Cortisol involvement in mechanisms of behavioral inhibition

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Abstract
We studied whether baseline cortisol is associated with post-error slowing, a measure that depends upon brain areas involved in behavioral inhibition. Moreover, we studied whether this association holds after controlling for positive associations with behavioral inhibition scores and error-related negativity (ERN) amplitudes that cortisol and post-error slowing may share. Healthy female volunteers performed a flanker task. Cortisol was independently positively associated with post-error slowing and the ERN, supporting hypotheses that cortisol is involved in behavioral inhibition. Additionally, cortisol mediated an association between ERN and more post-error slowing, which suppressed a direct association between ERN and less post-error slowing. The results are relevant, not only for researchers of behavioral inhibition, but also for researchers of the basic mechanisms of the ERN and post-error slowing, and may bring those literatures together.

Descriptors: Post-error slowing, Cortisol, Error-related negativity, Behavioral inhibition system

In humans, levels of the hormone cortisol have been related to punishment sensitivity (van Honk, Schutter, Hermans, & Putman, 2003), and it has been hypothesized that high levels of cortisol are involved in mechanisms of behavioral inhibition, perhaps by inhibiting dopaminergic approach (i.e., reward-seeking) systems (e.g., Tops, van der Pompe, et al., 2004; Tops, 2004). Behavioral inhibition, the inhibition of the initiation of behavior or behavioral responding in the context of novelty, signs of threat, or social evaluation (Fox, Henderson, Marshall, Nichols, & Ghera, 2005) may be part of a submissive behavioral repertoire that includes inhibition of aggression, which has also been linked to increased cortisol levels (e.g., Putman, Hermans, & van Honk, 2007; van Honk et al., 1998). At least in children, behavioral inhibition has been consistently related to high baseline cortisol levels (Fox et al., 2005). Effects of cortisol administration or stress-induced cortisol increases have been studied on behavioral and electroencephalographic (EEG) measures of approach-inhibition such as free recall positivity bias (Tops et al., 2003, 2004), frontal EEG asymmetrical activity (Schmidt, Fox, Goldberg, Smith, & Schulkin, 1999; Tops et al., 2005; Tops, van Peer, Wester, Wijers, & Korf, 2006), rotational behavior (Tops, Wijers, Koch, & Korf, 2006) and congruity between observed emotional facial expressions and performed hand gestures (Roelofs, Elzinga, & Rotteveel, 2005; Roelofs et al., 2009; van Peer et al., 2007). The results have not been consistently affirmative across studies and measures, for instance, effects on facial expression–hand gesture congruity has been found to be absent (van Peer et al., 2007) or independent of facial valence (Roelofs et al., 2005).

Individual differences in punishment sensitivity and behavioral inhibition can be measured by the Behavioral Inhibition System (BIS) scale (Carver & White, 1994). This scale is derived from the theory postulated by Gray (1982, 1989) and proposes two interacting motivational systems: the behavioral approach system (BAS) and the behavioral inhibition system (BIS). According to Gray, the BIS is sensitive to signals of punishment and reward omission and inhibits behavior that may lead to aversive or harmful outcomes. In contrast, the BAS is proposed to be sensitive to positive signals of reward. In addition, these two motivational systems are proposed to depend on separate, but interacting, neural circuits: the BIS was proposed to comprise septal cholinergic projections that inhibit dopaminergic behavioral approach systems. In most recent formulations of the theory, BIS maintains vigilance for unexpected stimuli, conflict, and incongruity in the environment, directs attention to such stimuli when detected, and resolves conflict by inhibiting ongoing action in order to facilitate the processing of these stimuli and biasing action toward defensive behavior (Gray & McNaughton, 2000).

In this study, we investigated whether cortisol levels are related to a behavioral measure of inhibition: post-error slowing. Paradigms such as the flanker task produce such a post-error
slowing effect; subjects typically slow down after committing an error to avoid making a subsequent mistake (Rabbit, 1966), which has been suggested to reflect activity of the BIS (Kleiter & Schwarzenbacher, 1989) and correlated positively to BIS scores (Boksem, Tops, Kostermans, & De Cremer, 2008), trait distress reactivity (Larson, Fair, Good, & Baldwin, 2010; Luu, Collins, & Tucker, 2000), trait worry (Compton, Lin, et al., 2008) and, in adolescents, to ratings of behavioral inhibition obtained in childhood (Fox, 2010). Developmental and brain imaging research suggests that post-error slowing reflects a mechanism of response inhibition (Gupta, Kar, & Srinivasan, 2009; Marco-Pallarés, suggests that post-error slowing reflects a mechanism of response inhibition (Gupta, Kar, & Srinivasan, 2009; Marco-Pallarés, Camara, Münte, & Rodriguez-Fornells, 2008). Indeed, slowing after errors and after failures to inhibit responding have been related to right inferior frontal gyrus (IFG) activation (Hester, Barre, Mattingley, Foxe, & Garavan, 2007; Li et al., 2008; Marco-Pallarés et al., 2008) and lesions of the right inferior frontal sulcus reduced post-error slowing (Molenbergs et al., 2009). Areas of neural activation have been found in the right IFG, anterior insula (AI), and anterior cingulate cortex (ACC) that are common to inhibiting responses, approach, and emotions (Avila, Parcet, & Barrós-Loscertales, 2008; Shafritz, Collins, & Blumberg, 2006; Stone, Connolly, Wynne, Alhusaini, & Garavan, 2009). Of these areas the right IFG/AI, which is part of the BIS (McNaughton & Corr, 2004), has previously been shown to be critical for motor response inhibition (Aron, Robbins, & Poldrack, 2004; Chikazoe, Konishi, Asari, Jimura, & Miyashita, 2007).

The flanker task is also used in studies of the error-related negativity (ERN), which may reflect the BIS (Amadio, Master, Yee, & Taylor, 2008). The ERN is a negative event-related potential with a fronto-central scalp distribution, peaking 60–110 ms after an error response (Falkenstein, Hohnsbein, Hoermann, & Blanko, 1990; Gehring, Coles, Meyer, & Donchin, 1990). Just like the BIS, the ERN is thought to reflect inhibition of dopaminergic systems in response to punishment, reward omission, and performance errors (Gray, 1982, 1989; Holroyd & Coles, 2002). Although associations between ERN and post-error slowing have not always been found, they have been reported in children, adolescents, and adults (Gehring, Goss, Coles, Meyer, & Donchin, 1993; Ladouceur, Dahl, & Carter, 2007; Scheffers & Coles, 2000; West & Travers, 2008) and in both between- and within-subject designs (Debener et al., 2005). The ERN is thought to be generated in the ACC, an area that is also involved in autonomic and hormonal control (Critchley, 2005). ERN amplitudes have been associated with BIS scores (Amadio et al., 2008; Boksem, Tops, Wester, Meijman, & Lorist, 2006; Boksem et al., 2008), cortisol levels (Tops, Boksem, Wester, Lorist, & Meijman, 2006) and cortisol responses to social evaluative threat in high BIS subjects (Cavanagh & Allen, 2008). Indeed, the ERN, BIS, and cortisol responses have all been related to social evaluative threat (Cavanagh & Allen, 2008; Hajcak, Moser, Yeung, & Simons, 2005). Moreover, adolescents who showed high behavioral inhibition in childhood displayed enhanced ERN amplitudes and post-error slowing: ERN amplitude moderated the relationship between early behavioral inhibition and later clinically significant anxiety disorders (Fox, 2010; McDermott et al., 2009).

In the present study, we investigated the relationship in healthy student subjects between basal salivary cortisol level and post-error slowing during a flanker task. We focused on basal levels just before the start of task performance because stress-induction and fatigue may impact on task performance in unintended ways. Indeed, at least in children, behavioral in inhibition has been consistently related to high baseline cortisol levels but less consistently to increased cortisol responses to stress (Fox et al., 2005). Similarly, in adults high BIS scores were related to decreased ERN amplitude and deficits in task performance during stress (Cavanagh & Allen, 2008). We studied whether cortisol is positively associated with post-error slowing. Moreover, we studied whether this association holds after controlling for positive associations with BIS scores and ERN amplitudes that cortisol and post-error slowing may have in common. Confirming this hypothesis would provide converging evidence of an association of cortisol with behavioral inhibition, using a measure that has the advantage over previously used measures that it has been shown to depend upon activity in brain areas that are known to be important in aspects of behavioral inhibition.

Methods

Subjects

Eighteen healthy right-handed female participants, between 18 and 27 (M = 20, SD = 3.6) years of age, were recruited from the university population. They were paid for their participation and had normal or corrected-to-normal vision. None of the subjects worked night shifts or used prescription medication. Written informed consent was obtained prior to the study.

Task

We used a version of the Eriksen Flanker Task (Eriksen & Eriksen, 1974). On each trial, a five-letter string was presented. The central letter was the target, the remaining letters the flankers. The stimuli used for targets and flankers were the letters H and S. The assignment of letter to response hand was balanced between participants. During the entire task, a fixation mark was displayed 0.14 degrees above the target letter. On congruent trials, the target letter was the same as the flankers (SSSSS); on incongruent trials the target letter differed from the flankers (SSHSS or HHHHH). Forty per cent of the trials consisted of incongruent stimuli, and 60% consisted of congruent stimuli. Congruent and incongruent trials were presented in random order.

The stimuli were presented on a 17-inch monitor. The letters were white against a black background, and each letter had a height and width of 0.24° visual angle. Eriksen and Eriksen (1974) showed that reaction times and error rates were highest when letters were presented close together. Therefore, we presented letters 0.05° apart. The complete five-letter string had a width of 1.43° visual angle.

In addition, flankers were presented 100 ms prior to target onset to maximize the expected flanker compatibility effect (Kopp, Rist, & Mattler, 1996). Target and flankers disappeared simultaneously at the moment a response was made. In case no response was given, targets and flankers disappeared after 1200 ms. The interstimulus interval was 3 s. Participants received seven blocks of 400 trials. Each block had a total duration of 20 min.

Questionnaires

Behavioral Inhibition System. We used the BIS subscale from the Dutch version (Franken, Muris, & Rassin, 2005) of the BIS/BAS-scale created by Carver and White (1994) to assess dispositional behavioral inhibition. This BIS scale (range: 7–28)
comprises seven items which subjects endorse on a 4-point scale from 1 ("very true for me") to 4 ("very false to me"). Cronbach’s alpha was .76.

Salivary Cortisol
Saliva samples were taken with a Salivette (Sarstedt Inc., Rommelsdorf, Germany). Analyses of salivary cortisol were performed in the biochemical laboratory of the University of Trier. Saliva samples were stored at −20°C until analysis. Cortisol concentration in saliva was measured using a time-resolved fluorescence immunoassay, as described in detail in Dressendorfer, Kirschbaum, Rohde, Stahl, and Strasburger (1992).

Procedure
Subjects were instructed to abstain from alcohol 24 h before the experiment and from caffeine containing substances 12 h before the experiment. At arrival at the laboratory at 12.00 hours, subjects were given written task instructions where after they were trained in performing the task for 15 min. Following the application of the electrodes, subjects were seated in a dimly lit, sound-attenuated, electrically shielded room at 1.20 m from the screen. Their index fingers rested on touch-sensitive response boxes. Subjects were instructed to lift their finger from the response button as quickly as possible when a target was presented, maintaining a high level of accuracy. Immediately before task performance, on average 45 min after arriving in the laboratory, a saliva sample was collected.

Electrophysiological Recording and Data Reduction
The EEG was recorded using 60 Sn electrodes attached to an electro cap (Electro-Cap International, Inc., Eaton, OH). All electrodes were referenced to averaged earlobes. The electro-oculogram (EOG) was recorded bipolarly from the outer canthi of both eyes and above and below the left eye, using Sn electrodes. Electrode impedance was kept below 5 kΩ. EEG and EOG were amplified with a 10-s time constant and a 200-Hz low pass filter, sampled at 1000 Hz, digitally low pass filtered with a cut-off frequency of 70 Hz, and online reduced to a sample frequency of 250 Hz.

All ERP analyses were performed using the Brain Vision Analyzer software (Brain Products, Gilching, Germany). ERPs were averaged off-line. The data was further filtered with a 0.53-Hz high-pass filter and a slope of 48 dB/oct and a 40-Hz low-pass filter with a slope of 48 dB/oct. Out-of-range artefacts were rejected and eye movement artefacts were corrected, using the Gratton, Coles, and Donchin method (Gratton, Coles, & Donchin, 1983). A baseline voltage over the 100-ms interval preceding the response was subtracted from the averages.

Data Analysis
Performance. For the different stimulus conditions, mean reaction times were calculated. Correct reactions occurring within a 150–1000-ms interval after stimulus presentation were considered as hits. Erroneous reactions occurring within this interval were considered as errors. Responses outside of this interval (also non-responses) were considered misses. To investigate strategic changes after error detection, we analyzed reaction times on correct trials following an error minus reaction times on correct trials following a correct response (i.e., post-error slowing; Kleiter & Schwarzenbacher, 1989; Rabbit, 1966). As we found no difference in post-error slowing for congruent and incongruent n−1 trials, our measure of post-error slowing includes both.

ERPs. Mean ERN amplitudes were calculated at Cz, where visual inspection showed this component was maximal. We quantified the ERN on error trials as the most negative peak occurring in the 100 ms following an erroneous response. For statistical analyses, we used the average amplitude of the ERN in a time window starting 20 ms before the peak until 20 ms after the peak. Measuring the amplitude as an area around the peak makes the data more reliable, as it reduces the impact of extreme data-points. We processed the ERP elicited by correct trials in exactly the same manner. In addition, to arrive at a measure that reflects activity associated with error-processing only, we created difference waves by subtracting amplitudes elicited on correct trials from those elicited by incorrect trials, creating a ‘difference ERN,’ which we will refer to as ERN.

Statistical Analyses
For the present analysis, we used data from a study of time-on-task effects on task engagement that we present elsewhere (Tops & Boksem, 2010). To study time-on-task effects, the subjects had to perform the flanker task for an exceptionally long time (2.5 h). Because from previous studies we know that performance and ERN amplitudes deteriorate quickly during prolonged performance of the present task (Boksem, Meijman, & Lorist, 2006; Tops, Boksem, et al., 2006), as do associations between ERN and measures of individual differences (Luu et al., 2000) and, indeed, post-error slowing was only significant in the first block, we analyzed and report only data from the first block. Only the first block is comparable to other studies that do not study time-on-task effects; it does not show fatigue effects that may interfere with behavioral inhibition processes, and it is closest in time to the cortisol measurement. We performed regression analyses of cortisol level as dependent variable and post-error slowing, BIS, and ERN amplitude as independent variables. In an additional analysis, we used post-error slowing as dependent variable and cortisol, BIS, and ERN amplitude as independent variables. We did not assume directionality of relationships, but merely used the regression analyses to investigate which variables independently related to cortisol levels and post-error slowing. All statistical tests of significance were two-tailed.

Results
Reaction times on incongruent flanker trials (M = 488 ms, SD = 56) were longer than on congruent trials (M = 428, SD = 64; F(1,17) = 130.95, p < .001). Also, error rates were lower on congruent trials (3.1%) compared to incongruent trials (10.4%; F(1,17) = 20.07, p < .001). The overall error rate was 6.7%, which amounts to 26.8 (SD = 15) erroneous responses on average per subject. Almost all of these error-trials (18.2 on average, SD = 9.5; range 7–35) were also included in the ERN analyses, indicating a very low loss of data in the ERP analysis due to signal artefacts. Reaction times and errors were not related to BIS scores, ERN amplitude, or cortisol levels. Misses were very rare, 0.8% = 3.2 trials on average per subject, and not included in the analyses.

Mean post-error slowing was 23 ms (SD = 35; t(17) = 2.82, p < .05). Mean BIS score was 21.8 (SD = 3.6). Mean ERN amplitude was −7.59 μV (SD = 6.57); mean ERN amplitude on
Higher cortisol was associated with more post-error slowing (Table 1; Figure 1), higher BIS scores (trend level), and a more negative ERN amplitude (Table 1; Figures 1 and 2). Figure 1A shows that individuals with higher cortisol levels showed post-error slowing, while individuals with low cortisol levels showed no post-error slowing; Figure 1B shows that individuals with higher cortisol levels showed clear ERNs, while some individuals with low cortisol levels evidenced no ERN. We performed a regression analysis of cortisol level as dependent variable and post-error slowing, BIS, and ERN amplitude as independent variables. We did not assume directionality of relationships, but merely investigated which variables independently related to cortisol levels. Table 1 shows that only ERN and post-error slowing remained related to cortisol levels, while the original trend-level correlation of BIS score with cortisol level no longer approaches significance. Similarly, to also study association of ERN and BIS with post-error slowing, we performed a regression analysis of post-error slowing as dependent variable and cortisol, BIS, and ERN amplitude as independent variables. Table 1 shows that the ERN but not BIS was associated with slowing. Larger ERN amplitudes were associated with less post-error slowing.

Figure 3A presents a depiction of the relationships between cortisol, post-error slowing, and ERN in terms of Pearson’s and partial correlations. The similarities of the partial correlations in this figure with those in Table 1 shows that BIS score has little influence on those relationships. The partial correlations in Figure 3A show that ERN amplitude appears significantly associated with both post-error slowing and cortisol, even though this association between ERN and post-error slowing did not show up using only Pearson’s correlations. Apparently, the positive path through cortisol cancels out the significant negative direct association between ERN and post-error slowing, observed when partialling out this correlation with cortisol. We tested the significance of this inconsistent mediator/suppressor effect of cortisol on the relationship between ERN and post-error slowing (MacKinnon, Krull, & Lockwood, 2000) using the MacKinnon, Lockwood, Hoffman, West, and Sheets (2002) distribution of products method. As cortisol proved to be significantly related to both ERN amplitude and post-error slowing, this mediation test provides a good balance of Type I error and statistical power in small samples (MacKinnon et al., 2002). This analysis showed that cortisol mediates a positive association between ERN and post-error slowing, while the direct relation between ERN and slowing is actually negative (P = −13.28, p < .05).

Based on MacKinnon et al. (2000), a suppressor is a third variable that increases the predictive validity of another variable (or set of variables) by its inclusion in a regression equation. A suppressor effect would be present when the direct and mediated effects of an independent variable have opposite signs. If the opposite direct and mediated effects are of similar magnitude, then they will cancel each other out in the overall relationship (e.g., the Pearson’s correlation will be close to zero). However, including the third suppressor variable in the regression equation will make the direct effect evident and at the same time demonstrate that the third variable mediated an opposite, suppressor effect. This is the situation described above and depicted in the top diagram of Figure 3, where including cortisol as a predictor made the direct association between larger ERN and less post-error slowing evident; mediation analysis showed that cortisol is a suppressor of a negative association between ERN amplitude and post-error slowing. Statistically, suppression is equivalent to inconsistent mediation and confounding, and differs from “negative confounding” only on conceptual grounds (i.e., it depends on the hypotheses studied; MacKinnon et al., 2000).

Many studies relating ERN amplitude to individual differences measures of temperament or emotionality used the ERN on error trials, instead of the difference between the ERN on error and correct trials (e.g., Boksem, Tops, et al., 2006, 2008; Hajcak, McDonald, & Simons, 2004; Luu et al., 2000; McDermott et al., 2009; Tops, Boksem, et al., 2006). It has been suggested that measures of temperament relate better to the ERN on error trials than to the difference ERN because such measures

### Table 1. Regression Analyses with Cortisol Level or Post-Error Slowing as Dependent Variable

<table>
<thead>
<tr>
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<th>Beta</th>
<th>t</th>
<th>p</th>
<th>r</th>
<th>Partial r</th>
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</thead>
<tbody>
<tr>
<td>Dependent: Cortisol</td>
<td>R² = .70, F(3,14) = 9.36, p &lt; .01</td>
<td></td>
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<tr>
<td>Post-error slowing</td>
<td>−.86</td>
<td>−4.15</td>
<td>.001</td>
<td>.66*</td>
<td>.77</td>
</tr>
<tr>
<td>ERN</td>
<td>−.07</td>
<td>−0.40</td>
<td>.694</td>
<td>.39</td>
<td>.12</td>
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<tr>
<td>BIS</td>
<td>.02</td>
<td>.92</td>
<td>.376</td>
<td>.23</td>
<td>.26</td>
</tr>
</tbody>
</table>

Note: p-values apply to both t-tests and partial correlations; r: Pearson’s correlation between independent variable and cortisol level (*p < .05; 'p < .10); Partial r: partial correlation between independent variable and cortisol level after partialling out the variance of cortisol that is explained by the other independent variables. ERN: amplitude on error trials minus on correct trials, inversely signed such that higher positive values mean larger amplitude.
tend to relate to larger ERN amplitudes on correct trials, as well (Endrass, Klawohn, Schuster, & Kathmann, 2008; Hajcak et al., 2004; Tops, Boksem et al., 2006). Indeed, in the present study BIS score correlated with ERN magnitude on error trials ($r = .53, p < .05$) but tended to correlate in the same direction to the ERN on correct trials ($r = .34, p > .10$) which rendered the correlation with the difference ERN nonsignificant ($r = .27, p > .10$). The ERN on error trials was highly correlated to the difference ERN ($r = .82, p < .001$) and related to the other measures similarly but less strongly compared to the difference ERN. However, Figure 3B shows that the ERN on error trials appears to mediate the relationship between BIS and cortisol: it is associated with both, and including it as predictor in the analyses almost completely abolishes the trend-level association between BIS and cortisol. However, because most of the associations in Figure 3B do not reach significance, we only present this pattern of associations so that larger studies in the future may investigate whether the ERN on error trials really mediates an association between BIS and cortisol. This particular association may be important but is not the main interest in the present study.

Discussion

We found that cortisol related positively to post-error slowing and ERN amplitude. The association between cortisol and post-error slowing was independent from the association between cortisol and ERN amplitude, while a positive trend-level association between BIS scores and cortisol seemed to be mediated by their associations with a larger ERN on error trials. Although we cannot really address this suggested mediation by ERN of an association between BIS and cortisol because of lack of power, we note mounting evidence that attention or error monitoring mediates the relation between behavioral inhibition and stress (Cavanagh & Allen, 2008; Fox, 2010; McDermott et al., 2009). The association between BIS and the ERN on error trials replicates previous studies in which BIS scores were related to error-trial ERN amplitude (Amodio et al., 2008; Boksem, Tops, et al., 2006, 2008) and also to the ERN in response to performance feedback (Balconi & Crivelli, 2010; De Pascalis, Varriale, & D’Antuono, 2010). In addition, trait low behavioral control has been related to smaller ERN amplitude (Stahl & Gibbons, 2007). Most importantly, the positive association between cortisol and post-error slowing is consistent with hypotheses that cortisol is involved in behavioral inhibition (e.g., Tops et al., 2005).

The present results suggested an association between ERN amplitude and decreased post-error slowing. The ERN is thought to be generated in areas of the ACC related to adaptive post-error changes in response behavior, such as improvement in response speed following an error, which suggests increases in cognitive control (Kerns et al., 2004). However, the literature on ACC activity in relation to post-error changes in behavior is inconsistent (Hester et al., 2007). Similarly, many studies found no association between the ERN and post-error slowing, although positive associations have been reported in children, adolescents, and adults (Gehring et al., 1993; Ladouceur et al., 2007; Scheffers & Coles, 2000; West & Travers, 2008) and in both between- and within-subject designs (Debener et al., 2005), indicating that a relation exists between ERN amplitude and increased post-error slowing. The present results suggest that a mechanism of cortisol-related post-error slowing may suppress the relationship between ERN amplitude and decreased slowing: an association between ERN and decreased slowing was only revealed after controlling for cortisol levels. Cortisol mediated a positive association between ERN amplitude and post-error slowing.
Consistent with post-error speed being controlled or influenced not only by ACC but also by other mechanisms, post-error slowing often is not related to better accuracy; indeed, it has even been related to less efficient performance (e.g., Carp & Compton, 2009; Compton, Robinson, et al., 2008; Notebaert et al., 2009). There are indications that post-error slowing is related to orienting and arousal responses to errors, and to failure to disengage from the error (e.g., Carp & Compton, 2009; Compton, Robinson, et al., 2008). For instance, slowing occurs after unexpected events whether they are correct or erroneous responses (Notebaert et al., 2009; Núñez Castellar, Kühn, Fias, & Notebaert, 2010). Errors are followed by increased autonomic arousal (Hajcak, McDonald, & Simons, 2003; Kleiter & Schwarzenbacher, 1989), potentiated defensive startle reflex (Hajcak & Foti, 2008), and increased cortical arousal, as measured by changes in EEG alpha power (Carp & Compton, 2009). The results by Carp and Compton (2009) indicated that, whereas after correct responses subjects transiently disengage during the intertrial period, after errors they failed to disengage, and this predicted increased post-error slowing. Indeed, depression is associated with a decrease in accuracy, increased slowing and inability to disengage after errors (Compton, Lin, et al., 2008; Tucker, Luu, Frishkoff, Quiring, & Poulsen, 2003), and ERN amplitude predicted post-error slowing only among depressed participants in a Stroop task condition involving negative words (Compton, Lin, et al., 2008). In addition, post-error slowing dramatically increased and performance became more error prone with a decreasing response stimulus interval (Dudschig & Jentzsch, 2009), providing further evidence for the idea that error evaluation can produce substantial interference with subsequent trial processing, particularly when there is insufficient time between the error and the subsequent event. In contrast, in a task in which an error on a difficult “lure” trial predicted that the same lure would be repeated between two and seven trials later, such that effects of post-error failure to disengage were unlikely to interfere with performance on the next lure trial, post-error slowing was related to increased accuracy on the next lure trial (Hester et al., 2007).

By what mechanisms may cortisol increase post-error slowing and ERN amplitude? One possibility is that individuals who began the task with greater levels of cortisol were also more aroused or engaged in the task itself, which may be reflected in larger ERN amplitudes and more post-error slowing (Tops, Boksem, et al., 2006). Second, the ability of cortisol to increase dopaminergic activity may be implicated (Pruessner, Champagne, Meaney, & Dagher, 2004; Tops, van Peer, Wijers, & Korf, 2006). The ERN is thought to be generated by dopaminergic mechanisms, and ERN amplitude is increased and decreased by dopaminergic stimulants and blockers, respectively (de Bruijn, Hulstijn, Verkes, Ruitg, & Sabbe, 2004; de Bruijn, Sabbe, Hulstijn, Ruitg, & Verkes, 2006; Zirnheld et al., 2004). As these manipulations left performance and post-error speed largely unaffected, they may abolish relationships between ERN amplitude and post-error slowing. At the same time, at the cortical control level, dopamine is thought to be involved in behavioral constraint and inhibition (Sallet & Rushworth, 2009; Tops, Boksem, Luu, & Tucker, 2010; Tops & Boksem, 2010; Tucker, Luu, & Pribram, 1995) and in the looping and iterative processing of old, redundant, or threatening information thought to cause post-error slowing (Kleiter & Schwarzenbacher, 1989; Tucker, Luu, & Pribram, 1995). We suggest the hypothesis that cortisol may stimulate behavioral inhibition by facilitating cortical dopaminergic function.

Alternatively, along the line of suggestions that trait ERN amplitude relates to stress reactivity or susceptibility to developing social anxiety (Cavanagh & Allen, 2008; McDermott et al., 2009), trait ERN amplitude may relate to cortisol levels. Such a relationship could be explained by the involvement of ACC and IFG/AI areas in autonomic and hormonal regulation (e.g., Critchley, 2005; Libezon et al., 2007; Suzuki et al., 2009; Wang et al., 2005). However, those suggestions that trait ERN amplitude relates to stress reactivity or susceptibility to developing social anxiety (Cavanagh & Allen, 2008; McDermott et al., 2009) are inconsistent with a study in which ERN amplitude and error correction predicted less emotional reactivity to stress in daily life.

1It should be noted that dopaminergic agonists and antagonists appear to acutely increase and decrease plasma cortisol levels, respectively (Fuller et al., 1983; Kitchen, Kelly, & Turner, 1988).
Perhaps relations between adaptive cognitive control and decreased emotional reactivity to stress may have higher likelihood of being detected in studies using the difference ERN measure, which reflects efficient error detection (Compton, Robinson, et al., 2008). In contrast, the likelihood of finding relations between post-error slowing, ERN, and increased stress susceptibility may be increased by differentiating individuals on the basis of variables such as behavioral inhibition or cortisol level, and by using the ERN on error trials (Cavanagh & Allen, 2008; McDermott et al., 2009). Individual differences in negative affectivity, stress reactivity, and obsessive compulsive symptom severity appear not to relate strongly to the difference ERN because they tend to relate to the ERN on correct trials as well (Endrass et al., 2008; Hajcak et al., 2004; Tops et al., 2006). Perhaps this relationship with the ERN on correct trials reflects a similar mechanism as proposed above for post-error slowing, such as a failure to disengage mechanisms of error-processing and increased accuracy bias triggered or primed by errors on previous trials. The adaptive performance monitoring function best reflected in the difference ERN may reflect the activity of cognitive dorsal ACC and related network areas implicated in proactive action control and emotion regulation, while the emotional reactive and inhibitory function best reflected in the ERN on error trials may reflect the activity of ventral/rostral ACC and related ventral networks including the IFG/AI implicated in reactive motor control (Critchley, 2005; Tops, Boksem, et al., 2010; Tucker et al., 1995).

It has been reported that post-error slowing (Jentzsch & Leuthold, 2006) and ERN amplitude (Gehring et al., 1993) were larger when instruction stressed accuracy rather than speed. Post-error slowing is usually explained by strategic control adjustments towards a more conservative response threshold (Jentzsch & Duschig, 2009). Using functional magnetic resonance imaging (fMRI), Ivanoff, Branning, and Marois (2008) showed that emphasizing the speed of a perceptual decision at the expense of its accuracy lowers the amount of perceptual evidence-related activity in the IFG/AI that is gathered before responding. Moreover, this speed-accuracy difference in activity correlated with a behavioral measure of speed-accuracy difference in decision criterion. The IFG seems involved in withholding responses when increased processing is needed for accurate responses (Leitman et al., 2010), which may also be its role in slowing processes such as post-error slowing. Indeed, right IFG activation is related to post-error slowing after errors (Marco-Pallarés et al., 2008) and failures to inhibit responding (Li et al., 2008), and lesions of the right inferior frontal sulcus reduced post-error slowing (Molenberghs et al., 2009). In the study in which an error on a difficult “lure” trial predicted that the same lure would be repeated between two and seven trials later, such that effects of post-error failure to disengage were unlikely to interfere with performance on the next lure trial, post-error slowing was related to increased accuracy on the next lure trial; the slowing and the increased accuracy were predicted by activity in right IFG/AI, middle frontal gyrus, and ACC (Hester et al., 2007).

Neuroimaging studies implicate the IFG/AI in behavioral inhibition and anxiety. Authors have argued that this area is involved in the restraining of inappropriate responses (Garavan, Ross, & Stein, 1999) and judging the appropriateness of facial affect (Kim et al., 2005). Left IFG/AI has additionally been associated with dopamine release in response to negative emotional stimuli (Badgaiyan, Fischman, & Alpert, 2009). Avila et al. (2008) presented infrequent stop signals after generating a dominant response set for reward and found activation of right IFG that seemed to antagonize activity in dopaminergic areas (the dorsal striatum and the medial prefrontal cortex). In a reinforcement learning task, activation of IFG/AI was more pronounced for risk-averse participants, suggesting that this region also serves to inhibit risky choices (d’Acremont, Lu, Li, Van der Linden, & Bechara, 2009). The right IFG/AI has also been consistently associated with social anxiety disorder, and the AI with anxiety in general (Etkin & Wager, 2007). Of note, the insula has been implicated in the regulation of automatic arousal and neuroendocrine responses to psychological stress (Craig, 2005; Libezon et al., 2007; Suzuki et al., 2009; Wang et al., 2005). The right IFG/AI may have an alarm function as part of its critical role in the switching between internally and externally oriented control modes (Sridharan, Levitin, & Menon, 2008) and consistently shows error-related activity (Wittfoth, Küstermann, Fahlé, & Herrmann, 2008) consistent with an alarm function of error-related brain signals (Tucker et al., 2003). In short, the IFG/AI may be the interface where behavior inhibition including post-error slowing, error-processing, and cortisol regulation interact.

Starting from the developmental research by Jerome Kagan and colleagues, the physiological parameters that have classically been associated with behavioral inhibition are high cortisol levels and relative right frontal EEG activity asymmetry (Fox et al., 2005). The tendency for right lateralization of inhibition-related activity in the IFG/AI may underlie these associations. BIS scores and behavior inhibition in adults (Balconi & Mazza, 2009; Harmon-Jones & Allen, 1997; Peterson, Gable, & Harmon-Jones, 2008; Shackman, McNemarin, Maxwell, Greischar, & Davidson, 2009; Wacker, Chavanon, Leue, & Stemmler, 2008) and behavioral inhibition in children (see Fox et al., 2005) have been related to relative right frontal activity. It has been suggested that this frontal asymmetry reflects asymmetrical AI/IFG activity (Craig, 2005; Tops & Boksem, 2010; Tucker et al., 2003) and a meta-analysis of emotional faces processing found a relation between approach vs. avoidance dimensions and left vs. right IFG (Fusar-Poli et al., 2009). Indeed, a recent study using source modeling found support for activity in right IFG explaining the association between BIS scores and relative right frontal EEG activity (Shackman et al., 2009). Moreover, frontal asymmetrical activity has been related to cortisol levels (see Tops et al., 2005), and exogenous cortisol has even been found to affect this asymmetry (Tops et al., 2005; Tops, Wijers, et al., 2006; Tops, van Peer, et al., 2006).

The present study has obvious limitations and should be regarded as preliminary. All results are correlational, and no causality can be inferred. The number of subjects was small, and replication in a larger sample is warranted before firm conclusions can be drawn. Cortisol measurement depended on one saliva sample, limiting its reliability as a measure of individual differences. Moreover, associations with stress-induced cortisol responses or other measures of hypothalamic-pituitary-adrenal cortex function were not addressed. The inclusion of only female students as subjects prevents generalization to other groups such as males or childhood behavioral inhibition. On the other hand, the present study was guided by theory and replicates previous findings; furthermore, it extends and sheds new light on previously reported associations by demonstrating an association between cortisol and post-error slowing, a measure of behavioral inhibition of which brain substrates have recently been discov-
ered, and suggesting opposing influences on the relationship between the ERN and post-error slowing. Moreover, suggesting a link between cortisol, behavioral inhibition, and this brain mechanism may help integrating this literature including associations with frontal asymmetrical activity, and suggest directions for further research.

Heightened orienting towards and decreased disengagement from threat, more post-error slowing, and larger ERN magnitude seem to characterize people who stay behaviorally inhibited from childhood into adulthood and have an increased likelihood of developing a social anxiety disorder (Fox, 2010). Fox (2010) proposed that behaviorally inhibited individuals are highly concerned over making mistakes, and that this overconcern (and perhaps their history in receiving negative feedback in certain situations) contributes to the emergence of heightened anxiety. Individual differences in concern over mistakes has been related to social concerns, increased attention focused on the mistake, a sense of pressure to overcome the mistake, difficulty disengaging from the mistake, and difficulty concentrating (see Frost et al., 1997). The associations between cortisol, post-error slowing, ERN, and BIS warrant further investigation of the involvement of brain systems of behavioral inhibition that may include the IFG/AI. Better understanding of such systems may generate options for treatment and prevention of anxiety disorders (McDermott et al., 2009). Moreover, we think the results are relevant, not only for researchers of behavioral inhibition, but for researchers of the basic mechanisms of the ERN and post-error slowing as well, and may bring those literatures closer together.

REFERENCES


ness is associated with increased social avoidance behavior in social phobia. *Biological Psychiatry, 65,* 336–343.


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