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1 General Introduction

“Working memory, age 32, appears to be alive and in remarkably robust health.”
(R.H. Logie & M. D’Esposito, Cortex 2007)

This citation closes the editorial of a special issue, dedicated to the topic working memory published by the Journal Cortex last year (Logie and D’Esposito, 2007). This citation reflects two very important facts relevant to this thesis: First, the “young” working-memory concept differs from the quite older short-term memory idea in some way, and second, working memory, although broadly investigated, still fascinates researchers.

Purpose of this introduction is to briefly outline the working-memory concept, the historical development, especially conceptual differences between short-term and working memory. Furthermore, theoretical and neurobiological background of the two experiments presented in chapter 2 and 3 will be given.

Since the early discoveries of Holmes and Ebbinghaus that only a limited number of items can be kept in mind after a single presentation (Holmes, 1871, Ebbinghaus, 1964, 1885), short-term memory has widely been investigated. During this early research period, most investigations focused on the capacity of short-term memory in form of reproduced items. This information was used to develop the first tests for memory span, which later served as subtests for the first intelligence tests (Binet and Simon, 1905, Binet and Simon, 1908, Wechsler, 1944). During the first half of the 20th century short-term memory research was even more practically motivated and there was a lack of theoretical short-term memory concept. Miller’s often cited work “The magical number seven, plus or minus two: Some limits on our capacity for processing information” established a milestone in experimental research of short-term memory processes (Miller, 1956). The capacity of working memory is extremely restricted by time limits and storage amount (Miller, 1956, Cowan, 2001). Neuropsychological testing had revealed that the average digit span of healthy adults contained seven items and that this number can be enhanced by chunking of individual items to larger
perceptual or conceptual associations, like four numbers “1, 9, 7, 4” into one date “1974”.

In 1968 Atkinson and Shiffrin proposed their well-known *multi-store memory model*, based on the prior assumptions of Waugh and Norman (Waugh and Norman, 1965). Waugh and Norman, maintaining the early terminology of James (1890), distinguished between primary memory as a transient store of limited capacity and secondary memory as a relatively permanent store of unlimited large capacity. The Atkinson and Shiffrin model also postulated different stores for short-term and long-term memory with a key assumption that the short-term store serves as a gateway to the long-term store via rehearsal processes. Already Atkinson and Shiffrin regarded their short-term store as working memory, which was responsible for encoding processes, search strategies and other control processes. In current specifications the working-memory system temporarily stores information and organizes or manipulates these incoming data to carry out complex cognitive tasks such as comprehension, learning, and reasoning. Working memory is assumed to be involved in the selection, initiation, and termination of information processes such as encoding, storing, and retrieving data. This description of working-memory functions demonstrates that the conception of working memory goes far beyond the simple temporary storage of information.

The following paragraph describes the well-established working-memory model of Baddeley and Hitch, which has strongly influenced this field of research for more than thirty years (Baddeley, 1992). In 1974, Baddeley and Hitch proposed a *three component-model of working memory*, which was an alternative version to the short-term store in Atkinson and Shiffrin’s multi-store memory model (Atkinson and Shiffrin, 1968). The initial three-component-working-memory model of Baddeley and Hitch included the following components: the *central executive* assumed to be an attentional controller with a supervising function aided by two subsidiary “slave” systems: the *phonological loop*, responsible for the short-term storage of speech-based (verbal) information, and the *visuo-spatial sketchpad*, capable of holding visual/spatial information, respectively. During the following research period this theoretical framework has given a good account for a wide range of psychological data. The phonological loop is the best investigated and developed component of this model. Several psychological phenomena, for example the *phonological similarity effect*, the *word-length effect* or the *effect of articulatory suppression* can be explained by this
concept. However, some phenomena were not well captured by the original model, so that in the year 2000 the episodic buffer was integrated as a fourth component into the initial model (Baddeley, 2000). The episodic buffer is assumed to be a temporary storage system of limited capacity, which is capable of storing information in a multidimensional code. Thus this system is able to bind information from the subsidiary systems (phonological loop and visuo-spatial sketchpad), and from long-term memory into a unitary episodic representation. It is assumed that the episodic buffer is controlled by the central executive in forms of conscious awareness, evaluating information, and if necessary, manipulating and modifying it.

Since the early nineties the neurobiological correlates of working-memory functions in humans have been investigated using modern neuroimaging techniques. Working-memory tasks generally evoke a consistent brain activation pattern, including extended frontal and parietal cortex activation. However, specific patterns of brain activation depend on the type of storage material (verbal, spatial, and object), the type of executive function, e.g. continuous updating, memory for temporal order, or manipulation of information, and, of course, on interactions between material and type of executive function. The principles by which working-memory representations might be organized in the brain have been discussed controversially in the literature, especially for the prefrontal cortex (PFC). Three main hypotheses have been tested: The first is that the frontal cortex is domain-specific, or in other words, is organized by the type of information (Goldman-Rakic 1987, Wilson 1997). The second hypothesis is that working-memory representations are organized by the type of processing operation, for example executive versus storage processes. Combining these two hypotheses logically leads to the third hypothesis, which supposes that working-memory representations might be organized both by process type and by information/material type. Much evidence, especially from lesion studies or studies with patients who underwent brain surgery, supports the first “domain-specific” hypothesis (Gazzaniga and Sperry, 1967, Gazzaniga, 2005). For example, impairment of the visuo-spatial sketchpad as a result of a right-hemisphere aneurysm leads to poor performance in mental rotation task, while no impairment of the phonological loop was observed (Hanley et al., 1991). A stronger left lateralization for verbal-working-memory tasks has often been replicated and spatial-working-memory tasks seem to favour the right hemisphere. Furthermore, a dissociation has been proposed for spatial versus object memory between dorsal and
ventral PFC, as well as for the left-right dissociation (for overview see Marshuetz and Bates, 2004).

In contrast to the above mentioned studies favouring the first domain-specific hypothesis that working-memory representations in the PFC are organized according to material type (Smith et al., 1996), recent meta-analyses and reviews revealed that representations are more specialized with regard to the demands for a particular executive process. For example the dorsolateral prefrontal cortex (DLPFC) is more active during executive processes and the ventral prefrontal cortex in storage-related processes (Owen, 2000, Wager and Smith, 2003). However, Wager and Smith do explicitly not exclude that effects of material type in the PFC exist, for example the left frontal PFC, including Brodman Area (BA) 44, 45, and 46, is relatively selective for verbal information, or the right lateral frontal cortex (BA 9) is specialized for object storage. Moreover, the meta-analysis of Wager and Smith corroborated the double dissociation between spatial maintenance in the parietal cortex (dorsal stream) and object maintenance in the temporal cortex (ventral stream) (Wager and Smith, 2003).

During the last decades not only the working-memory model has been redefined and conceptually enlarged, but also several external “subject” factors have been identified, influencing working-memory functions, for example age or use of substances (de Fockert, 2005, Chen and Li, 2007, Yucel et al., 2007). This dissertation investigated biological and psychological factors which might contribute to working-memory function in two experiments using functional Magnetic Resonance Imaging (fMRI). The first study, described in chapter 2, investigated sex differences for a visuo-spatial-working-memory task and the influence of sex steroid hormones on brain activation. The second study, described in chapter 3, deals with the impact of mood disorders on verbal-working-memory functions.

In the first study healthy subjects performed a mental rotation paradigm. Mental rotation describes the ability to mentally rotate two-dimensional and three-dimensional objects. Therefore mental rotation implies the active manipulation of objects in mind, which is depending on visuo-spatial memory functions (visuo-spatial sketchpad). The mental rotation process contains several cognitive operations, including creating a mental image of a specific object, rotating the object mentally until a comparison can be made, comparing the objects, deciding if the objects are the same or not, and reporting this decision. The mental rotation process was primarily investigated by Roger Shepard
and Jacqueline Metzler in 1971 (Shepard and Metzler, 1971). Their research revealed that the reaction time for the decision whether two objects were the same or not depends on the angle of rotation. That means it takes proportionally longer, if the original object has to be rotated in a stronger degree to reach a decision. During the last decade neuroimaging studies provided valuable insights into the neurobiology of mental rotation (Zacks, 2008). These studies revealed that the parietal/occipital cortex is of particular importance for the mental rotation process (Tagaris et al., 1996, Alivisatos and Petrides, 1997, Richter et al., 1997, Jordan et al., 2001). Consistent activation has been reported especially in the superior parietal lobule, intraparietal sulcus and adjacent areas, including BA 7, 19, 39, and 40. Besides the dominant role of the superior parietal and occipital cortex for mental rotation, activation has also been reported in motor regions in the precentral cortex and in the prefrontal cortex (Zacks, 2008).

Compared to some other cognitive tasks, mental rotation is a well established sexual dimorphic task (for a good overview of the field sex differences in cognition see Kimura, 1999). Neuropsychological studies revealed that men outperform women on this kind of spatial task. The meta-analysis of Voyer et colleagues demonstrated that sex differences in mental rotation are a stable phenomenon in contrast to some other spatial tasks (Linn and Peterson, 1985, Voyer et al., 1995). Thus it has been supposed that basic biological differences underlie these observed time-constant performance differences between men and women. So, the aim of the first study was to investigate basic biological differences, namely the influence of gender and the impact of sex steroid hormones during different menstrual cycle phases on brain activation during a mental rotation task.

The second study of this dissertation determines the impact of clinical depression on verbal-working-memory function. For this reason brain activation of recently remitted depressed patients was compared to brain activation of healthy, never depressed control subjects performing a verbal n-back task. The n-back task is one of the most popular experimental designs for functional imaging studies investigating working-memory functions (Owen et al., 2005). In the most typical variant of the n-back task, subjects are briefed to monitor a series of verbal (e.g. letters and words) or nonverbal stimuli (e.g. faces, objects, and pictures), and to decide if the current stimulus has been presented 1, 2 or 3 trials before. This kind of task contains several processes, like on-line monitoring, continuous updating, manipulation of remembered information,
and decision making. Furthermore, some studies included a 0-back condition, which is supposed to be a control condition with no working-memory demand. During the 0-back condition, subjects are asked to respond whenever a specific target stimulus appears. In a second common variant of this task, subjects are required to monitor the location of specific stimuli, e.g. objects or faces, and to respond, if a stimulus is in the same location as the one presented n-back before. A recent meta-analysis, including 24 n-back studies, found six cortical regions consistently activated across all studies (Owen et al., 2005). Bilateral and medial posterior activation was found in the parietal cortex, including the precuneus and the inferior parietal lobules (BA 7, 40). The premotor cortex was also bilaterally activated (BA 6, 8). Activation was observed in the dorsal cingulate/medial premotor cortex, including supplementary motor area (SMA, BA 32, 6). In the frontal cortex, the meta-analysis revealed bilateral activation of the rostral prefrontal cortex or frontal pole (BA 10), bilateral activation of the DLPFC (BA 9, 46) and mid-ventrolateral prefrontal cortex or frontal operculum (BA 45, 47). These six regions were also activated when only n-back studies, including verbal material and identifying the n-back stimulus (N=12), were considered. In addition, the meta-analysis revealed more subcortical activation in medial and lateral cerebellum and thalamus for this kind of studies.

Neuroimaging studies of mood disorders reported significant metabolic and structural alterations in the brain of patients, suffering from Major Depressive Disorder (MDD), especially in the prefrontal and limbic cortex (Sheline, 1996, Drevets, 2000b, Videbech, 2000, Videbech and Ravnhilde, 2004, Frodl et al., 2006). Furthermore, some abnormalities even persist when patients recover and clinical symptoms like depressed mood or reduced drive have subsided (Drevets, 2000a, Holthoff et al., 2004, Neumeister et al., 2005). Besides emotional disturbances, cognitive dysfunction is a core symptom of MDD according to the Diagnostic and Statistical manual of Mental Disorders (4th edition) (DSM-IV) and daily experiences of MDD patients (APA, 2000). While neuropsychological studies revealed that in the acute state of a MDD several cognitive domains are affected (Veiel, 1997, Zakzanis et al., 1998, Airaksinen et al., 2004), less is known about cognitive function in the remitted state. The impact of depressed mood on working-memory function is controversially debated (Channon et al., 1993, Zakzanis et al., 1998, Landro et al., 2001, Harvey et al., 2004, Christopher and MacDonald, 2005, Rose and Ebmeier, 2006). Recent fMRI studies observed deviations in patterns of brain
activation in acute MDD patients, performing working-memory tasks, compared to healthy controls (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006). This dissertation investigates in experiment 2, if this influence of mood on brain activation patterns persists in the euthymic state of MDD.
2 Experiment – Mental Rotation

The influence of the biological factors sex, menstrual cycle, and sex steroid hormones on visuo-spatial-working-memory functions in women and men performing a mental rotation task.

2.1 Introduction

Better performance of men compared to women on tests of spatial abilities is well documented in the (neuro)psychological literature (Linn and Peterson, 1985, Voyer et al., 1995). This holds in particular for the Mental Rotation Test (Shepard and Metzler, 1971, Vandenberg and Kuse, 1978, Peters et al., 1995). Mental rotation implies the active manipulation of objects in mind which is based on visuo-spatial memory functions (visuo-spatial sketchpad) (Baddeley, 1992). During the last 35 years, stable effects concerning sex differences in mental rotation have been reported to the advantage of men (Masters and Sanders, 1993, Lehmann, 2000, Geiser et al., 2006). However, it still remains unclear which biological and/or environmental factors may underlie women’s poorer task performance. Among influencing factors, sex hormones seem to be of major relevance, with both human and animal studies providing evidence for a biological explanation (Collaer and Hines, 1995, Christiansen, 2001, Kimura, 2002, Lacreuse, 2006). Other potential factors contributing to this gender effect include environmental influences, such as learning effects, or sex stereotypes.

Effects of sex hormones, especially testosterone, have been investigated in several behavioural studies (Hooven et al., 2004). Studies on testosterone supplementation in testosterone-deficient states, in transsexuals, and in healthy women suggest a positive influence of testosterone on visuo-spatial abilities and spatial memory (Collaer et al., 2002, Van Goozen et al., 2002, Hines et al., 2003, Orwoll et al., 2006). A single sublingual administration of 0.5 mg testosterone is sufficient to improve visuo-

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1 Reprinted from Neuropsychologia, Vol. 45(14), Schöning S. et al., Functional anatomy of visuo-spatial working memory during mental rotation is influenced by sex, menstrual cycle, and sex steroid hormones (pp. 3203-14) Copyright © 2007, with permission from Elsevier.
spatial abilities in healthy young women (Aleman et al., 2004). Spatial memory in women may also benefit from testosterone intake (Postma et al., 2000). Cherrier et al. reported an enhancement of spatial and verbal memory after six weeks of intramuscular testosterone in elderly men (advanced age is known to be associated with a state of relative testosterone deficiency) (Cherrier et al., 2001). Working-memory function might also be improved by testosterone supplementation (Janowsky et al., 2000). Female-to-male transsexuals demonstrate enhanced performance on tests of visuo-spatial ability after three months of androgen treatment, while male-to-female transsexuals perform less well under androgen deprivation (Van Goozen et al., 1994, Van Goozen et al., 1995, Slabbekoorn et al., 1999).

Taken together, these studies suggest a positive correlation between testosterone and spatial abilities, with an almost linear increase in several studies. However, other studies found a curvilinear relationship or sex-specific associations between testosterone and cognitive abilities (Moffat and Hampson, 1996, Muller et al., 2005, Thilers et al., 2006). This suggests that an optimal hormonal state for performance is not necessarily associated with the highest levels of testosterone.

Modulating effects of sex steroid hormones on visuo-spatial abilities are not limited to testosterone. Estradiol also exerts effects on some aspects of memory and attention in animals (Lacreuse, 2006), as well as on several cognitive processes in humans (Maki and Resnick, 2001). Effects of female sex hormones on cognitive abilities have been demonstrated by variations along the menstrual cycle. Women might perform better in spatial tests during the menstruation, as compared to other phases of the menstrual cycle in which estradiol and progesterone are present in higher concentrations (Hampson, 1990b, Hampson, 1990a, Silverman and Phillips, 1993, Hausmann et al., 2000).

Using modern functional imaging techniques, the neural networks underlying mental rotation and visuo-spatial-working-memory processes have been investigated during the last decade, and still new questions arise, e.g. concerning the influence of rotation angle on imagined spatial transformation (Keehner et al., 2006) and of social variables (Berns et al., 2005). As a main result, the parietal cortex emerged as a core region for mental rotation processes (e.g. Tagaris et al., 1996, Alivisatos and Petrides, 1997, Richter et al., 1997, Jordan et al., 2001). This was to be expected from neuropsychological work showing selective deficits in mental rotation in patients with
Mental Rotation

parietal lesions (Ditunno and Mann, 1990, Tomasino and Rumiati, 2004, Tomasino et al., 2004). In addition, precentral, medial frontal, and temporal regions are part of the mental rotation network (Cohen et al., 1996, Barnes et al., 2000, Richter et al., 2000, Kosslyn et al., 2001). Of interest here, sex and menstrual cycle were probed in some previous studies among other factors influencing these networks and their functions. The overall regional pattern of activations seems to be similar in men and women, and task-related activation differences occur within these cortical regions (Thomsen et al., 2000, Jordan et al., 2002, Weiss et al., 2003). Remarkably, these differences persist when behavioural measures are comparable for both sexes (Jordan et al., 2002, Weiss et al., 2003).

In only very few recent imaging studies, the cycle phase was taken into account at all (Gizewski et al., 2006, Halari et al., 2006). To the best of our knowledge, almost no available fMRI study examined men as well as the same women in two different phases of their menstrual cycle. An exception is the study of Dietrich et al. al, who investigated two phases of menstrual cycle in six females and six males and reported no sex differences for the low estradiol phase, but prominent differences for the high estradiol phase (Dietrich et al., 2001). Moreover, previous fMRI studies are limited by the fact that the cycle phase was determined by self-reports only, and not by serum hormone analysis. Halari et al. found that males activated the left middle temporal gyrus and the right angular gyrus more than women during menstruation in addition to the regions of the mental rotation network (Halar i et al., 2006). Gizewski et al. investigated females in the ovulatory cycle phase and found stronger activation in frontal and parietal cortex in men, whereas females showed stronger activation of the other frontal areas and left fusiform gyrus (Gizewski et al., 2006). Despite the limitations discussed above, these studies indicate that the phase of menstrual cycle plays an important role and needs to be considered.

Our functional MRI study was designed to investigate the influence of sex, phase of the menstrual cycle, and sex steroid hormone levels on cognitive performance and related fMRI signals. In order to probe sex differences, a sexually dimorphic mental rotation task was used. To overcome limitations of many previous studies, women were investigated in two well-defined phases of their menstrual cycle, confirmed by sex steroid level analysis. Moreover, every participant underwent substantial clinical and neuropsychological testing, to control for possible confounding factors. In brief, this
study was designed to probe the following three hypotheses: a) Mental rotation performance and fMRI signals during mental rotation are related to sex differences between men and women. According to previous observations men should outperform women in mental rotation task. b) Sex differences might vary according to the phases of the female menstrual cycle. Based on previous observations, we expected a poorer performance on the mental rotation task in the midluteal phase of the menstrual cycle. c) Sex steroid hormone levels, in particular testosterone levels, should correlate with the level of activation in brain areas involved in mental rotation. This relationship might be more clearly expressed in the natural range of testosterone values to be found in men.

2.2 Methods

2.2.1 Subjects

After providing written informed consent, 34 (20 females, 14 males) healthy, right-handed, heterosexual subjects participated in the study. None had a history of a serious medical illness or any neurological or psychiatric disease. All women had a regular menstrual cycle (self-report) and any form of hormonal treatment was excluded. Women were examined twice in randomized order, once during the early follicular phase (1-3 days after onset of menses), once in the midluteal phase adjusted for cycle length (individual cycle length – 7 days with a range of +/- 2 days).

Serum analyses confirmed the target phases of menstrual cycle in the 12 women reported here. Six female subjects had to be excluded from further analyses because the self-reported cycle phase could not be confirmed by sex steroid hormone analysis: four women were in the self reported luteal phase and two women in the self reported early follicular phase. In two women a second fMRI scan could not be acquired. Two men with hormone levels exceeding the normal range were also excluded. Therefore, 12 women and 12 men from the initially larger sample were included in the final analysis (for details see Table 1). Handedness was assessed by the Edinburgh handedness inventory (Oldfield, 1971). Women and men did not differ significantly with respect to age (32 ± 5.63), visuo-spatial intelligence with a mean of 121 and a standard deviation of 12.17 as measured by the Grundintelligenzentest Skala 2 (CFT 20, Weiss et al., 2003)
and verbal intelligence with a mean of 114.50 and a standard deviation of 12.75 as assessed by the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B, Lehrl et al., 1995). Both are German standard tests to estimate general intelligence. All participants had more than twelve years of education.

All procedures were approved by the Ethical Review Board of the Medical Board of Westfalia and the Medical Faculty of the Westfälische Wilhelms Universität Münster, Germany, and in accordance with the 1964 Declaration of Helsinki.

### Table 1

Mean and standard deviation (S.D.) for age and intelligence in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women (early follicular)</th>
<th>Women (midluteal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (Mean ± S.D.)</td>
<td>33.2 ± 6.2</td>
<td>30.8 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Intelligence, more visuo-spatially based estimate measure (CFT), (Mean ± S.D.)</td>
<td>124.0 ± 10.2</td>
<td>118.3 ± 13.7</td>
<td></td>
</tr>
<tr>
<td>Intelligence, more verbally based estimate measure (MWT-B), (Mean ± S.D.)</td>
<td>117.0 ± 11.9</td>
<td>112.0 ± 13.6</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2.2 Sex steroid hormone assessment

A venous blood sample was drawn to determine testosterone, estradiol, progesterone, luteinizing (LH) and follicle-stimulating hormone (FSH). All blood samples were taken between 2 and 5 pm right before scanning in order to minimize known day-time variation (Chappell, 2005). Hormone levels were measured by standard immunofluorimetric assays.

Men were excluded from further analysis if serum testosterone levels were below 2.3 ng/ml and above 7.9 ng/ml. Women were diagnosed as being in the early follicular phase of menstrual cycle if onset of menstrual bleeding occurred within the last 1-3 days and both serum estradiol and progesterone levels were ≤ 70 pg/ml and < 1 ng/ml, respectively. Women were characterized as being in the midluteal phase of
menstrual cycle if onset of menstrual bleeding was observed at individual cycle length minus 7 days (+/- 2 days) before the examination. All included women had estradiol levels ≥ 66 pg/ml and progesterone levels ≥ 4 ng/ml. FSH and LH levels were within the normal range of premenopausal women in all female subjects (FSH < 15 IU/L, LH < 20 IU/L).

### 2.2.3 Experimental design

Stimuli consisted of 3D-objects, extracted from the standard Mental Rotation Test (Peters et al., 1995), (provided by W.L.). The 3D-objects were presented in pairs in either congruent (50%) orientation or as mirror images (50%), and stimuli were presented in different rotated angles (see Figure 1). Stimuli were presented with Presentation Software® (Version 0.81, 2004, Neurobehavioral Systems Inc., Albany, CA, USA) and a MR compatible projection.

Three task conditions, (a) mental rotation of 3D-objects, (b) control condition, and (c) rest condition were administered in pseudo-randomized order. Before scanning all subjects were familiarized with the task. During the mental rotation task the participants were asked to mentally rotate the 3D-objects and to decide if they were congruent or not. No overt response was required during scanning to avoid any movement artefacts. The control condition contained the same kind of 3D-stimuli, in this case non-rotated (i.e. in identical projection), and the participants simply had to look at them (see Figure 1). In the rest condition the participants saw a white fixation cross on a black screen. Each condition lasted 30 s, within each experimental and control condition, five pairs of objects were presented for 5 s.

![Figure 1: 3D-objects adapted from Peters et al. (1995). First row: experimental condition (equal pair). Second row: experimental condition (unequal pair). Last row: control condition.](image)
followed by a pause of 1 s. Two equivalent versions of the mental rotation task were applied to the women, in a counterbalanced order across the two cycle phases. Each version was randomly administered to half of the men. After completing the fMRI scan, subjects were tested for mental rotation performance and strategy outside the scanner. To test mental rotation abilities, a redrawn paper and pencil version of the mental rotation task was applied to all subjects (Vandenberg and Kuse, 1978, Peters et al., 1995). In this test a target figure is compared to four stimuli and subjects have to identify those two stimuli that can be matched to the target figure by mental rotation. The Mental Rotation Test contains 2 x 12 problems with a time limit of three minutes for each part. Women performed two different, yet equivalent, versions of the Mental Rotation Test in both phases of menstrual cycle.

A non-standardized, self-developed questionnaire was used to ask for mental rotation strategies. It included the following questions: (i) whether figures were mentally rotated as a whole, (ii) whether figures were mentally rotated in parts, and (iii) whether rotation steps were internally verbalized (answers yes, no). Multiple answers were allowed.

### 2.2.4 Scanning procedures

MRI data were acquired in a 3-Tesla whole body Scanner (Intera T 3.0, Philips, Best, NL), equipped with master gradients (nominal gradient strength 30 mT/m, maximal slew rate 150 mT/m/ms). A circularly polarized transmit/receive birdcage head coil with a high frequency (HF) reflecting screen at the cranial end was used for spin excitation and resonance signal acquisition. Functional images were acquired using a T2*-weighted single shot echo planar (EPI) sequence (whole brain coverage, echo time (TE) = 50 ms, repetition time (TR) = 3000 ms, flip angle 90°, slice thickness 3.6 mm without gap, matrix 64 x 64, FOV 230 mm, in-plane resolution 3.6 mm x 3.6 mm). 36 transversal slices orientated to the commissura anterior - commissura posterior (AC-PC) line were acquired.
2.2.5 Functional data analysis

Functional MRI data were analyzed using Statistical Parametric Mapping (SPM) standard routines and templates (www.fil.ion.ucl.ac.uk/spm). The first ten images of each session (30 s pre-stimulus interval) were discarded, to allow for saturation effects of the Blood Oxygen Level Dependency (BOLD) signal. The remaining images were realigned, normalized to map the Montreal Neurological Institute (MNI) template, and resliced to a voxel size of 2 mm x 2 mm x 2 mm. Gaussian smoothing was performed using a 9 mm kernel. Data were filtered with a high-pass filter (cut-off period of 128 s). A boxcar function convolved with the canonical hemodynamic response function implemented in SPM2 was used to model BOLD-responses for the mental rotation task. In a first level fixed-effects analysis, we obtained one statistical parametric map and corresponding contrast images for each subject, reflecting the contrasts of interest. Mental rotation of 3D-objects was compared to passive viewing of the same 3D-objects. The individual contrast images were entered into a second level random-effects analysis.

Using one-sample t-tests, activations within each of the three study groups were determined. To compare brain activations between groups, two-sample t-tests were used. A paired t-test was applied to compare brain activation between women in the two phases of menstrual cycle. Finally, individual sex steroid levels for testosterone, estradiol, and progesterone were entered into the simple regression analysis (correlation), implemented in SPM2 for each group.
2.3 Results

2.3.1 Hormone assessment

As expected, testosterone levels were higher in men (5.10 ± 1.45 ng/ml) than in women. Female testosterone levels did not differ significantly between phases of the menstrual cycle. Women in the early follicular phase had 0.34 ± 0.13 ng/ml and women in the midluteal phase 0.37 ± 0.13 ng/ml of testosterone. Estradiol and progesterone levels showed the expected increase from the early follicular to the midluteal phase of menstrual cycle. Estradiol levels increased from 46.75 ± 11.10 pg/ml to 129.00 ± 56.81 pg/ml, and progesterone levels increased from 0.65 ± 0.15 ng/ml to 10.02 ± 5.70 ng/ml. Estradiol and progesterone levels in men were 36.00 ± 6.30 pg/ml and 0.70 ± 0.20 ng/ml, respectively (for details see Figure 2).

![Figure 2: Serum hormone levels in men and women (mean and standard deviation).](image)

2.3.2 Behavioural Results

Mental rotation ability, tested outside the scanner, showed the following. Overall, men solved more problems correctly in the standard Mental Rotation Test than women in both phases of the menstrual cycle (for details see Figure 3). This advantage was significant comparing men to women in the midluteal phase of menstrual cycle ($T = -2.150$, $df = 22$, $p = 0.043$, $d = 0.82$), but failed significance relative to the early follicular phase of cycle ($T = -1.387$, $df = 22$, $p = 0.179$, $d = 0.56$). For the women, the small difference between the menstrual cycle phases with slightly better performance
during menstruation was not significant (T = 0.936, df = 11, p = 0.369, d = 0.22). Concerning individual strategies to solve the mental rotation task, most participants in each group affirmed rotation of the figure as a whole (9 men, 10 women in the early follicular phase, and 9 women in the midluteal phase). Fewer subjects, overlapping with the subjects reporting the first “Gestalt” strategy, used partial rotation as an additional strategy. Concerning verbalization, 6 men, 8 women in their early follicular phase, and 11 in their midluteal phase, affirmed verbalization of rotation steps. Chi-Square test with Fisher’s exact correction for each item revealed no significant differences between groups. No significant correlation between mental rotation performance and hormonal data were observed in men and women in both cycle phases.

2.3.3 fMRI Results

2.3.3.1 Within group activation pattern for the mental rotation task

For women in both phases of menstrual cycle as well as for men, the key cortical areas known to be involved in mental rotation were indeed activated (see Figure 4). The superior and inferior parietal cortex was bilaterally activated in all groups ($p_{\text{uncorr.}}<0.001$, cluster size $\geq 20$ voxels). All groups showed extended bilateral activation of the medial and lateral frontal cortices. The occipital lobe was also bilaterally activated in all groups. The same holds true for the cerebellum (for details see Table 2).
Figure 4: Activated brain areas during the mental rotation task in a) men, b) women during the early follicular phase, and c) women during the midluteal phase of the menstrual cycle. Random-effects analysis rendered on the surface of the canonical template image used by SPM2 (p<0.001, cluster size ≥ 20 voxels).
Table 2  Patterns of cortical activation for the mental rotation task for a) men, women b) in the early follicular phase, and c) the midluteal phase of menstrual cycle, as obtained by a random effect analysis ($p_{uncorr.}<$0.001, cluster (C-)size $\geq$ 20 voxels).

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</tbody>
</table>
2.3.3.2 Between group comparisons: Sex differences

Group comparisons revealed significant differences between men and women in both phases of the menstrual cycle ($p_{uncorr.} < 0.005$, cluster size $\geq 20$ voxels) (for details see Table 3). During mental rotation, activation of the left inferior parietal lobe (BA 40) was significantly stronger in men compared to women in both cycle phases (see Figure 5). The same holds true for activation of the left fusiform gyrus and lingual gyrus. Compared to women in the midluteal phase, men also showed stronger activation of the right insula and precentral gyrus.

Relative to men, women in the early follicular phase of the menstrual cycle showed stronger cerebellar activation, and more activation of the left precentral, right middle frontal gyrus, and the postcentral gyrus than men. Women in the midluteal phase showed more activation than men in the left superior temporal and superior frontal gyri, and in the right caudate nucleus.

![Figure 5: Comparison of mental rotation between a) men versus women during the early follicular phase, and b) men versus women during the midluteal phase of the menstrual cycle as obtained by random-effects analysis ($p_{uncorr.} < 0.005$, cluster size $\geq 20$ voxels), projected on the canonical template image used by SPM2. Activation in a) at MNI-coordinate $x = -44$, $y = -34$, $z = 38$, b) at MNI-coordinate $x = -38$, $y = -29$, $z = 40$.]

2.3.3.3 Within group comparisons between phases of the menstrual cycle

The paired t-test revealed significantly different activations between the two phases of the menstrual cycle for the mental rotation task ($p_{uncorr.} < 0.005$, cluster size $\geq 20$ voxels) (see Table 3). Women showed significantly larger activation in the left superior temporal gyrus in their early follicular phase than in their midluteal phase. In contrast,
in the midluteal phase women revealed stronger activation in the left middle temporal gyrus, left lentiform nucleus and thalamus, left and right cingulate gyrus, corpus callosum, right middle temporal gyrus, superior occipital and angular gyrus, right middle and superior frontal gyrus.

**Table 3** Comparison of mental rotation between men and women during both phases of their menstrual cycle (two sample t-test, a-d) and between women in the early follicular and midluteal phase (paired t-test, e-f) as obtained by random-effects analysis ($p_{uncorr.}<0.005$, cluster size $\geq 20$ voxels).

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Brodmann Area</th>
<th>MNI -Coordinates $x$ $y$ $z$</th>
<th>Cluster Size</th>
<th>$Z$</th>
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<tr>
<td><strong>a) Men vs. women (early follicular)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left fusiform gyrus, lingual gyrus, declive</td>
<td>19</td>
<td>-28 -66 -12</td>
<td>56</td>
<td>3.38</td>
</tr>
<tr>
<td>Left inferior parietal lobe, postcentral gyrus</td>
<td>2, 40</td>
<td>-48 -42 56</td>
<td>103</td>
<td>2.97</td>
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<tr>
<td><strong>b) Men vs. women (midluteal)</strong></td>
<td></td>
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<tr>
<td>Left fusiform gyrus, lingual gyrus</td>
<td>19</td>
<td>-28 -66 -10</td>
<td>115</td>
<td>4.01</td>
</tr>
<tr>
<td>Left inferior parietal lobe, postcentral gyrus</td>
<td>2, 40</td>
<td>-38 -30 42</td>
<td>44</td>
<td>3.03</td>
</tr>
<tr>
<td>Right insula, precentral gyrus</td>
<td>13, 44</td>
<td>42 2 6</td>
<td>67</td>
<td>2.97</td>
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<td><strong>c) Women (early follicular) vs. men</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left cerebellum</td>
<td>-</td>
<td>-8 -52 -42</td>
<td>25</td>
<td>3.37</td>
</tr>
<tr>
<td>Left precentral + postcentral gyrus</td>
<td>3, 4</td>
<td>-46 -16 58</td>
<td>24</td>
<td>2.99</td>
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<tr>
<td>Left precentral + postcentral gyrus</td>
<td>4</td>
<td>-30 -30 70</td>
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<tr>
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<td>40 20 50</td>
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Table 3 continued

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<th>Cluster size</th>
<th>Z</th>
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<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
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<tr>
<td><strong>d) Women (midluteal) vs. men</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left superior temporal gyrus</td>
<td>22</td>
<td>-36</td>
<td>-52</td>
<td>10</td>
</tr>
<tr>
<td>Left superior frontal gyrus</td>
<td>9, 10</td>
<td>-2</td>
<td>60</td>
<td>32</td>
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<tr>
<td>Right caudate nucleus</td>
<td>-</td>
<td>18</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>e) Women (early follicular) vs. women (midluteal)</strong></td>
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</tr>
<tr>
<td>Left superior temporal gyrus, inferior parietal lobe</td>
<td>22, 40, 42</td>
<td>-64</td>
<td>-36</td>
<td>22</td>
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<tr>
<td>Left medial frontal gyrus</td>
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<td>-12</td>
<td>62</td>
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<tr>
<td><strong>f) Women (midluteal) vs. women (early follicular)</strong></td>
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<tr>
<td>Left middle temporal gyrus</td>
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<td>-34</td>
<td>-48</td>
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<tr>
<td>Left brainstem</td>
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<tr>
<td>Left + right anterior cingulate gyrus</td>
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<td>Left cingulate gyrus</td>
<td>24</td>
<td>-6</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Left lentiform nucleus, thalamus</td>
<td>-</td>
<td>-14</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Right anterior cingulate gyrus</td>
<td>24, 32</td>
<td>10</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>Right middle temporal gyrus, superior occipital gyrus, angular gyrus</td>
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<td>38</td>
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<td>20</td>
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<td>Right middle frontal gyrus</td>
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<td>50</td>
<td>28</td>
<td>30</td>
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<tr>
<td>Right superior frontal gyrus</td>
<td>6, 8</td>
<td>25</td>
<td>16</td>
<td>28</td>
</tr>
</tbody>
</table>

2.3.3.4 Correlation of steroid hormones and brain activity

SPM analysis demonstrated correlations between cortical activation during mental rotation and testosterone levels in men and in women in the early follicular phase of
No significant correlation was observed during the midluteal phase. In men, the correlation was significant in the left supramarginal gyrus and inferior parietal lobe (BA 40) (for details see Figure 6). In women in their early follicular phase areas in the right caudate body exhibited correlations.

A different pattern of cortical activation correlating with serum hormone concentrations was observed for estradiol. In men, a significant correlation was only found in the left parietal cortex (BA 7, 31). For women in both menstrual cycle phases, far more extended cortical regions correlated with estradiol. Women in the early follicular phase revealed significant correlation in the left fusiform and inferior temporal gyrus (BA 20, 37), right inferior and superior parietal lobe including the precuneus (BA 7, 40), inferior and middle frontal gyrus (BA 11, 47), and postcentral gyrus (BA 1, 2, 3). For women in the midluteal phase, estradiol levels correlated significantly bilaterally with activation in the superior parietal lobe (BA 7), and frontal gyrus (BA 6), and the right inferior parietal lobe (BA 40). Correlations were also observed in the left postcentral gyrus and the left fusiform gyrus (BA 37).

For progesterone, few correlations were observed. A significant correlation was observed in the left frontal cortex (BA 8 and 9) in women in the early follicular phase. In the midluteal phase no such correlations were found. Men showed one spot of significant correlation in frontal cortex, however, in a distinct right-sided rostral region (BA 10) (for details see Table 4).
Table 4  Brain regions showing a significant correlation with serum hormone levels within groups (p\textsubscript{uncorr.}<0.005, cluster size ≥ 20 voxels).

<table>
<thead>
<tr>
<th>Anatomical Region</th>
<th>Brodmann Area</th>
<th>MNI-Coordinates</th>
<th>Cluster Size</th>
<th>Z</th>
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<tr>
<td></td>
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<td>x   y   z</td>
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<tr>
<td><strong>Correlation with testosterone</strong></td>
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<tr>
<td><strong>a) Men</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Left supramarginal gyrus, inferior parietal lobe</td>
<td>40</td>
<td>-48  -54  34</td>
<td>42</td>
<td>4.04</td>
</tr>
<tr>
<td><strong>b) Women (early follicular)</strong></td>
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<tr>
<td>Right hemisphere, white matter</td>
<td>-</td>
<td>30   -24  38</td>
<td>57</td>
<td>3.91</td>
</tr>
<tr>
<td>Right caudate body</td>
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<td>14   14   14</td>
<td>32</td>
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<td></td>
<td>20</td>
<td>10   10   10</td>
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<td>2.63</td>
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<tr>
<td><strong>c) Women (midluteal)</strong></td>
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<td>-     -     -</td>
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<td>-</td>
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<tr>
<td><strong>Correlation with estradiol</strong></td>
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<tr>
<td><strong>a) Men</strong></td>
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<tr>
<td>Left precuneus, superior parietal lobe</td>
<td>7, 31</td>
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<tr>
<td><strong>b) Women (early follicular)</strong></td>
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<tr>
<td>Left fusiform gyrus, inferior temporal gyrus</td>
<td>20, 37</td>
<td>-46  -46  16</td>
<td>26</td>
<td>2.93</td>
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<tr>
<td>White matter and caudate body</td>
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<td>142</td>
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Table 4 continued

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<th>Cluster Size</th>
<th>Z</th>
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<td></td>
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<td>y</td>
<td>z</td>
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<td>Right superior parietal lobe</td>
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<td>50</td>
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<tr>
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<td>Right middle frontal gyrus</td>
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<td>4</td>
<td>50</td>
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**c) Women (midluteal)**

<table>
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<tr>
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<td>z</td>
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<td>Left precuneus, superior parietal lobe, postcentral gyrus</td>
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<td>-60</td>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td>-50</td>
<td>66</td>
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<td>37</td>
<td>-44</td>
<td>-42</td>
<td>-14</td>
</tr>
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<td>Left cingulate gyrus, middle + medial + superior frontal gyrus</td>
<td>6, 32</td>
<td>-22</td>
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<tr>
<td>Right middle frontal gyrus</td>
<td>6</td>
<td>36</td>
<td>4</td>
<td>48</td>
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**Correlation with progesterone**

**a) Men**

<table>
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<th>MNI-coordinates</th>
<th>Cluster Size</th>
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<tr>
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<td>Right superior + medial frontal gyrus</td>
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<td>64</td>
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**b) Women (early follicular)**

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<td>38</td>
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<td>Right middle temporal gyrus, white matter</td>
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<td>-60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>-64</td>
<td>12</td>
</tr>
<tr>
<td>Right parietal lobe/frontal lobe, white matter</td>
<td>-</td>
<td>24</td>
<td>-30</td>
<td>44</td>
</tr>
<tr>
<td>Right precentral gyrus, middle frontal gyrus, white matter</td>
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<td>32</td>
<td>16</td>
<td>38</td>
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**c) Women (midluteal)**

<table>
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</table>
2.4 Discussion

This functional MRI study was designed to investigate the influence of sex, phase of the menstrual cycle, and sex steroid hormone levels on cognitive performance and related fMRI signals in a sexually dimorphic, namely mental rotation task. Three hypotheses concerning a) general sex differences during mental rotation, b) cycle-dependence of sex differences and c) the role of sex steroid hormones were probed.

The general activation pattern for the mental rotation task obtained fits well with previous fMRI studies: strong bilateral activation in the parietal cortex, which is an established core region for mental rotation processes, see (e.g. Tagaris et al., 1996, Alivisatos and Petrides, 1997, Richter et al., 1997, Jordan et al., 2001). We also observed activation in precentral, and temporal regions that are known to be part of the mental rotation network (Cohen et al., 1996, Barnes et al., 2000, Richter et al., 2000, Kosslyn et al., 2001). In contrast to many previous studies, we did not limit our analysis to a priori defined regions of interest, but rather assessed significant activations across the whole brain. With this approach, we also found activation in the occipital lobe and the cerebellum, as was previously shown for mental rotation (Molinari et al., 2004).

In the between group analyses, we were able to confirm our first hypothesis (sex differences). Men and women in both menstrual cycle phases exhibited significant differences in patterns of brain activation. Previous studies reported significant activation differences between the sexes in frontal, temporal, and parietal brain areas, which are in line with our results. In men versus women, we observed stronger activation in the left intraparietal sulcus. Jordan et al. had also reported higher activation in this area in men, and additionally in the right precentral sulcus, left precentral gyrus, and right parieto-occipital sulcus (Jordan et al., 2002). Here we demonstrated higher parietal activation in men as compared to women in both distinct cycle phases. We also confirmed inferior temporal activation (BA 20) in men and in women, however this was specific for the early follicular phase of the menstrual cycle. More posterior, occipito-temporal activation in the fusiform gyrus was observed in all groups. These regions are primarily associated with object and object-part identification (Carpenter et al., 1999), and thus we did not expect sex or sex-hormone driven differences.

Regarding prominent activation in women vs. men, several reports highlighted differences in frontal and/or temporal brain areas, which has been interpreted to reflect a
more analytic, serial, or verbal strategy (Thomsen et al., 2000, Weiss et al., 2003, Hugdahl et al., 2006). Left brain areas were previously noted with increased activation in females. We found the left superior frontal gyrus to be significantly more active during the midluteal phase only, coinciding with a non-significant tendency toward a more verbal strategy during this cycle phase. Thus, it is tempting to speculate that strategy may indeed play a role. In line with our results, women were slightly overrepresented as users of non-rotational strategy in a large sample including 1724 subjects (Geiser et al., 2006). Jordan et al. (2002) found increases in inferior temporal gyrus and middle occipital gyrus activation in women. Hughdal et al. found stronger activation in the inferior frontal gyrus, as did Weiss et al. (however, right-sided, Weiss et al., 2003). In total, results on the neurobiology of sex differences in mental rotation and associated cortical networks are still heterogeneous, which might be due to different methodological approaches, including participant selection in terms of high performers (Weiss et al., 2003), averaging across women in different hormonal states, stimulus and task specifics such as letter or shape rotation (Jordan et al., 2002), or procedures of data analysis strategies using special masking procedures of a priori selected regions. Overall, our results are in accordance with the previous studies reporting sex specific brain activation. The differential brain activation was most pronounced in frontal (women greater than men) and inferior parietal brain areas (men greater than women).

In accordance with our second hypothesis, we demonstrate differences across menstrual cycle in women. The main finding is that several regions exhibit enhanced activation when female hormones are at their peak (midluteal phase). The cortical areas thus modulated by estradiol include the middle temporal and cingulate gyri bilaterally, as well as additional frontal and occipital right-sided areas. These modulated areas are higher-order association cortices, yet not part of the classical mental rotation areas (frontal and parietal cortices). One possible explanation might be that estrogen might unspecifically enhance vasoreactivity and therefore lead to an enhanced activation of activated areas (see also Dietrich et al., 2001). The left superior temporal gyrus was significantly more activated during the early follicular phase. This cortical region is best known for its involvement in auditory and linguistic functions, and its activation might reflect that additional functional networks are recruited.

Very few prior imaging studies have taken into account different menstrual cycle phases in premenopausal women. Here we focus on the previous work concerning
mental rotation. Halari et al. studied women in the low estradiol, early follicular phase of the menstrual cycle (Halari et al., 2006), and found no statistically significant differences compared to men. Gizewski et al. (2006) compared cerebral activation patterns during mental rotation in women during the high estradiol, midluteal phase to men. Using a priori defined regions of interest, they reported stronger activation in women in the right superior frontal cortex, right inferior and medial temporal, and left fusiform gyrus. Women in the high estradiol phase had less activation in the right precentral, right medial frontal, and bilateral inferior parietal cortex (Gizewski et al., 2006). Our data suggest stronger differences in the left brain, in superior frontal (BA 9, 10) and superior temporal regions (BA 22). In accordance, we also found less activation in precentral (BA 44) and parietal regions (BA 40) in women, the latter confined to the left side in our data. However, both studies cited above only investigated women in one defined phase of the menstrual cycle.

The early fMRI study of Dietrich et al. including women in both cycle phases is an exception. The number of activated voxels was compared in rather small samples (6 men, 6 women) (Dietrich et al., 2001), and no significant differences were observed between women in the low estradiol phase and men. Women in the peak estradiol phase of menstrual cycle activated more voxels than men (Dietrich et al., 2001). Our results support these earlier findings of Dietrich et al. and substantiate them in a larger sample. Our results not only add to and refine previous fMRI findings regarding cortical activation in women. The differences we report in functional activation are even more striking since behavioural performance did not significantly differ between the cycle phases in these identical women. Particularly, the conjunction of the fMRI data with psychological data suggests that biological factors may play an important role in the modulation of cortical activity across sex and menstrual cycle.

This brings us to our third hypothesis (correlation of activation with circulating sex hormone levels). Apart from organizational effects in early development, sex steroid hormones have important influences on cognitive function in animals and humans (Christiansen, 2001, Maki and Resnick, 2001, Kimura, 2002, Lacreurse, 2006). Our study revealed regionally specific correlations of fMRI signal with testosterone and estradiol.

We observed regionally specific correlations with testosterone in men, less significant in women in their early follicular phase and not at all during their midluteal
phase. These positive correlations were located in the left inferior parietal cortex in men, a key region for mental rotation tasks. While heavily interconnected with lateral prefrontal regions, the parietal cortex is the key cortical region for processing of spatial information, for spatial integrations and operations (Culham et al., 2006). The correlation of testosterone levels in men with this key region essential to spatial processing might be one of the biological reasons for males’ superior performance in visuo-spatial tasks, where no correlation was observed in women. Of course, these interpretations are limited by the fact that correlational data cannot establish causal relations, which is difficult in any non-interventional study. Therefore here we can only present the association of hormones and brain activity during mental rotation as an indirect indicator. More direct evidence comes from clinical and experimental studies on testosterone administration which suggest a causal relationship between testosterone and spatial abilities (Collaer et al., 2002, Van Goozen et al., 2002, Hines et al., 2003, Aleman et al., 2004, Orwoll et al., 2006). To fully understand the role of testosterone for mental rotation abilities, further research is necessary. For example Newman et al. suggested, that interactions of testosterone with social situation, especially social status, might be relevant to cognitive performance assessed by a mental rotation task (Newman et al., 2005). Other issues warranting clarification are the relationship between brain activity, steroid hormones, and increasing age (Thilers et al., 2006), or, more generally, whether an optimal testosterone level exists (Moffat and Hampson, 1996, Muller et al., 2005).

In addition to testosterone, regional correlations of estradiol with BOLD signal were also investigated. Women in both cycle phases and men exhibited significant, regionally specific, positive correlations, especially in frontal and parietal areas relevant to mental rotation processes. As stated above, it is in principle not possible to infer causal relationships from this type of data alone. However, our results might suggest a potentially causal influence of estrogen on cerebral neurons, which could be mediated by intracellular neural estrogen receptors such as ER-alpha and ER-beta, as well as by dendritic, presynaptic, or glial estrogen receptors (Shughrue and Merchenthaler, 2000, Maki and Resnick, 2001, McEwen, 2001, Sherwin, 2003). Dietrich et al. suggested that not only neuronal, but also vascular effects might be responsible for menstrual cycle effects in fMRI (Dietrich et al., 2001). To date, investigations on hormonal influences on the BOLD effect are scarce. It seems possible that neurovascular coupling might be
altered by hormone-induced cerebral vasodilatation and blood flow changes. For example, it is known that estradiol increases the concentration of vasodilatory nitric oxide (NO) through an Akt/PKB–enzyme dependent pathway and the endothelial isoform of nitric oxide synthase (eNOS) (Pelligrino and Galea, 2001, Florian et al., 2004). Unfortunately, fMRI can not differentiate between neurovascular and neuronal effects. Further research focusing on this topic will have to include additional validation, e.g. electrophysiological measures (Logothetis and Wandell, 2004).

Regarding progesterone, previous investigations did not report an influence of progesterone on mental rotation (Hausmann et al. 2000). We report few correlations within the left frontal cortex in women and right frontal cortex in men. However, regional inconsistencies across groups and rather small cluster volumes suggest that these correlational findings between brain activation and progesterone might be false positive findings here.

Before closing, we briefly come back to our behavioural data. Although our sample is rather small compared to many cognitive-psychological studies, we confirm sex differences in mental rotation performance. In accordance with our second hypothesis, men solved more problems than women in the standard Mental Rotation Test, which reached significance for the comparison between males and females in their midluteal phase. This is in line with previous behavioural studies, and supports formerly presented cycle effects in women for spatial abilities. Hausmann et al. found that during the luteal phase, when estradiol levels are high, women performed poorly on tasks of spatial ability (Hausmann et al., 2000). Concerning mental rotation strategies, previous research indicates that a more analytic strategy might be used in some of the items of the mental rotation test, especially in those items that are different in shape (Vandenberg and Kuse, 1978, Peters et al., 1995, Geiser et al., 2006). In line with our results, in a large group of more than 1700 participants, differences in mental rotation strategies between sexes were only subtle. As a caveat the limited sample size might have obscured potential additional behavioural differences between groups. The same holds true for the correlational data between mental rotation performance and hormonal levels.

To summarize, we demonstrate details on sex and sex hormone driven differences in the cerebral neurobiology underlying mental rotation function. Our study again confirms the functional neuroanatomy of the mental rotation cortical network:
bilateral parietal and frontal cortex regions are key areas. Our main results on sex differences of the present study overall are in line with previous reports. We suggest that differences between studies with respect to the exact cortical location of maximal differences might be due to different sample sizes and populations, experimental tasks, and statistical approaches such as whole-brain analysis versus region-of-interest based analysis. The strength of the present study lies in the assessment of sex steroid hormone levels in addition to neuropsychological and functional imaging investigations. Our subjects were well characterized with respect to different intelligence measurements based on verbal and visuo-spatial material, clinical testing, and their general mental rotation ability assessed with the standard Mental Rotation Test. The rigorous exclusion of participants with abnormal hormonal values and/or values indicating that cycle phases of interest were not met in women led to very homogenous samples. This kind of work – to the best of our knowledge – has not yet been presented. This, and the fact that we examined the same women twice, allows us to draw some important conclusions that could not be drawn from previous work. Since we did not observe differences in mental strategy or performance between women in different phases of the menstrual cycle, and since significant correlations of testosterone and estradiol with cortical regions exist in a sex-, hormone- and region-specific fashion, our results point towards an important role of biological factors such as hormone levels in mental rotation.

Limitations of our study design include the still limited sample size and the fact that men were measured once and women twice. This has been taken into account by a counterbalanced study design; however future studies should integrate repeated measurements in all groups. Another limitation is the fact that behavioural data were obtained right after, but not during functional imaging. However, this approach was chosen to reduce potential movement during scanning and no data set had to be excluded due to motion artefacts. Although several studies provided evidence that sex steroids might influence cognition, to the best of our knowledge we are the first to correlate sex hormone levels with brain activity in areas relevant to mental rotation for this kind of visuo-spatial memory task. Sex steroid hormone level might therefore be another factor influencing brain activation patterns during mental rotation. This study thus provides further insights into the complex interactions between sex hormones, brain activation and behaviour. It emphasizes the importance of considering sex, phase of the menstrual cycle, and hormonal status of subjects in functional imaging studies.
3 Experiment – N-back ²

The influence of clinical depression on the working-memory network in euthymic MDD patients and healthy subjects performing a verbal n-back task.

3.1 Introduction

Major Depressive Disorder is one of the most prevalent psychiatric disorders leading to a dramatic reduction of quality of life, increased mortality risk (Cuijpers and Smit, 2002, Alonso and Lepine, 2007) and causing a significant individual and economic burden being the most costly brain disorder in Europe (Sobocki et al., 2006, von Knorring et al., 2006).

Neuropsychological deficits of different functional domains are well documented for the acute phase of a depressive episode (Burt et al., 1995, Veiel, 1997, Zakzanis et al., 1998, Landro et al., 2001, Ravnikilde et al., 2002, Airaksinen et al., 2004, Castaneda et al., 2008). However, the nature of these deficits, the cognitive domains affected, as well as the severity of cognitive impairments are still a matter of ongoing debate. Compared to the acute phase of major depression, even less is known about the neurocognitive profile of patients who recovered from depression. Several studies reported lasting deficits in some cognitive domains (Marcos et al., 1994, Paradiso et al., 1997, Kessing, 1998, Austin et al., 2001, Paelecke-Habermann et al., 2005) such as executive functions, and attention (Trichard et al., 1995, Paelecke-Habermann et al., 2005, Smith et al., 2006). The influence of clinical depression on working-memory function is still under debate (Channon et al., 1993, Zakzanis et al., 1998, Landro et al., 2001, Harvey et al., 2004, Christopher and MacDonald, 2005, Rose and Ebmeier, 2006).

As we know from clinical experience, MDD patients often complain about problems with thinking and concentration (Nair et al., 1999). While impairments of

² Parts of this study published in “Working-Memory fMRI reveals cingulate hyperactivation in euthymic major depression”, Schöning S. et al., Hum Brain Mapp, in press. Copyright © 2008 Wiley-Liss, Inc.
working memory in the acute phase of MDD have previously been reported, studies focusing on this crucial cognitive function in remitted depression are rare. Subtle deficits were reported for strategic aspects of a spatial working-memory task (Weiland-Fiedler et al., 2004).

Working memory is an extensively researched psychological concept dealing with the temporary storage and processing of information (Baddeley, 1992, Baddeley, 2003). Intact working memory is essential for every day functioning. Working-memory tasks require several cognitive processes, such as on-line monitoring, continuous updating, manipulating stored information, and decision making, which might all be affected by MDD. The neuronal processes underlying working-memory function have widely been investigated with neuroimaging techniques (Wager and Smith, 2003, Owen et al., 2005). In healthy subjects, the verbal n-back task activated a bilateral network consisting of dorsolateral and ventrolateral prefrontal cortex, lateral premotor cortex, dorsal cingulate and medial premotor cortex, frontal poles, and medial and lateral posterior parietal cortex (Owen et al., 2005). Task-related activity was shown to be correlated with working-memory load. Especially dorsolateral and left inferior regions of the prefrontal cortex show a linear relationship between activity and task complexity (Braver et al., 2001).

To date, only a few imaging studies investigated working memory in major depression, almost exclusively focusing on the acute phase (Harvey et al., 2005, Matsuo et al., 2006, Rose et al., 2006, Walter et al., 2007a, b, Fitzgerald et al., 2007). These studies revealed abnormalities in cortico-limbic networks fundamentally involved in the pathophysiology of major depression (Mayberg, 1997, Dougherty and Rauch, 2007). Compared to healthy control subjects, a stronger activation was observed in the limbic system and lateral prefrontal cortex of MDD patients, in the absence of significant behavioural differences (Matsuo et al., 2007, Fitzgerald et al., 2007). For example, Matsuo et al. reported stronger left dorsolateral and anterior cingulate cortex activation in 15 MDD patients performing a visuo-spatial task, while healthy controls failed to show cingulate activation (Matsuo et al., 2007). Harvey et al. used a verbal variant of the n-back task and compared 10 MDD patients with 10 controls (Harvey et al., 2005). Both groups showed similar activation, but the lateral prefrontal cortex and the anterior cingulate were activated more strongly in MDD patients. Rose et al. investigated 10 MDD patients and 10 healthy controls with an n-back task and also reported anterior
cingulate differences in load-dependent activation between patients and controls (Rose et al., 2006). Using a longitudinal design, Walsh et al. reported greater load-response in the verbal-working-memory network of patients (Walsh et al., 2007). Taken together, these studies indicate that an acute episode of MDD is associated with abnormal cortico-limbic activation in working-memory tasks, mainly characterized by hyperactivation of lateral prefrontal and cingulate areas. Almost nothing is known as to whether this hyperactivation observed in the acute phase is a state-dependent phenomenon, and whether or not brain activation normalizes when depressive symptoms are no longer predominant.

While the above studies often failed to find differences on behavioural measures, Walter et al. found behavioural differences between 12 partially remitted patients and controls in a delayed match-to-sample working-memory task (Hamilton, 1960, Walter et al., 2007b). However, patients scored relatively high on the Hamilton depression rating scale (HDRS, mean = 18.2) (Hamilton, 1960, Walter et al., 2007b). The authors also reported stronger activation in the dorsolateral prefrontal cortex for the highest cognitive load condition, and in the ventromedial prefrontal cortex for the control condition.

To the best of our knowledge, no fMRI study yet has investigated working-memory function in a large group of completely euthymic unipolar depressed patients. Thus, the goal of this study was to investigate working-memory function, in particular cortico-limbic activation during working-memory performance, in euthymic MDD patients. We hypothesized that behavioural working-memory performance of euthymic MDD patients is almost equal to healthy controls. We expected neurobiological differences in cortico-limbic brain regions such as cingulate gyrus and prefrontal areas between euthymic patients with MDD and controls.
3.2 Methods

3.2.1 Subjects

In total, 56 subjects were recruited for this study. Twenty-eight inpatients from the Department of Psychiatry of the University of Muenster or the Landschaftsverband Westfalen-Lippe (LWL)-Clinic Muenster (16 female, 12 male subjects), fulfilling DSM-IV criteria for a MDD (APA, 2000), participated in this study (for details see Table 5). A diagnosis of either first (N = 9) or recurrent episode (N = 19) of unipolar depression was verified using the Structured Clinical Interview for DSM-IV Disorders (SCID, German version) (Wittchen et al., 1997), in addition to clinical assessment by two board-certified specialists in Psychiatry. After treatment of the acute phase of MDD, patients achieving a euthymic state as defined by Hamilton Depression Scale (HDRS \( \leq 8 \)) and as confirmed by two board-certified specialists in Psychiatry were included. The following additional inclusion criteria were applied: age between 18 and 55 years, no treatment with electroconvulsive therapy during the previous depressive episode, no history of any other serious medical or neurological disease, no serious head injury, no suicidal tendency, no benzodiazepine treatment three days before scanning, and no MRI contraindications. All patients were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and had more than twelve years of education. Details about the pharmacological and psychotherapeutic treatment are presented in Table 6.

Twenty-eight healthy, right-handed control subjects, recruited by advertisement in the local newspaper, were 1:1 matched to the patients according to sex and age (+/- 3 years). Education level, both in terms of years of education and highest graduation level, was also balanced between groups. All control subjects underwent an initial telephone screening to ensure matching criteria, to exclude medical and neurological diseases, or MRI contraindications. The standardized SCID-I-Interview was performed to exclude any current or previous psychiatric disorders (Wittchen et al., 1997).

All procedures were approved by the local Institutional Ethical Review Board. The ethical standards of the Declaration of Helsinki were met and all participants provided written informed consent.
Table 5  Mean and standard deviation (S.D.) for age, intelligence, BDI, HDRS assessed at time of testing, number of depressive episodes and hospitalizations, and days of hospitalizations.

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 28)</th>
<th>Patients (N=28)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender ratio (f/m)</td>
<td>16 f /12 m</td>
<td>16 f /12 m</td>
<td>χ² = 0, df = 1, p =1</td>
</tr>
<tr>
<td>Age</td>
<td>33.42 ± 9.62</td>
<td>34.18 ± 10.62</td>
<td>t = -0.26, df = 54, p &gt; 0.05</td>
</tr>
<tr>
<td>Intelligence, MWT-B- Score</td>
<td>32.14 ± 2.27</td>
<td>31.04 ± 2.85</td>
<td>t = 1.61, df = 54, p &gt; 0.05</td>
</tr>
<tr>
<td>Beck depression inventory (BDI)</td>
<td>2.54 ± 3.12</td>
<td>8.92 ± 6.24</td>
<td>t = -4.85, df = 39.71, p &lt; 0.001</td>
</tr>
<tr>
<td>Hamilton depression rating scale (HDRS)</td>
<td>-</td>
<td>3.64 ± 2.63</td>
<td>-</td>
</tr>
<tr>
<td>Number of depressive episodes</td>
<td>-</td>
<td>2.54 ±1.75</td>
<td>-</td>
</tr>
<tr>
<td>Number of hospitalization</td>
<td>-</td>
<td>1.54 ± 0.70</td>
<td>-</td>
</tr>
<tr>
<td>Days of hospitalization</td>
<td>-</td>
<td>75.61 ± 34.60</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6  Treatment characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 28)</th>
<th>Patients (N = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No medications</td>
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<td>1</td>
</tr>
<tr>
<td>Antidepressant only</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Antipsychotic only</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Antidepressant and antipsychotic</td>
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<td>11</td>
</tr>
<tr>
<td>Antidepressant and mood stabilizer</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Antidepressant, antipsychotic, and mood stabilizer</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
3.2.2 Materials and procedures

The working-memory task was the first part of a larger fMRI and neuropsychological study of memory processes in euthymic MDD. We used a classical letter variant of the n-back task (Braver et al., 1997). Before entering the scanner, a detailed task instruction was given, and participants were familiarized with the n-back task until they succeeded in the training trials. A standardized brief instruction announced the start of the task in the scanner. Working-memory load was manipulated in three levels (0-, 1-, and 2-back), presented in a block design. During the 0-back condition, subjects had to press the response button of a MRI-compatible response box if the target letter “X” appeared on the screen. In the 1-back condition subjects had to decide if the actual letter on the screen was identical to the previous letter. During the 2-back condition, subjects had to decide if the actual letter was identical to the letter presented two trials before (see Figure 7). Subjects responded with their right hand, using the index finger for targets and middle finger for non-targets.

Each active n-back condition lasted 36 s, and n-back blocks were presented in a fixed order (1-0-2-0-1-2) to each subject. White letters were presented in the centre of a black screen for 500 ms, with an interstimulus interval (ISI) of 2500 ms (Presentation Software®, Version 0.81, 2004, Neurobehavioral Systems Inc., Albany, CA, USA). Only orthographically distinct uppercase consonants were used (B, C, D, F, G, H, J, K, M, Q, R, S, T, V, X, Z). Each letter sequence consisted of 12 consonants, including one
third targets. During fMRI scanning, a short instruction announced the n-back type. All n-back conditions were separated by a pause of 21 s, during which participants had to look at a white fixation cross on a black screen.

As part of the larger study protocol, all patients and control subjects underwent neuropsychological testing, such as the Mehrfachwahlwortschatz-Test (MWT-B) as an estimate of verbal intelligence (Lehrl et al., 1995) and the Beck Depression Inventory (BDI) (Beck et al., 1961).

3.2.3 Scanning procedures

MRI data acquisition was performed in a 3 Tesla whole-body scanner (Intera T 3.0, Philips, Best, NL), equipped with master gradients (nominal gradient strength 30 mT/m, maximal slew rate 150 mT/m/ms). A circularly polarized transmit/receive birdcage head coil with an HF reflecting screen at the cranial end was used for spin excitation and resonance signal acquisition. Functional images were acquired using a T2* weighted single shot echo planar (EPI) sequence (whole brain coverage, TE = 38, TR = 3000 ms, flip angle 90°, slice thickness 3.6 mm without gap, matrix 64 x 64, FOV 230 mm, in-plane resolution 3.6 x 3.6). 36 transversal slices orientated to the AC-PC line were acquired.

3.2.4 Behavioural data analysis

During fMRI scanning, responses and response latencies (in ms) for the n-back performance were recorded. Behavioural results were acquired from all 28 patients. Data from three control subjects were omitted due to technical difficulties. Performance is reported as accuracy rate (percentage of correct answers) for each n-back condition. Repeated-measures analyses of variance (ANOVA), with one between-subject factor (group: two levels) and one within-subject factor (working-memory load: three levels), were performed for accuracy rate and response latency.
3.2.5 Functional data analysis

Functional MRI data were analyzed using SPM5 standard routines and templates (www.fil.ion.ucl.ac.uk/spm). The first ten images of each session (30 s pre-stimulus interval) were discarded to allow for saturation effects of the BOLD signal. The remaining images were realigned, normalized, and resliced to a voxel size of 2 mm x 2 mm x 2 mm. Gaussian smoothing was performed using a 9 mm kernel. Data were filtered with a high-pass filter (cut-off period of 128 s). A boxcar function convolved with the canonical hemodynamic response function implemented in SPM5 was used to model BOLD-responses for the working-memory task. In a first-level fixed-effects analysis, we obtained one statistical parametric map and corresponding contrast images for each subject, reflecting the contrasts of interest (0-, 1-, 2-back and instruction). Furthermore, 2-back was compared to 1-back and 0-back. The individual contrast images were entered into a second-level random-effects analysis, to obtain activation maps across subjects. One-sample t-tests were used to determine activation for the different conditions within the two groups (0-, 1-, 2-back-contrast: p < 0.05, corrected for family wise error (FWE), cluster size ≥ 15 voxels; 2vs0-, 2vs1-back-contrast: p < 0.05, corrected for false discovery rate (FDR), cluster size ≥ 15 voxels). Based on previous findings (Harvey et al., 2005, Matsuo et al., 2007) and our hypotheses of higher cingulate and prefrontal activation, differences between patients and controls were calculated in the cingulate gyrus and in the dorsolateral (BA 9, 46) and ventrolateral (BA 45, 47) prefrontal cortex, using two-sample t-tests (p < 0.05, FDR corrected, cluster size ≥ 15 voxels). The regions of interest (ROIs) in the bilateral cingulate cortex (anterior, medial and posterior part) as well as the inferior, middle, and superior dorsolateral frontal gyrus were defined according to the Anatomical Automatic Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002), as implemented in the Wake Forest University (WFU) PickAtlas Toolbox (Maldjian et al., 2003). In addition, a whole-brain analysis was performed to confirm the results of the ROI analysis. Within each group, a correlation analysis between between behavioral data (response latency and accuracy) and task-related activity was performed across all voxels and all conditions. Clinical variables (HDRS and days of hospitalization) were additional variables for the patient group.
3.3 Results

3.3.1 Behavioural Results

No significant differences between groups were observed. Analysis of variance on accuracy and response latency revealed a significant main effect of working-memory load \( (F_{(2,102)} = 12.64, p < 0.001) \) and \( (F_{(2,102)} = 32.65, p<0.001) \), respectively. As expected, accuracy decreased and response latency increased from 0-back to 2-back condition (see Figure 8, 9). However, no main effects of group \( (F_{(1,51)} < 1, p > 0.05) \) or interactions between group and working-memory load \( (F_{(2,102)} < 1, p > 0.05) \) emerged for accuracy or response latency. Furthermore, no significant differences were observed as a function of verbal intelligence (MWT-B) (two-sample t-test, \( T = 1.61, df = 56, p > 0.05 \)).

![Figure 8: Behavioural data for accuracy rate (percentage of correct answers, mean ± standard error) with varying working-memory load in patients and control subjects reveal a significant effect of working-memory load, but not of group or interaction.](image1)

![Figure 9: Behavioural data for response latency (mean ± standard error) in all load conditions in patients and control subjects. A main effect of working-memory load condition was observed, but no effect of group or interaction.](image2)

3.3.2 Activation patterns across load conditions and groups

For each group, activation was investigated for each load condition of the working-memory task separately. Healthy controls and patients activated the brain areas relevant
for a verbal-working-memory task, as expected from the literature (Wager and Smith, 2003, Owen et al., 2005). In both groups, we observed an increase of brain activation from the 0-back to 2-back condition (for details see Figure 10). Activation increased with working-memory demand, in particular with respect to the bilateral activation of the inferior and middle frontal cortex. Activation was found in the medial frontal and inferior frontal gyrus, insula, pre- and postcentral gyrus, inferior parietal lobule, and cerebellum in both groups.

**Figure 10:** Group activation in a) patients and b) controls for the 0-, 1-, and 2-back condition (one sample t-test, p<0.05, FWE corrected, cluster size ≥ 15 voxels). Random-effects analysis rendered on the surface of the canonical template image used by SPM5.

### 3.3.3 Regions activated with increasing working-memory load

#### 3.3.3.1 Activation increases from 0-back to 2-back

Common to both groups were the following effects. First, we observed extended activation clusters of the inferior, middle, superior and medial frontal cortex, including typical verbal working-memory regions, such as parts of the medial frontal cortex, dorsolateral and ventrolateral prefrontal cortex (BA 9, 46, 45, 47) (see Figure 11). Next,
we found activation of the insula, supplementary motor area, temporal lobe and cerebellum. Finally, there was strong activation in the parietal lobe, in the inferior and superior parietal lobule (BA 7, 40), the angular and supramarginal gyrus extending to the superior and middle occipital gyrus (BA 19, 18). However, while healthy controls showed only few activated clusters in the cingulate cortex, parahippocampal gyrus, and hippocampus, patients activated large parts of the cingulate cortex (BA 24, 32, 33), and parahippocampal gyrus (BA 27, 28, 35, 36) in the 2vs0-back contrast.

3.3.3.2 Activation increases from 1-back to 2-back

For the 2vs1-back contrast (for details see Figure 12), healthy controls activated few and small clusters in the inferior frontal cortex and the superior frontal cortex (BA 6). Activation was also found in the precuneus, inferior and superior parietal lobule. In patients, we observed the following. There were large activated clusters in the inferior, middle, medial and superior frontal gyrus (BA 6, 8, 9, 44-47). Parallel to the 2vs0-back contrast, the cingulate cortex (BA 24, 32, 33), parahippocampal gyrus (BA 35, 36) and hippocampus were significantly activated. Significant activations were also observed in the insula, pre- and postcentral gyrus, temporal and occipital lobe and cerebellum. Finally, in the parietal lobe, the angular and supramarginal gyrus, the inferior and superior parietal lobule and precuneus were bilaterally activated (BA 7, 39, 40).
3.3.4 Correlation analysis

No significant correlation (p < 0.05, FDR corrected, cluster size $\geq 15$ voxels) between brain activation and behavioural measures (accuracy and response latency) were observed, neither in patients nor in controls. One exception concerned a small correlation of accuracy with the right inferior frontal lobe in patients for the 2-back condition (MNI-coordinate 32/34/12). Moreover, no significant correlations (p < 0.05, FDR corrected, cluster size $\geq 15$ voxels) between brain activation and clinical variables such as Hamilton scores or days of hospitalization were found in patients.

3.3.5 Between-group comparisons

As we expected group differences in specialized working-memory areas, particularly prefrontal areas and the cingulate cortex, a ROI-analysis (two-sample t-test, p < 0.05, FDR corrected, cluster size $\geq 15$ voxels) was performed between groups. In the cingulate cortex, both the 2vs0-back and the 2vs1-back contrast revealed stronger activation of the anterior and posterior cingulate cortex (BA 24, 32, 23, 31) for patients than healthy controls. Unlike patients, healthy controls showed no increased cingulate activation (Figure 13, 14). In the prefrontal cortex, especially in the dorsolateral (BA 9, 46) and ventrolateral (BA 45, 47) prefrontal cortex, no significant differences between patients and controls were found.
In a confirmatory whole-brain analysis (p < 0.0005, uncorrected for multiple comparisons, cluster size ≥ 15 voxels) the cingulate difference between groups for both the 2vs0-back and the 2vs1-back contrast was corroborated (for details see Figure 15).

For the 2vs0-back contrast significant activation in a) were observed in the cingulate cortex (BA 24, 31, 32) extending into the medial frontal gyrus (BA 6), parahippocampal gyrus (BA 35), middle temporal (BA 21) and paracentral gyrus (BA 2, 3, 4). Healthy controls did not show any significant activation relative to patients. For the 2vs1-back contrast significant activation in a) were observed in the cingulate cortex (BA 24, 31) extending into the inferior parietal lobule, supramarginal gyrus (BA 40), angular gyrus (BA 39), medial frontal gyrus (BA 6), and pre- and postcentral gyrus (BA 2, 3, 4). Healthy controls did not show any significant activation relative to patients.
3.4 Discussion

Cognitive impairments are an important characteristic of major depression. Modern neuroimaging methods indicate that dysfunction of cortico-limbic networks plays an important role in the pathophysiology of both affective and cognitive symptoms in MDD (Dougherty and Rauch, 2007). In the acute episode of depression, brain metabolism is significantly altered, with pathological changes in the dorsolateral prefrontal and limbic cortex at rest and during cognitive activation (Drevets, 2001, Ebmeier et al., 2006, Fitzgerald et al., 2006, Greicius et al., 2007). Much less is known about brain function when depressed patients reach the euthymic mood state. Neuropsychological data suggest that cognitive deficits persist in certain domains, and thus might represent more a trait than a state characteristic (Paelecke-Habermann et al., 2005). This study investigated cortico-limbic networks involved in working-memory function in recently remitted patients with major depression. We explored whether dysfunctional activation of the lateral prefrontal and cingulate cortex would still be present in the euthymic phase of major depression, as had previously been reported for the acute episode of major depression (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006, Walter et al., 2007b).

In line with previous reports, we found the classic working-memory network activated in the n-back task (Wager and Smith, 2003, Owen et al., 2005). With increasing working-memory demand, strong activation was observed in both patients and controls, in the dorsolateral and ventrolateral prefrontal cortex, middle frontal cortex, and precentral gyrus. Both groups also showed activation in the parietal cortex, of the angular and supramarginal gyrus, inferior and superior parietal lobule, precuneus, and superior occipital gyrus. Activation was also observed in the temporal cortex, whose role for working-memory processes is as yet poorly understood, and subject of current research (Axmacher et al., 2007, Picchioni et al., 2007).

A novel and interesting finding is that our data point to a deviance of the working-memory network in patients with MDD even in the euthymic state. So far, altered cortico-limbic activity during working-memory tasks has only been reported in severely depressed patients, mainly in the acute phase of major depression. The majority of these studies did not find behavioural deficits between patients and controls (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006). Patients in the acute phase
performing working-memory tasks showed hyperactivation of the dorsolateral prefrontal cortex (DLPFC) (Harvey et al., 2005, Matsuo et al., 2007) and anterior cingulate cortex (ACC) (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006). These findings were taken as evidence for the recruitment of additional resources to fulfil the cognitive demands of a given task.

In the present study, patients in the euthymic state showed hyperactivation of the cingulate cortex as part of the limbic system, while lateral prefrontal hyperactivation was not observed relative to healthy controls. Both of these areas on the lateral and medial surface of the prefrontal cortex are known to play a central role in the pathophysiology of depression. Baseline functional-imaging studies demonstrated metabolic and regional blood flow abnormalities in major depression, in particular, a decreased metabolism in DLPFC and increased metabolism in orbitofrontal cortex (Dougherty and Rauch, 2007). This cortico-limbic network also revealed abnormal function when challenged by cognitive tasks such as working memory, most prominently evident as an increase of lateral prefrontal and limbic activity (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006). As a major result, our data indicate that metabolic abnormalities in the limbic system persist even in the euthymic state of MDD, while lateral cortical abnormalities normalize. Our results might reflect an earlier normalization of lateral prefrontal function occurring prior to possible similar changes in limbic areas in the course of remission.

The role of the limbic ACC has been controversially discussed in depression and recovery, playing an important role in both cognitive and emotional processing. The dorsal subdivision of the ACC subserves many cognitive functions, including working memory, and is highly interconnected with other regions involved in working memory, such as the above-mentioned DLPFC (Devinsky et al., 1995, Bush et al., 2000). This dorsal ACC region is involved in task complexity, mental effort or attentional processes (Mulert et al., 2005, Mulert et al., 2007), conflict monitoring and error processing (Carter et al., 1998, Carter et al., 1999, Botvinick et al., 2004, Kerns et al., 2004, Bioulac et al., 2005, van Veen and Carter, 2006, Michelet et al., 2007, Sohn et al., 2007). On the other hand, the rostral part of the ACC subserves emotional processing, especially for the assessment of emotional information and the regulation of emotional responses (Whalen et al., 1998). This part is highly interconnected with the amygdala, hippocampus, hypothalamus, nucleus accumbens, and orbitofrontal cortex. Alterations
of (rostral) ACC metabolism have been associated with depressive symptoms, their severity, and treatment response in MDD patients (Mayberg et al., 1997, Milak et al., 2005, Chen et al., 2007, Konarski et al., 2007). Moreover, brain imaging studies revealed altered brain activation of the rostral part of the ACC for emotional tasks in depressed patients (Frodl et al., 2007, Mitterschiffthaler et al., 2008). In this study, we observed an activation increase of the ACC with increasing working-memory load in patients, which seemed to involve both the dorsal and the rostral part. Our findings corroborate cingulate cortex hyperactivation observed in patients in the acute depressive episode (Harvey et al., 2005, Matsuo et al., 2007). Here, we demonstrate that cingulate hyperactivation during working-memory performance is still present when affective symptoms such as depressed mood or reduced drive are much relieved or have even subsided. As in acute depression, we might now hypothesize that enhanced recruitment of these cerebral resources is necessary to fulfil the cognitive demands of the given task. Enhanced recruitment might be necessary as baseline metabolism is decreased in the ACC even after recovery from depression (Holthoff et al., 2004).

As mentioned above, behavioural performance was not significantly different between euthymic MDD patients and healthy controls. This was expected on the basis of results from fMRI studies with acute depressed patients (Harvey et al., 2005, Matsuo et al., 2007). It is thus unlikely that behavioural differences between patients and healthy controls are responsible for the observed differential activation pattern. However, our study used a block design and only two levels of task difficulty, which might be not sensitive enough to detect subtle disturbances of working-memory capacity. Some other limitations of our study also need to be mentioned. We cannot fully exclude medication effects, since our patients did receive psychiatric treatment to modern standards of care. However, no differences of performance were noted and previous studies on the effects of antidepressant medication revealed that pharmacological treatment leads to an attenuation or decrease of limbic activation in response to emotional stimuli rather than to an increase of ACC activation, as observed in the present study (Sheline et al., 2001, Fu et al., 2004, Harmer et al., 2006, Arce et al., 2008). Although the above evidence points towards an attenuating effect of antidepressants on brain activation, we cannot completely exclude an opposite effect in a working-memory task, but this seems rather unlikely. Furthermore, patients were only
included when depressive symptoms were considerably reduced. We did not assess the time course of brain activation during the course of recovery, so additional studies need to clarify if further changes occur when more time elapses after the acute depressive episode. A strength of our study is the high number of well-characterized patients, for whom strict exclusion criteria were met.

To summarize, we demonstrated that even after clinical improvement of affective symptoms, abnormal cingulate activation was associated with a classical working-memory task in patients compared to healthy controls. In contrast to patients in the acute depressive episode, limbic ACC hyperactivation, but no lateral prefrontal hyperactivation, occurred in patients in the euthymic state. Our data might reflect a different lateral prefrontal and limbic pace of normalization, a trait marker of changes in neuronal networks after an episode of MDD, or a compensatory mechanism to maintain adequate working-memory performance.
4 Global Discussion

“Thinking is done by the cells of the brain behind the forehead… if the forehead cells do not know how to think, the mind cannot make use of memories. We say that such person is a fool.” (Overton, 1897)

This early statement of Overton well describes what happens if working memory does not work sufficiently (Overton, 1897). Many daily lapses, like forgetting a name during a conversation after somebody new has been introduced, forgetting a phone number, leaving the key in the flat, miscalculating prices in the grocery store, or losing the thread in a conversation after an interposed question, are due to brief working-memory disturbances. Serious consequences, for example regular underestimation of time constraints, inability to handle more than one task at time, failure of concentration on important issues and to ignore minor information might be a result of a permanent working-memory dysfunction. These few examples reflect that working-memory is essential for daily functioning, especially for building memory traces and social interactions. The goal of this dissertation was to investigate factors of influence on this crucial cognitive function using functional MRI.

The combination of modern non-invasive neuroimaging techniques and neuropsychological experiments offer the great opportunity for a better understanding of basic biological processes underlying cognition. During the last years, several neuroimaging studies have probed conceptual assumptions and neurobiological correlates of working memory in the human brain (e.g. Van der Linden et al., 1999, Collette and Van der Linden, 2002, Wager and Smith, 2003, Owen et al., 2005, Suchan, 2008, Zacks, 2008). Still, the question of the neural basis of working memory is not solved and many open questions remain (D'Esposito, 2007, Linden, 2007). However, this dissertation focused on possible influencing factors on verbal- and visuo-spatial-working-memory functions, rather than to examine different theoretical assumptions concerning working memory. Therefore we used the well-established working memory model of Baddeley as underlying theoretical framework (Baddeley, 1992). The major advantage of using this conceptual framework is the description of defined
neuroanatomical networks for the phonological loop, visuo-spatial sketchpad, and the central executive.

To date, some factors have already been identified, which have significant impact on working-memory function. A clear association has been demonstrated between age and working memory. Working-memory performance worsens significantly with increasing age (de Fockert, 2005, Chen and Li, 2007). Several studies have also investigated the influence of different substances on working-memory function. These studies revealed that nicotine and nicotinic agonists have a positive effect on cognitive function, including working-memory tasks. This might have therapeutic relevance, e.g. to improve working-memory impairment in cognitive disorders (Levin et al., 2006). Besides these positive effects, opposite effects are reported for the chronic use of alcohol, cannabis, inhalants, opiates, psychostimulants, and ecstasy. Across substances, long-term sequelae of substance abuse are neuropsychological impairments of executive (inhibitory) control, working memory, and decision making (Yucel et al., 2007). Of interest here and in line with the data presented in chapter 2, sex steroid hormones are supposed to have significant influence on working-memory function (Janowsky et al., 2000, Cherrier et al., 2001; Aleman et al., 2004).

This dissertation investigated the biological factors gender and sex hormones in the first experiment (cf. chapter 2), and the psycho-biological factor mood, in particular depression, in the second study (cf. chapter 3). Two different tasks were chosen to investigate both subsidiary slave systems: a mental rotation task was applied to investigate visuo-spatial-working-memory functions, in particular manipulation processes, and a verbal n-back task was chosen to study verbal-working-memory functions, in detail updating processes. Despite non-comparable study subjects in the two experiments, different task conditions, somewhat different working-memory demands, the classical working-memory network was activated in both studies, including mainly fronto-parietal areas. These common activations are in line with previous investigations (Wager and Smith, 2003). In their meta-analysis Wager and Smith could show that the posterior parietal cortex (BA 7) is involved in all types of executive functions. In both studies of this dissertation the posterior parietal cortex was strongly activated, although using verbal and visuo-spatial material, respectively. This result underlines its “material unspecific” role for working-memory processes. For the

mental rotation task we observed a bilateral dorsolateral/ventrolateral prefrontal and medial frontal brain activation pattern in all groups (BA 6, 9, 44-47), however with broad activation clusters in the left hemisphere. Similar brain activation patterns were also observed for the verbal-working-memory task in the second study. This again reflects that similar underlying processes are activated for both tasks. The following two paragraphs will summarize and discuss each experiment in more detail.

Recent observations indicate that sex and level of steroid hormones may influence cortical networks associated with specific cognitive functions, in particular visuo-spatial abilities (Janowsky et al., 2000, Cherrier et al., 2001, Aleman et al., 2004, Hooven et al., 2004). The first study of this dissertation probed the influence of sex, menstrual cycle, and sex steroid hormones on 3D mental rotation and brain function. Twelve healthy women and twelve men were investigated. Menstrual cycle and hormone levels were assessed. The early follicular and midluteal phase of the menstrual cycle were chosen to examine short-term cyclical changes.

Parietal and frontal areas were activated during mental rotation in both sexes. Significant differences between men and women were revealed in both phases of the menstrual cycle. In men, we observed a significant correlation of activation levels with testosterone levels in the left parietal lobe (BA 40). In women, a cycle-dependent correlation pattern was observed for testosterone: brain activation correlated with this male hormone only during the early follicular phase. In both cycle phases the female brain activation pattern was significantly correlated with estradiol in frontal and parietal areas.

Therefore the first study provided evidence that fMRI-related activity during performance of a visuo-spatial-working-memory tasks varies across sex and phase of the menstrual cycle. The variation might partly be explained by better task performance in men, but our results indicate that further explanations like basic neuronal or neurovascular effects modulated by steroid hormones must be considered. Both estradiol and testosterone levels may influence fMRI signals of cognitive tasks, which should affect selection of subjects for future fMRI studies.

The second study tested the impact of depressive mood on verbal-working-memory function. It has been demonstrated that differential mood states can influence cognitive processing (Clore and Huntsinger, 2007, Mitchell and Phillips, 2007). From everyday life we know that in sad mood states it is hard to concentrate and to fulfil
cognitive ambitious tasks. A recent study demonstrated that psychosocial stress induces working-memory impairment in healthy subjects (Schoofs et al., 2008). Major depressive disorder is a clinical relevant disease being one of the most prevalent psychiatric disorders, leading to a dramatic reduction of quality of life and increased mortality risk (Cuijpers and Smit, 2002, Alonso and Lepine, 2007). Thus individual and economic consequences go far beyond disturbances by normal sad mood and stress (Sobocki et al., 2006, von Knorring et al., 2006). While cognitive impairments are well documented for the acute episode of Major Depressive Disorder (MDD), less is known about cognitive function in the euthymic state. For working memory, dysfunctional activation of lateral prefrontal and cingulate cortex has been reported in the acute episode (Harvey et al., 2005, Matsuo et al., 2007, Rose et al., 2006). The second study investigated verbal-working-memory function and its neurobiological correlate in euthymic MDD patients, particularly whether dysfunctional activation persists when depressive symptoms improve. In the absence of significant behavioural differences we observed comparable overall patterns of brain activation in patients and control subjects. As expected, both groups showed stronger activation of the typical fronto-parietal working-memory network with increasing memory load. However, significant hyperactivation of the cingulate cortex was observed in euthymic patients, while lateral prefrontal activation was comparable between patients and controls. We conclude that working-memory challenge in the euthymic state of MDD reveals a dissociation of lateral prefrontal and cingulate brain function. Cingulate function, which is important for both emotional and cognitive processing and their integration, is still abnormal when mood is restored. This could reflect a different speed of normalization in prefrontal and limbic cortices, persistent systematic changes in neuronal networks after an episode of MDD, or a compensatory mechanism to maintain working-memory performance.

In sum, this dissertation reflects the influence of biological and psychological factors on working-memory networks for a verbal and visuo-spatial task or, using the more process-related view, a significant impact on particular updating (n-back) and manipulating (mental rotation) processes. But demonstrating significant influences on working-memory function is only a first step. To date, we can only hypothesize about the meaning of differential brain activation patterns between women and men or altered brain activation in patients who suffered from depression. Several interpretations are possible. The role of cognitive effort, a possible need for recruitment of cognitive
resources, the role of persistent cortical changes after critical life events and so forth still need clarification (cf. Perlstein et al., 2004).

Further research on this topic should also try to integrate basic molecular aspects underlying working-memory activity (Dash et al., 2007). Moreover, it is necessary to integrate the working-memory system in a broader memory concept, as recent imaging studies showed that the medial temporal lobe, known for its crucial role for semantic and episodic long-term-memory processes, might be involved in working-memory tasks (Ezzyat and Olson, 2008).
Kognitive Funktionen können durch verschiedene biologische und psychologische Faktoren beeinflusst werden. Mittels moderner, nicht-invasiver bildgebender Verfahren ist es möglich, zugrunde liegende neurobiologische Prozesse in vivo zu untersuchen. Die vorliegende Dissertation untersuchte in zwei Studien a) den Einfluss von Geschlecht und Sexualhormonen auf die Fähigkeit, mental Objekte im visuell-räumlichen Arbeitsgedächtnis zu rotieren und b) die Bedeutung klinisch relevanter Depression für eine verbale Arbeitsgedächtnisaufgabe mittels funktioneller Magnetresonanztomografie (fMRT).


In der zweiten Studie dieser Dissertation wurde der Einfluss von klinischer Depression auf die Funktion des verbalen Arbeitsgedächtnisses untersucht. Depressive Patienten weisen Defizite in verschiedenen kognitiven Dimensionen auf (Zakzanis et al., 1998). Es liegen bislang kaum Befunde zur Frage vor, ob diese kognitiven Defizite
im remittierten Zustand persistieren, bzw. welche kognitiven Funktionen davon betroffen sind. In Bezug auf das Arbeitsgedächtnis wurde mittels fMRT bei akut depressiven Patienten im Vergleich zu gesunden Kontrollprobanden eine Mehraktivierung des präfrontalen Cortex und des Cingulums gezeigt, wobei die Verhaltensleistung in beiden Gruppen vergleichbar war (Harvey et al., 2005, Matsuo et al., 2007).

In der zweiten Studie dieser Dissertation wurden 28 euthyme, unipolar depressive Patienten (HDRS $\leq 8$) und 28 gesunde, nach Alter, Geschlecht und Bildungsstand gematchte Kontrollprobanden untersucht. Im MRT (Gyroscope Intera 3,0 Tesla, Philips, Best, NL) wurde eine funktionelle Untersuchung mit einem geblockten, verbalen n-back-Paradigma durchgeführt (single shot EPI, 36 Schichten, TR 3 s, TE 38 ms, Voxel von 3.6 mm Kantenlänge). Die funktionellen Daten wurden mittels SPM5 (Wellcome Department of Cognitive Neurology, London) ausgewertet.


Zusammenfassend konnte in dieser Dissertation also gezeigt werden, dass kognitive Funktionen, insbesondere des verbalen und visuell-räumlichen Arbeitsgedächtnisses, durch Faktoren wie Geschlecht und hormonelle Einflüsse sowie durch affektive Erkrankungen beeinflusst werden können.
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8 Lebenslauf