To approach or to avoid : neurobiological mechanisms in social anxiety

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Chapter 7

Cortisol administration enhances the coupling of midfrontal delta and beta oscillations.

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Abstract

There is increasing evidence that the strength of the relation between slow (SW) and fast (FW) wave activity in the EEG is associated with specific motivational states and their corresponding neuroendocrine patterns. Enhanced correlations between SW and FW have been related to anxiety, behavioral inhibition and high basal cortisol levels. However, the direct effects of cortisol on SW–FW coupling have not been experimentally studied yet. The present study investigated whether cortisol administration increases SW–FW coupling. Resting state EEG recordings were obtained from 40 right-handed healthy male participants with extreme low or high scores on a behavioral inhibition scale, after placebo and cortisol (50 mg) administration. As expected, delta–beta correlation tended to be higher in high compared to low behaviorally inhibited (BIS) participants. In addition, cortisol resulted in an increase in correlation between SW (delta) and FW (beta) activity compared to placebo, especially in high BIS participants. Consequently, the group differences were most pronounced after cortisol administration. These results suggest that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. This is in line with findings associating cortisol with behavioral inhibition and anxiety.

Introduction

There is increasing evidence that the strength of the relation between slow (SW) and fast (FW) wave activity in the EEG is associated with specific motivational states and their corresponding neuroendocrine patterns. Enhanced SW-FW correlations have been related to anxiety and behavioral inhibition (Knyazev & Slobodskaya, 2003; Knyazev et al., 2003; Knyazev, Savostyanov, & Levin, 2004; Knyazev, Schutter, & Van Honk, 2006), and were recently found in healthy participants with high basal cortisol levels (Schutter & Van Honk, 2005). This is an important finding, as high cortisol levels have been related to behavioral inhibition and anxiety (e.g., Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998). Based on this finding, Schutter & Van Honk (2005) suggested that cortisol enhances SW-FW coupling. However, the direct effects of cortisol on SW-FW coupling have not been experimentally studied yet. In the present study we therefore investigated whether cortisol administration increases SW-FW coupling.

Slow and fast EEG waves are thought to be generated by separate neural systems with different functional properties (e.g., Başar, 2006; Başar, Başar-Eroğlu, Karakaş, & Schürmann, 2000; Klimesch, 1999; Laufs et al., 2003; Lopes da Silva, 1991). Although the specific brain regions involved in these systems and their specific functions are still not completely understood, some authors have suggested that SW (delta and theta) oscillations are EEG correlates of motivational and emotional processes associated with subcortical activity, whereas FW (alpha and beta) oscillations are correlates of more cognitive processes associated with cortical activity (e.g., Ray & Cole, 1985; Robinson, 1999, 2000, 2001; Knyazev, 2007; Knyazev & Slobodskaya, 2003; Neuper & Pfurtscheller, 2001).

Higher relative amplitudes of a given frequency in the EEG indicate more activity in the underlying system, which renders it more likely that a behavioral pattern associated with this system will occur. Relative SW and FW EEG spectral power may thus serve as a measure of predisposition to a specific behavioral style (Knyazev & Slobodskaya, 2003). Consistent with this proposition, measures of extraversion and behavioral activation have been found to be *positively* related to (SW) delta power, and *negatively* to (FW) alpha power. In contrast, neuroticism, behavioral inhibition and trait anxiety have been found to be *negatively* related to (SW) delta and theta power, and

positively to (FW) alpha and beta power (Knyazev, Slobodskaya, & Wilson, 2002; Knyazev et al., 2003, 2004).

Moreover, the relationship between SW and FW spectral power may provide insight into the balance or interaction of activity in the underlying systems. For example, relatively high SW-to-FW ratios have been associated with increased risk-taking behavior and impulsivity in healthy participants (Schutter & Van Honk, 2005) and patients with attention deficit hyperactivity disorder (see e.g., Loo, Hopfer, Teale, & Reite, 2004 for an overview). In addition, the strength of the reciprocal relationship between (FW) alpha and (SW) delta oscillations (in averaged evoked potentials as well as in resting state EEG) has been found to be *negatively* related to extraversion and behavioral activation (Knyazev et al., 2003; Robinson, 1999, 2001) and to be *positively* related to neuroticism, behavioral inhibition and trait anxiety (Knyazev & Slobodskaya, 2003; Knyazev et al., 2003, 2004, 2006). Similarly, experimental manipulation of anxious apprehension (by giving participants random negative feedback on their performance) has been found to significantly increase the correlation between (SW) delta and (FW) beta spectral power (i.e., delta-beta coupling) compared to baseline (pre-feedback) measurements (Knyazev et al., 2006).

Because these motivational states (i.e., behavioral inhibition and anxiety on the one hand and behavioral disinhibition or impulsivity on the other hand) have been associated with specific neuroendocrine patterns, Schutter and Van Honk (2004, 2005) hypothesized that the strength of the relationship between SW and FW activity may also vary as a function of these neuroendocrine patterns. The results of two studies supported this hypothesis: Administration of testosterone, a drug with clear disinhibitory and anxiolytic properties (e.g., Hermans, Putman, Baas, Koppeschaar, & Van Honk, 2006; Svensson, Akesson, Engel, & Soderpalm, 2003; Van Honk et al., 2004), to healthy volunteers was found to result in a decrease in the relation between delta and beta spectral power, resulting in a non-significant delta-beta correlation (i.e., delta-beta *de*coupling) (Schutter & Van Honk, 2004). In contrast, comparing the delta-beta correlation in healthy participants with high and low basal cortisol levels, Schutter and Van Honk (2005) found high cortisol levels to be associated with a significant delta-beta correlation (i.e., delta-beta coupling), whereas low cortisol levels were associated with the absence of delta-beta coupling. The latter finding is in agreement with findings associating the stress-hormone cortisol with behavioral inhibition and anxiety (e.g.,

Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998) and suggests that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. Such finding supports the important influence of cortisol on cognition, emotion processing and behavior (e.g., De Kloet et al., 1999; Erickson, Drevets, & Schulkin, 2003). However, Schutter and Van Honk (2005) used a cross-sectional design and, as a result, the group differences between participants with high and low cortisol levels may be due to factors other than basal cortisol levels as well. To enable more conclusive interpretations of the effects of cortisol on the correlation between delta and beta activity, it needs to be shown whether cortisol *administration* can also increase this correlation.

The main purpose of the present study was to investigate whether cortisol can enhance delta-beta coupling by administration of cortisol in a within-subject, placebo-controlled design. In line with the findings of Schutter and Van Honk (2005), we expected to find an increased delta-beta correlation after cortisol compared to placebo. Second, since cortisol has been proposed as an endocrinological marker for behavioral inhibition and anxiety (e.g., Korte, 2001; Sapolsky, 1990; Van Honk et al., 1998), and since increased delta-beta coupling has been found in high anxious participants under conditions of uncertainty (Knyazev et al., 2006), we aimed to investigate whether high delta-beta coupling would also be associated with high behavioral inhibition.

Materials and Methods

Participants

Forty male students recruited from the University of Leiden participated in the study for financial or course credit. We selected a priori 20 students with low scores (≤ 16) and 20 students with high scores (≥ 21) on the Behavioral Inhibition Scale (BIS: Carver & White, 1994).[1] All participants were right-handed, had a bodyweight between 60-85 kg, and were screened to exclude any psychiatric disorder, clinical significant medical disease, past head injury with loss of consciousness > 5 min, and use of medication. All participants provided written informed consent prior to participation in

[1] Cutoff scores for these groups were based on the lower third and the upper third of the distribution of BIS scores (range 9-28, M = 18.5, SD = 3.6) in a sample of 153 male students.

the study, which was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Procedure

The participants were given a 50 mg hydrocortisone or placebo capsule in a double-blind, within-subject crossover design. The order of cortisol or placebo administration was random and balanced within the high and low BIS groups. The two experimental sessions were one week apart. On the days of testing, participants arrived at the laboratory where, after a short introduction, drug administration took place at 12.30 or 14.30 h, followed by a resting period of one hour to allow for the cortisol to take effect.[2] During this period, participants completed questionnaires and the electrodes for the EEG measurements were placed. Subsequently, the experiment started with the measurement of resting state EEG, followed by a number of additional cognitive tests of which the results will be reported elsewhere (Van Peer et al., 2007). Resting state EEG was measured in a series of eight 1-minute recording periods, while the participants sat quietly with eyes opened and closed in counterbalanced trials (i.e., OCOCOCOC or COCOCOCO). With eyes opened, participants were instructed to look at a fixation point in front of them. Participants sat in a dimly lit, air-conditioned and sound-attenuated room, while the experimenter sat in an adjacent room where the EEG apparatus was located.

Psychophysiological data reduction and analysis

The electroencephalogram (EEG) was recorded from 19 scalp locations according to the international 10-20 system and referred on-line to C3/C4. Vertical electro-oculogram (EOG) was recorded bipolarly from the supraorbital and the infraorbital ridge of the right eye, and horizontal EOG from the outer canthi of both eyes. The ground electrode was located at Fpz. EEG impedances were kept below 5 kΩ. The EEG and EOG signals were digitized at 500 Hz. Signals were processed offline using Brain Vision Analyzer software (version 1.05, Brain Products GmbH, 1998-2004).

[2] Cortisol can readily cross the blood-brain barrier (see e.g. Herbert et al., 2006 for a review), and cortisol administration has been shown to significantly affect cognitive and emotional processes (e.g. Kirschbaum et al., 1996; Lupien et al., 1999; van Peer et al., 2007; Putman et al., 2007; Reuter, 2002) as well as resting state EEG (e.g. Tops et al., 2005; 2006) from one hour after administration, when cortisol levels reach their maximum (e.g. Reuter, 2002; see also van Peer et al., 2007).

Signals were re-referenced to an average-ears reference and filtered with Butterworth Zero Phase Filters (low cutoff: 0.1 Hz (12 dB/oct), high cutoff: 100 Hz (12 dB/oct), notch filter: 60 Hz). Subsequently, the data of each 1-minute recording period were segmented into 1.024 second segments, with 50% overlap. Rough artifact rejection (+/- 200 μV), followed by automatic ocular correction using a standard procedure (Gratton et al., 1983) and automatic artifact rejection (+/- 100 μV, lowest allowed activity 0.5 μV for 100 ms) were performed on these segments. The designation of an artifact in one of the leads resulted in the removal of that epoch for all channels in order to ensure that the remaining data were identical for all sites in time. A Fast Fourier Transform (FFT, full spectrum method, Hamming window of 10% length) was applied to chunks of artifact-free data to obtain estimates of spectral power density (μV^2/Hz). Spectral power density values for each electrode were averaged across all epochs within a single baseline and calculated for the delta (1-3 Hz), and beta (14-30 Hz) frequency bands. A natural log transformation (ln) was performed on these values to normalize the distribution. The data from one participant were excluded from the analyses because of deviant ln delta power density in both the placebo and cortisol condition (*Z*-score > 3.5 in both conditions).

Since a specific aim of the present study was to extend the findings of previous studies, we focused our primary analyses on the midfrontal (Fz) electrode, for which the effects on delta-beta correlation have been reported to be specifically pronounced (Schutter & Van Honk, 2004, 2005). However, in order to explore the topographic distribution of the effects of cortisol administration and group differences in delta-beta correlation, we performed additional analyses including more lateral and posterior electrodes (F3, F4, C3, Cz, C4, P3, Pz, P4).

Statistical analyses were performed on the Pearson correlation between ln average power density in the delta and beta frequency bands across all recording periods (i.e., the delta-beta correlation), at each electrode in each condition. To test for significant differences between correlations, we used Fisher's *r* to *r'* transformation to normalize the distribution of correlation coefficients, which allows the use of a *Z*-test to compare the correlations. To test the effect of cortisol administration we calculated a *Z*-score for non-independent groups, and to test for group differences we calculated a *Z*-score for independent groups (see Clark-Carter, 1997). Given our specific directional

hypotheses for increased delta-beta correlations associated with cortisol and trait inhibition, condition and group effects were tested with a one-tailed alpha of .05.

Salivary cortisol measures

To verify the effect of cortisol administration and to check for possible differences in baseline cortisol, saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were obtained before capsule intake and at the start of the EEG recording (one hour after capsule ingestion). Biochemical analysis of free cortisol in saliva was performed using a competitive electrochemiluminescence immunoassay (ECLIA, Elecsys 2010, Roche Diagnostics), as described elsewhere (Van Aken et al., 2003).

Results

Salivary cortisol

Salivary cortisol (nmol/L) measures were skewed and therefore natural log transformed before statistical analysis. As expected, unbound levels of cortisol did not differ between conditions before capsule intake (placebo: M = 9.1, SD = 3.0; cortisol: M = 9.4, SD = 2.8; t (37) = - 0.74, p = .47), but were significantly increased (t (37) = - 18.69, p < .001) one hour after cortisol administration (M = 173.4, SD = 142.3) compared to placebo (M = 6.8, SD = 1.7). There were no significant differences in salivary cortisol values between the low BIS and high BIS participants (all p > .20).

Delta-beta correlation

Effect of cortisol administration

First, to test the hypothesis that cortisol administration can enhance midfrontal delta-beta coupling, we calculated the delta-beta correlation at Fz electrode after placebo and cortisol administration for all participants (see Table 7.1). In line with our expectations, cortisol administration resulted in a near-significant increase in the midfrontal delta-beta correlation compared to placebo (Z = -1.57, p = .058).

Extended analyses including more lateral and posterior electrodes (F3, F4, C3, C4, Cz, P3, P4, and Pz) showed that cortisol administration tended to increase the delta-beta correlation on all electrodes (except for a decrease at F4), but besides the midfrontal

Table 7.1. Delta-beta correlation (Pearson correlation between mean ln delta power density and mean ln beta power density at Fz electrode) and mean (SD) delta and beta activity (ln power density at Fz electrode) after placebo and cortisol administration for all subjects and for the low BIS and high BIS subgroups.

Measure	Placebo			Cortisol		
	All (n = 39)	LBIS (n = 19)	HBIS (n = 20)	All (n = 39)	LBIS (n = 19)	HBIS (n = 20)
Delta-beta correlation	R =.29	R =.08	R = .50*	R = .51**	R =.31	R = .70**
Ln delta power	2.52(0.3)	2.55(0.3)	2.48(0.2)	2.49(0.2)	2.51(0.2)	2.47(0.3)
Ln beta power	-.97(0.4)	-.94(0.4)	-.99(0.4)	-.99(0.5)	-.98(0.5)	-.01(0.5)

* p < .05, ** p < .001

(Fz) electrode this effect only approached significance at P3 (Z = -1.35, p = .09) (see Figure 7.1, panel A).

Interestingly, in contrast to the effects on the *correlation* between midfrontal delta and beta power, paired samples t-tests showed no differences between the placebo and cortisol conditions in either mean ln delta power (t (38) = 0.80, p = .43) or mean ln beta power (t (38) = 0.94, p = .36) at Fz (see Table 7.1), nor at any of the other electrodes.

Behavioral inhibition

To test the hypothesis that high behavioral inhibition is associated with increased midfrontal delta-beta coupling, the delta-beta correlation at Fz electrode was calculated separately for high and low BIS-groups in the placebo and cortisol conditions (see Table 7.1 and Figure 7.2). As expected, the midfrontal delta-beta correlation in the placebo condition was significant in the high BIS group, but not the low BIS group (Group difference: Z = - 1.33, p = .09). Although cortisol administration resulted in a general increase in midfrontal delta-beta correlation compared to placebo (see above), this correlation remained non-significant after cortisol administration in the low BIS group (see Table 7.1). The group difference was borderline significant after cortisol administration (Z = - 1.57, p = .058).

In line with the findings on Fz, results of the extended analyses (including F3, F4, C3, C4, Cz, P3, P4, and Pz) showed significant delta-beta correlations for high BIS, but not for low BIS participants on all electrodes in the cortisol condition, and on almost all electrodes (except for a lower correlation at F3, F4, and C4) in the placebo condition (see Figure 7.1). However, besides the effect at Fz, in both conditions the group difference

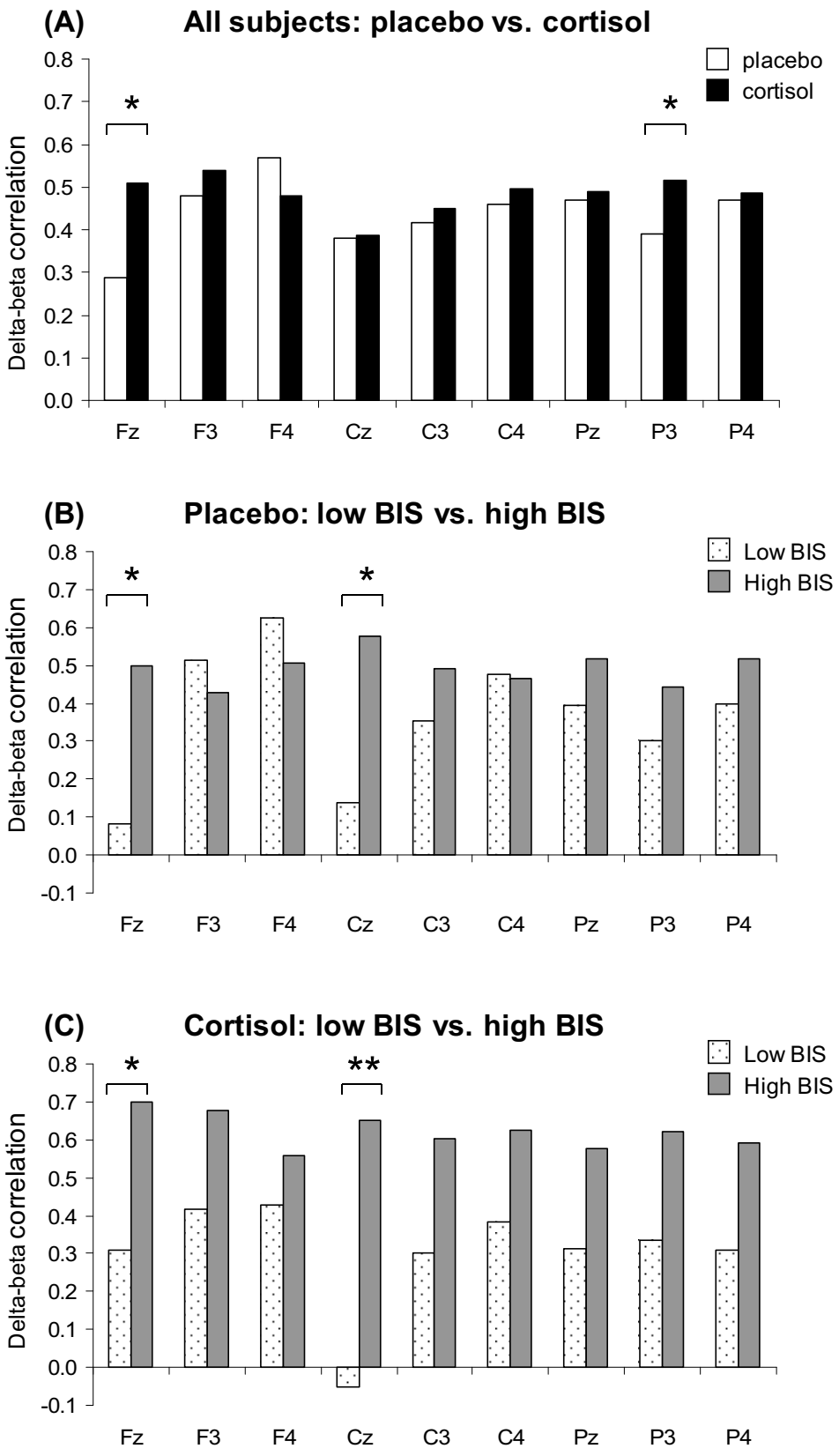

Figure 7.1. Delta-beta correlations (Pearson correlation between mean ln delta power density and mean ln beta power density) by electrode site for (A) all participants (n = 39) after placebo and cortisol administration, (B) low BIS (n = 19) and high BIS (n = 20) participants after placebo administration, and (C) low BIS and high BIS participants after cortisol administration. *$p < .10$ **$p < .05$ (1-tailed)

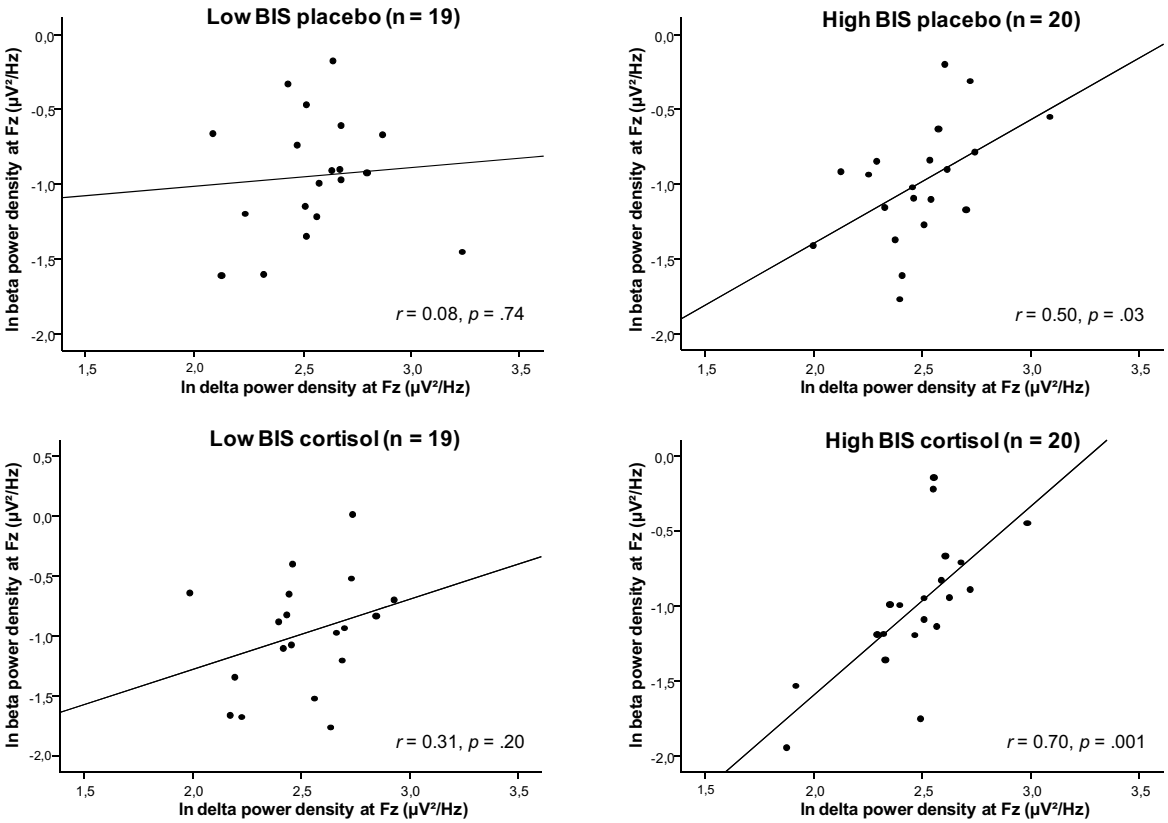

Figure 7.2. Significant midfrontal delta-beta correlation in high BIS participants (right), but not in low BIS participants (left) after placebo and cortisol administration. Cortisol administration (bottom) resulted in a general increase in delta-beta correlation compared to placebo (top) for all participants.

only approached significance at Cz (placebo: Z = -1.49, p = .07; cortisol: Z = -2.38, p <.01) (see Figure 7.1, panels B and C).

High and low BIS participants did not differ in either mean ln delta power (placebo: t (37) = 0.86, p = .39; cortisol: t (37) = 0.57, p = .57) or mean ln beta power (placebo: t (37) = 0.40, p = .69; cortisol: t (37) = 0.17, p = .87) at Fz (see Table 7.1), nor at any of the other electrodes.

Discussion

This study shows that cortisol administration tended to increase the correlation between midfrontal/central delta and beta spectral power in healthy male participants, especially in a group with high self-reported levels of behavioral inhibition, suggesting that cortisol can modulate brain activity, increasing a pattern that has been associated with anxiety and behavioral inhibition. These results replicate and extend previous findings of Schutter and Van Honk (2005) showing an increased correlation between

midfrontal delta and beta power in participants with high compared to low basal cortisol levels. The present study is the first to show that a within-subject experimental manipulation of cortisol can increase this correlation. These results make it unlikely that these differences can be attributed to other factors than cortisol.

Interestingly, cortisol administration only increased the *correlation* between midfrontal delta and beta power, and did not change mean (i.e., group) delta or beta power. This implicates that the increased correlation cannot be attributed to a general increase or decrease of activity in the neural systems underlying these oscillations (for example due to changes in arousal), and suggests that only the coherence between activity in the underlying systems is affected.

The present findings are important considering the increasing interest in the effects of cortisol administration on human emotion processing and behavior (e.g., De Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000; Putman et al., 2007a; Soravia et al., 2006; Tops et al., 2003, 2004; Van Peer et al., 2007). Despite this interest and though present brain states may influence subsequent processing (e.g., Başar, 2006), so far little was known about the effect of cortisol on brain activation associated with motivational states. Although studies investigating EEG frontal asymmetry (a widely studied index associated with the balance between approach and avoidance motivation, see e.g., Davidson, 1992) have shown a significant relationship between high endogenous cortisol levels and extreme right frontal asymmetry in primates (Kalin et al., 1998a) and infants (Buss et al., 2003), this relationship has not been reported for healthy human adults, and studies on the effects of acute cortisol administration in adults have produced mixed results (Tops et al., 2005, 2006). Given the now growing evidence showing a consistent relationship in healthy human adults between the strength of the midfrontal delta-beta correlation and different neuroendocrine patterns associated with motivational states (Schutter & Van Honk, 2004, 2005; present study) this measure can be considered a promising alternative or addition to the frontal asymmetry measure.

We also found significant differences in delta-beta correlation between participants with low and high scores on the Behavioral Inhibition Scale (Carver & White, 1994), especially at Cz after cortisol administration. The fact that the group difference was most pronounced after experimental manipulation of cortisol levels is consistent with findings of Knyazev et al. (2006), who reported a significantly higher delta-beta correlation in high trait anxious participants compared to low trait anxious

participants after an experimentally manipulated increase in uncertainty. These findings suggest that these group differences become more pronounced after experimental increase of anxious motivational states.

In the present study, the delta-beta correlation was highest in the high BIS group after cortisol administration. Interestingly, in a subsequently administered reaction time task measuring approach and avoidance tendencies to happy and angry faces, we found relatively facilitated avoidance reactions to angry faces only in these same high BIS participants after cortisol administration, as evidenced by relatively faster reaction times and increased positive event-related potentials when participants avoided an angry face (Van Peer et al., 2007). This suggests that high delta-beta correlations are associated with a reaction pattern related to social anxiety (e.g., Bögels & Mansell, 2004), though this needs direct confirmation.

All reported findings were most pronounced on the midfrontal and central electrode sites, confirming previous findings of Schutter and Van Honk (2004, 2005) suggesting that the midfrontal electrode site is most sensitive to neuroendocrine manipulation and individual differences in delta-beta correlation. These locations are in line with studies investigating SW-FW ratio in attention deficit hyperactivity disorder also reporting most pronounced differences at midline frontal and central electrode sites (Fz and Cz: see Lubar, Swartwood, Swartwood, & Timmermann, 1995).

Finally, it should be noted that although delta-beta correlations were strong and significant only in the high and not the low BIS group, especially after cortisol administration, these differences only approached statistical significance at midfrontal and central electrodes. This may be due to a lack of power in our study. Thus, studies including larger participants samples are needed to replicate our findings.

Based on theories associating SW with (subcortically driven) motivational or emotional processes and FW with cortically driven cognitive processes, several authors have suggested that enhanced SW-FW correlations reflect increased communication between subcortical and cortical brain regions, or even increased cortical control over subcortical drives (Knyazev & Slobodskaya, 2003; Robinson 2001; Schutter & Van Honk, 2005). Although such interpretation of the delta-beta correlation as an index of the information exchange between subcortical and cortical brain systems is still speculative, it fits with the findings of numerous direct and indirect connections between cortical and subcortical systems (e.g., Barbas, 2000), and is in agreement with the notion that

these connections play an important role in the regulation of emotion and behavior (e.g., LeDoux, 2000; Ochsner & Gross, 2005; Phelps, 2006). Hence, this interpretation may be used as a heuristic model, although

future studies are needed to further investigate the functional significance of the delta-beta correlation. For example, the identification of the functional neuroanatomical correlates of increases or decreases in delta-beta correlation (using functional Magnetic Resonance Imaging or Transcranial Magnetic Stimulation) may help to define the brain systems that relate to shifts in anxious/ inhibited or impulsive motivational states, and yield more insight in to what extent this correlation is related to inhibitory or excitatory connections. In addition, studies with within-subject measures of delta-beta correlation related to task performance may provide more insight into the relation between this motivational state and subsequent emotion processing and behavior.

In sum, the present study shows that cortisol administration tended to increase the correlation between SW (delta) and FW (beta) activity compared to placebo. In addition, delta-beta correlation was higher in high compared to low behaviorally inhibited subjects, and this group difference was most pronounced after cortisl administration. These results suggest that cortisol can modify brain activity, increasing a pattern associated with anxiety and behavioral inhibition. This finding is particularly interesting in the light of notions that cortisol prepares the organism for adaptive stress reactions and facilitates cognitive processes or behavior that is most relevant to the situation (e.g., De Kloet et al., 1999; Tops et al., 2006).