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DISSERTATION

Effects of Mineralocorticoid Receptor Stimulation on Cognitive Functions in Depressed and Healthy Subjects

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Abstract

Background:

People suffering from major depression often show impaired cognitive function while cortisol secretion is increased. Being predominantly expressed in the hippocampus and prefrontal cortex, mineralocorticoid receptors (MRs) are linked to cognitive function and cortisol inhibition. Therefore, we investigated whether specific fludrocortisone-induced stimulation of MRs 1) improves cognitive functions and 2) decreases cortisol levels in participants with major depression and healthy controls.

Methods:

In this randomized, double-blind, within-subject, cross-over study design, twenty-four drug-free depressed patients and twenty-four age-, sex- and education-matched healthy controls were treated with either 0.4 mg fludrocortisone or placebo. Cognitive functions were assessed between 2:00 and 5:00 pm; cortisol secretion was measured during cognitive testing using saliva samples.

Results:

1) Fludrocortisone-induced stimulation of central MRs improved verbal memory and executive function across groups. No treatment effect on other cognitive domains emerged. In verbal memory and psychomotor speed tests, people with major depression achieved worse results than their healthy counterparts did.

2) Fludrocortisone-induced stimulation of MRs decreased cortisol secretion across groups. We found a significant correlation between cortisol inhibition and verbal memory performance.

Conclusions:

In conclusion, MR stimulation reveals a possibility to improve cognitive functions in depressed and in healthy participants, which might be partly explained by cortisol inhibition.

Zusammenfassung

Hintergrund:
Depressive Störungen gehen häufig mit Beeinträchtigungen der kognitiven Leistungsfähigkeit und gesteigerter Kortisolsekretion einher. Zentrale Mineralokortikoidrezeptoren (MR) spielen eine wichtige Rolle bei kognitiven Funktionen und der Regulation des Kortisolhaushalts. Im Gehirn werden MR überwiegend im Hippocampus und im präfrontalen Kortex exprimiert, zwei Hirnareale, die eng mit der kognitiven Leistungsfähigkeit und der Hemmung der Kortisolsekretion assoziiert sind. Wir untersuchten die Effekte von Fludrokortison, einem MR-Agonisten, auf die kognitive Leistungsfähigkeit und Kortisolsekretion. Unsere primäre Fragestellung war, 1) ob durch die Stimulation der MR die kognitiven Funktionen verbessert und 2) die Konzentration des freien Kortisols gesenkt werden können.

Methodik:
In dieser randomisierten, doppelblinden, intraindividuellen Cross-Over-Studie erhielten 24 unmedizierte depressive und 24 gesunde alters-, geschlechts- und bildungsparallelisierte Probanden 0,4 mg Fludrokortison oder ein Plazebo. Zwischen 14:00 und 17:00 Uhr wurde zum einen die kognitive Leistungsfähigkeit mit Hilfe standardisierter Testverfahren und zum anderen die Kortisolsekretion mittels Speichelproben und anschließender Speichelkortisolbestimmung erfasst.

Ergebnisse:
1) Die Fludrokortison-induzierte Stimulation zentraler MR verbesserte die verbale Gedächtnisleistung und die Exekutivfunktionen sowohl bei depressiven Patienten als auch gesunden Kontrollprobanden. Weitere kognitive Domänen wurden durch die MR-Stimulation nicht beeinflusst. Bezüglich der verbalen Gedächtnisleistung und der psychomotorischen Geschwindigkeit erzielten die depressiven Teilnehmer schlechtere Ergebnisse als die gesunden Kontrollprobanden.
2) Die Fludrokortison-induzierte Stimulation zentraler MR reduzierte die Kortisolsekretion sowohl bei depressiven Patienten als auch gesunden Kontrollprobanden. Darüber hinaus bestand über beide Gruppen hinweg eine signifikante Korrelation zwischen der Inhibition der Kortisolsekretion und der verbalen Gedächtnisleistung.

Zusammenfassung:
Die gezielte Stimulation zentraler MR eröffnet eine neue Möglichkeit zur Verbesserung der kognitiven Funktionen bei depressiven und gesunden Probanden. Dieser Effekt könnte unter anderem auch auf die Absenkung der Kortisolkonzentration zurückgeführt werden.

1. INTRODUCTION

The debate on the occurrence of cognitive deficits in major depression has for a long time concerned researchers. With new biological theories emerging on aetiology and pathophysiology of depressive disorders, the development of the mineralocorticoid receptor-to-glucocorticoid receptor disbalance-hypothesis based on characteristic hypothalamus-pituitary-adrenal axis dysregulations has introduced new clinical and preclinical approaches to the understanding of psychiatric symptomatology. By placing the mineralocorticoid receptor family into the centre, latest lines of research cast a new light on the close connections between stress-system dysregulations, depressive disorders and, of course, cognitive impairments.

To further understand the role of mineralocorticoid receptors in cognitive deficits, this study aimed to investigate whether specific receptor stimulation would improve cognitive performance in the context of major depression, and in how far findings could be transferred to non-depressed participants.

To outline the adopted working model and research questions entailed, this chapter introduces important concepts of depressive disorders and cognitive systems. The theoretical basis for the interrelations and psychopathology of cognition in major depression is established using the concept of hypothalamus-pituitary-adrenal axis dysregulation in major depression.

Latest epidemiological data stress the importance of research on the fields of aetiology, pathophysiology and especially therapy of depressive disorders.

In international studies, lifetime prevalence of clinically relevant depressive disorders measures up to 20% (S3-Guideline/National Disease Management Guideline Unipolar Depression, effective 2012). In Germany, lifetime prevalence is estimated to be 11.6% in total, females 15.4% and males 7.8% (Busch 2013). Affective disorders will take second place of widespread diseases by 2020 according to WHO (Stiftung Deutsche Depressionshilfe 2009).

Depressive disorders are associated with a high burden of disease (Global Burden of Disease Study 2000, “GBD 2000”). International studies to assess QALY-rankings show that for females, depression ranks fourth and in males they rank seventh (GBD 2000). In the population aged between 15 and 44 years,

depressive disorders rank second for females and third for males (GBD 2000). Economical analyses reveal that direct costs caused by depressive disorders in Germany amount to 4.6 billion euros (Stiftung Deutsche Depressionshilfe 2009).

These facts underline the social, economic and of course political relevance of depressive disorders und emphasize the increasing demand for sufficient research to improve therapy.

1.1 Major Depressive Disorder

According to DSM-5, major depressive disorder (MDD) is the model disease for the category “Depressive Disorders” (DSM-5 2013).
In order to diagnose major depressive disorder, Criteria A to E have to be met for at least 2 consecutive weeks. The combination of Criteria A – C characterises a major depressive episode.

Criterion A comprises of a variety of symptoms common in depression:

- Depressed mood for most of the day on almost every day of an episode; it can be either reported by the patient or observed by others
- Anhedonia, which is defined as loss of interest and pleasure of activities that were usually enjoyable for the same time period as mentioned above
- Considerable weight loss or weight gain without personal efforts or intentions; DSM-5 requires a change of 5% of body weight in a month's time
- Insomnia or hypersomnia
- Psychomotor retardation or agitation as reported by others
- Fatigue or loss of energy
- Feeling of worthlessness or excessive inappropriate feeling of guilt, which might appear in the context of delusional symptoms
- Cognitive impairments concerning memory, concentration or thinking
- Ideas of death, suicidal ideations, suicide attempts or specific plans for committing suicide

A total of at least 5 symptoms of Criterion A, necessarily including depressed mood and/or anhedonia, have to have occurred anew in the present episode and have to last for at least 2 weeks.

Criterion B requires the symptoms mentioned under Criterion A to cause clinically significant distress or impairment of social or other important areas of functioning.

Criterion C excludes symptoms which are demonstrably caused by other medical conditions or substance side effects.

Criterion D excludes causal influences of schizophrenia spectrum and other psychotic disorders.

Criterion E excludes any hypomaniac or maniac episodes, unless they were substance-induced or caused by other medical diseases.

To characterize a depressive episode more precisely, it is important to know whether it is the first in the patient's life or if the patient already suffered from a depressive episode before, no matter if previously diagnosed or based solely on self-observation. In technical terms, it is to be decided whether the present episode is regarded as single or recurrent.
Secondly, severity of acute psychopathological symptoms has to be assessed. According to DSM-5, acute severity is given if full criteria for a major depressive episode are met. The same is necessary for the diagnosis of psychotic symptoms. If for example severity is rated "mild", the depressed is still able to work sufficiently or manages his household more or less appropriately.
If no more criteria are met, the degree of remission has to be specified. Full remission is defined by absence of symptoms for at least 2 months or presence of up to 2 symptoms in a mild degree.

In some cases a major depressive episode can exhibit additional symptoms, for example anxious or atypical features. Psychotic symptoms can be either mood-congruent or mood-incongruent.

First-degree relatives of a depressed patient show 2 – 4-fold higher risk of developing major depression than the unrelated average population does. Heritability is estimated to be approximately 40%, with neuroticism as a personality trait accounting to a considerable amount. Neuroticism is defined as a negative affectivity. It is supposed to make individuals more susceptible to stressful events. Stressful life-events and especially adverse experiences in childhood increase risk as well (DSM-5 2013).

The first depressive episode is usually developed at a younger age, with incidence peaking in the twenties. However, a first depressive episode also in older people is not uncommon (DSM-5 2013).
The number of episodes per life varies from one to continuous repetition without any full remission in between. If a depressive episode occurred quite acutely, chances are higher for successful treatment or even spontaneous recovery. The probability of full remission is decreased if any or all of the following three are given: a severe grade, the onset at a young age and recurring episodes. The longer the duration of remission, the lower the risk for relapse (S3-Guideline/National Disease Management Guideline Unipolar Depression, effective 2012).

As described above, symptoms of impaired cognitive functions are among those defining depressive episodes. Most pronounced disturbances concern memory, attention and executive functions (Majer 2004, Ravnkilde 2002, Basso & Bornstein 1999). To conceptualize these, the following chapter introduces current theories of cognitive systems and outlines the applied working model.

1.2 Cognitive Functions

Mental functions – which include perception, memory, attention, concentration, thinking and more – are commonly referred to under the collective term of “cognitive functions” (McGraw-Hill Concise Dictionary of Modern Medicine 2002).

Memory

Memory is defined as the ability of the central nervous system (CNS) to retain afferent information selectively over a short or long period of time and to retrieve it when required (Zetkin 2005).

Memory can be structured by implementation of various systems, each emphasizing a different perspective. For this study, the memory concept of Squire et al. was applied (Squire 2004).

(1) Memory can be analysed regarding its temporal order. Basically, it is divided up into short-term memory (or primary memory) and long-term memory (or secondary or tertiary memory).

Short-term memory, which includes working memory, is anatomically a function of prefrontal and parietal cortex (Goldman-Rakic 1987). It is confined by means of duration of memory storage and by the amount of information, which can be memorized. As Helmstaedter et al. comprehensively depicted, short-term memory spans a time range of a maximum of up to 60 seconds. In order to make information available beyond one minute, it has to be consolidated, a process that is anatomically attributed to the hippocampus, and moved to long-term memory consecutively (see Helmstaedter 2001 for further information). The amount of information retainable is estimated at 7 +/- 2 items, which is rather constricted (Miller 1994). Recent research understands short-term and long-term memory not as two separate, somewhat dichotomous systems, but emphasizes the concept of parallel working (Markowitsch 1999).

Long-term memory is conceptualized with emphasis on the specific kind of stored information (Squire 2009). Basically, it can be divided up into declarative (or explicit) and nondeclarative (or implicit) memory.

Declarative memory stores facts. It is located in the medial temporal lobe, containing the hippocampus and diencephalon. It further can be subtyped into episodic and semantic memory (Tulving 2002). Episodic memory stores information that is connected to a certain situation, for example, word-learning tests in neuropsychological examinations. Its memory content can be retrieved consciously and it is the only memory system directed towards the past (Tulving

& Markowitsch 1998). Semantic memory, on the other hand, contains abstract knowledge of facts and is more unrelated to particular situations, for example linguistic proficiencies. Again, the two systems should not be separated but rather regarded as interrelated (Tulving & Markowitsch 1998).
Nondeclarative, or implicit, memory is composed of procedural memory (i.e. skills and habits), priming, classical conditioning and nonassociative learning (Squire 1998). Procedural memory is associated with the striatum. Priming is a part of neocortex functions. Classical conditioning can be divided into emotional responses and skeletal musculature functions. The amygdala forms the anatomical correlate of emotional responses, whereas skeletal musculature functions are connected to the cerebellum. Finally, nonassociative learning is represented in reflex pathways. In contrast to declarative memory, nondeclarative memory usually operates in the unconscious (Squire 1998).

(2) Memory can be analysed regarding the type of information stored. Basically, verbal information is discriminated from nonverbal, spatial information. Both temporal memory systems, short-term and long-term memory, are affected by this category (Baddeley 1991). Verbal information is supposed to be stored mainly in the left hemisphere, whereas spatial information is supposed to be processed by the right hemisphere primarily (Kelley 2002, Postle 1999, Smith 1996).

Executive Functions

The term “executive functions” subsumes basic cognitive functions, like decision making, planning, working memory and abstract thinking. Executive functions enable the person to react to his environment appropriately by initiating, planning, controlling and terminating complex responses. These functions are anatomically related to the prefrontal cortex and to associated fronto-striatal loops. Disrupted executive functions may result in diminished abilities concerning work, everyday life or relationships (Margraf 2012, Ayd 2000).

Working Memory

Working memory involves the temporary storage of spatial and non-spatial information after perception in order to actively select and process it due to its importance (Margraf 2012, Ayd 2000).

Attention

According to DSM-5, attention is defined as the ability to focus in a sustained manner on one activity (DSM-5), which enables a person to improve his cognitive capacity. Attention is linked to working memory (Margraf 2012).

Psychomotor Activity

Psychomotor activity is a collective term which refers to verbal and nonverbal behaviour like reaction time, speed of movement and flow of speech (Ayd 2000).

To integrate the presented cognitive functions (1.2) and the concept of major depression (1.1) – and especially impaired cognition as a common feature of major depression – and to establish the theoretical basis for the integration of mineralocorticoid receptors, the functional system of the hypothalamus-pituitary-adrenal axis and its relation to mineralocorticoid receptors, depressive disorders and cognition is outlined in the following chapter.

1.3 The Hypothalamus-Pituitary-Adrenal Axis

A common neurobiological finding in major depression is a dysregulated hypothalamus-pituitary-adrenal axis function.

The hypothalamus-pituitary-adrenal axis (HPA axis) fulfils several tasks of vital importance in vertebrates, for example within mammalian development and adaptation to physiological and psychological stressors (Denver 2009). The current debate regards the HPA axis as having already been present in the earliest vertebrates and as having been preserved to a large extent by

evolutional selection due to its crucial role for vertebrate life. As reported by Oelkers et al., people will die if it does not work properly (Oelkers 1996).

Basically, the HPA axis accomplishes two substantial functions. First, it is a constant provider of glucocorticoids, expressed in the diurnal circadian pattern of cortisol secretion. Secondly, it is the neuroendocrine correlate of adaptation to physical and psychological stress.

1.3.1 The Hierarchical Structure of the Hypothalamus-Pituitary-Adrenal Axis

Basically, the HPA axis is a hierarchically structured signalling cascade.

Top regulation is constituted by a main circadian oscillator localised in the suprachiasmatic nucleus of the hypothalamus (Chan 2010). In a pulsatile rhythm, the 41 AA neuropeptide corticotropin-releasing hormone (CRH) and arginine vasopressine (VP) are transported via axons to the eminentia mediana, after having been synthesised and stored in the parvocellular neurons of hypothalamic Ncl. paraventricularis (PVN). If an action potential as a stimulus reaches this area, exocytotic release leads to the secretion of these hormones, which are finally transported to the capillary bed of the adenohypophysis, where they primarily bind CRHR1 receptors – a G-coupled protein receptor – of basophilic corticotropes, ultimately stimulating the secretion of adrenocorticotropic hormone (ACTH) (Krieglstein 2004).

36 AA monomeric ACTH, besides being synthesised together with a variety of different hormones, is released into central blood circulation. Its functions are 1) the stimulation of cortisol synthesis in the adrenal gland, 2) the provision of basic substances for hormone syntheses, like cholesterol and progesterone, and 3) maintaining the anatomical structure of the adrenal gland (Krieglstein 2004).

Final target structure of the axis is the adrenal gland’s zona fasciculata. The adrenal gland is comprised of two anatomically divergent structures, cortex and medulla. The cortex is divided up into three morphologically and functionally

distinct cellular strata, zona glomerulosa, zona fasciculata and zona reticularis (from the surface to the inside). Zona glomerulosa produces mainly mineralocorticoids, zona faciculata produces mainly glucocorticoids, i.e. cortisone and its active metabolite cortisol, and zona reticularis synthesizes sex hormones, mostly androgens. Target zona fasciculata constitutes approximately 70% of the cortex of a full grown person, containing radially arranged cell strings as well as sinus (Krieglstein 2004). ACTH now enhances the expression of enzymes of the synthesis and secretion of steroid hormones by activating membrane-bound G-coupled MC2 receptors (Smith & Vale 2006).

Thus, the target of the HPA axis is synthesis and secretion of cortisol. After release, roughly 90% of cortisol is bound to corticosteroid-binding globulin (CBG), a transport protein (Lang 2007), and with much lower affinity to serum albumin (de Kloet 2005) due to its poor solubility. But only the rest of less than 5%, the so-called free cortisol, is biologically active and able to bind glucocorticoid and mineralocorticoid receptors to be fully effective in the target tissues (de Kloet 2005).

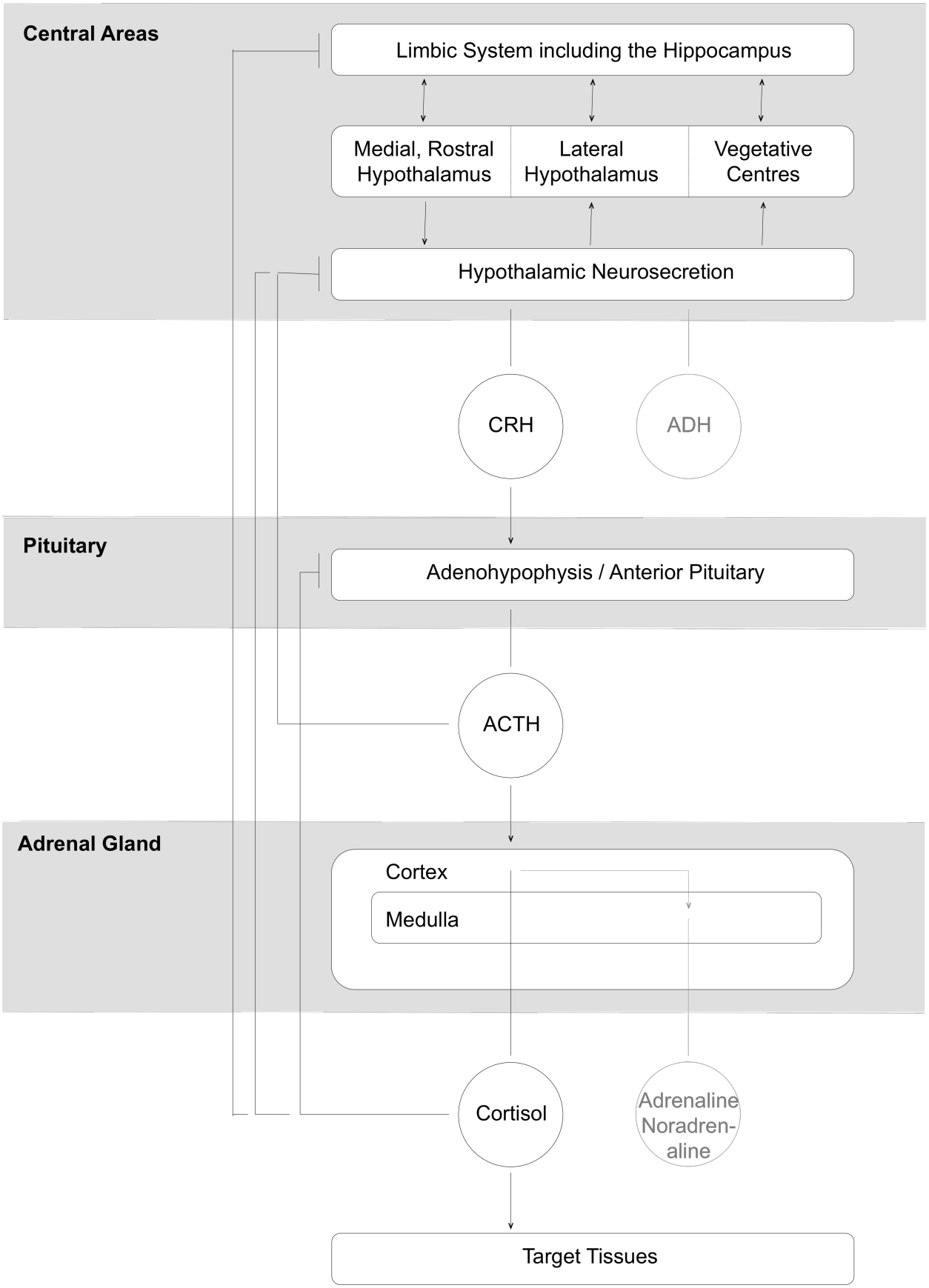

Figure 1: Idealized representation of the HPA axis (modified after Silbernagl 2007)

1.3.2 Regulation of the Activity of the Hypothalamus-Pituitary-Adrenal Axis

In general, the activity of the HPA axis is regulated on a hormonal and on a neuronal level (Smith & Vale 2006).

Hormonal Regulation

Besides several hormone-mediated feedback systems of the HPA axis, the glucocorticoid-induced negative feedback is the most prominently discussed mechanism of regulation.
Currently, two separate systems are employed: a delayed-genomic and a fast-nongenomic feedback.

To initiate the delayed-genomic feedback system, glucocorticoids bind to cytosol MRs and GRs. Cytosolic MRs maintain basal cortisol levels whereas GRs are activated following periods of acute stress when cortisol secretion is elevated. Basically, both receptor types interact with glucocorticoid responsive elements (GREs) and transcriptional factors to modulate genomic activity (for detailed information see below) (Smith & Vale 2006).
To terminate an acute stress reaction, free cortisol binds to hippocampal, pituitary and adrenal MRs and GRs. Via projections, GC-release in the hypothalamus is decreased, thus restricting cortisol secretion. Therefore, the organism is enabled to modulate HPA function if necessary, and to decrease high cortisol concentrations, to prevent tissue integrity damage (Sapolsky 2000). Slow, genomic effects need at least 1 – 2 hours to occur after corticosterone binding (Shors 2009).

The fast-nongenomic feedback system is supposed to react to the frequency of glucocorticoid secretion (Smith & Vale 2006) (see below).

Neuronal Regulation

The PVN is innervated by numerous projections, ranging from GABA-ergic neurons of the hypothalamus, structures from the limbic system, and cell groups in the lamina terminalis to brain stem catecholaminergic neurons (Smith & Vale 2006).

1.3.3 Target Receptors for Glucocorticoids

Glucocorticoids, like cortisol, bind and stimulate two receptors, type I receptors (or mineralocorticoid receptors) and type II receptors (or glucocorticoid receptors). Both receptors belong to the biochemical superfamily of steroid receptors, constituting a subgroup of nuclear receptors (Grossmann 2004).
However, both receptor types differ significantly in distribution and in functional terms (Table 1 gives a synopsis of basic information on both receptors).

	Type I Receptors Mineralocorticoid Receptors		**Type II Receptors Glucocorticoid Receptors**
	Cytosolic	**Membrane-bound**	
Localisation	Central main localisation: Hippocampus, limbic system		Central main localisation: Hypothalamus, Corticotrophes
	Anatomically mainly restricted to the central nervous system and the kidneys		Anatomically widespread throughout the central nervous system and periphery (ubiquitous)
Affinity for GC	High affinity for GC	Lower affinity for GC	Lower affinity for GC
	K_d = 0.5 nM		K_d = 5.0 nM
Saturation	ca. 70% in the nadir of the cycle (= basal GC levels)	Peaks of the cycles (= max. GC levels)	Peaks of the cycles (= max. GC levels)
		Physical or psychological stress	Physical or psychological stress
Agonist	Aldosterone		Dexamethasone
	Fludrocortisone		RU 28362
Antagonist	Spironolactone		RU 38486
	RU 26752		

Table 1: Synopsis of MRs and GRs

1.3.4 Mineralocorticoid Receptors

The mineralocorticoid receptor, or type I receptor, can be detected in the CNS, with highest densities in the hippocampus and limbic structures (Ahima 1991). Predominant MR expression was found in all hippocampal pyramidal cell fields, in dentate gyrus granule cell layer, lateral septum and amygdala. Finally, cortex-located MRs were detected, being quite restricted to superficial layers (Patel 2000). In comparison to the GR, its distribution is anatomically quite restricted to the central nervous system (Joëls 2008).

Basically, the 984 amino acid (AA) protein is composed of three distinct domains, the N-terminal domain (NTD), the DNA binding domain (DBD), which interacts with nuclear DNA to exert its function, and the C-terminal ligand binding domain (LBD), which mediates the binding between receptor and ligand (i.e. GC) (Berardelli 2012).

Cytosolic MRs, being steroid receptors, reside mostly in the cell's cytosol. This chemical status is stabilized by the binding of several proteins like 90-kDa hsp and corepressors like nuclear receptor corepressor (NCoR) and the silencing mediator of the retinoid and thyroid receptor (Berardelli 2012). The corepressors' main function is to inhibit transcriptional processes by activating histone deacetylase proteins (Berardelli 2012).

Ligand binding, however, induces conformational changes in the MR mediated by the LBD. Thus the balance of coregulators is altered and coactivators like steroid receptor coactivator-1 can enhance the transcriptional activity of MRs (Berardelli 2012).
When free cortisol binds to the MR, a conformational change is induced, and this finally leads to dissociation of MR molecules and bound proteins. This is followed by translocalization to the nucleus, binding of specific DNA regions and eventually transcription of target genes (Berardelli 2012).

Cytosol MRs in the brain show a 10-fold higher affinity to cortisol than GRs do. The current explanation for this phenomenon focuses basically on the role of the

enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). Type 1 is widely spread throughout limbic brain structures and catalyses the chemical transformation from cortisone to cortisol, whereas the type 2 isoenzyme of 11β-HSD, which in contrast inactivates glucocorticoids by downgrading cortisol, could not yet be detected in these areas, suggesting that central MRs lose their selectivity for mineralocorticoids like aldosterone and bind glucocorticoids instead, because of high local concentrations (Berardelli 2012).
The GC affinity of the MRs entails the consequence that cytosol MRs are highly saturated at quite moderate to low serum levels of cortisol. 50 – 70% of hippocampal MRs are occupied during the nadir of the diurnal cycle from 2 – 9 pm (Berardelli 2012). Therefore, one limiting factor and functional implication is the amount of receptors available.

As described above, MRs play an important role in the GC-mediated feedback control of the HPA axis. High GC affinity of MRs suggests that MRs are supposed to serve as proactive feedback in the nadir of the cycle, i.e. maintain basal cortisol levels by recruiting tonic neuronal GABA-erg inhibitory projections to the hypothalamic PVN. In other words, MRs ensure the steady inhibition of the hypothalamus and therefore restrict the level of activity of the HPA axis during the nadir of the circadian cycle (Berardelli 2012). Additionally, this thesis was supported by the finding that high dosage fludrocortisone administration (fludrocortisone is a potent MR agonist) could significantly reduce serum cortisol levels in the nadir, whereas high dosage spironolactone administration (spironolactone is a potent MR antagonist) significantly increased serum cortisol levels in the nadir and the peak of the circadian cycle (Oitzl 1995, Dodt 1993, Born 1991). Accordingly, intraventricular administration of an MR antagonist in rats resulted in an increased adrenocortical stimulation, when confronted with novel situations (de Kloet 2005). These effects – in general basal HPA activity – appear to be mediated by hippocampus-located MRs (de Kloet 2013), since a number of studies in rats showed that high MR expression in the hippocampus led to lower basal and lower stress-induced activation of the HPA axis, but elevated levels of free corticosterone (de Kloet 1998). On the other hand, old rats show a decrease in MR and GR expression and increased basal HPA activity as well as prolonged stress-induced ACTH release (de Kloet 1998). Finally, tricyclic

antidepressants increase the expression of MRs in the hippocampus and decrease basal and stress-induced HPA activity (de Kloet 2005), whereas MR blockade results in an increase of CRH and VP (Berardelli 2010, Arvat 2001, Heuser 2000).

Recently, membrane-bound MRs in limbic neurons are at the centre of attention. Being accessible only from the outside of a cell (Olijslagers 2008), these low-affinity receptors – very much in contrast to cytosol MRs – become activated in correspondence with corticosterone peaks of the hourly corticosterone excretion bursts during the ultradian cycle and in situations of acute stress (Joëls 2009) and employ rapid non-genomic pathways (de Kloet 2013).
Presynaptic membrane-bound MRs of hippocampal CA1 neurons facilitate fusion of glutamate-containing vesicles with the presynaptic membrane (Olijslagers 2008) and consecutively increase the probability of glutamate release (Joëls 2009) by utilizing the ERK1/2 pathway.
Postsynaptic membrane-bound MRs, when activated by corticosterone, decrease K-conductance by activating G-coupled proteins (Joëls 2009, Olijslagers 2008). Each presynaptic release of a glutamate-containing vesicle causes a so-called miniature excitatory postsynaptic current (mEPSCs) at the postsynaptic side, which is interpreted as being a sign of increased excitability (Karst 2005). In short, these reversible fast-acting mechanisms are supposed to raise excitability and therefore long-term potentiation (LTP) in order to improve encoding of information in stressful events, thus enhancing adaptive behaviour (Joëls 2008).
In animal studies, membrane-bound MR activation altered search patterns in Morris Water Maze navigation experiments (de Kloet 2005). Moreover, they were discussed in regard to having a possible influence on cell proliferation and cell death in dentate gyrus (de Kloet 2005).

1.3.5 Glucocorticoid Receptors

In contrast to MRs, GRs are ubiquitously distributed in the CNS, and have their highest density in the PVN and pituitary gland (de Kloet 2013, Funder 1997, Jacobson 1991). Hippocampal GRs are highly expressed in CA1 and CA2 cells,

whereas cortical GRs were detected in all layers (Patell 2000). However, MR-GR-co-localization is found in the hippocampus (de Kloet 2005).

Being bound only in phases of high corticosterone concentrations because of low affinity, GRs become activated in correspondence to the peaks of the diurnal cycle and in stressful situations (de Kloet 2013). In these cases, hippocampal output is decreased.

GR activation results in the termination of stress reactions, the mobilization of energy resources necessary for this purpose, as well as recovery and the consolidation, but not retrieval, of relevant information (de Kloet 2005).

GC binding can lead to a variety of responses on the receptor level. As described in Section 1.3.2 here, MR and GR homo and heterodimers and monomers interact with GRE to exert their effects. In the case of termination of a stress reaction and in the recovery phase, GR monomers interact with stress-induced transcription factors (TF) to decrease their transcriptional activities, thus repressing stress-induced CRH and VP syntheses (de Kloet 2005). On the cellular level, GR activation alters the expression of genes regulating cell metabolism, cell structure and synaptic transmission. Ultimately, macromolecules like enzymes, receptors, growth factors and cell-adhesion factors are affected. Therefore, de Kloet et al. interpret GRs as structural modulators in limbic areas (de Kloet 2005). On the behavioural level, GR antagonists inhibit GR dimerization and DNA binding, impairing memory consolidation. Thus, GR activation is necessary for retaining of information in long-term memory. To sum up, GRs have little effect on resting cells, but become active when cells are driven from their resting potential (de Kloet 2005).

In conclusion, it is important to note that the timing of events seems to be a crucial factor. Therefore, it is no longer surprising that memories from emotionally arousing situations can easily become long-lasting (de Kloet 2005). Moreover, recent studies emphasize the MR-to-GR relationship and disbalance in diseases.

1.3.6 The Hypothalamus-Pituitary-Adrenal Axis as Part of Human Homeostasis

Biological Rhythms

Measuring the cortisol blood concentration over the course of a day as a reflection of physiological HPA activity reveals hourly cortisol secretory bursts as well as a characteristic circadian progression of mean blood levels (Joëls 2009). Pulsatile secretion of cortisol ensures constant provision of glucocorticoids, which are necessary for the maintenance of cell integrity and function (Joëls 2009). Membrane-bound MRs and GRs are saturated to the maximum during bursts. Mean blood concentrations of cortisol after hourly secretions constitute another biological rhythm, which spans roughly 24 hours, and is characterized by an undulating concentration curve. The peak of the free serum cortisol concentration is observed in the morning (approximately 8:00 am), whereas the nadir is reached around midnight (Otte 2003). Showing high to very high affinities for binding free serum cortisol, cytosol MRs are usually reported to be extensively occupied by human cortisol during the peak of the circadian rhythm, thus making it far more difficult for an agent to bind and stimulate MRs. In contrast, as reported by Spencer et al., in the nadir of the cycle, a maximum of only 69% of hippocampal cytosol MRs are occupied, so that 1) the feedback activity of the HPA axis is mainly controlled by MRs, and therefore 2) the external application of MR binding substances is expected to attain maximum response (Figure 2) (Spencer 1993).

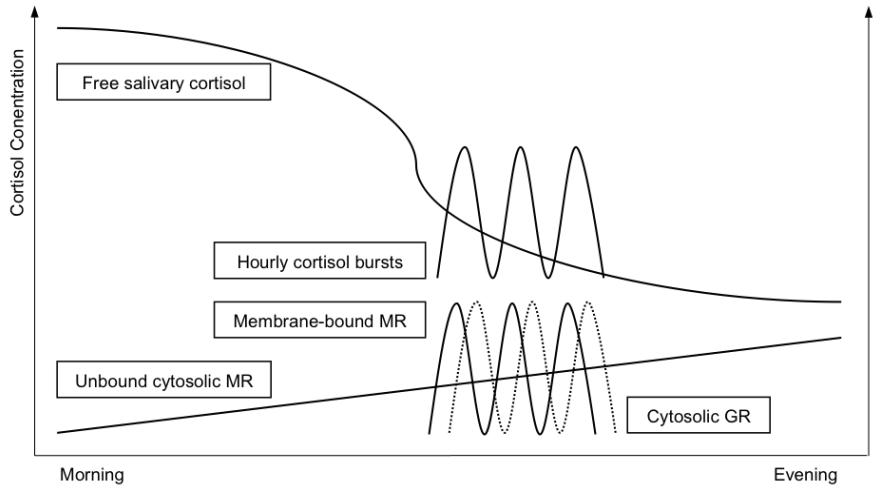

Figure 2: Free serum cortisol levels and MR/GR occupation over time (modified after Joëls 2009)

Exposition to Stress

Stress, as it is defined by de Kloet et al., is a real or perceived threat to homeostasis. To reconstitute homeostasis in the presence of stressors, a balanced “stress response” is required. This term subsumes a variety of regulative responses that are induced to improve a subject’s chance of survival. Biological regulative responses can be discriminated according to the temporal order of events as well as the biological subsystem that is affected, like molecular, cellular, hormonal and behavioural changes (de Kloet 2005).

Early Responses to Acute Stress

If a situation is perceived as stressful, CRH and VP secretion in the PVN is increased, leading to enhanced secretion and blood levels of cortisol among other substances (like catecholamines and neuropeptides) (Smith & Vale 2006, de Kloet 2005). So called “rapid changes” are induced by transmitters such as noradrenaline to enable the organism to react to the stressor adequately. This includes enhanced excitability on the cellular level, ultimately heightening the individual’s arousal, vigilance and attention (Smith & Vale 2006, de Kloet 2005).

Late Responses to Acute Stress

It is not only important to react to stressors quickly; there must also be the capability to terminate these adaptive mechanisms sufficiently. On the average, blood cortisol levels are reconstituted to initial conditions around 2 hours after onset (Smith & Vale 2006, de Kloet 2005). Underlying mechanisms are corticosteroid-induced alterations of gene expression via MRs and GRs (Smith & Vale 2006, de Kloet 2005).

Whereas MR activation plays a crucial role in the adaptation to stress, GR activation primarily acts as a suppressor of the stress system and leads to a normalization of the processes involved, to promote homeostasis. In this way, GR-mediated regulations can be interpreted as the rescission of rapid noradrenaline-induced changes (Smith & Vale 2006, de Kloet 2005). Moreover, on the behavioural level, storage of information is enhanced for future use, possibly by increasing Ca-influx into neuronal cells (Diamond 2007, Joëls 2006).

Responses to Chronic Stress

Chronic stress, when examined in studies, is often referred to as repetitive exposition to stress over an extended time period (usually lasting weeks) or confronting organisms with several different stressors per day (de Kloet 2005).
Most studies indicate that chronic exposure to stress impairs cognitive functions, especially hippocampus-dependent memory (de Kloet 2013).
On the behavioural level, animals show increased fear motivation (de Kloet 2005).
On the cellular level, changes in the hippocampus, amygdala and prefrontal cortex were observed. Long expositions to elevated glucocorticoid levels caused impaired cell proliferation and regeneration (Wong 2004, Pham 2003). In contrast to GRs, which are supposed to be rather unaffected by chronic stress, MRs seem to be involved in dentate gyrus neurogenesis and cell death. The induction of MRs by several antidepressant drugs might have an effect on hippocampal neurogenesis (Santarelli 2003). A more indirect approach showed that X-ray-induced blockade of neurogenesis decreased treatment effects of antidepressants (Santarelli 2003). Woolley et al. and Magarinos et al. could show that chronic stress causes atrophy of dendrites of CA3 neurons in the hippocampus and a decrease in the number of synaptic contacts (Magarinos 1996, Woolley 1990). Vyas et al. reported hypertrophy of basolateral amygdala due to chronic stress (Vyas 2002), a feat contrasting hippocampal changes. Finally, neuroplasticity in form of LTP is reduced in hippocampal neurons (Alfarez 2003). Some authors report cellular regeneration in the course of weeks after chronic stress exposure (Heine 2004).

1.4 Hypothalamus-Pituitary-Adrenal Axis Dysregulation in Depression

As de Kloet famously concluded, elevated cortisosteroid levels and disruption of mood, cognition and behaviour are highly associated (de Kloet 2013, de Kloet 2005).

In major depression, the most consistently described biochemical feature is the hyperactivity of the hypothalamus-pituitary-adrenal system, resulting in hypercortisolaemia (Checkley 1996). Although not highly specific, this

endocrinological finding, which is observed in roughly 50% of the depressed (Checkley 1996), can differentiate MDD from other stress-related psychiatric disorders like PTSD, which in short is characterized by a combination of CRH hyperactivity and peripheral hypocortisolaemia (Handwerger 2009). In comparison to healthy subjects, depressed patients show a greater range of cortisol values and a more distorted diurnal secretion pattern (Yehuda 2011).

As a side note, even in patients with somatic disorders accompanied by elevated cortisol levels, for example Cushing's disease, hypercortisolaemia alters monoaminergic transmission and impairs cognition, both similar to changes in MDD (de Kloet 2005).

In both Cushing's disease and MDD, hippocampal hypotrophy, but not cell destruction, was reported (Müller 2001, Sheline 1999). In chronic stress, elevated GC levels are triggered by CRH and VP, whereas in MDD this control mechanism seems to be impaired (de Kloet 2005). Therefore, de Kloet et al. conclude that HPA dysregulations like Cushing's disease and chronic exposure to stress can unveil preexisting pathological features, ranging from genomic to behavioural affections (de Kloet 2005). On the other hand, correction of hypercortisolaemia decreased depressive symptomatology significantly (Dorn 1997).

Among other things, the hallmark of biochemical function analyses is the combined dexamethasone-suppression/corticotropin-releasing test (dex/CRH-test). Dexamethasone is a potent GR agonist and by application suppresses cortisol levels, whereas CRH is the ACTH releasing factor, which increases cortisol levels via ACTH stimulation. In depression, dexamethasone-induced suppression is insufficient, whereas cortisol response to CRH-application is increased. Taken together, all endocrinological test results support the hypothesis of a central feedback resistance at the level of the PVN and pituitary (Holsboer 1996). In post-mortem human brains, elevated CRH and VP levels in the PVN were observed (Purba 1996, Raadsheer 1994) and along those lines, Heuser et al. could show in a clinical trial that in fact CRH and VP do drive HPA activity (Heuser 1994). As Ising et al. argue, this might be due to impaired

corticosteroid signalling in the CNS in the sense of downregulated negative feedback, increasing CRH and VP levels (Ising 2005). This is supported in preclinical studies demonstrating decreased MR and GR levels in the hippocampus as a consequence of increased corticosterone in animals (de Kloet 2005) and a reduction of hippocampal and prefrontal cortex MR in post-mortem brains of depressed patients (Lopez 1998). Klok et al. quantify the reduction of MR mRNA transcripts in the hippocampus, inferior frontal gyrus and cingulated gyrus as being about 30 – 50% (Klok 2011).

Healthy subjects who carry a familial, i.e. genetic, risk for the development of depression, already show feedback resistance, mild hypercortisolism and cortisol responses, which were intermediate between patients with an acute depressive episode and risk-free healthy control subjects (Modell 1998, Holsboer 1995). These subtle changes of HPA activity can be interpreted as a symptom of drive and feedback imbalances, which are already present before the clinical manifestation of a depression (Holsboer 1995). In this case, expression of MR and GR variants, defects in proteins like TF and GRE and alterations of target genes might cause dysregulations that increase the susceptibility to chronic stressors (Holsboer 1995).

Longitudinal studies with repeated dex/CRH-test performances led to results showing that if HPA dysregulations persist, 1) patients do not respond well to pharmacological antidepressant treatments, and 2) even in case of clinical remission, patients are at high risk of relapse (Zobel 2001). Therefore the combined dex/CRH-test can be regarded a prognostic surrogate marker (Zobel 2001). In contrast, successful treatment normalizes HPA function (see below) (Pariante 2004, Raison & Miller 2003, Holsboer 2000, Holsboer & Barden 1996).

A plethora of psychopharmacological studies supported this hypothesis. CRHR1 antagonists improve the symptomatology of depression while intra-cerebroventricular CRH-application induced anxiety and a depression-like phenotype (de Kloet 2005). MR antagonists worsened the therapeutic efficiency of antidepressants (Holsboer 1996).

On the other hand, administration of tricyclic antidepressants or citalopram restores 5HT and noradrenaline transmission in the brain and increases GR and especially MR levels in parallel with the normalization of the HPA function (Reul

1993, Seckl 1992), thus restoring the MR-to-GR balance. Reul et al. argue that amitriptyline-induced increase of limbic MR might be the initial step for a successful HPA normalization, decreasing plasma cortisol levels (Reul 1993). MR overexpression in the forebrain decreases symptoms of anxiety and decreases HPA activity in mice (Rozeboom 2007).

The restoration of the physiological negative feedback system in the course of an antidepressant treatment is more closely related to the improvement of specific psychopathological symptoms, namely cognitive impairments of working memory, than to the overall depressive syndrome (Reppermund 2007). This is also reflected in the finding that cognitive impairments often continue to exist even after the remission of overall psychopathology. Therefore, it has been argued that cognitive impairments are not an epiphenomenon of depression but a representation of distorted functional networks (Majer 2004, Austin 2001, Mcallister-Williams 1998).

1.5 Mineralocorticoid Receptor and Cognitive Functions in Depression

As described above, cognitive impairments are common among depressed persons. Most pronounced impairments affect memory, attention and executive function (Majer 2004, Ravnkilde 2002, Basso & Bornstein 1999). A closer look reveals that it is not a feature of necessity, because the impaired memory system depends rather on the subtype and most of all the severity of depression (Austin 2001, Pelosi 2000). For example working memory, which is associated with functioning prefrontal cortical areas, is impaired mostly in moderate to severe depression (Austin 2001, Pelosi 2000). This is in line with the findings described above, which propose a dissociation of general depressive psychopathology and cognitive function regarding underlying mechanisms. Therefore, Zobel et al. conclude that cognitive impairments are much more strongly correlated with the aetiology of depression than symptoms of mood and behaviour might be (Reppermund 2007, Zobel 2004).

Studies focusing on anatomical-morphological features link memory deficits in depression to hippocampal dysfunction associated with reduced hippocampus

volumes (Hickie 2005, Lupien 1998). They argue that chronically elevated glucocorticoid levels damage cellular integrity of the hippocampus, possibly by reducing LTP and causing dendritic atrophy. Associations between reduced hippocampal volumes and decreased general cognition (as measured by the MMSE) and verbal and spatial memory were observed (Hickie 2005, Lupien 1998). However, these effects were thought to be mediated mainly by GRs (Kim & Diamond 2002).

A series of pharmacological studies examined the effects of varying blood cortisol concentrations.

Hinkelmann et al. compared a population of depressed persons to healthy controls regarding cortisol concentrations and effects on cognitive domains (Hinkelmann 2009). Depressed patients showed higher salivary cortisol levels in combination with impaired verbal, spatial and working memory and selective attention in comparison to healthy controls. They detected a negative correlation between salivary cortisol concentration and in particular hippocampus-related cognitive domains (verbal and visuospatial memory) and prefrontal-executive function.

Lupien et al. reported a higher sensitivity of working memory to changes of cortisol concentrations compared to episodic and declarative memory, suggesting higher sensitivity of prefrontal cortical areas (Lupien 1999). Animal studies supported this hypothesis by showing that the prefrontal cortex was especially vulnerable to chronically elevated cortisol (Wellman 2001).

In healthy young men, high-dosage hydrocortisone administration impaired working memory (as measured by the Digits Backwards Test) (Lupien 1999). In the elderly, Lupien et al. demonstrated improved or worsened verbal memory performance among decreased or increased cortisol levels, respectively (Lupien 2002).

Another hint to the outstanding interdependencies of HPA function and cognitive abilities concerns pharmacological treatment. In short, during antidepressant treatment, normalization of the HPA activity was associated with improvements in the cognitive domain, especially working memory (Zobel 2004). This finding concurs with endocrinological studies, in which reduction of peripheral cortisol

levels in patients with Cushing's syndrome led to improved verbal memory and increased hippocampal volume (Hook 2007, Starkman 2003).

Taken together, these findings strongly suggest the involvement of glucocorticoid receptors. It is widely accepted that GRs are incorporated into consolidation and retrieval of memories, because high cortisol levels facilitate consolidation while impairing retrieval (de Kloet 2005). However, these mechanisms could not yet sufficiently explain immediate effects of different cortisol levels on memory, especially rapidly induced changes and working memory performance. In this context, MRs come to the centre of attention.

Spironolactone-induced blockage of MRs in animals decreased spatial learning as measured by Morris Water Maze performance (Yau 1999, Oitzl 1992). Mice with artificially induced limbic MR deficiency suffered from impaired learning in Morris Water Maze and impaired working memory (Berger 2006). In a clinical trial, Otte et al. blocked MR pharmacologically in healthy subjects, which impaired hippocampus as well as prefrontal-dependent memory performance. Decreased visuospatial memory, selective attention and mental flexibility were reported (Otte 2007).

On the other hand, transgenic mice overexpressing MR benefited from improved short-term memory performance. Even adverse effects of high-dosage glucocorticoid application on cognition were blocked by an increase of the number of MRs (Ferguson 2007, Lai 2007). In an experiment conducted by Mitra et al., MR overexpression in the basolateral amygdala (BLA) reduced anxiety-like behaviour and reduced stress-induced corticosterone secretion, which confirmed inhibitory effects of MR activation on HPA activity (Mitra 2009). In adreno-ectomized rats which cannot synthesize glucocorticoids and mineralocorticoids among other hormones, spatial memory can be improved by applying aldosterone, an MR agonist (Conrad 1997). And finally, mifepristone, a GR antagonist, enhances MR cortisol binding and expression by blocking GRs, thus improving spatial working memory in patients with bipolar depression (Watson 2012).

2. RESEARCH QUESTIONS

In short, blockage of MR function was associated with decreased cognitive function, whereas stimulating MRs leads to improved cognitive performances, at least in some domains.

Since a considerable number of the studies mentioned above were conducted in preclinical settings (i.e. animal studies, post-mortem studies) or drew conclusions on MR-influences on cognition and on the aetiology of depressive disorders more indirectly, this study was developed to investigate whether these findings could be transferred to humans, validating current theories concerning HPA dysregulation in MDD and MR role in cognition.

In order to extensively cover the research questions, this study was designed to analyse influences of MDD on cognition (= Hypothesis I) first, before further investigations would address in detail effects of FC-induced MR stimulation on different cognitive domains (= Hypotheses II and III).

2.1 Hypothesis I

The first question which was derived was whether severe depressive psychopathology could be replicated with regard to cognitive performance. Therefore we wanted to know if depressed subjects performed worse in neuropsychological tests than their healthy counterparts.

Hypothesis I:
Participants suffering from major depression show impaired cognitive functions in comparison to healthy controls.

2.2 Hypothesis II

Secondly, we wanted to know whether fludrocortisone-induced MR stimulation would improve cognitive functions in depressed humans. Considering the background that depressed persons show impaired memory performance, decreased MR mRNA expression and increased HPA activity, we assumed that MR activation in this population might lead to enhanced cognitive abilities.

Hypothesis II:
Fludrocortisone-induced MR stimulation improves cognitive performance in depressed participants.

2.3 Hypothesis III

Considering that healthy participants, in contrast to depressed persons, would display physiological MR functioning, we assumed that further MR activation would not result in improved cognitive performance due to ceiling effects.

Hypothesis III:
Fludrocortisone-induced MR stimulation does not improve cognitive performance in healthy participants.

3. MATERIALS AND METHODS

3.1 Study Design

The present study has a double-blind, placebo-controlled, cross-over design.

To measure treatment effects of fludrocortisone within one subject, each volunteer had to complete two separate days of testing, with a time lag of two days in between. The procedures on both test days were equivalent. One test day represented the placebo and the other day the verum condition. 50% of the depressed (and their corresponding healthy control participants) received fludrocortisone on the first day and placebo pills on the second, whereas the other half was treated in the opposite order to minimise any undue learning effects in consecutive test evaluations. All neuropsychological tests were used in parallel forms, if available (for detailed information see 3.4).

The time schedule of a test day was conceived with due consideration given to the circadian rhythm of the pulsatile cortisol excretion in humans (see 1.3.6). Accordingly, in the nadir of the cycle, physiological MR occupation is lowest and therefore external application of an MR agonist is expected to attain maximum response, so that test day beginnings were scheduled at 2:00 pm (Otte 2003).

To stimulate MR, we applied fludrocortisone (FC), a potent MR agonist (see technical information). Based on previous studies, test takers received 0.4 mg FC (Manufacturer: Astonin H, Merck Serono GmbH, Germany) in form of pills at approximately 2:00 pm (0 minutes) (Otte 2003). Due to the galenic formulation of the agent, the maximal biological effect was expected to begin approximately 102 minutes after intake (see DRUGDEX), so that neuropsychological testing started at 4:00 pm. The duration of the pharmacological effect was estimated to be 60 minutes, this providing the time frame for all testing to be completed (Figure 3).

Systolic and diastolic blood pressure (BP) as a measure of free cortisol effects on periphery vessels was quantified at -10 minutes (baseline), +90 minutes and at

+180 minutes (Figure 3) by an automatic device (Carescape V100, GE Healthcare).

To measure peripheral cortisol levels and thereby the effect of FC-induced MR stimulation, we collected saliva samples using salivettes (Sarstedt AG, Nümbrecht, Germany). This is possible because saliva and plasma cortisol levels are highly correlated, so that analysing saliva samples provides a measure of circulating free cortisol levels (Vining & McGinley 1986). Participants were introduced to the correct technique of providing samples by the tester. In short, participants were advised not to eat, drink or smoke before using salivettes. To ensure the accuracy of application, all samples were collected in the presence of a tester. Cortisol levels were measured using radioimmunoassay (DRG, Marburg, Germany; inter- and intra-assay coefficients of variation <8%; detection limit = 0.5 ng/ml).

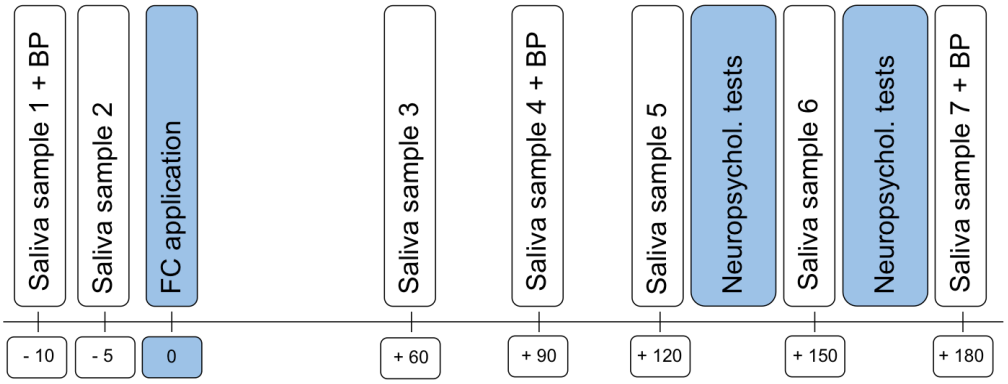

Figure 3: Schedule of a test day

3.2 Subjects

3.2.1 Recruitment of Depressed Participants

In order to recruit subjects affected by major depression, a special survey of in and outpatients regarding study participation was conducted in the “Klinik für Psychiatrie und Psychotherapie” of the Charité Campus Benjamin Franklin over a limited period of approximately 20 months from September 2011 to May 2013.

All study participants had to meet the following criteria:

Eligibility criteria:

- Males and females aged between 18 and 33 years
- Acute manifestation of major depressive disorder (based on ICD/DSM criteria) or
- Acute episode of recurring depressive disorder (based on ICD/DSM criteria) along with
    - Corresponding scores in the following questionnaires:
        - Mini International Neuropsychiatric Interview (M.I.N.I.)
        - Hamilton Depression Rating Scale (HDRS)
        - Montgomery–Åsberg Depression Rating Scale (MADRS)
        - Beck Depression Inventory (BDI-II)
- No intake of psychiatric or neurological medications or medications that reportedly influence HPA axis activity at the time of examination or for the previous 5 days
- No psychiatric or neurological comorbidities, except Anxiety Disorder
- No somatic comorbidities which reportedly influence HPA axis activity and which are not carefully regulated by medication
- Signed informed consent

Exclusion criteria:

- Axis-I and -III psychiatric and neurological co-morbidities, such as:
    - Acute suicidal tendencies
    - Psychotic symptoms
    - Substance-related addiction
    - organic, including symptomatic mental disorders and neurological disorders
- Taking of psychiatric or neurological medications on the days of testing
- Diseases that require oral or inhalative treatment with steroids
- Shift working at the time of examination
- Pregnancy, nursing

3.2.2 Sociodemographic examination

All participants provided information about age, sex, body height and weight, years of education and school leaving certificate, civil status, menstruation cycle, hormonal contraception, psychiatric and somatic morbidities, medications, substance abuse and history of depression in core family.

3.2.3 Psychometric examination

The presence of a depression was assessed by the scientific coordinator based on DSM criteria. The following psychometric tests were utilised:

3.2.4 Mini International Neuropsychiatric Interview

The “Mini International Neuropsychiatric Interview”, in short “M.I.N.I.”, was developed by David V. Sheehan and Yves Lecrubier in 1992 to provide an alternative to comprehensive diagnostic interviews for research purposes on the one hand, and short clinical screening tests for practical use on the other. Characterized by a quite simple execution, high sensitivity for MDD (concordance with SCID-P = 0.96), high specificity (concordance with SCID-P = 0.88), Positive Predictive Value of .87, as well as high inter-rater and test-retest reliability (kappa values 1.00 and .87, respectively), the M.I.N.I. has been internationally well established by now in clinical trials for the assessment of Axis-I disorders (Sheehan 1998).

It is comprised of 16 individual diagnostic modules (A – P). Each module represents one diagnostic category, for example Major Depressive Episode, Dysthymia, Suicidality, PTSD, Anorexia nervosa and many more. Thus, the M.I.N.I. also allows controlling for psychiatric comorbidities.

Every module consists of a grey box with a few pointed questions addressing the main symptom, as well as additional questions to specify possible afflictions. For

example, Module A is called “Major Depressive Episode”. The questions in the grey box are A1: “Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?” and A2 “In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?”. The interviewee is instructed to answer all questions with Yes or No. If he agrees with A1, the interviewer must continue and read out all questions that are subsumed under A3 in order to explore additional symptoms. At the end, the evaluation allows a comprehensive screening for a variety of disease symptoms, in principle. For this study the German Version 5.0.0 was applied (Ackenheil 1999).

3.2.5 Hamilton Depression Rating Scale

The “Hamilton Rating Scale for Depression” (HDRS), originally published by Max Hamilton in 1960, is widely applied in psychiatric clinics and research as the “gold standard” to measure severity of a depression. As Hamilton stated, the HDRS should not be utilised as diagnostic manual, but rather as an observation instrument of disease progression (Hamilton 1960).

The clinician-rated HDRS is comprised of 17 items, which cover the main symptoms that might occur during an episode of depression. In a later version ($HDRS_{21}$), four more items were added to subtype the depression. For example, Item 1 called “Depressed Mood (sadness, hopeless, helpless, worthless)” is read out to the patient. The interviewer then has to mark the answer that best characterizes the patient. In this category, 5 individual answers are given, from “0” for “Absent.” up to “4” for “Patient reports virtually only these states of feeling in his/her spontaneous verbal and nonverbal communication.”.

A total score between 0 and 6 does not indicate a depression, scores between 7 and 17 are supposed to indicate mild depression, 18 - 24 moderate depression and scores over 24 are regarded as an indicator of severe depression.

Internal consistency has been reported to be above .80 for $HDRS_{17}$. Total inter-rater reliability ranges from .80 to .98, test-retest reliability estimates up to .81

and validity ranges from .65 to .90. The HDRS is highly correlated with other clinician-rated measurements such as MADRS (Cusin 2010).

3.2.6 Montgomery–Åsberg Depression Rating Scale

Montgomery and Åsberg published the unidimensional “Montgomery–Åsberg Depression Rating Scale” (MADRS) in 1979 as a tool to measure treatment effects of antidepressant medications, mainly tricyclic antidepressants (Montgomery 1979).

The clinician-rated 10-item scale comprises 10 main symptoms of depression. Again, ready-made statements are read out to the interviewee and the examiner’s task is to mark the most appropriate of the answers given. Every category offers 7 answers, ranging from “0 = No sadness” to “6 = Looks miserable all the time; extremely despondent”. However, in contrast to the HDRS, this rating scale focuses almost exclusively on psychological aspects of depression (Fava 2002). A total score from 20 to 29 indicates moderate, above 30, severe depression.

Bech et al. reported an internal consistency for MADRS, which was even superior to that of HDRS. This was based on the very high correlation between all items (.95). Inter-rater reliability is above .89. The correlation with the HDRS ranges from .80 to .90 (Bech 2002).

3.2.7 Beck Depression Inventory

The “Beck Depression Inventory” (BDI) is regarded as the “gold standard” of self-rating scales and is therefore executed as an equivalent to the HDRS. Introduced by Aaron Beck in 1961 and revised twice (1978 and 1996) to adapt to the reworkings of DSM, the BDI-II measures the severity of 21 symptoms.

All questions concerning these 21 symptoms come with four possible answers. The test taker is advised explicitly to think of how he is feeling at the moment and

in the past two weeks. He has to pick which he thinks is the most appropriate option (for example: 1.) Sadness; possible answers: “0” = ”I do not feel sad.” to “3” = ”I am so unhappy or sad that I can’t stand it.”). Total scores from 10 to 18 indicate mild depression, from 19 to 29 indicate moderate depression and above 30, severe depression.

Test-retest reliability has been reported to be between .65 and .72 (Cusin 2010).

Characterization of Final Depressive Test Population

Seven males and 17 females could be recruited for this study, totalling 24 depressive subjects and averaging 26.46 years of age and 12.17 years of education at the point of testing. 15 participants were single, eight in a partnership and one lived separately. Mean BMI was 23.3 kg/m^2. Six participants had somatic comorbidities (hypothyroidism: 2; allergies: 1; myoma: 1; CHD: 1; Gilbert’s disease: 1). Two participants took L-thyroxine, one subject applied ointment for acne treatment and one took lorazepam as a one-off treatment.
17 participants received inpatient treatment at the “Klinik für Psychiatrie und Psychotherapie – Charité Campus Benjamin Franklin” at the time of testing, seven received non-hospital care.
All participants were German-speaking and did not suffer from impaired vision or hearing.

The estimated mean total score for BDI-II was 31.79, for $HAMD_{17}$ 24.8 and for MADRS 29.7 (Table 2).

In our study population, no drop-outs occurred.

3.2.8 Recruitment of Healthy Control Participants

Every depressed subject was matched with a healthy control subject in terms of sex, age (+/-3 years) and years of education (based on school-leaving certificate: Hauptschulabschluss, Realschulabschluss and Abitur). Female patients and matched controls were in corresponding phases of the menstruation cycle on both dates of testing.

Healthy controls were recruited via announcements displayed on boards at “Klinik für Psychiatrie und Psychotherapie”, Charité Campus Benjamin Franklin, Charité Campus Virchow-Klinikum, Charité Campus Mitte and postings in close proximity over a time period of altogether 15 months from February 2012 to May 2013.

All potential participants first underwent a telephone screening. Besides taking sociodemographic data, the subjects were examined for recent symptoms of Axis-I disorders, psychiatric-neurological histories, somatic morbidities, current medications and substance abuse. Furthermore, traumatic experiences in the life span were assessed. These common initial screening questions were applied only to preselect possible study participants. Detailed psychometric assessments as described in 3.2 followed separately.

To qualify as healthy controls, all test takers needed to meet the following criteria:

Eligibility criteria:

- Males and females aged 18 to 33 years
- No psychiatric or neurological diagnoses:
    - Corresponding scores in the following questionnaires:
        - Mini International Neuropsychiatric Interview (M.I.N.I.)
        - Beck Depression Inventory (BDI-II)
- No intake of psychoactive or neurological medications or medications that reportedly influence HPA axis activity at the time of examination or for the previous week

- No somatic comorbidities which reportedly influence HPA axis activity and which are not carefully regulated by medication
- Signed informed consent

Exclusion criteria:

- Any Axis-I, -II or -III psychiatric or neurological diagnosis
    - High scoring in one of the following questionnaires:
        - Mini International Neuropsychiatric Interview (M.I.N.I.)
        - Beck Depression Inventory (BDI-II)
- Traumatic experiences in the life span
- Taking of psychiatric or neurological medications
- Any acute somatic disease
- Any remitting somatic disease which reportedly influences HPA axis activity and which is not carefully regulated by medication
- Diseases that require oral or inhalative treatment with steroids
- Shift working at the time of examination
- Pregnancy, nursing

Characterization of Final Control Test Population

24 healthy, gender-, age- and education-matched control participants were included in this study. All participants were German-speaking and did not suffer from impaired vision or hearing.

Psychometric tests revealed very low scorings for the healthy population. All demographic variables are presented in Table 2.

Synopsis of Final Study Population

Table 2 summarizes all demographic and psychometric data obtained from the test population. Depressed and healthy control participants are compared.

Variable		Patients	Healthy Controls	p
Sex	Male	7	7	n. s.
	Female	17	17	(1.00)
Age	Mean	26.5	26.8	n. s.
	Stand. Dev.	3.1	3.5	(0.86)
	Min / Max	20 / 31	22 / 33	
Education				
- School leaving qualification	Abitur	17	17	
	Realschulab.	6	6	
	Hauptschulab.	0	1	
	ø Abschluss	1	0	
- Years of Education	Mean	12.0	12.1	n. s.
	Stand. Dev.	1.4	1.5	(0.79)
	Min / Max	9 / 13	9 / 13	
Civil Status	Single	15	17	n. s.
	Married / Part.	8	6	(0.81)
	Divorced	1	1	
Body Mass Index	Mean	23.3	23.2	n. s.
	Stand. Dev.	4.2	3.7	(0.99)
	Min / Max	16.1 / 33.0	18.7 / 35.6	
Smoker Status	Smoker	10	8	n. s.
	Nonsmoker	14	16	(0.55)
Contraceptive Pill		3	6	n. s. (0.29)
Somatic Comorbidities		6	4	n. s.
- Hypothyroidism		2	2	(0.38)
- others		4	2	
Medication		4	3	
- L-Thyroxine		2	2	
- Lorazepam		1	0	
- Timophtal		0	1	
- Ointment for acne treatment		1	0	
Type of Treatment	Inpatients	17		
	Outpatients	7		
Psychometric Examination				
- BDI-II	Mean	31.8	3.3	0.001
	Stand. Dev.	7.6	2.6	
	Min / Max	17 / 50	0 / 8	
- $HDRS_{17}$	Mean	24.8		
	Stand. Dev.	4.8		
	Min / Max	15 / 29		
- MADRS	Mean	29.7		
	Stand. Dev.	3.8		
	Min / Max	24 / 40		

Table 2: Synopsis of final study population

3.2.9 Organisational Matters, Compensation, Data Protection, Approval of the Ethics Committee and Informing the Participants

All tests were conducted by trained personal and took place in two standardised examination rooms at the “Kinik für Psychiarie und Psychotherapie” of the Charité, Campus Benjamin Franklin.

Every control subject was granted € 80.00 once after completing the entire testing procedure.

Each participant’s name was replaced by an individual code. All test results were stored and evaluated using only code names.

The study was approved by the Medical Councils’ Ethics Committee of Hamburg and Berlin. All participants were informed by the clinic’s scientific coordinator about the aim and the procedure of this study and had the possibility to pose questions. All participants were able to withdraw from participation at any time of testing without giving reasons. In this case, previously collected data were destroyed immediately.

Testing did not begin before participants gave their signed consent.

3.3 Structure of a Test Day

All neuropsychological tests were ordered in a specific manner, which was partly derived from the respective test manuals.

The VLMT opened the testing at 4:00 pm (ca. +120 minutes after fludrocortisone application) and was followed by the RCFT. To bridge inevitable latency times, TMT-A and B and the d2 Test succeeded the Immediate Recall of the Rey Figure. That was justified because both tests measure primarily different cognitive functions. With a 30 minutes delay, at 4:30 pm (ca. +150 minutes), the participant had to complete the VLMT (Trail 7). The next step was remembering the Rey Figure in the delayed recall condition (30 minutes after presentation), followed by the Digit Span Forwards and Backwards. The AMT closed each day of testing (Figure 4).

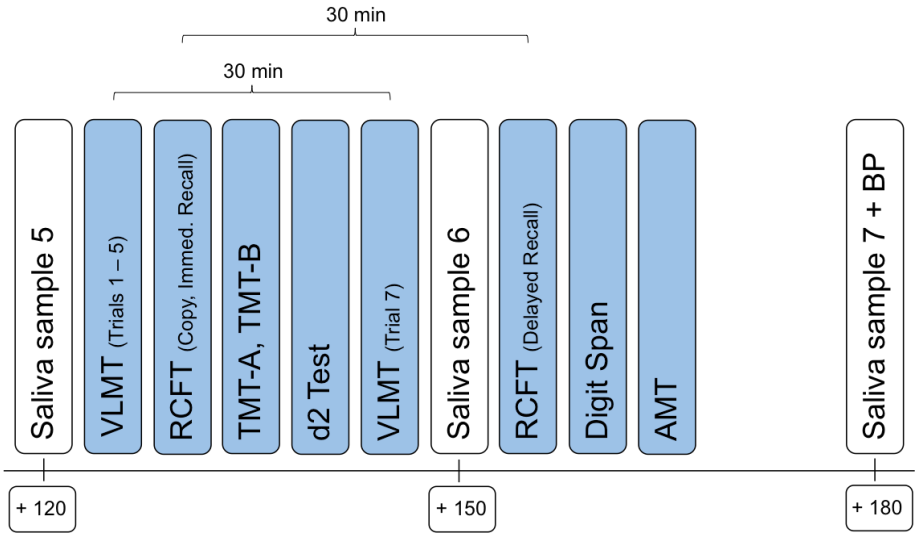

Figure 4: Schedule of neuropsychological tests

3.4 Neuropsychological Assessment of Cognitive Functions

Figure 5 displays the selection of neuropsychological tests.

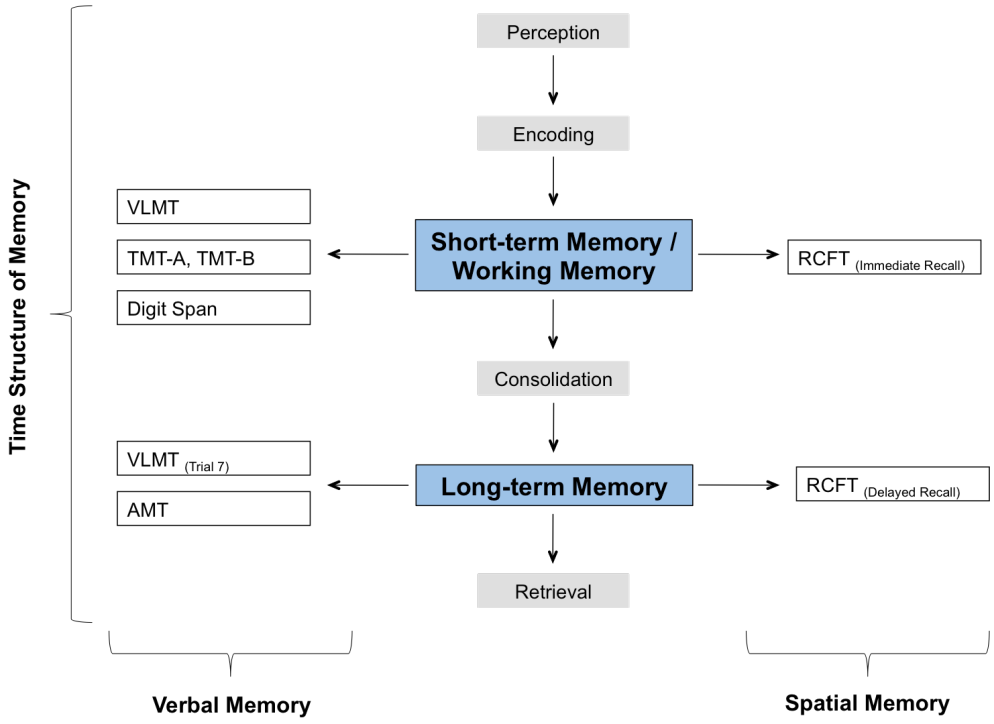

Figure 5: Selection of neuropsychological tests for the assessment of cognitive functions (excluding the d2 Test)

As described in 1.2, cognitive functions can be structured according to different perspectives. In order to emphasize the temporal structure of memory procession, it has become standard to talk of short-term and long-term memory (vertical axis). To outline the importance of the type of information, it has become customary to differentiate verbal from nonverbal, spatial memory (horizontal axis).

The selected neuropsychometric tests illustrate the main aspects of both classification systems, measuring short-term verbal ($VLMT_{Trials\ 1-5}$, Digit Span) and nonverbal memory ($RCFT_{Immediate\ Recall}$), as well as long-term verbal ($VLMT_{Trial\ 7}$, AMT) and nonverbal memory ($RCFT_{Delayed\ Recall}$). Additionally, one test was added to assess concentration endurance (d2 Test) (not shown in Figure 5), another to assess psychomotor speed (TMT).

3.4.1 Verbaler Lern- und Merkfähigkeitstest

The “Verbaler Lern- und Merkfähigkeitstest” (VLMT) is aimed to examine verbal declarative-episodic memory functions.
It collects sufficient data which allow a precise assessment of 1) learning performance and 2) memory consolidation.
Learning performance in terms of data acquisition is widely accepted as a representation of verbal short-term memory function, whereas memory consolidation and retrieval is regarded as a function of the long-term component of declarative-episodic verbal memory (Heimstaedter 1997).

The VLMT is comprised of three different parts.
First, a list of 15 different, semantically non-associated words is read out to the participant in a neutral manner, with a steady 2-second interval in between two words and without any particular emphasis on single terms. Then the study subject is requested to reproduce as many words as he or she can remember, without considering any specific order. This procedure is repeated 4 times, so that the “learning phase” spans 5 iterations altogether (Trials 1 to 5). The number of remembered words in Trial 5 is interpreted as a measure of short-term verbal memory (Heimstaedter 1997).
Following this, a list of distractor words is presented to the test taker in the way described above. The subject has to recall as many words as possible from the second list. Afterwards the test person is requested to recall as many words as he can remember from the first list, but this time, without prior presentation by the tester (Trial 6).
After a time delay of 30 minutes – and having passed different nonverbal memory tests in the meantime – the participant is asked to remember as many words as possible from the first word list (Trial 7), again. The number of remembered words at this stage is regarded as a reflection of long-term memory performance (Heimstaedter 1997).

The tester had to record each answer, regardless of its being correct or not.
Every correct answer received one point, adding up to a possible maximal score of 15 points per stage. Every mistake was recorded and classified as either false

positive (counting all the words which are not corresponding to either the first or the second word list), perseveration (recurring answers within one stage, no matter if the word was on the list or not) or “interferences” (all of the words which are not on the respective list). “False positive” answers and “perseverations” might occur in Trials 1 to 5, whereas all kinds of mistakes could only be made in Trials 6 to 7.

The VLMT is evaluated in two steps. First, the grand total of correct answers in Trials 1 to 5 is calculated to create the learning curve. Secondly, the total score for Trial 7 is calculated as a measure of long-term verbal memory function.

To minimise learning effects, a paralleled version of the VLMT was used on the second day of testing. As described by Lienert et al., retest-/parallel-form-reliability values range from r = 0.68 to r = 0.86 (Lienert 1961).

3.4.2 Rey-Osterrieth Complex Figure Test

The “Rey-Osterrieth Complex Figure” (RCFT), developed in 1941 by André Rey and standardized by Paul Alexandre Osterrieth in 1944, is a tool to test for visuospatial constructional abilities, visual memory (Spreen & Strauss 2006) as well as executive functions, including organizational and problem-solving skills, psychomotor and memory functions (Waber & Holmes 1986).
Measuring nonverbal, declarative, spatial memory functions, the RCFT can be regarded as a nonverbal equivalent of the VLMT.

The participant is presented with the complex figure and is given the task of copying it accurately on a separate sheet of paper in no more than five minutes (Copy). At this point, the tester is explicitly instructed not to accidentaly indicate the following recall of this figure from memory. Thus, the score achieved at this stage is a reflection of the precision of the participant’s work and is interpreted as a measure of visual-constructional ability (Spreen & Strauss 2006).
Following this, the template and the drawing are both removed and therefore invisible to the test person. After a time span of three minutes, the participant is

requested to re-draw the figure from memory. No time limit was set on this stage (Immediate Recall).
The same procedure is repeated after thirty minutes (Delayed Recall). Both immediate and delayed recall scores reflect the amount of memorized information over time (Spreen & Strauss 2006).

In this study, the most commonly used scoring system was applied to measure the accuracy of the subject's work (Taylor 1959).
Basically, Copy, Immediate and Delayed Recall are scored in the same manner.

The figure was divided up into eighteen distinct elements. Each element was rated in the following manner (quoted in Spreen & Strauss 2006):

- the element was constructed and placed correctly → 2 points
- the element was constructed correctly but placed incorrectly → 1 point
- the element was constructed incorrectly but placed correctly → 1 point
- the element was constructed and placed incorrectly → 0.5 points
- the element was not constructed or not recognizable → 0 points

The maximal score adds up to 36 points per stage.

The test was conducted using two parallel forms, Form A, which is the original Rey Figure, and Form B, which is the so-called Taylor Alternative Version. According to the depiction in Spreen & Strauss, alternate versions show comparable reliability coefficients (Spreen & Strauss 2006).
Test-retest reliability ranges from r = 0.76 to 0.89 (Lu 2003). Liberman et al. report that intra-rater reliabilities were .96, .99 and .96 and inter-rater reliabilities were .88, .97 and .96 for Copy, Immediate Recall and Deleyed Recall, respectively (Liberman 1994). To test for internal consistency, split-half and coefficient alpha reliabilities were calculated and measured as >.60 for Copy and >.80 for both recall conditions (Woodrome 2005, Berry 1991).

3.4.3 Trail Making Test

Introduced in 1944 as part of the Army Individual Test Battery with subsequent incorporation into the Halstead-Reitan Battery (Reitan & Wolfson 1985), the “Trail Making Test” (TMT) (original version: Reitan 1992) has become a frequently used neuropsychological method for the measurement of speed for attention (Solana 2010), visual search, psychomotor speed (Lu & Bigler 2000) and executive functions (Tombaugh 2004, McGrath 1997), such as cognitive flexibility (Fernández & Marcopulo 2008) and working memory (Sánchez-Cubillo 2009).

The TMT consists of two independent components, A and B.
In TMT-A, the test person is handed a single sheet of paper with 25 circles, each of them containing only one number from 1 to 25. All digits are randomly distributed over the entire worksheet. The task is to interconnect all numbers by making pencil lines in ascending order as fast as possible and without making errors. The aim of this test is to quantify processing speed by measuring the time needed to complete the task correctly (Bowie & Harvey 2006).
The second part of the test (TMT-B) combines numbers (from 1 to 13) with letters (A to L). The test taker is asked to connect all elements in ascending order, always alternating between numbers and letters. The time measurement allows one to draw conclusions about executive functions (Bowie & Harvey 2006).

In both test parts, scoring is expressed in terms of time in seconds needed to complete the task correctly.

If the test taker makes a mistake, he is instantly made aware of it by the tester, so that the time for correction has a direct influence on the test score.
First, individual scores for TMT-A and B are evaluated. As indicated by Heilbronner et al., the correlation between TMT-A and B only reaches a value of .31 (Heilbronner 1991). This is usually explained by the assumption that in version B,

1) The absolute length of the final line is about 57 cm longer than in A, and
2) B includes more visual interference (i.e. B includes more items than version A) (Woodruff 1995).

Therefore, it is important to keep in mind that a comparably lower score in TMT-B does not necessarily reflect a somewhat diminished cognitive ability; on the contrary, it reflects a higher demand on psychomotor speed (Gaudino 1995).

Secondly, time delays caused by the tester's interferences in the case of mistakes should be taken into consideration when evaluating. For this purpose, Lazek et al. recommend calculating the difference score TMT-B minus TMT-A. It is supposed to eliminate the variability that is caused by such interferences, at least to some extent (Lazek 1995).

High inter-rater reliabilities were reported (.94 and .90 for Part A and B, respectively) (Fals-Stewart 1991). Retest reliability of TMT-A ranged from .76 to .89 and of TMT-B from .86 to .94 (Wagner 2011).

3.4.4 d2 Test: Concentration Endurance Test

The "d2 Test: Concentration Endurance Test" was developed by Brickenkamp 1981 to assess sustained attention and visual scanning ability (Spreen & Strauss 1998).

The test is comprised of 14 individual lines. Each line contains 47 randomly distributed letters, either "d" or "p". Each letter is combined with one to four dashes, arranged either above or below the letter.
The task is to find and mark each "d with two dashes", no matter if both dashes are arranged above, below or one dash above and one below the "d". All other combinations of letters and dashes are to be neglected. A time limit of 20 seconds is set for each line. The crucial goal for the test person is to detect as many "d's with two dashes" as possible within the timeframe. The maximal score is 296.

The d2 Test is evaluated on the basis of different scores (Spreen & Strauss 1998):

1) The “Gesamtzahl (GZ)”/”Total Raw Score (TS)” refers to the total number of correctly identified letters, without considering mistakes. The maximal score is 298 points.
2) The subtraction of the number of errors from the TS yields the “Total Score minus Errors (TS-F)”.
3) The “Schwankungsbreite (SB)”/”Fluctuation (FL)” is calculated by subtraction of the score of the line with the lowest rate of correctly identified letters from the score of the line with the highest rate.

Brickenkamp et al. reported an internal consistency of >.80 and test-retest reliabilities ranging from .89 to .92 for the total score (Brickenkamp 1981). Data on practise effects are controversial and do not allow definitive evaluations (Sturm 1983).

3.4.5 Forward and Backward Digit Span

The “Forward and Backward Digit Span” is part of the Wechsler Adult Intelligence Scale (WAIS) and tests verbal working memory function (Wechsler 1987).

It is composed of two different sections. The first task is to repeat numerical series correctly in forward order to get 1 point. Stage 1 consists of only two digits, whereas the last one (Stage 8) is comprised of nine numbers. Each stage includes two series of the same length, with a total possible score of 16 points. In the second part, however, the orally presented numerical series has to be repeated correctly in reverse order. It is made up of seven stages of 2 series each, and with a maximal score of 14 points. Therefore, the highest result achievable adds up to 30 points.

As reported by Wechsler et al., the average reliability coefficient across age groups is .88 (Wechsler 1987).

3.4.6 Autobiographical Memory Test

Autobiographical memory is widely conceptualised as a subsystem of episodic-declarative memory (Tulving 2002). It is oriented towards the past and allows people to recall events from their life. In a study with suicide attempters, Williams and Broadbent observed the tendency towards overgeneralized answers when their participants were asked to recall specific events of their life. In this case, Williams and Broadbent introduced the “Autobiographical Memory Test“ (AMT) (modifications by Buss 2004), to assess the specificity of a participant’s memory recall of events from his past (Wingenfeld 2011, Schlosser 2009, Buss 2004, Williams & Broadbent 1986).

The modified version of the AMT used in the present study exists in two parallel versions. Each test version contains six different adjectives, two neutral, two positive and two negative expressions.

		Valency		
		Neutral	**Positive**	**Negative**
Version	**A**	concentrated	happy	angry
		busy	interested	hurt
	B	patient	safe	sad
		correct	successful	clumsy

Each participant was presented both versions, with each version being counterbalanced across the two treatment conditions. All participants were instructed orally and in written form, and had to pass a training trial.

The test person was presented with white cards with the following sentence: “Please remember a situation, in which you felt *concentrated*”. First, the test person had to read out loud the phrase. Then, he had to remember a specific situation in which he felt as described in the instruction and write it down.

The participant’s attention was drawn to the fact that not the content of the situation was important, but the specificity of its description. Specificity is defined by giving information on a certain time (the critical date must not exceed one day

and must not include the day of testing), location, persons involved and an explanation of the activity. All answers were evaluated by two trained raters independently, who were not informed about the treatment condition (inter-rater reliability >.82) (Buss 2004). If the statement affected at least two categories and both raters agreed on this, the answer was validated and awarded 1 point (i.e. considered specific). If not, the answer was regarded as categorical and received 0 points (i.e. considered not specific). Additionally, the tester had to record the time needed for recall, which is counted from the end of reading to the beginning of writing. If the participant needs more than 60 seconds, his memory is considered to be not “spontaneously recalled” and is thus not eligible for evaluation. Finally, all points were summed up to generate the test score (= total number of specific answers).

3.5 Statistical Analyses

All calculations were conducted using IBM SPSS Statistics 22.

To test for group differences regarding demographic variables Chi-square tests were run for categorical variables (for example sex and smoking) and one-way ANOVA for continuous variables (i.e. age, years of education and total psychometric test scores).

To test for group or treatment effects, separate ANOVAs in repeated-measures design were conducted with treatment (fludrocortisone versus placebo) as within-subjects factor and group (depressed versus healthy) as between-subjects factor for the dependent variables TMT-A (time), TMT-B (time), VLMT-measured long-term memory (Trial 7) and learning curve (sum score Trials 1 - 5), Digit Span Forward and Backward and d2 Test as well as blood pressure and cortisol secretion (including delta values: highest baseline cortisol value minus lowest value +/- FC). For RCFT, there were 2 within-subjects factors (time and treatment) and 1 between-subjects factor (group).

If necessary, exploratory post hoc tests were conducted to further analyse treatment effects.

$p \leq 0.05$ was established as significance level.

4. RESULTS

As described in 3.5, the two groups' sociodemographic data were compared using one-way ANOVA for continuous variables (like years of education and BMI) and Chi-square tests for categorical variables (like sex and smoker status). Since neither test revealed any significant demographic differences between the two groups (except for psychometry), it did not become necessary to conduct analyses of covariables in the test evaluations (see 3.2.8).

4.1 Verbaler Lern- und Merkfähigkeitstest

The VLMT was applied to measure verbal memory (Heimstaedter 1997). In short, the number of remembered words was noted in all trials. Results for the delayed recall condition (Trial 7) in percent are shown below, indicating verbal long-term memory performance.

		Healthy Controls	Depressed Subjects
Placebo	Mean	94.3	83.4
	Std. Error	3.6	3.6
Fludrocortisone	Mean	96.5	92.2
	Std. Error	2.8	2.8

Table 3: VLMT results in percent of depressed and healthy participants with and without fludrocortisone

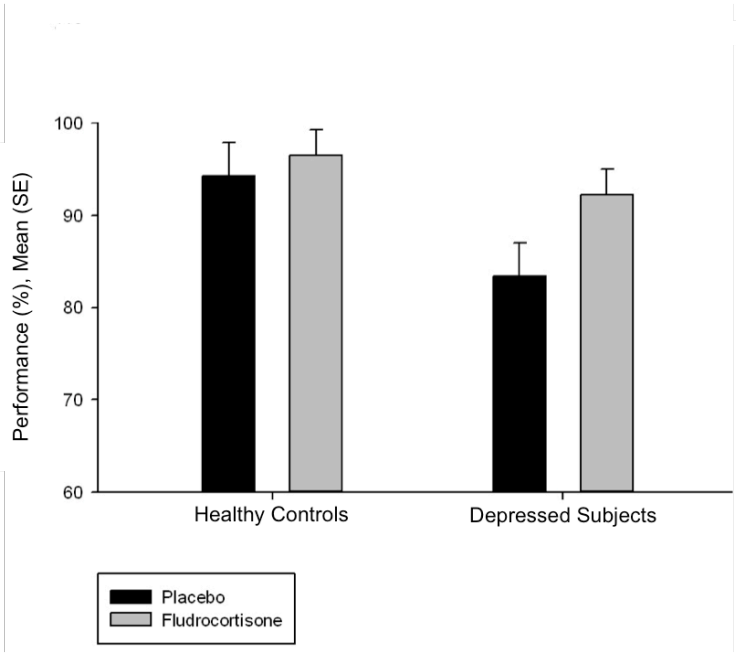

Figure 6: VLMT test performances of depressed and healthy participants after fludrocortisone or placebo. FC-application did show a significant improvement of performance across groups (F = 4.9, p = 0.03). Post hoc analyses revealed a significant treatment effect in the depressed population (F = 4.2, p = 0.05, effect size partial η^2 : 0.15), but not in the healthy control group (F = 0.36, p = 0.55). No significant effect of group (F = 2.6, p = 0.11) or treatment by group interaction (F = 1.8, p = 0.18) emerged.

As shown in Figure 6, repeated measures ANOVA with group (depressed versus healthy participants) as between-subjects factor and treatment (fludrocortisone versus placebo) as within-subjects factor revealed a highly significant treatment effect of FC (expressed in percentage of correctly remembered words) in the delayed recall condition (Trial 7), indicating improved verbal memory performance across groups after MR stimulation (F = 4.9, p = 0.03). Exploratory post hoc tests revealed a significant treatment effect in the depressed population (F = 4.2, p = 0.05, effect size partial η^2 : 0.15), whereas in the healthy control group, no treatment effect was detectable (F = 0.36, p = 0.55), indicating improved verbal long-term memory in depressed participants after FC administration. The effect of group (F = 2.6, p = 0.11) and the treatment by group interaction were not significant (F = 1.8, p = 0.18).

As described in 3.4.1, the complete VLMT execution included 7 trials in total. Analysing trials 1 to 5 allows conclusions to be drawn about the participants' learning curve. We found a significant effect of group ($F = 5.1$, $p = 0.03$), indicating impaired verbal-declarative learning in depressed in comparison to healthy participants. No effect of treatment ($F = 0.30$, $p = 0.59$) and no treatment by group interaction ($F = 0.21$, $p = 0.65$) emerged.

4.2 Rey-Osterrieth Complex Figure Test

The RCFT was included to measure nonverbal, spatial memory as a complement to the VLMT (Spreen & Strauss 2006).

			Healthy Controls	Depressed Subjects
Placebo	Copy	Mean	35.5	35.3
		Std. Error	0.2	0.2
	Imme. Rec.	Mean	26.3	25.4
		Std. Error	1.2	1.2
	Delay. Rec.	Mean	26.3	24.7
		Std. Error	1.1	1.1
Fludrocortisone	Copy	Mean	35.3	35.8
		Std. Error	0.2	0.2
	Imme. Rec.	Mean	27.6	25.3
		Std. Error	1.1	1.1
	Delay. Rec.	Mean	27.2	24.7
		Std. Error	0.9	0.9

Table 4: Test results for the RCFT in absolute numbers; Copy, immediate and delayed recall

Repeated measures ANOVA with group (depressed versus healthy subjects) as between-subjects factor and treatment (fludrocortisone versus placebo) and time (copy, immediate recall and delayed recall) as within-subjects factor revealed no significant effect of treatment ($F = 0.4$, $p = 0.52$), effect of group ($F = 2.6$, $p = 0.11$) or treatment by group interaction ($F = 1.4$, $p = 0.24$).

4.3 Trail Making Test

As outlined in 3.4.3, the Trail Making Test is a device to measure speed for attention (Solana 2010), visual search, psychomotor speed (Lu & Bigler 2000) and executive functions (Tombaugh 2004, McGrath 1997).

4.3.1 Synopsis of TMT - Test Results

TMT test results viewed in isolation and difference scores are shown below.

TMT - A

		Healthy Controls	Depressed Subjects
Placebo	Mean	21.8	27.0
	Std. Error	1.6	1.6
Fludrocortisone	Mean	21.9	26.8
	Std. Error	1.4	1.4

TMT - B

		Healthy Controls	Depressed Subjects
Placebo	Mean	49.9	62.0
	Std. Error	4.3	4.3
Fludrocortisone	Mean	48.6	56.4
	Std. Error	3.5	3.5

TMT - Difference Score

		Healthy Controls	Depressed Subjects
Placebo	Mean	28.2	85.0
	Std. Error	3.7	3.7
Fludrocortisone	Mean	26.7	29.7
	Std. Error	2.9	2.9

Table 5: Synopsis of test results for TMT-A, TMT-B and difference score in seconds

4.3.2 TMT - A

The TMT-A test result viewed in isolation.

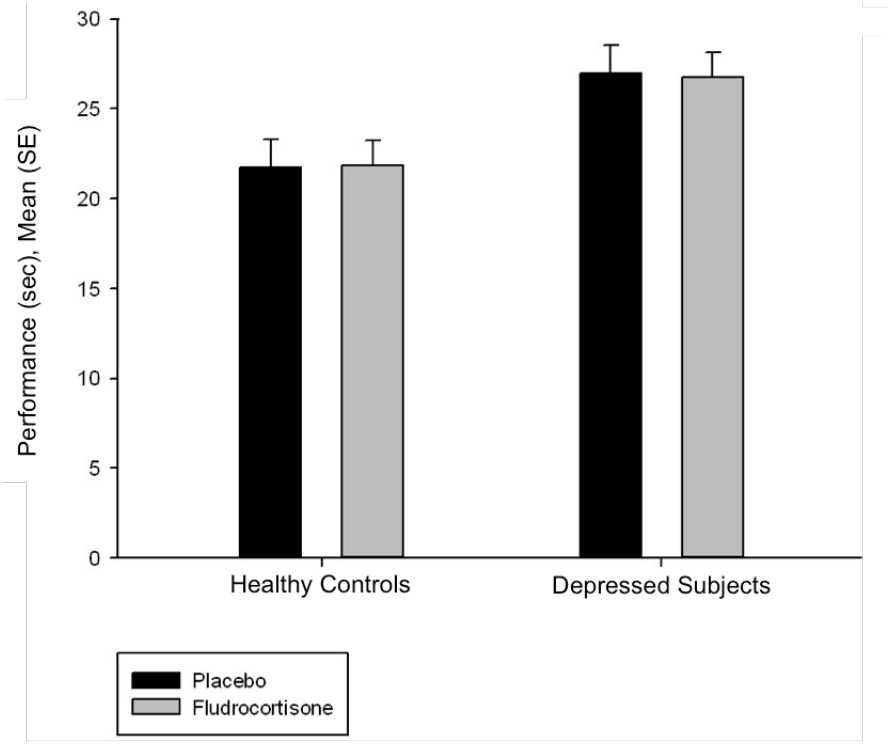

Figure 7: TMT-A test performances of depressed and healthy participants with and without FC-application viewed in isolation. A significant group effect emerged (F = 6.6, p = 0.01), indicating that depressed subjects needed more time to complete the task correctly than their healthy counterparts. There was no treatment effect on performance (F = 0.1, p = 0.96). No group by treatment interaction was observed (F = 0.05, p = 0.83).

As shown in Figure 7, repeated measures ANOVA with group (depressed versus healthy participants) as between-subjects factor and treatment as within-subjects factor revealed a significant effect of group (F = 6.6, p = 0.01) indicating that it took more time to complete the task in the patient group compared with healthy controls.

Differences in treatment conditions were not significant (F = 0.1, p = 0.96). No group by treatment interaction emerged (F = 0.05, p = 0.83).

4.3.3 TMT - B

The TMT-B test result viewed in isolation.

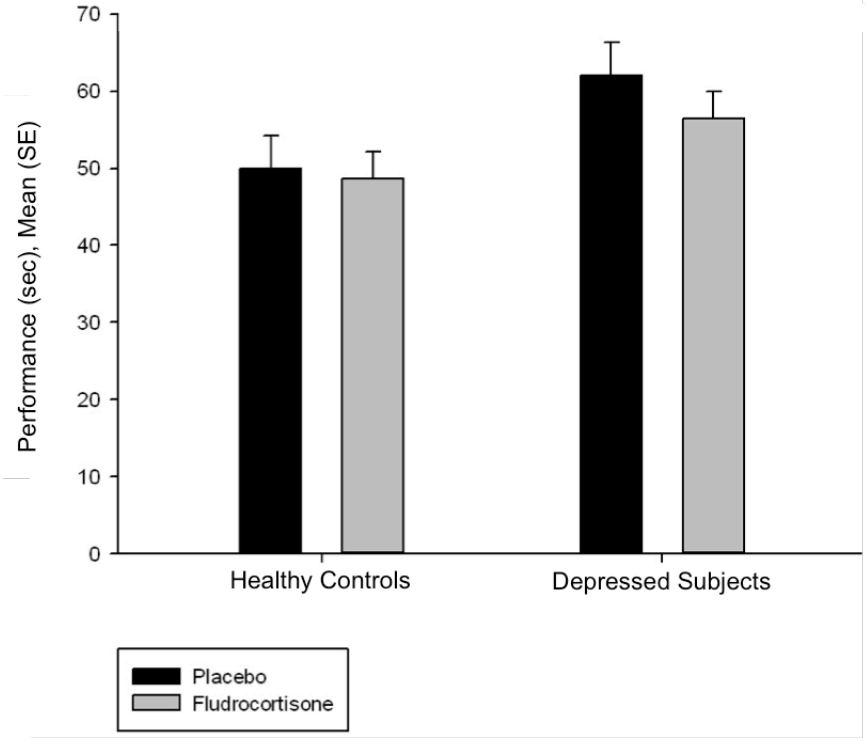

Figure 8: TMT-B test performances of depressed and healthy participants with and without FC-application viewed in isolation. The depressive study population needed more time to complete the task correctly than its healthy counterpart on trend level (F = 3.76, p = 0.059). There was no treatment effect on performance (F = 2.8, p = 0.1). No group by treatment interaction emerged (F = 1.07, p = 0.31).

Repeated measures ANOVA with group (depressend versus healthy participants) as between-subjects factor and treatment (fludrocortisone versus placebo) as within-subjects factor and revealed a group effect on trend level (F = 3.76, p = 0.059) indicating inferior performance of depressed compared with healthy participants. The main effect of treatment (F = 2.8, p = 0.1) and the group by treatment interaction (F = 1.07, p = 0.31) were not significant.

4.3.4 Difference Score ($Score_{TMT-B} – Score_{TMT-A}$)

Calculating the difference score (score TMT-B minus score TMT-A) allowed measuring executive function unaffected by psychomotor speed (for detailed information see 3.4.3).

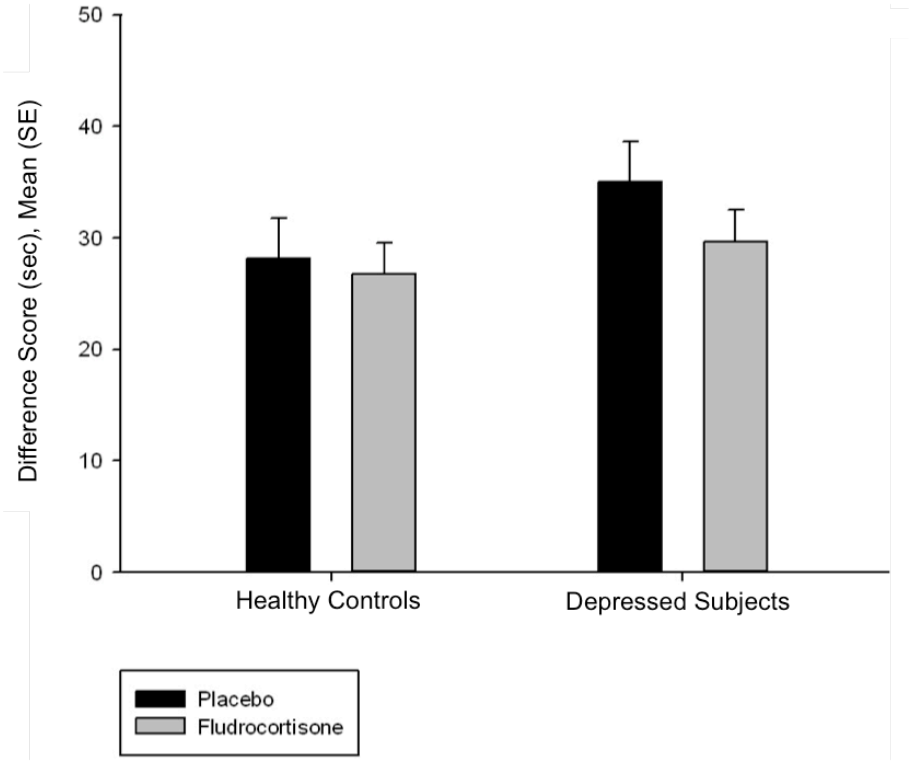

Figure 9: TMT-B – TMT-A difference score calculation revealed a significant effect of treatment with FC across groups (F = 4.4, p = 0.04). FC-application led to a decrease in time needed to complete the task correctly. FC-application in the depressed group caused significant changes in performance (F = 5.2, p = 0.03, effect size partial η^2: 0.18). The effect of group (F = 1.4, p = 0.24) and the group by treatment interaction (F = 1.0, p = 0.32) were not significant.

Repeated measures ANOVA with treatment as within-subjects factor and group as between-subjects factor revealed a significant effect of treatment (F = 4.4, p = 0.04), indicating improving effects of FC-induced MR stimulation on executive function across groups.

Exploratory post hoc tests revealed a significant treatment effect in the depressed population (F = 5.2, p = 0.03, effect size partial η^2 : 0.18), but not in the healthy control group (F = 0.35, p = 0.56).

The main effect of group (F = 1.4, p = 0.24) and the group by treatment interaction (F = 1.0, p = 0.32) were not significant.

4.4 d2 Test: Concentration Endurance Test

The letter cancellation test “d2 Test: Concentration Endurance Test” assesses sustained attention and visual scanning ability (Spreen & Strauss 1998).

		Healthy Controls	Depressed Subjects
Placebo	Mean	197.8	189.8
	Std. Error	9.0	9.0
Fludrocortisone	Mean	198.5	189.9
	Std. Error	9.8	9.8

Table 6: Results of the d2 Test in absolute numbers

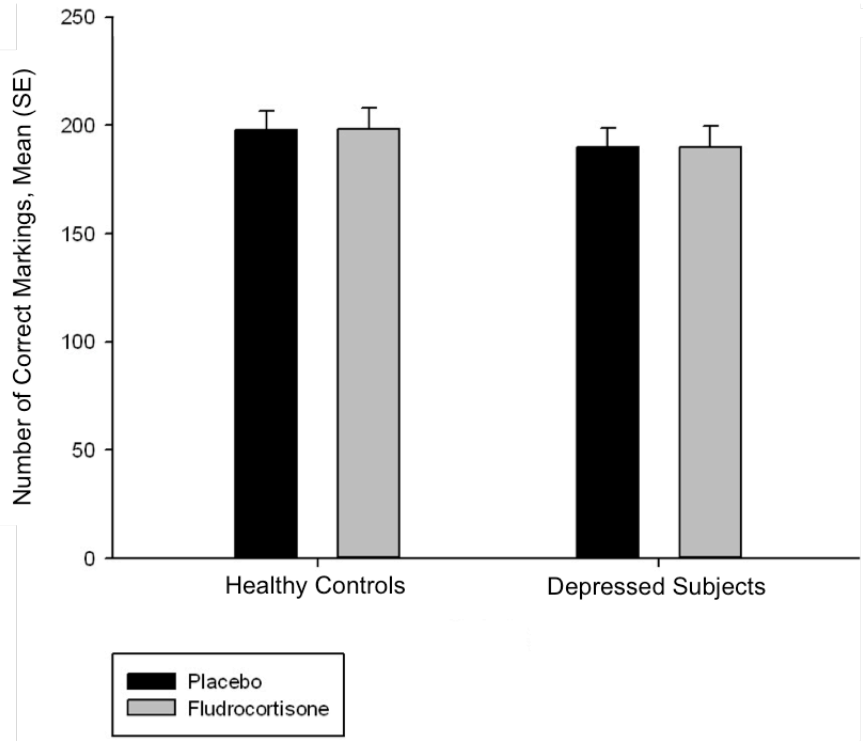

Figure 10: The results of the d2 Test for sustained attention and scanning ability. There was no significant effect of group or treatment ($F = 0.47$, $p = 0.5$ and $F = 0.006$, $p = 0.94$, respectively). No treatment by group interaction emerged ($F = 0.002$, $p = 0.96$).

As shown in Figure 10, repeated measures ANOVA with group (depressed versus healthy participants) as between-subjects factor ($F = 0.47$, $p = 0.5$) and treatment (fludrocortisone versus placebo) as within-subjects factor ($F = 0.006$, $p = 0.94$) did not reveal significant effects. The group by treatment interaction was not significant, either ($F = 0.002$, $p = 0.96$).

4.5 Digit Span Test

The Forward and Backward Digit Span Test was included to measure verbal working memory function (Wechsler 1987).

Forward Digit Span – Results

		Healthy Controls	Depressed Subjects
Placebo	Mean	10.0	9.3
	Std. Error	0.5	0.6
Fludrocortisone	Mean	10.0	9.9
	Std. Error	0.4	0.5

Backward Digit Span – Results

		Healthy Controls	Depressed Subjects
Placebo	Mean	8.0	7.7
	Std. Error	0.5	0.5
Fludrocortisone	Mean	8.3	7.8
	Std. Error	0.5	0.5

Table 7: Synopsis of test results of the Forward and Backward Digit Span tests in absolute numbers

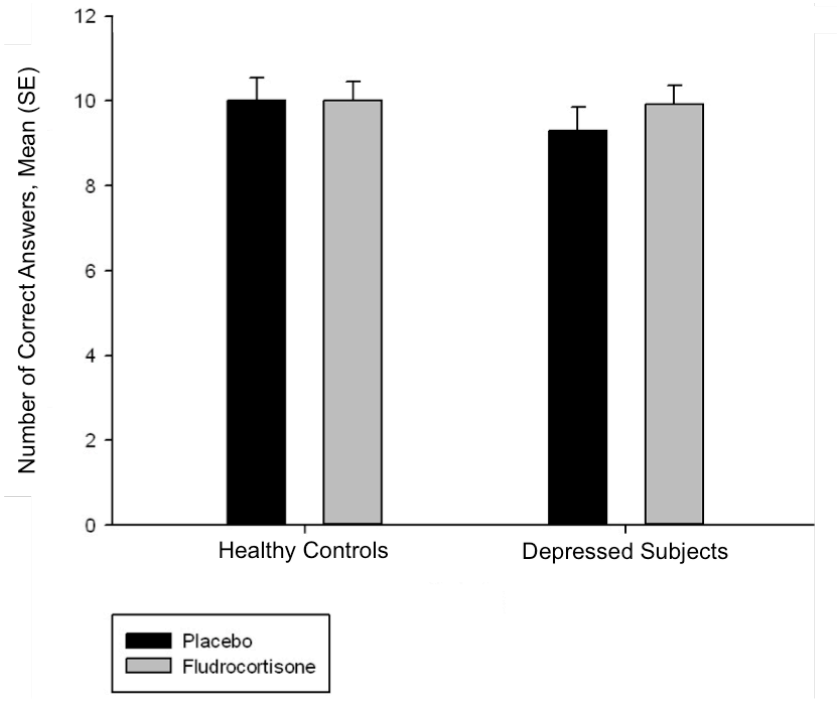

Figure 11

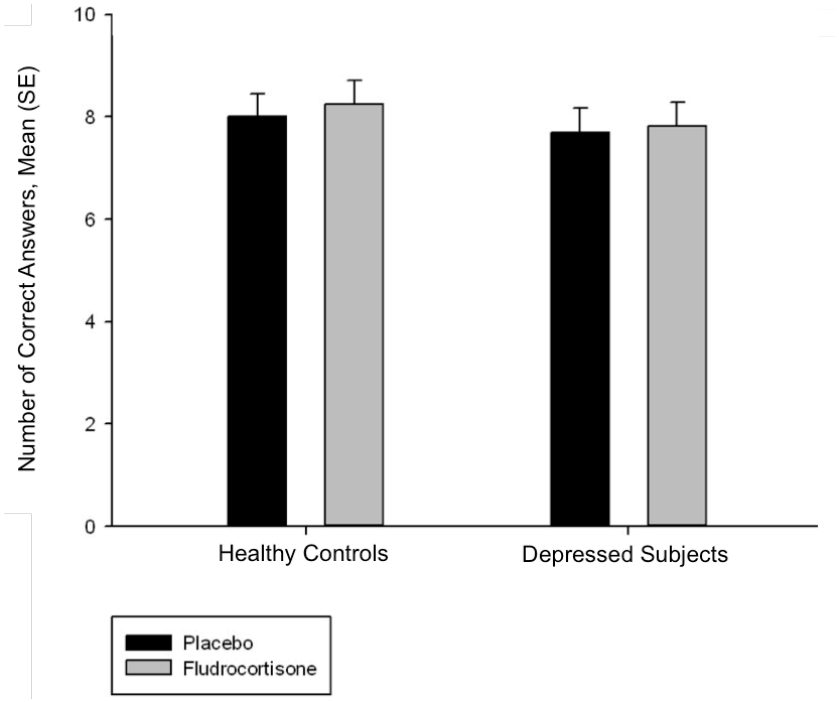

Figure 12

Figures 11 and 12: Results of the Forward and Backward Digit Span tests, respectively. No significant effect of group ($F = 0.36$, $p = 0.55$ and $F = 0.37$, $p = 0.55$), or of treatment ($F = 1.14$, $p = 0.29$ and $F = 0.52$, $p = 0.48$) was found in either test. No treatment by group interactions emerged ($F = 1.14$, $p = 0.29$ and $F = 0.05$, $p = 0.82$).

Repeated measures ANOVA did not reveal any effect of group (Forward Digit Span: F = 0.36, p = 0.55; Backward Digit Span: F = 0.37, p = 0.55) or of treatment (Forward Digit Span: F = 1.14, p = 0.29; Backward Digit Span: F = 0.52, p = 0.48). No significant treatment by group interaction was found in either test (Forward Digit Span: F = 1.14, p = 0.29; Backward Digit Span: F = 0.05, p = 0.82).

4.6 Autobiographical Memory Test

Numbers of specific answers of healthy and depressed participants are shown below.

		Healthy Controls	Depressed Subjects
Placebo	Mean	3.1	3.7
	Std. Error	0.4	0.4
Fludrocortisone	Mean	3.1	3.5
	Std. Error	0.4	0.4

Table 8: Absolute numbers of recalled specific memories in the AMT

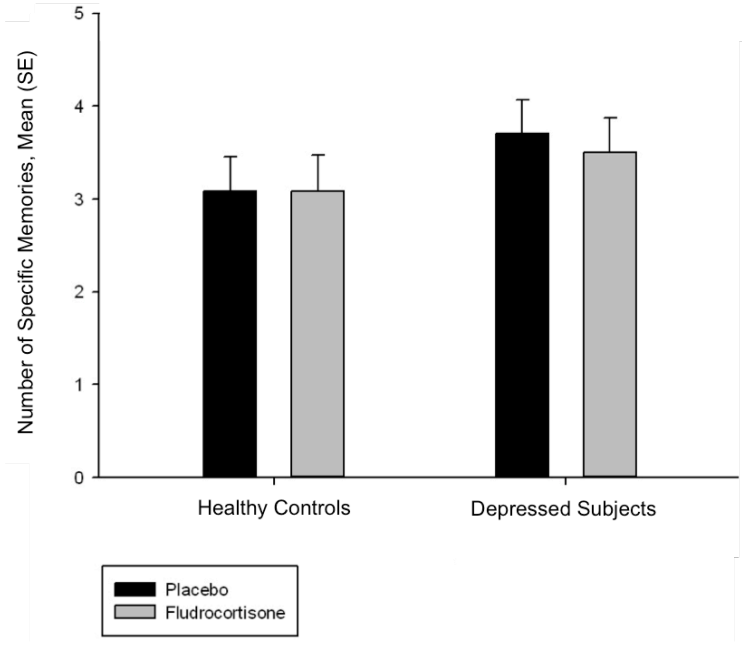

Figure 13: The amount of specific memories in the AMT. There were no significant group or treatment effects (F = 1.18, p = 0.28 and F = 0.21, p = 0.65, respectively). No significant treatment by group interaction emerged (F = 0.21, p = 0.65). No significant effect of group, treatment or interaction emerged with regard to a specific valency.

Repeated measures ANOVA with group (depressed versus healthy participants) as between-subjects factor and treatment (fludrocortisone versus placebo) as within-subjects factor revealed no significant effect of treatment ($F = 0.21$, $p = 0.65$), group ($F = 1.18$, $p = 0.28$) or treatment by group interaction ($F = 0.21$, $p = 0.65$). No significant effect of group, treatment or interaction emerged with regard to a specific valency.

4.7 Cortisol Secretion in the Course of a Test Day

Saliva cortisol levels were measured using salivettes. Time points were ca. 2:00 pm (double-measurement for baseline cortisol), 3:00 pm (+60 minutes), 3:30 pm (+90 minutes), 4:00 pm (+120 minutes), 4:30 pm (+150 minutes) and 5:00 pm (+180 minutes).

Repeated measures ANOVA with time (i.e. 7 measures) and treatment (fludrocortisone versus placebo) as within-subjects factor and group (depressed versus healthy participants) as between-subjects factor did not return significant treatment effects, group effects, nor group by treatment interaction.

Repeated measures ANOVA nevertheless detected a significant treatment by time interaction ($F = 9.5$, $p = 0.01$), which means that FC-application decreased salivary cortisol levels over time more effectively than the placebo did. In delta value analyses, ANOVA again detected a significant effect of treatment ($F = 8.2$, $p = 0.01$), supporting the finding mentioned above. Here, no group effect ($F = 0.2$, $p = 0.88$) or group by treatment interaction ($F = 0.8$, $p = 0.38$) was found.

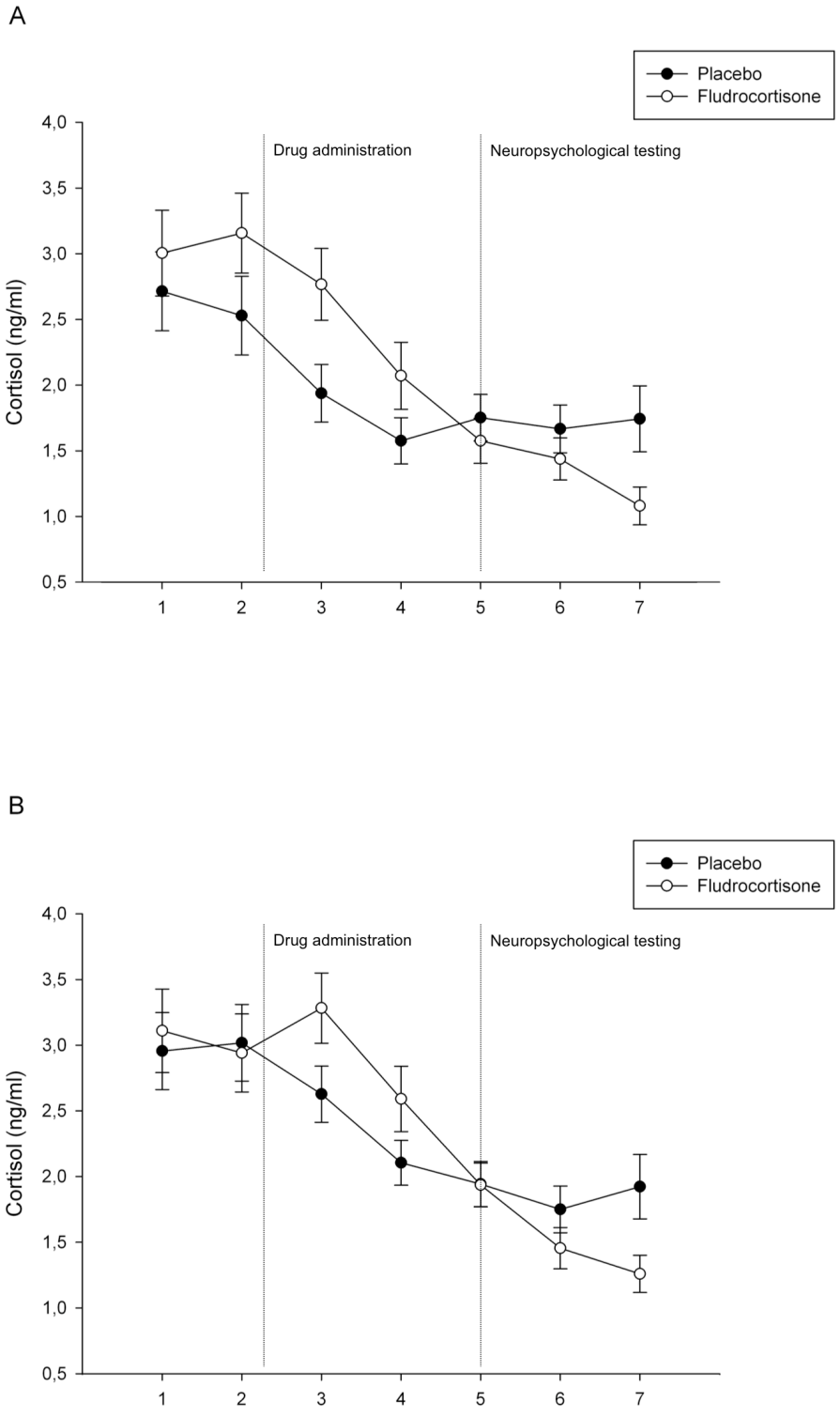

Figure 14: Saliva cortisol levels in the course of a test day

Key: **A** Healthy Controls **B** Depressed Subjects

1 = -10 min **2** = -5 min **3** = +60 min **4** = +90 min

5 = +120 min **6** = +150 min **7** = +180 min

4.8 Correlation Between Fludrocortisone-induced Cortisol Suppression and Cognitive Performance

FC-induced cortisol suppression (measured using delta values) and VLMT Trial 7 (= delayed recall), which is long-term declarative-verbal memory performance, were correlated (r = 0.45, p = 0.01). There was no correlation between FC-induced cortisol suppression and spatial memory, executive function, working memory or autobiographic memory.

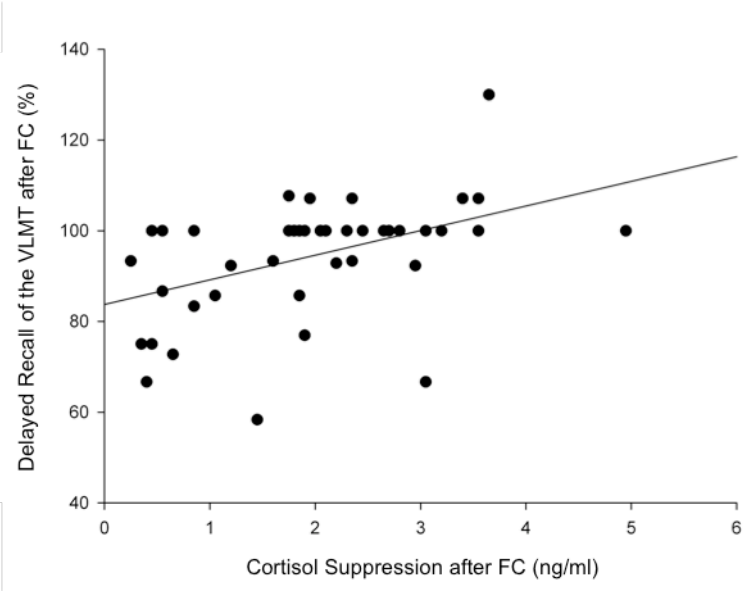

Figure 15: Correlation between FC-induced cortisol suppression and verbal memory function expressed in percentage of correct answers in the delayed recall condition of the VLMT for both groups (healthy controls: r = 0.60, p = 0.01; depressed participants: r = 0.41, p = 0.059; across groups: r = 0.45, p = 0.01).

4.9 Blood Pressure in the Course of a Test Day

Depressed and healthy subjects did not differ in systolic (F = 0.73, p = 0.39) or diastolic (F = 1.1, p = 0.29) blood pressure. FC-application did not influence blood pressure (systolic BP: F = 0.02, p = 0.88; diastolic BP: F = 0.19, p = 0.66). No group by treatment interaction emerged (systolic BP: F = 0.19. p = 0.66; diastolic BP: F = 0.24, p = 0.62).

5. DISCUSSION

A plethora of study results emphasizes the interrelationships between depressive disorders and cognitive functions. In the context of HPA dysregulations, recent findings strengthen the role of MRs on aetiology and psychopathology of depressive disorders. In short, preclinical investigations showed that decreased MR activity impaired cognitive functions, whereas enhanced MR activity improved cognitive functions. For the first time a clinical study has been designed, hypothesizing that those preclinical findings could be transferred to humans, so that pharmacological MR stimulation would enhance cognition acutely, especially in depressed persons. Results could influence future treatment of major depression, not only in the context of add-on strategies.

5.1 Subjects

This study was conducted with a remarkably homogeneous group of participants. With regard to age, school leaving qualification and total years of education, BMI, smoker status, civil status, phase in menstruation cycle and taking of contraceptive pills, the depressed and the healthy group did not differ significantly. For the interpretation of the results it is essential to consider that the study population was quite young. As described below, this very likely had consequences for test performance in the placebo condition as well as effects of MR stimulation, and thus limits conclusions about the elderly (for further information see below).

5.2 Hypothesis I

Participants suffering from major depression show impaired cognitive functions in comparison to healthy controls.

5.2.1 Declarative-episodic Verbal and Spatial Memory

Verbal Memory

The “Verbaler Lern- und Merkfähigkeitstest” (VLMT) measures verbal declarative-episodic memory. Hypothesis I was confirmed, since data presented in 4.1 indicate impaired learning in the depressed group when compared with the healthy group.

This result is in line with the general reported psychopathology of a modest to severe episode of major depression (see 1.1 and 1.5), with impaired episodic and working memory (Austin 2001, Pelosi 2000).

There are numerous reasons for impaired abilities, ranging from anatomical to functional mechanisms. As reported by Lupien et al. and Hickie et al., deficits of hippocampus-related memory functions in major depression are associated with reduced hippocampal volumes as a consequence of chronically elevated cortisol (Hickie 2005, Lupien 1998). The extent of volume reduction correlated with the degree of cortisol elevation and basal cortisol levels (Lupien 1998). Hickie et al. replicated this finding later for impaired visual and verbal memory performance (Hickie 2005). It is important to note that participants’ ages ranged from 28 to 82 years. Matched control subjects allowed controlling for age and physiological atrophy.

A plethora of studies emphasizes the influence of elevated free cortisol itself. Basically, poor cognitive performance was either related to increased cortisol levels in stress or after pharmacological interventions or in somatic comorbidities like Cushing’s disease, whereas improvement of cognitive performance was achieved with pharmacological blocking of GR (Hinkelmann 2009, Vythilingam 2004, Forget 2000, Newcomer 1999, Lupien 1997, Kirschbaum 1996).

The most striking finding in the context of this work was reported by Otte et al. Pharmacologically induced MR blockade led to decreased hippocampus and prefrontal-dependent memory performance, namely visuospatial memory, selective attention and mental flexibility in healthy participants (Otte 2007). In animal studies, blocking MRs resulted in poorer performance in Morris Water Maze performances (Berger 2006, Yau 1999, Oitzl 1992).

Underlying mechanisms could concern subtle changes of single proteins as well as complete neurons. On the one hand, cellular integrity of the hippocampus could be influenced, because chronically elevated cortisol levels cause dendritic atrophy by reducing LTP (de Kloet 2005). On the other hand, the decrease of MRs and GRs in conditions of chronically elevated cortisol has been reported (Joëls 2007), so that the downregulation of hippocampal MR could also contribute to cognitive impairments (López 1998).

However, imaging techniques were not applied in this study, so it is not deducible if impaired learning in the VLMT was really due to structural hippocampal affections, or if reasons predominantly concerned cell metabolism, which in turn would have made additional preclinical approaches neccessary.

Spatial Memory

The “Rey-Osterrieth Complex Figure Test” and the “Taylor Alternative Version” (RCFT) is a tool to test for visual memory (Spreen & Strauss 2006) and executive functions (Waber & Holmes 1986). Depressed patients did not display impaired spatial memory; that is, they did not perform worse, in comparison to healthy controls.

At the first look, this finding might be surprising, since other studies report decreased visuospatial abilities in major depression (for example Hinkelmann 2009 and others). However, a closer look reveals that impaired spatial declarative memory in depression as measured by the RCFT has been associated with contemporaneous presence of additional cognitive impairments as well as age and education of test persons.

Rosenstein et al. conducted a retrospective and a prospective study on the question in how far RCFT results were influenced by depression and neurological diagnoses. In the retrospective part of the study, the age ranged from 17 to 92 years. In the prospective part a group of depressed and a group of neurologically impaired and matched healthy controls were tested. All results taken together led to the conclusion that depressed and healthy subjects did not generally differ in

average RCFT performance, but age and education were significantly related to RCFT outcome (Rosenstein 1999).

A different aspect was highlighted by Elderkin-Thompson et al. A group of elderly depressed (mean age = 69.6 years, SD = 7.2) was compared to matched healthy controls (mean age = 73.0 years, SD = 7.9) using BQSS criteria for the evaluation of RCFT results. RCFT was performed as described in 3.4.2. The Boston Qualitative Scoring System (BQSS) is a manual that provides criteria for the evaluation of executive function and visuoconstructional ability during the copy and recall conditions of the RCFT (Stern 1999). Elderkin-Thompson et al. could show that in aged-depressed, planning, as part of executive function, mediated the influence of depression on RCFT performance, as expressed in per cent of correct elements in the delayed recall (Elderkin-Thompson 2003). Therefore we conducted further analyses with executive function in the placebo condition as control variable. However, no significant interaction effect emerged.

Another possible explanation for the dissociation of two hippocampal functions (i.e. verbal and visuospatial memory) refers to lateralization effects. fMRI and PET imaging emphasizes the importance of the quality of information for anatomical representations. For the creation of verbal memory, left dorsolateral prefrontal areas are additionally activated, whereas for generating spatial memory, right dorsolateral prefrontal areas are additionally utilized (Kelley 2002, Postle 1999, Smith 1996). This might add to the explanation why affections of verbal memory and affections of spatial memory – although both represented mainly in hippocampal structures – can occur unrelatedly.

To conclude, this study's population was homogeneously young and did not show impaired executive functions, two reasons that might explain, at least to some extent, why depressed did not differ from healthy controls in spatial declarative-episodic memory performance.

5.2.2 Verbal Working Memory

In our depressed and healthy study population, verbal working memory was not impaired. Test results did not differ between our two cohorts or from normative data for the age group 25 – 34 years (Mittenberg 1992).

Even if deficits in working memory are common in depression (see 1.5), recent studies underline the importance of age for test outcomes. Barch et al. conducted a study, comparing working memory and prefrontal cortex dysfunction in depressed patients, participants with schizophrenia and a matched healthy control group (Barch 2003). In contrast to schizophrenic patients, they could not show such an impaired working memory in depressed. Barch et al. discussed their findings with regard to age and alluded to mean years of age of the depressed group, which was 37.6 +/-12 (Barch 2003). Similar findings (none to minimal cognitive impairments in young depressed) had been reported before (Grant 2001, Purcell 1997). Cognitive deficits were reported to be more commonly detected in elderly depressed, with more severe impairments, too (Beats 1996, Elliott 1996, Palmer 1996).

Given the fact that we also examined a comparably young group of patients (mean years of age 26.5 +/-3.1), our findings are in line with study results mentioned above. We assumed that the young depressed could have mobilized resources, for example previously inactivated MRs, which elderly would not have been able to carry out to this extent.

5.2.3 Executive Function

Data presented in 4.3.4 show no significant group effect or treatment by group interaction.

A closer look at the literature concerning level of executive functioning in major depression reveals rather conflicting results. In a review on cognitive impairments in depression, Austin et al. analysed 86 studies and came to the conclusion that despite all differences in study design, execution and population, deducible overall results emphasize that cognitive impairments are highly correlated with age and with disease severity (Austin 2001).

To illustrate this, Beats et al. found that aged-depressed display the most prominent deficits in verbal fluency and cognitive set-shifting (Beats 1996). In a study sample of young and moderately depressed, Purcell et al. could not detect distorted motor speed or attentional set-shifting (Purcell 1997).

In contrast, studies by Channon and Green showed impaired executive function in young populations with mean age ranging from 20 – 40 years. The reservation must be made, however, that participants suffered from dysphoria instead of recurring depressive episodes (Channon & Green 1999, Channon 1996).

And on a more basic level, Watkins and Brown discuss why executive dysfunction does not appear to be fundamentally associated with depression, and therefore does not affect all patients (Watkins & Brown 2002).

Despite all results and their contradictions, what can be concluded is that age is associated with reduced cognitive performance. Based on Jorm et al., with age, mental processing slows down, and performance in difficult tasks is impaired, whereas mental inflexibility and susceptibility to distractions increase (Jorm 1986). Moreover, as described in 1.4, cognitive deficits often tend to persist after clinical remission of overall psychopathology. This progression of depressive disorders can be found in all age groups, but becomes more severe with age (Austin 2001). Against this background, our results appear to be rather conclusive, since our participants were comparably young. Again, it is reasonable to assume that effects of depression on cognition might be more pronounced in older subjects.

5.2.4 Autobiographic Memory

In this study, autobiographic overgeneralization was not detectable in the AMT (see 4.6). Reasons could be either the lack of overgeneralization in our depressed population, as it is not necessarily common in MDD, or the chosen execution of the AMT.

Although overgeneralized autobiographic memories often occur in patients with depressive disorders, studies with the AMT return rather inconclusive, sometimes even contradictory results (Williams 2007). Some studies could show that overgeneralized autobiographic memory in depressed using the AMT, whereas other studies failed to find a depression-specific effect (Williams 2007). A

possible explanation focuses on the differences in test executions, for example visual or oral presentation of cue words and variations in response time (Van Vreeswijk 2004).

In a review by Williams et al. from 2007, the authors conclude that overgeneralized autobiographic memory in MDD may be related to overall memory deficits, but this could not be explained by them (Williams 2007). In other words, overgeneralization and corresponding AMT results may occur in a depressive episode, but not for reasons of necessity. In the context of HPA dysregulations, Barnhofer et al. conducted a clinical study to investigate the relationship between cortisol levels in depressed patients and specificity of their autobiographic memory. The authors failed to show that overgeneralized autobiographic memory was determined directly by increased cortisol levels (Barnhofer 2005).

5.2.5 Psychomotor Speed

Evaluating Trail Making Test - A (TMT-A) results indicated slower psychomotor speed in depressed compared to healthy controls (see 4.3.2).

This finding replicates a plethora of previous studies with different preclinical, clinical and epidemiological approaches focusing on psychomotor disturbances in depressive disorders (for example Bennabi 2013, Hickie 2005, Mitchell 1996). Mitchell et al. correlated cortisol levels with performances in psychomotor examinations, Hickie and Naismith focused on the interrelationships between hippocampal volumes and cognitive impairments in depression, but with more emphasis on the role of Nucleus accumbens (Naismith 2007, Hickie 2005, Naismith 2002). An extensive review of psychomotor impairments in depression was published by Bennabi et al., which is in line with our findings (Bennabi 2013).

The presented data do not allow one to draw conclusions on underlying mechanisms. As described above, impaired MR function, cellular atrophy or involvement of hormones like CRH and cortisol might have contributed.

5.2.6 Attention

Attention as measured by the d2 Test was not impaired in depressed compared to healthy controls (see 4.4). It is important to note that attention, as a function of prefrontal cortex, might not always be severely impaired, especially not in young patients (Barch 2003). We assume that these results may be due to ceiling effects. The young study group could have made use of MR functioning in a way that covers possible impairments caused by depression. However, the presented data do not allow conclusions to be drawn and further investigations are needed to reveal potential compensation mechanisms.

5.3 Hypothesis II and Hypothesis III

Fludrocortisone-induced MR stimulation improves cognitive performance in depressed participants (= Hypothesis II).

Fludrocortisone-induced MR stimulation does not improve cognitive performance in healthy participants (= Hypothesis III).

5.3.1 Declarative-episodic Verbal and Spatial Memory

Verbal Memory

The VLMT measures verbal declarative-episodic memory, with learning performance as a reflection of short-term memory and memory consolidation and retrieval as a function of long-term memory (Heimstaedter 1997).

The data presented in 4.1 show a significant treatment effect, indicating improved delayed recall of the presented words after fludrocortisone administration across the groups. As mentioned in 4.1, treatment effects were much more pronounced in the depressed study group than in the healthy control group. Exploratory post hoc analyses confirmed a significant treatment effect in the depressed study population, but not in the healthy control group.

This result confirms the hypothesis that MRs are involved in memory processes. This seems to be plausible because a variety of findings support this interrelationship.

First, MR expression is highest in the hippocampus and prefrontal cortex (Berardelli 2012). As described above, the hippocampus is the anatomical-morphological correlate of declarative memory (see 1.2). Therefore, it is plausible that manipulation of MRs leads to hippocampus-dependent consequences as reflected by the altered VLMT outcome.

Several studies demonstrated that altering MR function results in different cognitive performances. As described above, spironolactone-induced decrease of MR function resulted in impaired learning in animals (Berger 2006, Yau 1999, Oitzl 1992) as well as in healthy humans (Otte 2007). By contrast, transgenic mice with an increased number of MR benefited from enhanced short-term memory and were not impaired by high-dosage glucocorticoid application, additionally (Ferguson 2007, Lai 2007). Adreno-ectomized rats treated with aldosterone showed improved spatial learning (Conrad 1997). In humans, mifepristone increased MR binding of cortisol by GR blocking with the result that spatial working memory improved in bipolar depression (Watson 2012). Thus our study complements these previous findings and demonstrates the acute beneficial effects of direct MR activation in humans for the first time.

At the moment a few possible explanations for this effect are available.

1) The observed effect could have been mediated by fast-acting, non-genomic effects of activated membrane-bound MR. As described in 1.3.4, membrane MRs are bound during concentration peaks of the ultradian cycle and during stress (Joëls 2009). Pharmacological application of the highly MR-selective FC imitates these conditions, a fact supporting this explanation. Activation might have led to increased presynaptic glutamate excretion and increased postsynaptic excitation in hippocampal neurons, thus strengthening synaptic plasticity (de Kloet 2013, Joëls 2009).

2) As described in 1.3.4, MR stimulation could have led to effective increase of long-term potentiation (LTP) in the hippocampus. According to de Kloet et al., LTP is the best investigated and documented biological substrate for learning and memory (de Kloet 2005). However, further studies are needed to untangle the effects of hormonal components, MRs and GRs, and not least the timing of events.

3) Being increased in major depression (Gold & Chrousos 2013, Holsboer & Ising 2010, Binder & Nemeroff 2010) and being associated with stress-related cognitive impairments (Wang 2011), CRH shifts to focus.
MR stimulation leads to decreased CRH secretion (Müller 2003). As depicted in 1.3.1, CRH regulates ACTH release and therefore triggers cortisol levels. We could show that FC-application decreased cortisol levels (see 4.7). Therefore, CRH decrease itself could have contributed to the improvement of verbal memory.

4) Ultimately, free cortisol itself has been reported multiple times in connection with cognitive performance. For example, Hinkelmann et al. reported the inverse relationship between free cortisol and memory performance with regard to verbal, spatial and working memory and selective attention (Hinkelmann 2009).
In this study, a strong correlation between cortisol suppression by FC and the results of the delayed recall in the VLMT (= percentage of correctly remembered words) was found, supporting this explanation. Nevertheless it is important to note that it cannot be deduced from our data whether cortisol is involved directly or is acting as a mediator.

To conclude, improvement of verbal memory by MR stimulation via FC-application in depressed seems to be rather plausible and is in line with earlier studies. Having been transferred from animal studies, this effect is demonstrated in humans for the first time.

Spatial Memory

The RCFT did not return any significant difference between depressed and healthy controls (see 4.2), neither in placebo nor in verum condition.

This goes in line with our findings that test results of the healthy population could not be improved by FC-application in any test. As described above, a possible explanation could be that test persons could have made use of an appropriate number and sufficient functioning of MRs, so that performance occurred on a ceiling level, making FC-application either negligible or not sufficient to significantly improve performance. This assumption is supported if normative data for the age range 20 – 29 years are considered. Both our healthy and depressed groups achieved test results (see 4.2) which are in the range of scores derived from the healthy, well-educated test population from test evaluation studies (Spreen & Strauss 2006).

Another note of importance refers to lateralization effects as explained in 5.2.1. Spatial and verbal declarative memory can be regarded as being rather dissociated, although both are associated with hippocampus formation.

In short, influences on verbal memory might not affect spatial memory in any case (Kelley 2002, Postle 1999, Smith 1996). Against this background, our findings of dissociated MR stimulation effects on declarative-episodic verbal and spatial memory performance do not appear improbable.

5.3.2 Psychomotor Speed

In our study sample, depressed persons showed slower psychomotor speed compared to healthy controls as measured by TMT-A (see 4.3). However, analyses of treatment condition did not reveal significant influences of MR stimulation on psychomotor speed, suggesting low or negligible involvement of MR.

5.3.3 Executive Function

Executive function was assessed using TMT-B minus TMT-A difference scores. Data presented in 4.3.4 show a significant treatment effect across groups, indicating improved executive function after FC-application. As shown in Figure 9, this effect was much more pronounced in the depressed group than in the healthy control group. Therefore, we conducted exploratory post hoc analyses, revealing a significant treatment effect in depressed subjects on executive function.

The prefrontal cortex is attributed to be the anatomical correlate of executive function (see 1.2). As described above, MR concentration is highest in hippocampus and prefrontal cortex (Berardelli 2012). Therefore it was reasonable to assume that changes of MR activity influence executive function.

Evidence can be drawn from the findings that executive function is among those systems which are most often impaired in depression (Rock 2013, Wagner 2012). MR – hippocampal and prefrontal – are downregulated in depression (Klok 2011). Taken together, these observations deliver a possible explanation for the result: stimulation of prefrontal MRs in an organism prevented from taking full advantage of sufficient MR functioning leads to improved executive function.

As described in 5.3.1, this effect could be explained by increased glutamate secretion. Lowy et al. could show in animal studies that acute stress – i.e. acute corticosterone elevations – increased glutamate concentrations in the synaptic gap of hippocampal neurons (Lowy 1995, Lowy 1993). This effect was shown in young and elderly rats. Just a few years later, the glutamate-increasing effect of acute stress was also shown in extrahippocampal areas, mainly prefrontal cortex and other structures of the limbic system (Reznikov 2007, Bagley 1997). However, Popoli et al. related these increased glutamate concentrations in the frontal and prefrontal cortex mainly to GR activation (Popoli 2011). Given the fact that fludrocortisone – despite its highly selective MR affinity – shows GR binding too, this explanation could be seen as a reason for this. GR affinity of FC is supposed to be approximately 150-fold lower than MR affinity (Agarwal 1999).

FC potency of GR activation ranges from very low to rather moderate, depending on the cited literature and on methodological questions (Grossmann 2004). In conclusion, FC-induced GR activation could explain improved prefrontal-associated executive function, at least to some extent.
Nevertheless, decreased CRH levels or even decreased plasma cortisol levels – despite our finding that cortisol suppression did not correlate with improved executive function – cannot be completely excluded from influence, because impairing effects of both hormones on cognition are well known (see 5.3.1).

To sum up, FC-induced MR stimulation improved executive function in depressed persons, whereas psychomotor speed did not appear to be affected in a similar manner.
As described in 3.4.3, the correlation of TMT-A and B reaches a value of only .31. One explanation focuses on differences regarding the number of items and total length of the line. Heilbronner et al. elaborated on the aspect that TMT-B viewed in isolation measures slightly different, more visual-perceptual processing-based abilities (Woodruff 1995, Heilbronner 1991). Nevertheless, Spreen et al. reached the conclusion that TMT-B is the more sensitive part of the test (Spreen & Strauss 2006). Despite all differences, TMT-A and B are highly sensitive to neurological and psychiatric damage of the brain (for detailed information see Spreen & Strauss 2006).
However, Lezak et al. underline the idea that scoring the TMT only with regard to time and not a combination of time needed and errors made, decreases reliability of the test, because identifying errors by the test taker and examiner might make use of different temporal resources (Lezak 1995). Therefore Lezak et al. recommended that one should use difference scores to improve reliability. Our results can be interpreted in this context, because it was only with the TMT-B minus A difference score where we found a significant effect of FC treatment, an effect which could not be detected by analysing TMT-A and B results in isolation.

5.3.4 Other Cognitive Domains

Concerning attention, working memory and autobiographic memory, no MR stimulation effect was found. We hypothesized that this might be explained by the assumption that the young study population could have made use of ceiling effects which could have covered possible impairments caused by MDD.
Again, our test results concur with respective normative data (Spreen & Strauss 2006, Spreen & Strauss 1998).
Against this background, the depressed participants could have compensated for impaired MR function, either by recruiting inactive MRs or different molecular mechanisms.
Regarding the healthy controls, they could have been able to utilize a sufficient number of MRs; their MR binding capacity is not altered and they therefore work on the ceiling level of their capability. In this respect, FC-application might have been unable, or perhaps insufficient in the case of this study, to further increase the MR function with effects on the cognitive domains mentioned above.

5.4 Fludrocortisone-induced Suppression of Cortisol

We could show that FC-induced MR activation suppressed plasma cortisol levels over time more effectively measured in saliva samples (see 4.7). As described above, MR stimulation decreases hypothalamic CRH release via inhibitory projections, thus reducing serum cortisol levels finally (for detailed information see 1.3.2). Our results confirm these inhibitory effects and concur with numerous previous studies (for example Baes 2014). It is important to note that differences of cortisol suppression between the depressed and the healthy group would have been more distinct at a later point in time, not included in our assessment.

5.5 Synopsis and Conclusion

To sum up, fludrocortisone-induced stimulation of MR acutely increased verbal declarative-episodic memory as well as executive function and decreased

salivary cortisol. These results not only support findings about the close interconnections of depression, cognitive systems and MR, but also provide a possible explanation for the phenomenon that FC add-on treatment in antidepressant pharmacotherapy was able to precipitate remission induction. Otte et al. conducted a proof-of-concept study, in which depressed patients treated with escitalopram for 5 weeks received additionally either fludrocortisone or placebo or spironolactone for the first 21 days. Patients who received fludrocortisone reached remission significantly faster than patients who received placebo or spironolactone. The FC-group went into remission in roughly 16.0 +/-2.6 days, whereas the placebo-group needed 22.2 +/-2.0 days (Otte 2009). Furthermore, in the whole study population, FC treatment reduced plasma cortisol levels significantly (Otte 2009). Therefore, both studies – the one by Otte et al. from 2009 and the study presented here – might contribute to the strategy of pharmacological add-on therapies and thereby improve pharmacological interventions in the end.

In contrast to the homogeneous, young groups of depressed and healthy participants in our study, elderly groups show (1) more prominent cognitive deficits in depression (Elderkin-Thompson 2004), (2) impaired HPA regulation (Otte 2003, Heuser 2000), and (3) a reduced expression of hippocampal MRs (Choi 2008, Reul 1991). Therefore it appears to be plausible that FC-application in elderly people might lead to more pronounced effects, not only with regard to verbal declarative-episodic memory and executive function, but presumably also with regard to spatial memory and attention.
Therefore, further studies are needed to disentangle the complex role of central MR in depression, HPA regulation and psychopharmacology.

With the rise of new biological theories on the aetiology of depression, MR function is shifted to the centre of attention. It becomes more and more obvious that alterations of MRs can contribute to, and to some extent, even explain psychopathology. Eventually, this might constitute – in the context of the MR-to-GR imbalance hypothesis of depression – one more piece of a complex and permanently growing jigsaw puzzle.

6. LIST OF LITERATURE

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7. LIST OF ABBREVIATIONS

11β-HSD	11β-Hydroxysteroid Dehydrogenase
AA	Amino Acid
ACTH	Adrenocorticotropic Hormone
AMT	Autobiographical Memory Test
ANOVA	Analysis of Variance
BDI-II	Beck Depression Inventory
BLA	Basolateral Amygdala
BMI	Body Mass Index
BP	Blood Pressure
BQSS	Boston Qualitative Scoring System
CBG	Corticosteroid-binding Globulin
CHD	Coronary Heart Disease
CNS	Central Nervous System
CRH	Corticotropin-releasing Hormone
dex/CRH-test	Dexamethasone-suppression/Corticotropin-releasing Test
DSM	Diagnostic and Statistical Manual of Mental Disorders
DBD	DNA-binding Domain
d2 Test	Concentration Endurance Test / Letter Cancellation Test
FC	Fludrocortisone
fMRI	Functional Magnetic Resonance Imaging
GBD	Global Burden of Disease Study
GC	Glucocorticoid
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Responsive Element
HDRS	Hamilton Depression Rating Scale
HPA Axis	Hypothalamus-pituitary-adrenal Axis
HSP	Heat Shock Protein
ICD	International Statistical Classification of Diseases and Related Health Problems
K_d	Dissociation Constant
LBD	C-terminal Ligand Binding Domain
LTP	Long-term Potentiation

MADRS	Montgomery-Åsberg Depression Rating Scale
MDD	Major Depressive Disorder
mEPSC	Miniature Excitatory Postsynaptic Current
M.I.N.I.	Mini International Neuropsychiatric Interview
MMSE	Mini-Mental State Examination
MR	Mineralocorticoid Receptor
NCoR	Nuclear Receptor Corepressor
NTD	N-terminal Domain
PET	Positron Emission Tomography
PTSD	Posttraumatic Stress Disorder
PVN	Ncl. Paraventricularis
QALY	Quality-adjusted Life Year
RCFT	Rey-Osterrieth Complex Figure Test
rm-ANOVA	Repeated Measures Analysis of Variance
SD	Standard Deviation
SE	Standard Error
TF	Transcription Factor
TMT	Trail Making Test
VLMT	Verbaler Lern- und Merkfähigkeitstest
VP	Arginine Vasopressine

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9. EIDESSTATTLICHE VERSICHERUNG

Ich, Michael David Kaczmarczyk, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Effects of Mineralocorticoid Receptor Stimulation on Cognitive Functions in Depressed and Healthy Subjects“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.icmje.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetz-buches) sind mir bekannt und bewusst.

Datum Unterschrift

10. CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Anteilserklärung an etwaigen erfolgten Publikationen

Michael David Kaczmarczyk hatte folgenden Anteil an den folgenden Publikationen:

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Beitrag im Einzelnen:

> Michael David Kaczmarczyk war verantwortlich für die Rekrutierung geeigneter depressiver Studienteilnehmer und gesunder alters-, geschlechts- und bildungsparallelisierter Kontrollprobanden (einschließlich des jeweils erforderlichen Screenings sowie der psychiatrischen und körperlichen Untersuchung), die Organisation und Koordinierung der Testtermine, die Durchführung der einzelnen Testungen einschließlich der notwendigen präanalytischen Verarbeitung der Speichelproben sowie die Aufarbeitung und Auswertung der Testergebnisse.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

Unterschrift des Doktoranden

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