involved. For instance,

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increased cortisol levels and especially cortisol hyperactivity are often observed in major depressive disorder (Chrousos, 2009), whereas relative hypocortisolism has been reported in patients with post-traumatic stress disorder (PTSD) (Daskalakis et al., 2016), atypical depression (Chrousos, 2009), and adults with adverse childhood experiences. However, it is important to note that cellular responses to glucocorticoids are strongly modulated by GR function and not solely determined by absolute levels of circulating cortisol. This implies that a functional read-out of GR sensitivity might be more informative when studying altered HPA axis regulation. To use cortisol-associated mRNA profiles as such a marker for GR sensitivity, it is important to determine the delay between cortisol secretion and mRNA expression of glucocorticoid responsive genes of interest. In previous studies, we have demonstrated that the *PERIOD* genes *PER1* and *PER3* show increased expression after hydrocortisone administration (Yurtsever et al., 2016), and increased *PER1* expression after psychosocial stress exposure (Schwaiger et al., 2016). While drug- (Arloth et al., 2015; Menke et al., 2016; Yurtsever et al., 2016) and stress-induced changes in mRNA expression have been studied in humans (Schwaiger et al., 2016), the association between cortisol secretion and gene expression under unstimulated conditions is insufficiently investigated. Given that little is known about the link between normal variation of cortisol secretion and gene expression throughout the day in humans, we aimed to assess the intra-individual coupling of cortisol levels and the expression of several glucocorticoid-responsive genes in peripheral blood under unstimulated conditions. The genes were selected from different glucocorticoid-regulated biological systems: *FKBP5* and *glucocorticoid-induced leucine zipper gene* (*GILZ*) as candidates of the HPA axis, the *PERIOD* genes (*PER1, PER2, PER3*) as candidates from the circadian system, and *serum deprivation-response protein* (*SDPR*) as a control gene. An important aim was to estimate time lags between cortisol secretion and gene expression to determine time windows where the largest degree of cortisol-mRNA coupling would be observed.

2. Materials and methods

2.1. Subjects and experimental design

Eighteen male participants were recruited from the general population (details see (Trifonova et al., 2013). Exclusion criteria, assessed by a nurse administered questionnaire on the experimental morning, were chronic or acute illnesses, intake of medication within the previous two weeks and a BMI < 18 or > 30. Samples were collected over a 6-week period with 3 experimental days per week in autumn to minimize the impact of seasonal cortisol fluctuations. For the final analyses, 17 donors (age range 19–38, mean 27.5 ± 6.7 years) were included; one participant was excluded because of insufficient RNA quantity and quality. Ethical approval of the study was obtained from the Luxembourg National Research Ethics Committee (CNER) and the National Data Protection Committee (CNPD) in accordance with the latest revision of the Declaration of Helsinki. All participants provided written informed consent.

The experimental design has been described elsewhere (Trifonova et al., 2013). Experimental procedure started from the time of arrival (from 07:00 h to 08:30 with a mean of 07:45 h). Participants remained at the institute for the whole experiment (from 7.5 h to 9 h), and were allowed to do normal ambulatory activity, receiving standardized meals at 09:00 h and 12:00 h.

2.2. Blood sampling and gene expression analysis

Blood sampling (Trifonova et al., 2013) and gene expression analysis (Yurtsever et al., 2016) were performed as previously published. Briefly, an indwelling intravenous cannula was inserted in the arm of the participants and EDTA blood was drawn every 15 min for the analysis of *FKBP5, GILZ, SDPR, PER1, PER2* and *PER3* mRNA. Gene expression was determined using whole blood. Stabilization of mRNA, total RNA isolation, cDNA synthesis and real-time PCR were performed under standardized conditions. Supplementary Table 1 shows the primer sequences and optimized PCR conditions. All primers were synthesized by Biomers (Ulm, Germany). For reliable analyses, each gene for each sample was examined in triplicates. MJ Opticon Monitor Analysis Software version 3.1 was used to compute the threshold cycle (Ct) values. Melting curve analyses for each PCR product revealed single melting peaks and no primer-dimer or artifacts. Ct values of all analyzed samples were normalized to the glyceraldehyde 3-phosphate dehydrogenase gene. The relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ relative quantification method (Schmittgen and Livak, 2008), with the last sampling time point as calibrator.

2.3. Statistical analysis

For the final analyses, data from 8:00 h to 16:00 h was used as the largest amount of data was available for the majority of donors during this time window. The association of cortisol concentrations in plasma and saliva with gene expression levels was determined via Pearson's product-moment correlation investigating time lags between -240 and + 240 min in 15 min intervals using IBM SPSS 24 (Armonk, New York). The correlations were calculated for each individual and then meta-analyzed for all subjects, using the DerSimonian-Laird (DSL) random-effect approach, implemented in the metacor package in R (Laliberte et al., 2010). The significance level was set to $\alpha < 0.05$. Additionally, Bonferroni corrected significant results (33 different time lags, 6 genes; $p < 0.00025$) are indicated.

3. Results

3.1. Cortisol profiles in plasma and saliva

Participants showed the typical diurnal cortisol pattern with a morning peak, a lunch-related peak and an afternoon nadir (See Trifonova et al., 2013 and Supplementary Fig. 1).

3.2. Correlation of plasma cortisol and gene expression levels

The expression of *FKBP5* followed plasma cortisol levels with the highest positive correlations observed at a lag of + 90 min ($r = 0.74$ (CI = 0.65-0.81), $p < 0.001$; Fig. 1A). A similar relation was observed for *GILZ* expression, with highest positive correlations observed at a lag of + 30 min ($r = 0.59$ (CI = 0.46-0.69), $p < 0.001$; Fig. 1B). A weak and negative correlation between plasma cortisol levels and *SDPR* expression was observed at a lag of -165 min (Fig. 1C). *PERIOD* genes did not show a clear correlation pattern with plasma cortisol (Figs. 1D-F).

3.3. Correlation of salivary cortisol and gene expression levels

Analyses with saliva cortisol revealed similar results. Salivary cortisol levels preceded the expression of *FKBP5* with the highest positive correlation observed at a lag of + 75 min ($r = 0.71$ (CI = 0.61-0.78), $p < 0.001$; Fig. 2A), while the highest positive correlation with *GILZ* expression was observed a lag of + 15 min ($r = 0.53$ (CI = 0.41-0.63), $p < 0.001$; Fig. 2B). There were no strong correlations of salivary cortisol levels with *SDPR* or *PERIOD* gene expression (Figs. 2C-F).

Supplementary Fig. 2 shows a representative participant, in whom plasma and saliva cortisol showed a close link with *GILZ* and *FKBP5* expression (Supplementary Fig. 2).

4. Discussion

The present study shows the temporal association between cortisol levels and the expression of six genes in whole blood of healthy participants under unstimulated conditions. This complements the results
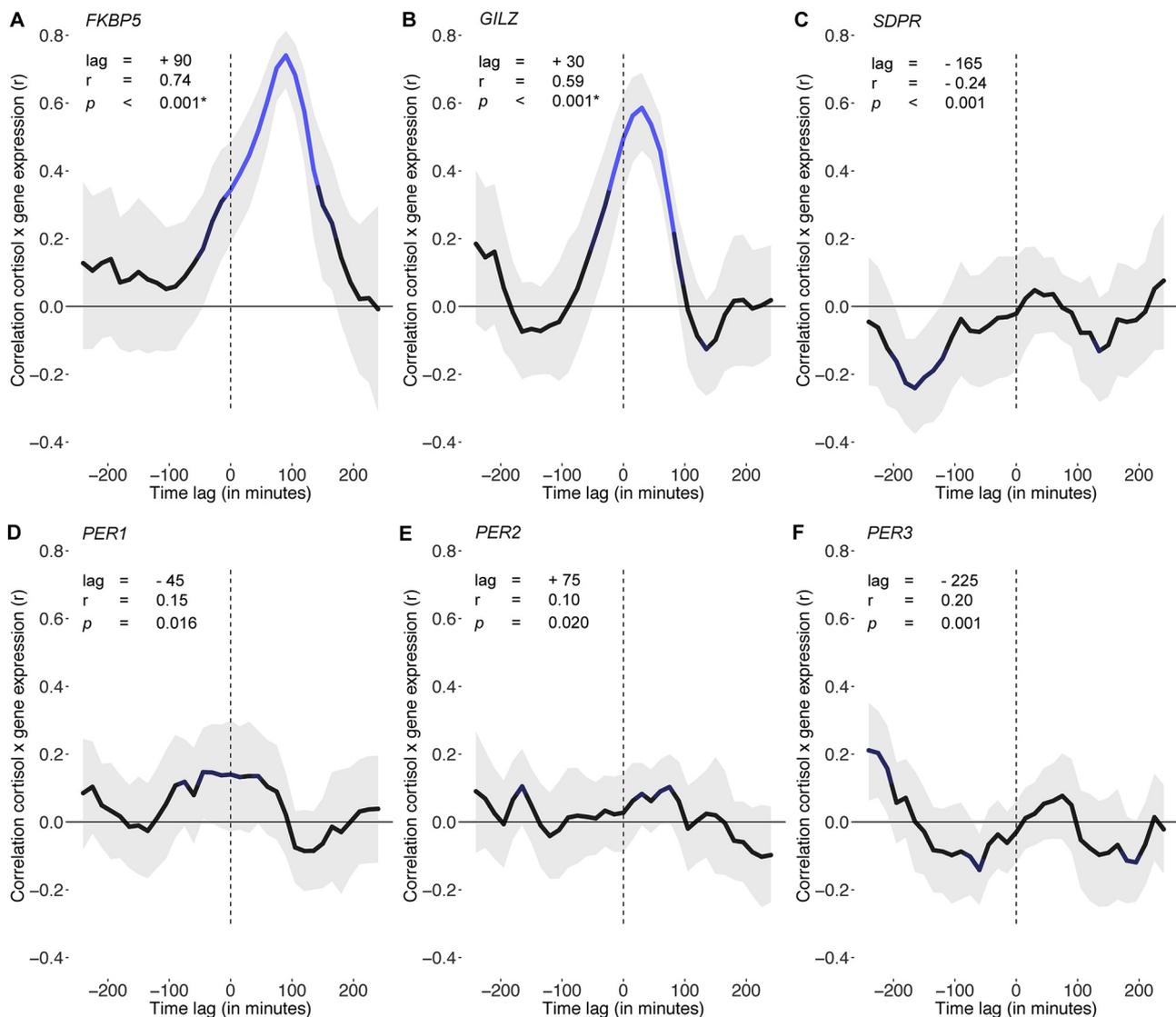
Fig. 1. Pearson's correlations between plasma cortisol and *FKBP5* (A), *GILZ* (B), *SDPR* (C), *PER1* (D), *PER2* (E) and *PER3* (F) expression. Correlations were calculated for each individual and then meta-analyzed. Time lags from -240 to + 240 min were assessed using 15 min intervals. Nominal significant correlations ($p < 0.05$) are displayed in dark blue, Bonferroni corrected results (33 different time lags, 6 genes; $p < 0.00025$) in bright blue. The grey shaded areas represent 95% confidence intervals. Correlation coefficient and the respective p value are given for the most significant lag for each gene. The dotted line indicates a lag of 0 min.

from studies investigating the link between cortisol release and gene expression following psychosocial stress (Schwaiger et al., 2016) or glucocorticoid administration *in vitro* and *in vivo* (Yurtsever et al., 2016; Arloth et al., 2015). We used a dense sampling scheme to capture cortisol and gene expression dynamics over the day. This enabled detailed analysis of time-lagged correlations, revealing strong positive correlations between both plasma and salivary cortisol levels and mRNA expression with gene-specific time lags. The strongest associations were observed for *FKBP5* and *GILZ,* whereas *PERIOD* genes and *SDPR* showed only weak correlations with cortisol concentrations.

The strongest positive correlations of *FKBP5* expression were determined at a lag of + 90 min with plasma cortisol, and at a lag of + 75 min with salivary cortisol. *GILZ* expression showed positive correlation as well, with a substantial shorter delay between cortisol and gene expression (+ 30 min plasma; + 15 min saliva). The shorter lag between gene expression and saliva cortisol compared to plasma cortisol refers a 15-minute delay, which is consistent with the result of Schlotz et al., 2008. This delay may be caused by the passive diffusion of free cortisol from blood to saliva.

Both *FKBP5* and *GILZ* are regulators of glucocorticoid actions and as

expected, showed strong positive correlations with cortisol levels in our study. *FKBP5*, coding for FKBP51, is one of the early target genes activated by ligand-bound GRs. When *FKBP5* is transcribed and translated, it resides in the cytoplasm to lower the affinity of GR for its ligand and to inhibit the nuclear translocation of the receptor. Therefore, GR activation of *FKBP5* results in an ultra-short negative feedback loop, where cellular sensitivity to glucocorticoids is decreased (Wochnik et al., 2005). Another early GR-target gene is *GILZ*, which is an essential mediator involved in the anti-inflammatory actions of glucocorticoids (Ayroldi and Riccardi, 2009). Through protein-protein interactions, GILZ inhibits the activity of various transcription factors (e.g. nuclear factor kappa B and activator protein 1) and regulates pro-inflammatory response genes in T cells (Ronchetti et al., 2015).

SDPR plays a critical role in caveolae biogenesis and is highly expressed in lung and fat tissues (Kovtun et al., 2015). Previous *in vitro* studies demonstrated that *SDPR* expression is stimulated by glucocorticoids (Rogatsky et al., 2003). However, the present study in humans does not support a strong coupling of *SDPR* expression with cortisol under unstimulated conditions. Similarly, *PERIOD* genes have been shown to contain GREs in their promoters, where GRs can bind
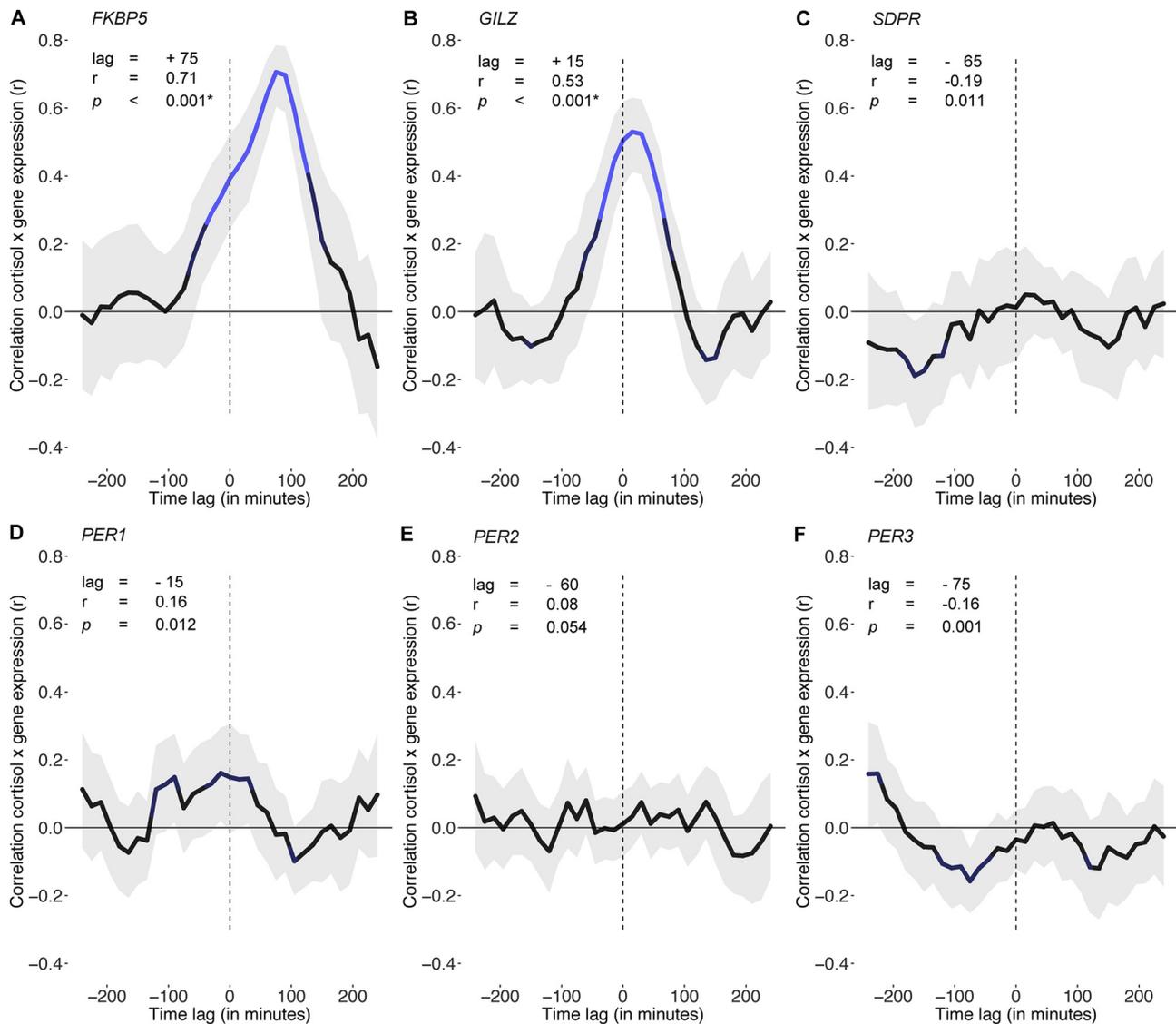
Fig. 2. Pearson's correlations between saliva cortisol and *FKBP5* (A), *GILZ* (B), *SDPR* (C), *PER1* (D), *PER2* (E) and *PER3* (F) expression. Correlations were calculated for each individual and then meta-analyzed. Time lags from -240 to + 240 min were assessed using 15 min intervals. Nominal significant correlations ($p < 0.05$) are displayed in dark blue, Bonferroni corrected results (33 different time lags, 6 genes; $p < 0.00025$) in bright blue. The grey shaded areas represent 95% confidence intervals. Correlation coefficient and the respective p value are given for the most significant lag for each gene.The dotted line indicates a lag of 0 min.

and regulate the transcription. Recently, we showed that glucocorticoid administration (Yurtsever et al., 2016) and psychosocial stress exposure (Schwaiger *et al.*, 2016) increased *PERIOD* gene expression. In the current study, we observed only weak correlations of *PERIOD* gene expression with cortisol, suggesting that a robust coupling between cortisol and *PERIOD* gene expression is only observed with stimulated cortisol release.

There are limitations of our study. In order to reduce the possible effects of sex-steroids on GR activation and therewith on the GR-target genes, only male subjects were included in the study. Furthermore, while the current study comprises a very dense sampling scheme to assess our research question, samples were only taken on one day, and the observed associations might not be stable over time. Lastly, only six candidate genes were selected for this study. Given the dense sampling scheme of > 30 sampling time points per individual, and the limited number of investigated genes, the study was well powered to detect medium to large associations, as observed for *GILZ* and *FKBP5*. However, for the investigation of smaller associations and a larger number of genes, bigger number of participants would be necessary.

In conclusion, we demonstrate that HPA axis-dependent gene

expression can also be investigated under unstimulated conditions. While strong and highly significant associations were observed between cortisol and the expression of *FKBP5* and *GILZ*, this was not the case for the other genes tested here. The present results indicate that the coupling between cortisol and *PERIOD* gene expression differs between *in vitro* and *in vivo* studies. Differences might also exist between stimulated and unstimulated conditions, as some genes might only show a response to more pronounced glucocorticoid levels, as released under acute stress, or in a pharmacological challenge. The reported gene-specific time-lags can guide research investigating glucocorticoid signaling assessing cortisol-associated modification of gene expression in healthy subjects and clinical samples, and highlight that cross-sectional designs are limited in capturing the full extent of these associations.

Declaration of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.033.

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