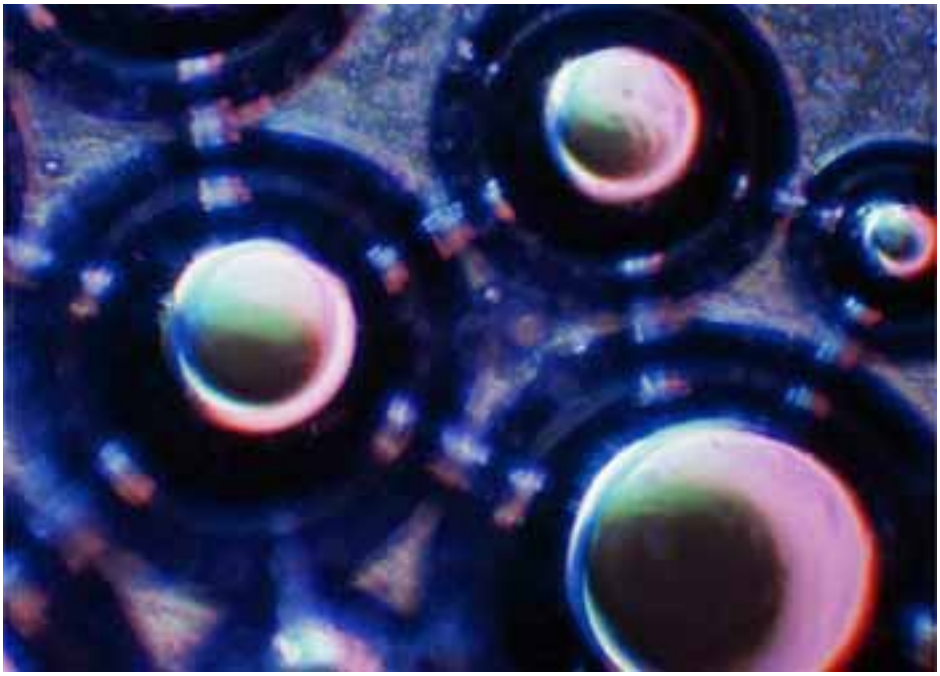
Hypothalamic-Pituitary-Adrenal (HPA) axis Activity in Schizophrenic Patients



December 2005

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Preface

This research project was performed as the last part of my study clinical and health psychology at the Erasmus University Rotterdam. I wanted to do an investigation that was related to neuropsychology in psychiatric patients. When I applied for an internship at the Erasmus MC Rotterdam, I learned that a research project in which physiological activity was measured in schizophrenic patients was about to start. I was given the opportunity to participate in the part of this project that investigated hormonal changes.

Because my internship only lasted several months, I was not able to use all data that would be gathered for the whole investigation. I hope that my assistance and this thesis will add to a satisfactory result of the whole research project.

I would like to thank Joke Tulen and Roelie Hempel for their support, comments and advises on my work. Thank you both for your patience and the nice and good cooperation. I would also like to thank Ingmar Franken for his supervision and comments on my thesis.

Most of all, I would like to thank my colleague and good friend Lisa Kolet for putting up with my grumbling and grousing, for the moral support, and for all the fun we had in the EMC. Thanks also to my roommate Sven Crama for making sure the candy pot was always filled and for calmly accepting the elastic-excesses that sometimes were inevitable! Even the snowman in front of our window had a permanent smile (made out of office supplies) on his face!

Finally, I would like to thank my family for having faith in me and supporting me during the whole process. Especially my father and my partner Michel, thanks for being there when I needed you. I will look back on these previous months as a valuable and unforgettable period of my life.

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Abstract

The course and outcome of schizophrenia seem to be influenced by stress and maladaptive coping, yet the process by which stress affects schizophrenia is far less clear. The hypothalamic-pituitary-adrenal (HPA) axis, and in particular the release of glucocorticoids, has been known to play an important role in stress regulation. The purpose of this study was to investigate HPA axis functioning in individuals with schizophrenia. We assessed the salivary cortisol day curve of first-episode schizophrenic inpatients (n = 18) and healthy controls (n = 15). Salivary samples were taken at five different time points during the day: directly after awakening, 30 minutes after awakening, at 12:00h, at 16:00h, and at 22:00h. Furthermore, the differences in cortisol levels between schizophrenic patients were related to symptom severity as measured by the Positive and Negative Syndrome Scale (PANSS).

Results indicated significantly heightened cortisol levels in schizophrenic patient as compared to controls, although both groups showed a diurnal pattern of cortisol secretion. The area under the curve showed a trend for higher total cortisol secretion in schizophrenic patients, but did not reach significance. Correlations between ratings on the PANSS subscales and different time points were not significant.

Our results provide evidence for a dysregulation of the HPA-axis in schizophrenic patients. The heightened cortisol levels might be a consequence of hypercortisolism. Further research is required to determine the relationship between HPA-axis functioning and clinical manifestation of schizophrenia.

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1. Introduction

1.1 Schizophrenia

Schizophrenia is a chronic and severe disorder that is a heavy burden for patients and their families. This mental illness is characterized by impaired psychological, social and occupational functioning, and it affects about 1% of the worldwide population. Even though this is a relatively small number compared to the prevalence of other mental disorders, the consequences for society in terms of money and care are large (Mueser & McGurk, 2004). The incidence of schizophrenia is the same across sexes and countries, although some ethnic groups seem to have a higher incidence of the disorder (Harrison, 1988; Selten & Sijben, 1994; Van Os, 1996a; Selten, Slaets & Kahn, 1997). The onset of the illness is usually between 16 and 30 years in men. Women tend to have a later onset and less intense course of illness, which may be associated with the effects of oestrogen and better social functioning before the onset of the illness (Mueser & McGurk, 2004).

Cognitive and emotional functioning is severely impaired in schizophrenia. However, clinical presentations of schizophrenia can be very different, and symptoms vary greatly between patients. A frequently used way of classifying symptoms is to distinguish between positive and negative symptoms. Positive symptoms can be divided into psychotic (e.g. hallucinations and delusions) and disorganized (e.g. bizarre behaviors and thought disorder). Negative symptoms include flat affect, anhedonia, weak social interaction and reduced quantity or content of speech (Gelder, Mayou & Geddes, 2005; Mueser & McGurk, 2004). The exact *Diagnostic and Statistical Manual* (DSM-IV) criteria for the diagnosis of schizophrenia are to be found in appendix I (APA, 2000).

Several factors are known to influence the risk of developing schizophrenia and the course of the illness. Genetic factors are very important as shown in twin studies, adoption studies and research on first-degree relatives (McGuffin, Owen & Farmer, 1995; Mäki et al., 2005). Other significant risk factors include prenatal and perinatal factors (e.g. maternal influenza, smoking during pregnancy, malnutrition) and sociodemographic factors (e.g. poverty, low social class, living in urban areas) (Nuechterlein et al., 1994; Mueser & McGurk, 2004).

1.2 Schizophrenia and stress

Stressful life events are also known to provoke the disorder. It is hypothesized that schizophrenic patients have an altered sensitivity to stress (Gispen-de Wied, 2000; Mäki et al., 2005). The vulnerability-stress model has often been used to conceptualize this

sensitivity to stress (Nuechterlein et al., 1994; Nuechterlein & Dawson, 1984). With this model, the authors propose a simplified device to organize the interaction between important factors that explain the aetiology and pathogenesis of schizophrenia (Figure 1). It contains three clusters: vulnerability, chemical and psychosocial factors. Vulnerability is determined by genetic and early environmental effects. Chemical factors, such as medication and substance abuse, can influence vulnerability by respectively reducing or worsening symptoms and chances of relapse. Stress has a negative effect on vulnerability and increases the chance of relapse. However, other psychosocial factors such as effective coping skills and social support can reduce the effects of stress on vulnerability.

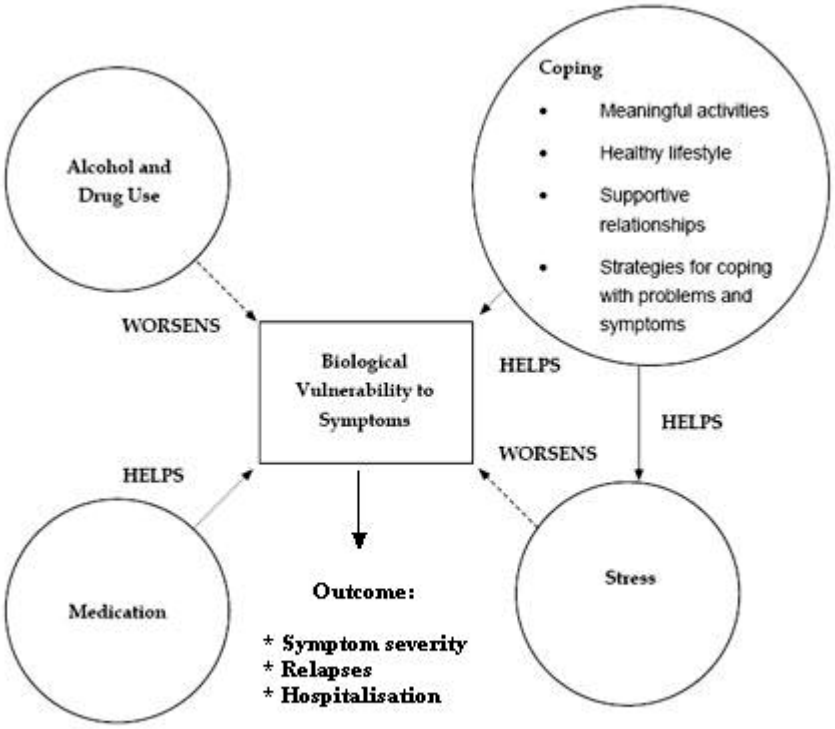

Figure 1. The Vulnerability Stress Model
(Based on Mueser & McGurk, 2004).

In short, this model describes schizophrenia as the result of a complex interaction between biologically determined factors and challenging environmental factors. From this perspective, maladaptive coping strategies and genetically determined vulnerability to stress may strongly influence the course of the illness. Following this vulnerability-stress approach,

it seems important to get a better insight in the different aspects of stress in schizophrenia. The present study will focus on an essential component of the stress system: the hypothalamic-pituitary-adrenal axis.

1.3 Neurobiological systems involved in stress

The most relevant biological systems regulating emotional functioning are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA-axis).
Both of these systems seem to be activated by the corticotropin-releasing hormone (Sapolsky, 1992; Gispen-de Wied, 2000).

The autonomic nervous system is responsible for the quick reaction to a potential stressor, which is referred to as the 'fight or flight' response. A stressor activates the sympathetic nervous system while the parasympathetic nervous system is inhibited. The adrenal medulla is stimulated to secrete epinephrine and norepinephrine. Because of these catecholamines, the heart rate increases, glucose enters the bloodstream and digestion, growth and reproduction are inhibited. This whole process takes place within a few seconds (Sapolsky, 1992).

The HPA-axis is responsible for the secretion of cortisol (also known as hydrocortisone). This is a slower process, which is regulated by a feedback system. The importance of this system is emphasized when prolonged stressors are present. Increased secretion of cortisol in reaction to a stressor helps the body to maintain homeostasis.

1.4 The Hypothalamic-Pituitary-Adrenal axis

Three chemical messengers are involved in the HPA-axis: corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and glucocorticoids (Figure 2). The system is activated when the brain perceives a stressor. The periventricular nucleus (PVN) of the hypothalamus secretes CRH, which binds to CRH receptors of the pituitary gland. The pituitary is hereby induced to produce and secrete ACTH. This hormone in turn stimulates the adrenal cortex to secrete glucocorticoids, including cortisol (Sapolsky, 1992; Walker & Diforio, 1997). In humans, this is the most important glucocorticoid that is secreted in reaction to stressors. Cortisol elevates blood sugar and enhances metabolism. These changes supply the organism with the energy to cope with stress. However, the consequence is that less energy is available for synthesis of proteins, including the proteins of the immune system. When the HPA-axis is activated only incidentally, this is not a

problem. In the case of chronic stress, however, elevated cortisol levels may lead to serious health problems (McEwen, 1998).

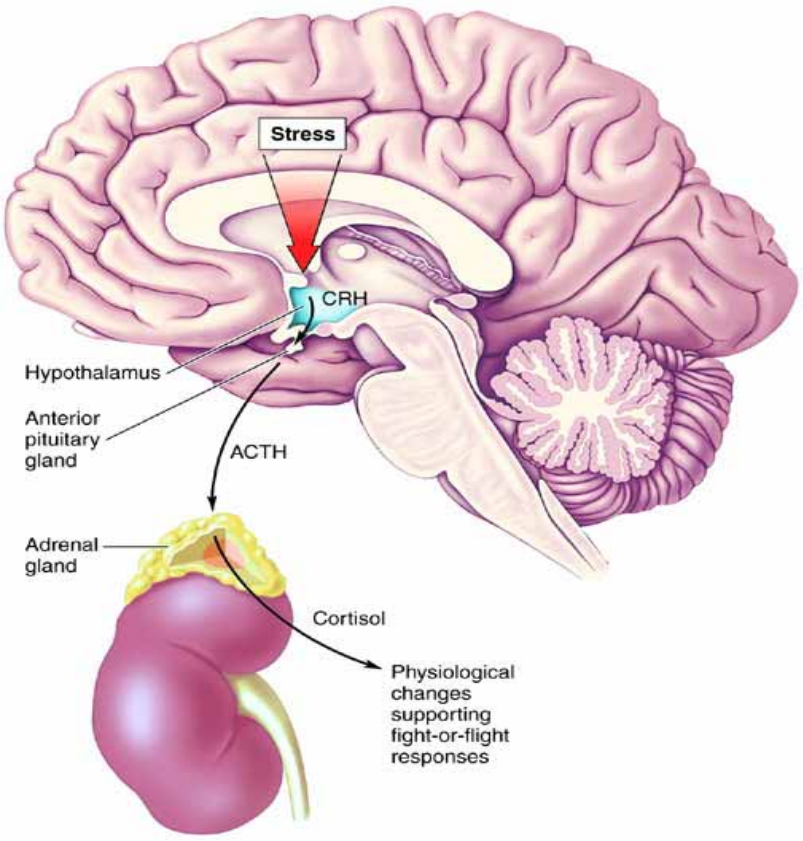

Figure 2. The Hypothalamic Pituitary Adrenal (HPA) axis.

1.5 Daytime cortisol curve and factors of influence on cortisol secretion

In an unstressed situation, the secretion of cortisol follows a circadian rhythm. Figure 3 shows an example of a diurnal profile of cortisol secretion in healthy adults. In the morning, shortly after awakening, the cortisol level elevates and reaches a peak in about 30 minutes. Soon thereafter the concentration of cortisol slowly decreases to reach its lowest value around midnight (Kirschbaum & Hellhammer, 1989, 1994).

Because of this circadian rhythm, the most important factor in obtaining reliable data of cortisol levels is to start measurements directly after awakening, instead of measuring at fixed time points. Pruessner et al. (1997) repeatedly measured free cortisol levels after awakening in children, adults and elderly subjects. In all subjects, free cortisol levels increased by 50 – 75% within the first 30 minutes after awakening. They found that premenopausal women showed a stronger increase and a delayed peak in cortisol secretion

as compared to men. Women taking oral contraceptives showed a trend towards lower early morning free cortisol levels. Cortisol levels were consistent over the measurement days. Later research from Wüst et al. (2000) confirms these findings. Both studies concluded that age, weight, smoking, sleep duration, time of awakening, alcohol consumption and the use of an alarm clock have little or no effect on baseline and peak cortisol levels.

However, later research showed that time of awakening and health status does influence cortisol secretion. In a sample of 179 subjects with a large age range (4 – 75 yrs) healthy subjects and early awakeners had larger morning cortisol responses compared to respectively subjects reporting health problems and late awakeners (Kudielka & Kirschbaum, 2003).

Furthermore, the diurnal pattern is not as stable as is assumed by most researchers. Smyth et al. (1997) found that in a sample of 109 healthy subjects only 51% showed the typical diurnal pattern in cortisol secretion. 31% of the participants showed different diurnal patterns on the two study days and 17% showed no significant diurnal pattern on any of the two days. A replication study confirmed these findings (Stone et al., 2001).

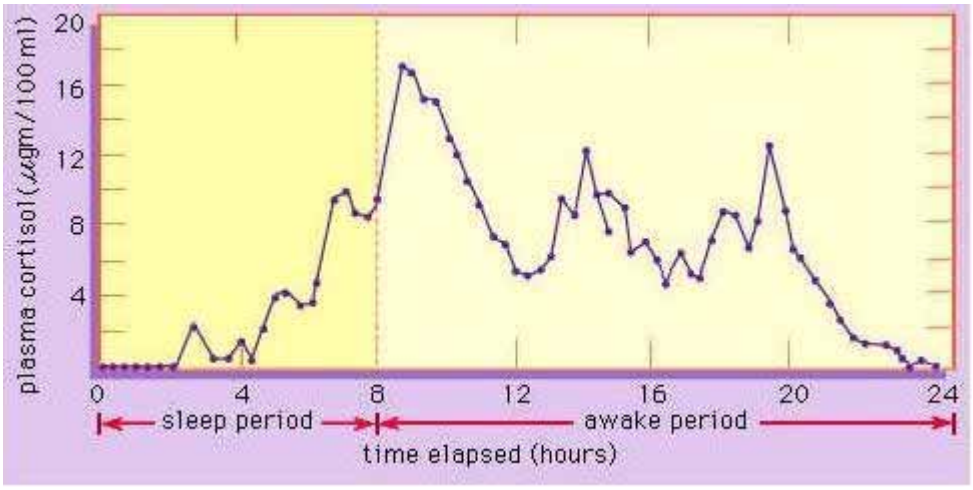

Figure 3. Circadian rhythm, a graphic depiction of cortisol values over a 24-hour period.

1.6 Feedback system and stress responsivity

In a well functioning HPA system, the rising cortisol level slows down the production of both CRH from the hypothalamus and ACTH from the adrenal glands. Cortisol induces this decrease because it binds to glucocorticoid receptors that are located in various regions of the brain. This completes the feedback loop and prevents the initial adequate stress response to a potential stressor to become damaging (Jansen et al., 1998). The

hippocampus is thought to play an important role in the modulation of the HPA-axis, because it contains a high density of glucocorticoid receptors (Walker & Diforio, 1997).

However, research has shown that in some people the HPA-axis is out of balance. Scientists distinguish between hypocortisolism and hypercortisolism: a persistent decrease or increase of cortisol levels, respectively. When the HPA-axis is activated too long, too frequently (i.e. chronic stress) or for no physiological reason (i.e. psychological stress) the elevation of cortisol can become harmful. Most importantly, prolonged elevation weakens the immune system and affects neurons in the hippocampus (Sapolsky, 1992). As a result people may become more susceptible to illness and can experience memory impairment. If, on the other hand, cortisol levels are persistently low, pain, fatigue and high stress sensitivity are reported (Fries et al., 2005).

1.7 HPA-axis activity in different patient groups

A lot of research on cortisol levels has been done with specific patient groups. This research shows evidence of a disturbance of HPA-axis activity in the diurnal cycle, as well as in relation to specific stressors. Clear evidence of HPA-axis hyperactivity has been found in patients with melancholic depression, alcoholism, and eating disorders (for an overview see Ehlert, Gaab & Heinrichs, 2001). Other areas in which HPA-axis malfunctioning was established are posttraumatic stress disorder, stress-related bodily disorders like idiopathic pain syndromes, and chronic fatigue syndrome. These conditions are associated with diminished HPA activity (i.e. hypocortisolism; for a review see Heim, Ehlert, & Hellhammer, 2000).

1.8 HPA-axis activity in schizophrenia

As previously discussed, the vulnerability stress model emphasizes the negative effect of stress on vulnerability in schizophrenic patients. Although the results are sometimes contradictory, several lines of evidence suggest that schizophrenic patients are also impaired in their biological response to stress. For instance, Gil-Ad et al. (1986) studied ten schizophrenic patients and eight age/sex matched control subjects. They found a normal diurnal pattern in plasma cortisol levels in both groups. However, the mean plasma cortisol levels in the schizophrenic patients were significantly higher than in the controls throughout the day. These results support the idea of hypercortisolism in schizophrenia. Ryan et al. (2004) measured plasma levels of the stress related hormones arginine vasopressin (AVP), adrenocorticotropin (ACTH) and cortisol in twelve drug naive patients with first episode

schizophrenia and age/sex matched healthy controls. Blood samples were taken at 20-minute intervals from 13:00 to 16:00 hours, since this time frame is thought to reflect the 24 h cycle. Schizophrenic patients secreted higher levels of ACTH and cortisol, as calculated by the area under the curve (AUC). This study also found evidence of pituitary-adrenal overactivity in patients with schizophrenia. Similar conclusions were drawn by Walsh and colleagues (2005). They measured cortisol and ACTH release in response to a dopamine antagonist (metoclopramide) that has the ability to release AVP. When a baseline sample was taken, first episode, drug naïve male patients with paranoid schizophrenia had higher levels of plasma cortisol and ACTH than controls. Metoclopramide-induced AVP responses were similar between patients and controls. These studies all provide evidence of hyperactivity of the HPA axis. However, there are several studies that are not in concordance with the “hyperactivity of the HPA-axis” hypothesis.

For example, Rao et al. (1995) found no difference in circadian cortisol secretion between drug-free schizophrenic patients who were not chronically hospitalized and healthy subjects. Likewise, several other studies investigating baseline cortisol levels as well as cortisol reactions to stressors found similar baseline levels in schizophrenic patient and controls. For instance, Elman et al. (1998) found a significant elevation of ACTH levels in patients, but no difference in baseline cortisol levels between patients and controls. More evidence is provided by Jansen and colleagues (2000) who found no difference between patients and controls in cortisol day profiles.

Apart from this research on baseline cortisol levels, investigators have also been interested in changes of HPA activity due to specific stressors. In a study done by Breier et al. (1987) no stress-induced elevations in plasma levels of cortisol, ACTH and growth hormone in reaction to lumbar puncture were found in schizophrenics, while depressed patients and healthy controls had elevations in all the neuroendocrine parameters. Albus et al. (1982) studied reactivity of autonomic functions to four standardized tasks: a cold pressor test, noise, mental arithmetic, and active relaxation. They found elevated baseline levels of cortisol and norepinephrine and a diminished cortisol response to stress. Jansen et al. (1998) found a significant cortisol response in controls, but not in schizophrenic patients when performing a public speaking task. Another study done by the same research team (Jansen et al., 2000) found lower cortisol responses in schizophrenic patients in reaction to a psychosocial stressor (i.e. public speaking task), although there was no difference in reaction to a physiological stressor (i.e. bicycle exercise). This suggests an impaired ability to psychologically and biologically adapt to the environment.

To summarize, it seems as though schizophrenic patients have a normal diurnal pattern of cortisol secretion, although it is not yet clear if their basal level of cortisol is elevated or not. At the same time their hormonal reactions to potential stressors seem diminished.

These discrepant findings may be due to several factors. In the first place, the use of medication may influence HPA-axis activity. Patients previously treated with neuroleptic medication showed decreased cortisol levels when switching to clozapine (Breier, Kirkpatrick & Buchanan, 1993; Hatzimanolis et al., 1998). However, Lee, Woo and Meltzer (2001) found no effect of clozapine on night time cortisol secretion during different sleep stages. Other research has indicated that haloperidol does not significantly affect cortisol secretion (Angelopoulos et al., 1997).

The symptomatology in schizophrenia is another factor that can possibly influence cortisol secretion. Findings on this subject are, once again, contradictory. Walder, Walker and Lewine (2000) suggest an association between higher cortisol secretion and more severe positive symptoms because cortisol increases dopamine activity. Furthermore, they state that this association cannot be attributed to the presence of mood disorders in some patients. Several other studies associate cortisol secretion with higher ratings of negative symptoms (Tandon et al., 1991; Kaneko et al., 1992; Zhang, et al., 2005). Ryan et al. (2004) found no significant correlation between cortisol and symptom severity.

1.9 Purpose of the study

The previously discussed research provides evidence for changes of cortisol levels in reaction to chronic stress. It shows that if a stress stimulus is not adequately responded to, stress may enhance disease susceptibility. This may happen when the HPA system is hyper- or hyporesponsive, or when it is not able to habituate. When this knowledge is integrated in the initial concept of the vulnerability stress model of schizophrenia, a (chronic) disturbance of cortisol secretion may increase the chance of relapse and worsen the outcome for schizophrenic patients.

The negative consequences of HPA-axis malfunctioning and the obscurity concerning the exact functioning of the stress mechanism in schizophrenic patients motivated us to do more research on these topics. The primary objective of the present study was to investigate HPA-axis activity in patients with first episode schizophrenia and control subjects by evaluating the differences in daytime salivary cortisol curves. To obtain a clear view of the cortisol day curve, multiple measurements of cortisol are essential. Therefore, we collected

salivary cortisol samples at five different time points of one day. Besides the comparison with healthy controls, the patient outcomes were also related to clinical parameters. We investigated this relationship between salivary cortisol levels and clinical symptom severity in schizophrenic patients by means of the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987, 1988). The aim of this study is to contribute to a better understanding of the stress system in schizophrenic patients in relation to severity of the disease.

Based on the research previously discussed, we expect patients to have an elevated baseline cortisol level as compared to controls, but the same diurnal pattern. Because of the possible influence of medication on cortisol, this factor was carefully documented and included in the analyses of the study. Furthermore, based on the contradictory findings in the literature, we propose an experimental hypothesis about the relationship between cortisol and symptom severity. We expect differences in HPA-axis activity between schizophrenic patients in relation to their clinical features as reflected by scores on the PANSS. More specific, we expect cortisol levels to be positively related to overall symptom severity. We also expect higher cortisol levels in patients with higher ratings of positive symptoms, because higher dopamine levels are associated with positive symptoms and cortisol augments dopamine secretion (Walker & Diforio, 1997; Walder, Walker & Lewine 2000).

2. Method

2.1 Subjects

Eighteen schizophrenic inpatients (age range 16 – 33 years, mean age 23.4 ± 5.3 years, 14 males and 4 females) and fifteen healthy controls (age range 18 – 27 years, mean age 20.9 ± 2.5 years, 14 males and 1 female) participated in the study. Due to practical difficulties, 5 patients were not able to collect all saliva samples in one day, although sample t1 and sample t2 were always collected on the same day. In this case, the remaining samples were taken on the next day. Two patients were excluded from all analyses because there was no psychiatric nurse available to guide the sampling, and the exact time of the sampling is therefore unreliable. Another patient was excluded from the ANCOVA due to one missing value. For the correlational analysis another two patients were excluded because of missing data on the PANSS.

Patients were selected from the Psychosis ward of the department of Psychiatry of the Erasmus MC (Medical Centre) in Rotterdam. Patients were eligible if they had the diagnosis of schizophrenic or schizophreniform disorder according to DSM-IV criteria (American Psychiatric Association, 2000), or if they suffered a first-episode psychosis that

suggested schizophrenia or schizophreniform disorder. A senior psychiatrist performed the psychiatric diagnoses and all patients experienced a psychotic episode at the time of the study. Twelve patients received medication: 4 patients used risperdone, 3 used haloperidol, 5 used olanzapine and 6 patients were medication-free at the time of the study.

Healthy controls were recruited via advertisements in the hospital and at the Erasmus University Rotterdam for participation in experimental research. Controls were compensated for their participation by a small amount of money or, in case of students, a course credit. Control subjects were in good health and drug-free at the time of testing and had no history of psychiatric illness as assessed by means of a health questionnaire (appendix II).

The study was approved by the Medical Ethical Committee of the Erasmus MC Rotterdam. Written informed consent was obtained from all subjects.

2.2 Cortisol samples

Many different paradigms have been developed to assess HPA-axis functioning in relation to specific stressors (e.g., Elhert et al. 2001). Basal HPA-axis activity is usually investigated by simply measuring cortisol levels. There are two frequently used methods for obtaining reliable and valid cortisol levels: measuring cortisol levels in saliva, and measuring plasma cortisol levels in blood. In the present study salivary samples were taken. The advantages of using salivary samples over blood samples are many. Firstly, this method is non-invasive and there is much less discomfort associated with obtaining a saliva sample than with obtaining a blood sample. Secondly, this method makes it possible for control subjects to obtain the samples at home without assistance (Clements & Parker, 1998). Finally, because proteins and protein bound molecules do not enter the saliva, only the active fraction of cortisol (free cortisol) is measured in the salivary samples. This fraction is capable of reaching the target tissue and elicits glucocorticiod effects (Kirschbaum & Hellhammer, 1994).

A disadvantage of the method is that minor elevations in plasma cortisol levels cannot be measured in saliva samples due to an enhanced conversion of cortisol to cortisone in saliva (Kirschbaum & Hellhammer, 1994). A second difficulty is that subjects overestimate their compliance in salivary cortisol sampling in the absence of objective monitoring. This may affect the quality of the data that are gathered (Kudielka, Broderick & Kirschbaum, 2003; Broderick et al., 2004).

2.3 Cortisol analysis

At the clinic, saliva samples were stored at -20 ºC, and transported afterwards to the laboratory for storage at -80 ºC and analysis. Salivary cortisol was assayed in duplicate by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (DRG Diagnostics, Germany). The lower limit of detection of this method is 1.14 ng/ml at the 95% confidence limit. All samples from an individual participant were analyzed in a single assay run.

2.4 Clinical State

Clinical symptoms in schizophrenic patients were assessed by means of the Dutch translation of the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987). The Dutch version of the PANSS comprises 32 items. It was designed to assess three main domains: the positive subscale (7 items), the negative subscale (7 items), and the general psychopathology (16 items). The total score is obtained by simply adding the scores on the three subscales. The PANSS is widely used in clinical and research settings, and is regarded as a reliable instrument for the assessment of psychopathology in schizophrenic patients (Kay, Opler, & Lindenmayer, 1988; Bell et al., 1992; Muller et al., 1998). The PANSS was translated in 1996 by Linszen, De Haan, Kuipers and Dingemans.

2.5 *Procedure*

Subjects were asked to collect five saliva samples for the cortisol measures at different time points: t_1: directly after waking when subject is still in bed; t_2: 30 minutes after waking; t_3: at 12:00h; t_4: at 16:00h; t_5: at 22:00h. Patients and controls received capped plastic vessels for the salivary samples. All subjects received a standardized verbal and written instruction for the sampling (appendix III). The exact time of collection was to be indicated on an enclosed form. A psychiatric nurse who had received standardized instructions assisted patients with the cortisol sampling. Controls were asked to gather the salivary samples at home and store the samples in the refrigerator until they could be returned. Previous research indicated that cortisol concentrations in unfrozen samples are stable during extended periods when exposed to widely varying temperatures and movement (Clements & Parker, 1998). Subjects were asked to follow their normal daily rhythm to avoid bias in cortisol levels as a result of extreme activities or a deviance of the normal daily patterns. Furthermore, subjects were asked not to eat or to brush their teeth during a half hour before the sampling. The latter to avoid contamination of saliva with blood caused by micro-injuries in the oral cavity. All

subjects were examined in the same season. A psychiatrist assessed symptom severity in schizophrenic patients by means of the PANSS on the study day.

2.6 Statistical analysis

Descriptive statistics were computed for cortisol levels and PANSS scores. To determine whether changes in the diurnal cycle of cortisol differed between schizophrenics and controls we performed a 2 X 5 repeated-measures analysis of covariance (ANCOVA-RM), with Group (schizophrenics vs. controls) as the between subjects factor and Time (t_1 – t_5) as the within subjects factor. Medication was included as a covariate. For this purpose, we relabelled medication into a dichotomous variable. When appropriate, results were corrected by the Greenhouse-Geisser procedure.

To measure the total cortisol secretion, we computed the area under the curve with respect to the ground (AUC_G) following the trapezoid formula. This method allows us to simplify the statistical analysis and increase the power of the testing without sacrificing the information contained in multiple measurements. Furthermore, the exact time of sampling is used for the AUC analysis. Therefore, this analysis provides us with the possibility to take samples that deviated from the instructed sampling time into account. The AUC_G values that were obtained by this method were analysed using an ANCOVA because this analysis allowed us to control for medication. The within subjects factor was the total cortisol secretion (AUC_G), and the between subjects factor was Group (schizophrenics vs. controls). A clear explanation of the method is given by Pruessner et al. (2003).

Pearson's product moment correlations were calculated to investigate the relationship between cortisol secretion and symptom severity. For this analysis we used the AUC values and the cortisol samples of 16:00 h (t_4) and 22:00 h (t_5). The latter two were used because these time points are not affected by the morning peak of cortisol secretion. All statistical measures were two-tailed, and the alpha was defined at 0.05. We used the Statistical Packages for the Social Sciences (SPSS) version 11.0 to analyse our data.

3. Results

3.1 Cortisol levels and diurnal pattern

Descriptive statistics for cortisol secretion are presented in Table 1. Patients and controls were matched in terms of their age (23.6 ± 5.4 vs. 20.9 ± 2.5 years respectively; t (28) = -1.74, p = 0.93). The Group (schizophrenics vs. controls) X Time ($t_1 – t_5$) ANCOVA-RM analysis showed a significant main effect for Group [F (1,27) = 4.29, $p < .05$]. This means that overall, the schizophrenic patients had significantly higher cortisol levels than the control subjects. As can been seen in Figure 4, this main effect is probably due to the differences in cortisol secretion between schizophrenic patients and controls at t_1 and t_5. Furthermore, there was a main effect for Time [F (2.4,64.7) = 14.34, $p < .001$]. Thus, both groups secreted different amounts of cortisol at the different time points. Visual inspection of Figure 4 clearly showed a diurnal pattern of cortisol secretion. No medication covariate effects were found [F (2.4,64.7) = .77, n.s.]. This indicates that medication did not influence cortisol secretion. Furthermore, no interaction was found between Group and Time [F (2.4,64.7) = 1.35, n.s.], indicating that cortisol secretion in schizophrenic patients and control subjects followed the same diurnal pattern.

Table 1. Descriptive statistics for cortisol secretion in schizophrenic patients and healthy controls.

Measures	Schizophrenic patients (n = 16)	Healthy controls (n = 15)
Directly after awakening (nmol/l)	16.6 (5.4)	11.0 (3.4)
30 min. after awakening (nmol/l)	18.0 (9.1)	16.8 (9.4)
12:00 h (nmol/l)	10.1 (3.8)	9.7 (5.2)
16:00 h (nmol/l)	8.8 (3.4)	7.1 (4.5)
22:00 h (nmol/l)	9.7 (6.2) [a]	4.3 (3.1)
AUC (min x nmol/l)	9238.4 (3276.9) [a]	7085.0 (2501.4)

Data are presented as mean (SD). AUC = area under the curve.

[a] n = 15 due to missing data.

3.2 Total cortisol secretion

The AUC analysis showed a trend towards significance for total cortisol secretion when controlled for medication [F (1,27) = 2.96, p = .097], with schizophrenic patients exhibiting a higher total cortisol secretion than control subjects. Again, no significant medication covariate effect was found [F (1,27) = .19, n.s.].

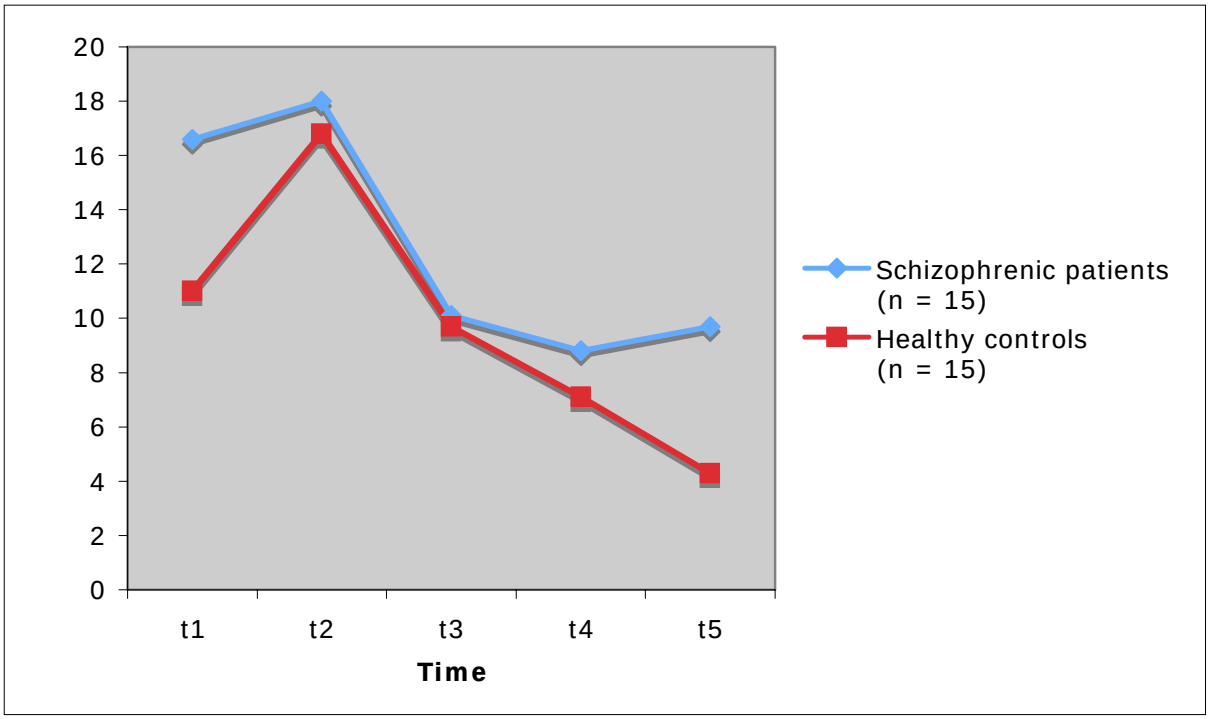

Figure 4. Mean cortisol secretion of schizophrenic patients and healthy controls at t_1: directly after awakening; t_2: 30 minutes after waking; t_3: 12:00h; t_4: 16:00h; t_5: 22:00h.

3.3 The relationship between cortisol and symptoms

Descriptive statistics for the PANSS scores are presented in Table 2. The results of the correlational analysis showed no significant relationship between cortisol secretion at 16:00 h or 22:00 h and positive symptoms, negative symptoms, general psychopathology, and overall symptom severity as indicated by the scores on the PANSS. Nor did it show significant correlations between the AUC values and the ratings on the different PANSS-scales (Table 3). This means that no evidence was found for a significant relationship between cortisol secretion and symptom severity. For illustrative purposes, we included scatterplots of the correlations between the cortisol measurements and the scores on the positive subscale (Figure 5a, b and c).

Table 2. Descriptive statistics for PANSS scores in schizophrenic patients.

	PANSS Symptom scale			
	Positive	Negative	General Psychopathology	Overall Symptoms
Schizophrenic patients (n = 16)	17.7 (6.2)	15.1 (4.9)	35.4 (12.1)	68.2 (18.6)

Data are presented as mean (SD).

Table 3. Correlation coefficients relating cortisol with symptom ratings on the PANSS.

	Symptom scale			
Cortisol	Positive	Negative	General Psychopathology	Overall Symptoms
Sample at 16:00 h[a]	.342 [c]	.152	.048	.178
Sample at 22:00 h[b]	.432 [d]	.140	.155	.275
AUC[b]	.435 [d]	-.001	-.005	.140

[a] n = 14.

[b] n = 13.

[c] p = .23

[d] p = .14

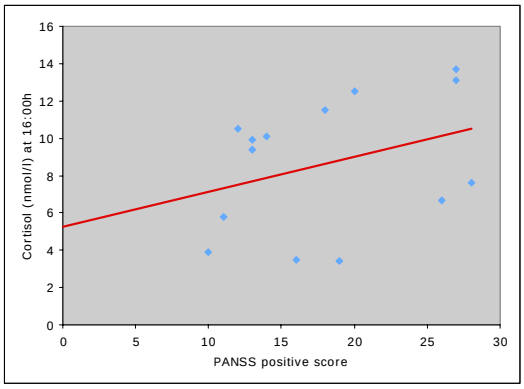

Figure 5a. Scatterplot of positive symptoms vs cortisol secretion at t_4 (16:00h).

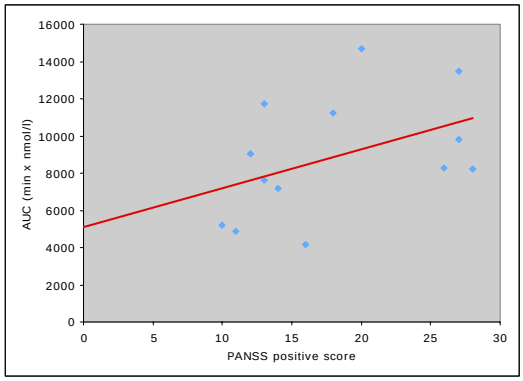

Figure 5c. Scatterplot of positive symptoms vs total cortisol secretion (AUC).

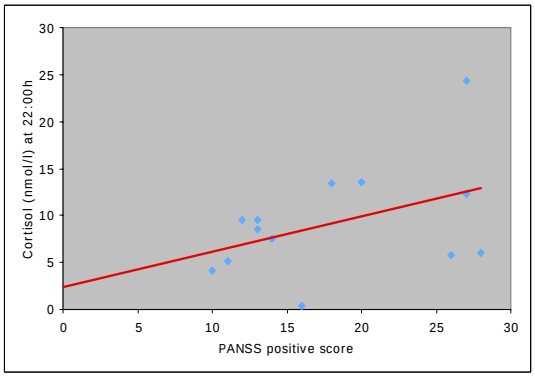

Figure 5b. Scatterplots of positive symptoms vs cortisol secretion at t_5 (22:00h).

4. Discussion

The present study was designed to investigate HPA-axis functioning in patients with schizophrenia. Stress and maladaptive coping seem to play an important role in the course of the illness. If these factors cause cortisol levels to be chronically heightened in schizophrenic patients, this may have a negative influence on symptom severity and increase the chances of relapse. This study was motivated by the lack of consensus from research up to date on this subject. By investigating a small part of the stress system in schizophrenic patients, we hoped to increase our understanding of the mechanisms underlying symptom severity and relapse in this disorder.

To get a clear view of HPA-axis functioning, we assessed salivary cortisol secretion. We compared the cortisol day curves of first-episode schizophrenic patients with those of healthy controls. Furthermore, the differences in cortisol levels between schizophrenic patients were related to symptom severity as measured by the PANSS. It was hypothesized that patients with schizophrenia would have elevated baseline cortisol levels, but the same diurnal pattern as healthy controls. Furthermore, we expected elevated cortisol levels to be positively correlated with ratings of overall symptom severity and ratings of positive symptoms.

Overall, patients showed a higher level of cortisol as compared to controls. Both groups displayed the same diurnal pattern. The correlational analysis yielded no significant relationships between symptom severity and cortisol levels.

4.1 Cortisol levels and diurnal pattern

Consistent with the predictions, the data obtained in this study indicated a normal diurnal pattern of cortisol secretion in schizophrenic patients and control subjects. On average, both groups showed the typical diurnal pattern with a morning peak about 30 minutes after awakening and a rapid decline until noon (12:00h). This decline slowly continued throughout the day. Cortisol secretion in schizophrenic patients differed from that observed in the control subjects, in that the mean cortisol values were higher. In other words, the diurnal pattern is preserved in schizophrenic patients, although at a higher level than in normal subjects.

These findings are in agreement with previous research. Gil-Ad et al. (1986) studied plasma cortisol levels in ten schizophrenic patients and eight age-matched controls. They took blood samples at 7:00h, 10:00h, 13:00h, 16:00h, 19:00h and 22:00h, and found higher basal cortisol levels in the schizophrenic patients. Ryan et al. (2004) took plasma cortisol samples from 13:00h to 16:00h with 20 minute intervals. The sample consisted of 12 drug

naïve patients with DSM-IV schizophrenia, and 12 age- and sex-matched controls. Patients had a higher mean cortisol AUC compared to controls. Walsh and colleagues (2005) investigated a patient sample that consisted of drug naïve, male patients with paranoid schizophrenia. Ten patients that experienced a first-episode psychosis were compared to 10 age-matched, healthy controls. A baseline plasma sample was taken at 13:00 h. They found an elevated level of plasma cortisol in the patients as compared to the controls. Despite the fact that these studies used another sampling method for obtaining cortisol measures and not all time points were similar, the results are in agreement with our findings.

Although there was no statistical difference between the diurnal patterns of the two groups, the overall higher cortisol level in schizophrenic patients seems to be mainly caused by deviating cortisol levels directly after awakening and at 22:00h, as shown in Figure 4. The morning peak seemed more distinct in the control subjects, as well as the decline at the end of the day. We know of one study that measured 24-hour cortisol secretion and only found significant differences at night (Van Cauter, 1991). Although this could not be concluded this strongly from our results, it could be hypothesized that especially nighttime cortisol secretion is deviant in schizophrenic patients.

4.2 Total cortisol secretion

Unexpectedly, the analysis of the total cortisol secretion revealed only a trend towards a higher total cortisol secretion in schizophrenic patients. Although the ANCOVA analyzes cortisol secretion over time and the AUC analyzes the total cortisol secretion, we would expect a potential difference to be revealed by both analyses. Therefore, it is not completely clear how to interpret these results. The absence of a significant result may be explained by the different methods of analysis. As previously discussed, large differences could only be observed directly after awakening and at 22:00h. The two groups did not show large differences 30 minutes after awakening, at 12:00h and at 16:00h. The difference between groups may therefore not reach significance when the total volume of cortisol is analyzed, although it does reach significance when measured over time. Insufficient statistical power due to small sample size may account for the failure of the total cortisol level to reach statistical significance. Nevertheless, the trend towards significance does imply a difference in total cortisol secretion for schizophrenic patients as compared to controls. These results are in line with the hypothesis that schizophrenic patients are impaired in their biological response to stress.

4.3 The relationship between cortisol and symptoms

Although the direction of the relations indicated a weak relationship between positive symptoms and cortisol secretion, none of the coefficients reached significance. Thus, there was no evidence for a relationship between HPA-axis functioning and the clinical manifestation of schizophrenia. The present findings could not support the hypothesis that elevated cortisol secretion is related to overall symptom severity. Nor could they support the existence of a significant relationship between heightened cortisol and positive symptoms. Again, it may be that the absence of significant correlations is due to the design and the small sample size. Insufficient statistical power may have hindered us in finding a relationship between symptom severity and cortisol levels.

The direction of the correlation, however, suggests that cortisol levels are higher when positive symptoms are present. This is in line with findings of Walder et al. (2000). They found significant positive correlations between cortisol levels and ratings of positive, disorganized and overall symptom severity. This may reflect the influence of cortisol on dopaminergic activity (Walker & Diforio, 1997). Further research on the relationship between HPA-axis activity and symptomatology is required.

4.4 Influence of medication on cortisol secretion

A large part of our patient group (65%) was medicated with an antipsychotic drug. By including medication in the main analysis, we hoped to control for potential medication effects. Research up to date shows a lack of consensus for the effect of antipsychotic drugs on HPA-axis functioning. Typical and atypical antipsychotic medication seems to reduce cortisol release (Walker & Diforio, 1997), and several studies found normalizing effects of clozapine on HPA-axis functioning (Breier et al., 1993; Hatzimanolis et al., 1998). However, chronic use of antipsychotics does not seem to affect cortisol levels (Meador-Woodruff & Greden, 1988). In addition, research indicates that haloperidol does not influence cortisol secretion (Angelopoulos et al., 1997; Ryan et al., 2004). Several patients also used a benzodiazepine. This drug has been known to reduce the effects of stress on cortisol (Breier et al., 1991). In the present study, use of medication was carefully documented and included as a covariate in the analyses. These analyses yielded no significant relationship between medication and cortisol secretion. Furthermore, this study found higher cortisol levels in schizophrenic patients. Since most antipsychotic drugs seem to have a normalizing effect on cortisol, this knowledge makes the conclusion that schizophrenic patients have higher baseline cortisol levels even more plausible. Nevertheless, since patients used different

types of medication and because medication was not included in the correlational analysis, we cannot completely exclude the possibility of unintended medication influences on our results.

4.5 Limitations

A number of critical remarks can be made with regard to the method of the current study. Firstly, because we intended to get a better view of the complete day curve, we instructed all subjects to collect the samples on the same day and to follow their normal daily rhythm. However, due to practical difficulties several patients did not collect all samples on the same day. This makes it difficult to draw valid conclusions. Those patients may have been exposed to different stress levels on the days the saliva samples were taken. Therefore, the quality of our data may have been influenced. However, several studies indicate that cortisol secretion has a moderate to high stability across days. The greatest variation in cortisol levels is observed in the morning hours (Pruessner et al., 1997; Schulz et al., 1998; Wüst et al., 2000). In the present sample, measurements in the first hours after awakening were always taken on the same day. This reduces the possibility of large variations in the gathered data to a minimum.

Secondly, it is possible that results have been influenced by non-adherence to the sampling instructions. Previous research indicates that self-report of compliance is overestimated in the absence of objective monitoring (Kudielka, Broderick & Kirschbaum, 2003; Broderick et al., 2004). This may result in data that significantly differs from compliant data. Therefore, electronic monitoring, and subject awareness of the monitoring is recommended. Unfortunately, no electronic monitoring devices were available for the present study. To compensate, psychiatric nurses, patients and controls were well instructed beforehand about the importance of compliance for obtaining an accurate day curve. Furthermore, the subjects were instructed to keep a record of the time of sampling on an enclosed form, even when the time of the actual sampling deviated from the instructed sampling time. They were told that deviance of the instructed time points could be accounted for, as long as it was indicated on the enclosed form. Therefore, large discrepancies between self-reported compliance and actual compliance are not likely.

A final critical note concerns the fact that patients were included in the study when they experienced a first-episode psychosis. These patients were diagnosed as being schizophrenic. However, this diagnosis will be reevaluated after a period of six months. This

study was completed before the diagnoses had been confirmed. It is therefore possible that the present study includes non-schizophrenic patients.

4.6 Conclusion and implications

The main result of this study supports the idea of hypercortisolism in schizophrenia. It is however not clear what causes the elevation of the cortisol levels in schizophrenic patients. There is a possibility that stress in early life caused a persistent sensitization of the HPA-axis in schizophrenic patients. This may lead to increased biological reactions to stress in adulthood (Mäki et al., 2005). Cortisol may not be able to bind to glucocorticoid receptors, there may be less glucocorticoid receptors, or glucocorticoid receptors may be less sensitive (Sapolsky, 2000). These alternatives remain to be examined. Furthermore, it is unclear how the present finding should be implemented in the vulnerability-stress model (Nuechterlein et al., 1994). This study does not clarify whether these heightened cortisol levels are associated with the genetic vulnerability for schizophrenia or with the clinical phenotype of the disease. Marcelis et al. (2004) described HPA-axis malfunctioning in schizophrenic patients, but not in first-degree relatives. They concluded that altered stress sensitivity was associated with the clinical state of schizophrenia. Therefore, it would be interesting to study the cause of HPA-axis malfunctioning in schizophrenia more thoroughly in future research.

In conclusion, although our results could not support the hypothesized relationship between cortisol and symptom severity, we found evidence for heightened day time cortisol levels in schizophrenic patients as compared to healthy controls. The hypothesis of a dysregulation of the HPA-axis in schizophrenic patients is supported. However, it would be premature to conclude that heightened cortisol levels are a marker of vulnerability in schizophrenic patients. The present study offers perspectives for future research. This research should include a larger sample of preferably unmedicated patients. If possible, it would also be advisable to measure the cortisol day curve several following days in the presence of an electronic monitoring device.

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Appendices

Appendix I: Diagnostic criteria for schizophrenia DSM IV

Diagnostic criteria for Schizophrenia

A. *Characteristic Symptoms*: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if succesfully treated):
   (1) delusions
   (2) hallucinations
   (3) disorganized speech (e.g., frequent derailment or incoherence)
   (4) grossly disorganized or catatonic behavior
   (5) negative symproms, i.e., affective flattening, alogia, or avolition

   Note: Only one Criterion A symptom is required if delusions are bizzare or hallucinations consist of a voice keeping up a running commentary on the person's behaviot or thoughts, or two or more vices conversing with each other.

B. *Social/occupational dysfunction*: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. *Duration*: Continuous signs of the disturbance persist for at least 6 months. This 6 months period must include at least 1 month of symptoms (or less if succesfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the distrubance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. *Schizoaffective and Mood Disorder exclusion*: Schizoaffective Disorder and Mood Disorder With Psychotic Features have been ruled out because either (1) no Major Depressive, Manic, or Mixed Episodes have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and

residual periods.

E. *Substance/general medical condition exclusion*: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. *Relastionship to a Pervasive Developmental Disorder*: If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of Schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if succesfully treated).

Classification of longitudinal course (can be applied only after at least 1 year has elapsed since the initial onset of active-phase symptoms):

Episodic With Interepisode Residual Symptoms (episodes are defined by the reemergence of prominent psychotic symptoms); *also specify if:* **With Prominent Negative Symptoms**

Episodic With No Interepisode Residual Symptoms

Continuous (prominent psychotic symptoms are present throughout the period of observation); *also specify if:* **With Prominent Negative Symptoms**

Diagnostic criteria for 295.30 Paraniod Type

A type of Schizophrenia in which the following criteria are met:

A. Preoccupation with one or more delusions or frequent auditory hallucinations.

B. None of the following is prominent: disorganized speech, disorganized or catatonic behavior, or flat or inappropriate affect.

Diagnostic criteria for 295.10 Disorganized Type

A type of schizophrenia in which the following criteria are met:

A. All of the following are prominent:
   (1) disorganized speech

(2) disorganized behavior
(3) flat or inappropriate affect

B. The criteria are not met for Catatonic Type.

Diagnostic criteria for 295.20 Catatonic Type

A type of Schizophrenia in which the clinical picture is dominated by at least two of the following:

(1) motoric immobility as evidenced by catalepsy (including waxy flexibility) or stupor
(2) exessive motor activity (that is apparantly purposeless and not influenced by external stimuli)
(3) extreme negativism (an apparently motiveless resistance to all instructions or maintenance of a rigid posture against attempts to be moved) or mutism
(4) peculiarities of voluntary movement as evidenced by posturing (voluntary assumption of inappropriate or bizarre postures), stereotyped movements, prominent mannerisms, or prominent grimacing
(5) echolalia or echopraxia

Diagnostic criteria for 295.90 Undifferentiated Type

A type of schizophrenia in which symproms that meet Criterion A are present, but the criteria are not met for the Paranoid, Disorganized, or Catatonic Type.

Dagnostic criteria for 295.60 Residual Type

A type of schizophrenia in which the following criteria are met:

A. Absence of prominent delusions, hallucinations, disorganized speech, and grossly disorganized or catatonic behavior.
B. There is continuing evidence of the disturbance, as indicated by the presence of negative symprtoms or two or more symptoms listed in Criterion A for Schizophrenia, present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

Appendix II: Personal Details and Health Questionnaire

Naam:	Ppn:	Datum: - -2005

Erasmus MC centrumlocatie
Afd. Psychiatrie
Dr. Molenwaterplein 40
3015 GD Rotterdam
Onderzoek: Psychosen2

Personalia deelnemer

NAAM : ……..

ADRES : ……..

POSTCODE : ……..

WOONPLAATS : ……..

TELEFOON : ……..

BEROEP/ OPLEIDING : ……..

GEBOORTEDATUM : ……GESLACHT: M/V

NATIONALITEIT (afkomst): ……

LENGTE : ……m

GEWICHT : ……kg

DOMINANTE HAND : Links/ Rechts LENZEN : Hard/ Zacht

GENOTSMIDDELEN : * Alcohol : …. Consumpties/ week
* Koffie : …. Consumpties/ dag
* Sigaretten : …. Aantal/ dag

Heeft u vandaag alcohol, koffie of sigaretten gebruikt? ja/nee Zo ja, hoeveel?
.....

MEDICATIE GEBRUIK
.....
.....

Naam:	Ppn:	Datum: - -2005

Erasmus MC centrumlocatie
Afd. Psychiatrie
Dr. Molenwaterplain 40
3015 GD Rotterdam
Onderzoek: Psychosen

REKENINGNUMMER

BANK/GIRO : ……...

FISCAALNUMMER : ……...

REISKOSTEN VER-
GOEDING : …….

AANTEKENINGEN/
OPMERKINGEN :……...

……...

……...

…….

…….

…….

…….

…….

…….

FORMULIER GEZONDHEIDSTOESTAND

Naam:……

Datum:……

Lijdt of heeft u ooit geleden aan een der onderstaande ziekten, respectievelijk, heeft of had u een der onderstaande klachten?:

- Neurologische aandoeningen? Ja / Nee
- Hoofd- nekletsel? Ja / Nee
- Hart- en vaatziekten, bijvoorbeeld een te hoge bloeddruk? Ja / Nee
- Astma, bronchitus, hoesten, kortademigheid? Ja / Nee
- Hoofdpijn, duizelingen, flauwten, toevallen? Ja / Nee
- Duizeligheid bij het opstaan uit een stoel of uit bed? Ja / Nee
- Kleurenblindheid? Ja / Nee
- Gehoorproblemen (doofheid / slechthorendheid)? Ja / Nee

Lijdt u of heeft u ooit geleden aan een psychiatrische ziekte? Ja / Nee

Zo ja, welke?

…….....

.....

Komen er psychiatrische aandoeningen bij u in de familie voor? Ja / Nee

Zo ja welke, en bij welk familielid?

……

…….....

Gebruikt u medicijnen? Ja / Nee

Zo ja, welke?

.....…

…….....

Appendix III: Sampling Instruction and Registration Form

Controles

Instructie verzamelen speeksel

Geachte heer/mevrouw,

U bent uitgenodigd deel te nemen aan het onderzoek “Emotionele kwetsbaarheid bij schizofrenie: de invloed van emoties op de startle reflex en affectieve interferentie”. In dit onderzoek worden een aantal dingen onderzocht. Eén van de dingen waar we naar willen kijken is cortisol.

Wat is cortisol?

Cortisol is een stofje wat in het menselijk lichaam zit, bijvoorbeeld in het speeksel. Het heeft te maken met spanning: er komt meer cortisol in het speeksel wanneer er iets spannends (leuks of vervelends) gebeurt. Daarom willen we een aantal keer wat speeksel van u verzamelen. Het speeksel zal worden gebruikt om zogenaamde cortisolconcentraties te kunnen bepalen. De cortisolspiegel wisselt over de dag. Het is dus van belang om op verschillende tijdstippen op de dag speeksel te verzamelen.

Cortisol in het speeksel.

In het onderzoek gaan we proberen vast te stellen hoeveel cortisol er in uw speeksel zit. Om dat te kunnen bepalen hebben we u 5 buisjes meegegeven/opgestuurd. We willen u nu vragen om de dag *voordat* u aan het onderzoek gaat deelnemen in het ziekenhuis, 5x wat speeksel in één van de buisjes te verzamelen.

1. De voorbereiding.

Allereerst: het is belangrijk dat u een schone mond heeft om zo zuiver mogelijk speeksel te kunnen verzamelen. We willen u dan ook verzoeken om een *half uur* voor de speekselafname niet meer te eten. Daarnaast verstoren zuivelproducten (zoals melk of yoghurt) de samenstelling van het speeksel nogal. Het verzoek is dan ook om *één uur* voordat het speeksel wordt verzameld geen zuivelproducten (melk e.d.) meer te drinken of te eten.

2. Wanneer speeksel te verzamelen??

Het is belangrijk dat er op *één dag op vijf vaste tijdstippen* speeksel wordt verzameld:
1. Meteen bij het ontwaken,
2. Een half uur na het ontwaken,
3. Om 12:00 ’s middags,
4. Om 16:00 ’s middags,
5. En om 22:00 uur ’s avonds.

Het is de bedoeling dat de speekselafname de dag voor het onderzoek in het Erasmus Ziekenhuis gebeurt. Het is van groot belang dat de eerste afname echt vlak na het ontwaken gebeurt. De tweede afname gebeurt 30 minuten na het ontwaken.

3. Speeksel verzamelen.

Dit is eenvoudig. In de gesloten envelop treft u vijf buisjes aan. Ieder buisje heeft een etiket. Op ieder etiket ziet u rechtsonder een nummer: 1 t/m 5:

1. Buisje 1 is het buisje voor bij het wakker worden. Tip: leg het buisje met een pen voordat u gaat slapen naast uw bed, zodat u meteen speeksel kunt verzamelen nadat u wakker bent geworden.
   Als u wakker wordt zet u meteen het buisje met nummer 1 tegen uw onderlip. Het is belangrijk dat u hier snel mee bent. Laat dan wat speeksel in het buisje lopen. Als het speeksel tot de onderkant van het etiket komt heeft u genoeg. Stop de dop erop. Kijk dan op de wekker of horloge en zet de tijd op het bovenste etiket.
2. U kunt zich nu gewoon gaan wassen en aankleden, maar eet alstublieft nog niet!! Want precies 30 minuten later is buisje twee al aan de beurt.
3. Buisje twee een half uur na het wakker worden; Dertig minuten na buisje 1 mag u in buisje 2 wat speeksel verzamelen, weer tot de onderkant van het etiket. Dop erop en dan kunt u ontbijten!! De buisjes kunnen bij voorkeur in de vriezer bewaard worden, maar als die niet in de buurt is, dan mag het ook in de koelkast bewaard worden.
4. buisje 3 mag om 12:00 uur gevuld worden,
5. buisje 4 om16:00 uur,
6. en tot slot buisje 5 om 22:00 uur

Op buisje 1 zit een extra etiket. Wilt u hierop aangeven hoe laat u wakker bent geworden en dus het speeksel heeft verzameld?

4. Hoe krijgt u het speeksel in het buisje?

Het is het makkelijkst om voldoende speeksel te verzamelen, wanneer u het buisje tegen de onderlip aanzet en steeds een kleine hoeveelheid speeksel in het buisje laat lopen. Het is de bedoeling dat het speekselniveau tot de onderkant van het etiket komt. Dit is zonder het schuim dat soms boven het speeksel zit. Nadat er voldoende is verzameld, gelieve het buisje goed af te sluiten (dop erop).

5. De buisjes bewaren.

Nadat een buisje is afgesloten, kan het buisje het beste in het vriesvak worden bewaard zodat de kwaliteit van het speeksel zo optimaal mogelijk blijft. Als er geen vriezer in de buurt is kunnen de buisjes ook korte tijd worden bewaard in een koelkast. Het is dan wel belangrijk om zo snel mogelijk de buisjes uit de koelkast in de vriezer te plaatsen.
Wilt u zo vriendelijk zijn om de buisjes mee te nemen op de onderzoeksdag? Dan zullen wij ze in het ziekenhuis in de vriezer opbergen.

Hartelijk dank voor uw tijd en inzet!

Patiënten

Instructie verzamelen speeksel

Geachte heer/mevrouw,
U bent uitgenodigd deel te nemen aan het onderzoek “Emotionele kwetsbaarheid bij patiënten met een eerste-episode psychose”. In dit onderzoek worden een aantal dingen onderzocht. Eén van de dingen waar we naar willen kijken is cortisol.

Cortisol in het speeksel.
In het onderzoek gaan we proberen vast te stellen hoeveel cortisol er in uw speeksel zit. We willen u nu vragen om de dag *nadat* u aan het onderzoek met de computer heeft deelgenomen, 5 keer wat speeksel in buisjes te verzamelen.

1. De voorbereiding.
Allereerst: het is belangrijk dat u een schone mond heeft om zo zuiver mogelijk speeksel te kunnen verzamelen. We willen u dan ook verzoeken om een *half uur* voor de speekselafname niet meer te eten. Het is echter niet de bedoeling dat u uw tanden poetst voor de speekselafname. Daarnaast verstoren zuivelproducten (zoals melk of yoghurt) de samenstelling van het speeksel nogal. Het verzoek is dan ook om *één uur* voordat het speeksel wordt verzameld geen zuivelproducten (melk e.d.) meer te drinken of te eten.

2. Wanneer speeksel te verzamelen??
Het is belangrijk dat er op *één dag op vijf vaste tijdstippen* speeksel wordt verzameld:
6. Meteen bij het ontwaken,
7. Een half uur na het ontwaken, (vóór het ontbijt)
8. Om 12:00 ’s middags (vóór de lunch),
9. Om 16:00 ’s middags,
10. En om 22:00 uur ’s avonds (vóór het tandenpoetsen).

Het is van groot belang dat de eerste afname echt vlak na het ontwaken gebeurt. De tweede afname gebeurt 30 minuten na het ontwaken.

3. Speeksel verzamelen.
Ieder buisje heeft een etiket. Op ieder etiket ziet u rechtsonder een nummer: 1 t/m 5:
7. Buisje 1 is het buisje voor bij het wakker worden. De verpleging zal u bij het wekken het buisje geven, zodat u meteen kunt spugen.
   Als u wakker wordt zet u meteen het buisje met nummer 1 tegen uw onderlip. Het is belangrijk dat u hier snel mee bent. Laat dan wat speeksel in het buisje lopen. Als het speeksel tot het zware streepje komt heeft u genoeg. Stop de dop erop. De verpleging zal de tijd noteren.
8. U kunt zich nu gewoon gaan wassen en aankleden, **maar eet alstublieft nog niet!!** Want dertig minuten na buisje 1 mag u in buisje 2 wat speeksel verzamelen, weer tot het zwarte streepje. Dop erop, de verpleging noteert de tijd en dan kunt u ontbijten!!
9. buisje 3 mag om 12:00 uur gevuld worden;
10. buisje 4 om 16:00 uur;
11. en tot slot buisje 5 om 22:00 uur.

De verpleging zal u helpen met het verzamelen van het speeksel en op het registratieformulier noteren op welke tijd er precies speeksel is verzameld.

4. Hoe krijgt u het speeksel in het buisje?

Het is het makkelijkst om voldoende speeksel te verzamelen, wanneer u het buisje tegen de onderlip aanzet en steeds een kleine hoeveelheid speeksel in het buisje laat lopen. Het is de bedoeling dat het speekselniveau tot de zwarte streep komt. Dit is zonder het schuim dat soms boven het speeksel zit. Nadat er voldoende is verzameld, gelieve het buisje goed af te sluiten (dop erop).

Wat is cortisol?
Cortisol is een stofje wat in het menselijk lichaam zit, bijvoorbeeld in het speeksel. Het heeft te maken met spanning: er komt meer cortisol in het speeksel wanneer er iets spannends (leuk of vervelend) gebeurt. Daarom willen we een aantal keer wat speeksel van u verzamelen. Het speeksel zal worden gebruikt om zogenaamde cortisolconcentraties te kunnen bepalen. De cortisolspiegel wisselt over de dag. Het is dus van belang om op verschillende tijdstippen op de dag speeksel te verzamelen.

Hartelijk dank voor uw tijd en inzet!

Naam:	Ppn:	Datum / /2005

Erasmus MC centrumlocatie
Afd. Psychiatrie
Dr. Molenwaterplein 40
3015 GD Rotterdam
Onderzoek: Psychosen2

Registratieformulier verzamelen speeksel

Buisje 1: direct na het ontwaken

Tijd: .....

Buisje 2: 30 minuten na het ontwaken

Tijd: .....

Buisje 3: 12:00 uur ’s middags

Tijd: .....

Buisje 4: 16:00 uur ’s middags

Tijd: .....

Buisje 5: 22:00 uur ’s avonds

Tijd: .....