FUNCTIONAL INVESTIGATIONS INTO THE RECOGNITION MEMORY NETWORK, ITS ASSOCIATION WITH GENETIC POLYMORPHISMS AND IMPLICATIONS FOR DISORDERS OF EMOTIONAL MEMORY

DISSERTATION

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I

Für meinen Vater.

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NOTE

The following parts of this thesis have already been published or submitted (chapter 4, 5 and 6).

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5-HT	5-Hydroxytriptamine (Serotonin)
5-HTT	Serotonin Transporter
5-HTTLPR	5-HT Transporter-linked Polymorphic Region
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	Analysis of Variance
BA	Brodmann Area
BDNF	Brain Derived Neurotrophic Factor
BOLD	Blood-Oxygen-Level-Dependent
CA	Cornu Ammonis
CaMKII	Ca2+/calmodulin-dependent protein kinase II
CaMKIV	Ca2+/calmodulin-dependent protein kinase IV
cAMP	Cyclic adenosine monophosphate
corrKnow	Correct Know
corrRej	Correct Rejection
CorrRem	Correct Remember
CREB	cAMP response element binding protein
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DNA	Deoxyribonucleic Acid
DPSD	Dual Process Signal-Detection
E-LTP	Early Long Term Potentiation
EPI	Echo Planar Imaging
EPSP	Excitatory Postsynaptic Potential
fMRI	functional Magnetic Resonance Imaging
FWE	Family Wise Error
H.M.	Patient Henry Molaison
IPL	Inferior Parietal Lobe
l/l	homozygote long alleles of the 5-HTTLPR
LEA	Lateral Entorhinal Area
LTM	Long Term Memory
LTP	Long Term Potentiation
MAPK	Mitogen-activated protein kinase
MDMA	3,4-Methylenedioxymethamphetamin Ecstasy
MEA	Medial Entorhinal Areas
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging

XIII

mRNA	Messenger Ribonucleic Acid
MTL	Medial Temporal Lobe
NMDA	N-methyl-D-aspartate
NT	Neurotrophin
p75^{NTR}	p75 Neurotrophin Receptor
pC/PCC	Precuneus/Posterior Cingulate Cortex
PCR	Polymerase Chain Reaction
PDP	Process-Dissociation Procedure
PFC	Prefrontal Cortex
PHC	Parahippocampal Cortex
PKA	Protein Kinase A
PPI	Psychophysiological Interaction Analysis
PRC	Perirhinal Cortex
PTSD	Posttraumatic Stress Disorder
R/K	Remember-Know
ROC	Receiver Operator Characteristics
ROI	Region of Interest
s	short allele of the 5-HTTLPR
SERT	Serotonin Transporter
SLC6A4	Solute carrier family 6, Member 4 (5-HTT Gene
SMA	Supplementary Motor Area
SNP	Single Nucleotide Polymorphism
SPM	Statistical Parametric Mapping
STM	Short Term Memory
TE	Echo Time
TR	Time of Repetition
Trk	tropomyosin-related kinase
UVSD	Unequal Variance Signal-Detection
Val66Met	Valine to Methionine Substitution at codon 66 of the BDNF Protein
WMS	Wechsler Memory Scale

1 INTRODUCTION

In 1953, a young patient with severe epileptic seizures underwent a brain surgery to remove most parts of his medial temporal lobes (MTL). After surgery, this patient, called H.M., showed severe memory deficits which were particularly pronounced in declarative memory, the memory for facts and events of human life. He suffered from severe anterograde amnesia. This means, no new information or event could be transferred into his long term memory, hence, he could not remember any event that happened after the surgery. In a first description of the case, Scoville and Milner (1957) described the extent of the removed tissue in the MTL. The resection was carried out from the temporal poles extending 8 cm posteriorly in the bilateral MTL. Most parts of the bilateral hippocampi, parahippocampal gyrus, the whole entorhinal cortex and the amygdala were removed.

However, the performance in procedural memory and working memory was not affected, e.g. H.M. could learn new motor skills but he could not remember that he had learned it. Scoville and Milner (1957) concluded that removal of the hippocampus causes the severe declarative memory deficit, but leaves other memory systems intact. Through his whole life, H.M. became 82 years old, he taught the memory scientists about the organization and the neurobiological basics of memory, because he repeatedly was a subject in neuroscientific memory research (Corkin, 2002). At the time of his death in 2008, we knew much more about the organization of memory systems and the brain regions that are involved in episodic memory than in the 1950ies. Other patients with brain lesions also contributed to this knowledge, but H.M. belongs to the most prominent ones.

He also demonstrated how such a memory deficit severely impairs human life. His whole life he was reliant on his parents or relatives. At the end of his life, he lived in an institution where he died on December 2th, 2008. He could not remember the persons that he got to know after the surgery, he could not learn new facts about the world and he could not remember events that occurred after surgery. He lived in the past. "For the next 55 years [after surgery], each time he met a friend, each time he ate a meal, each time he walked in the woods, it was as if for

the first time." (obituary for Henry Molaison, H.M., New York Times, December 4th 2008).

Those descriptions emphasize the importance of a functional declarative memory for human life. You may wonder if a person without episodic memories misses a great part of his or her personality. Or whether a person without knowledge about the world around is unsecure and feels unsafe? Certainly, the loss of the declarative memory caused a great interference in H.M.s life.

A very surprising result from studies assessing H.M.s recognition performance is that he had comparable recognition rates to healthy controls, when the procedures to assess recognition were adapted to his skills (Freed & Corkin, 1988; Freed, Corkin, & Cohen, 1987). Thus, it could be suggested that recognition not only depends on the MTL and the hippocampus, but may use a process that relies on different brain structures and may not be related to episodic memory.

Recent research, which has been focused on recognition memory, has revealed that two processes contribute to recognition of previously encountered items: recollection and familiarity (Aggleton & Brown, 1999; Eichenbaum, 2006; Eichenbaum, Yonelinas, & Ranganath, 2007; Rugg & Yonelinas, 2003; Skinner & Fernandes, 2007; Squire, Stark, & Clark, 2004; Wixted, 2007a; Yonelinas, 2001a; Yonelinas, 2002). Recollection is based on remembering the temporal and spatial embedding of the learned item in the study context, whereas familiarity is often described as a feeling of knowing: one could know that a certain item has been encountered before, but has no recollection of additional context information of the study event. In the last decades extensive research has taken place to achieve knowledge about the nature of recollection and familiarity and to reveal the anatomical and functional substrates of recognition memory in the brain. There are opposing views about the relationship between recollection and familiarity and about the association with hippocampal function (Wixted, 2007a; Yonelinas, 2002). One possibility to achieve more clarity about the relationship between the two processes is the investigation of influences by genetic variables on hippocampal structure and function in relation to recognition memory. Additionally, there is almost no knowledge about how brain regions are working in concert in a network supporting either familiarity or recollection.

The current thesis is concerned with those problems and, therefore, will investigate brain networks underlying recollection and familiarity. Furthermore, the

modulation of a variation in the gene encoding the Brain Derived Neurotrophic Factor (BDNF), which is known to be strongly involved in memory consolidation (Egan, et al., 2003), on recognition memory is measured with respect to the different processes of recollection and familiarity.

Finally, the impact of the BDNF gene variation alone and in interaction with variations in the gene that encodes the serotonin transporter on the structure of the hippocampus is evaluated.

2 THEORETICAL BACKGROUND

2.1 Memory

"Learning is the acquisition of new information or knowledge. Memory is the retention of learned information." (Bear, Connors, & Paradiso, 2007, page 740). This sentence already includes the features by which memory as a process is commonly described in psychological science. The main stages of the memory process are Encoding, Storage, and Retrieval. Although the present work is concerned with the latter, the other stages will also be touched on throughout the thesis.

2.1.1   Memory systems and their neural correlates

There are different types of memory which can be ordered by content and by the time they are lasting. Additionally, psychologists use different terms according to the type of memory testing.

First, looking at the time component, at least two phases of memory storage can be divided (McCraty, Barrios-Choplin, Rozman, Atkinson, & Watkins, 1998). When attention is paid, sensory information is transformed into short term memory (STM) that lasts on the order of seconds to hours and is vulnerable to disruption. Through a rehearsal process STM is transferred into long-term memory (LTM) that can be recalled for days, months, or years and is characterized by stability and robustness, but is also modifiable over time (Atkinson & Shiffrin, 1968; Shiffrin & Atkinson, 1969). The process by which STM is transformed into LTM is called consolidation and it is supposed that molecular processes of early and late Long-Term Potentiation (LTP) parallel those components (Lynch, 2004).

In terms of long-term memory content, explicit (declarative) memory is contrasted to implicit (non-declarative, procedural) memory (Cohen & Squire, 1980; Squire, 2004; Tulving, 2002). Declarative memory consists of information that is explicitly stored and retrieved and requires conscious recall. In contrast, implicit memory is not based on the conscious recall of information, but on implicit learning. Implicit memory is further subdivided into procedural memory, associative learning (conditioning) and into priming processes (Schacter & Tulving, 1994). Procedural

learning and memory is primarily employed in learning motor skills and basically depends on normal function of the cerebellum and the basal ganglia (Squire, 2004).

Conditioning processes are primarily influenced by associative learning and strongly depend on amygdala function. There exist strong interconnections between declarative and associative learning, which are supposed to form emotional memories (Eichenbaum & Cohen, 2001).

Priming is defined by a change in the ability to identify or produce an item as a result of a specific prior encounter with the item (Tulving & Schacter, 1990) and is further subdivided into perceptual and conceptual priming. Perceptual priming is modality specific and does not depend on semantic or elaborative encoding of an item at the time of study, whereas conceptual priming is not modality specific and benefits from semantic encoding. There is strong evidence that perceptual priming depends on occipital lobe function, more precisely on blood flow reductions in extrastriate visual cortex, whereas conceptual priming is related to the prefrontal and temporal cortex (Schacter & Buckner, 1998).

Declarative or explicit memory can be divided into episodic and semantic memory (Squire, 2004; Tulving, 2002). The latter comprises our knowledge about the world, about facts and meanings which can be consciously retrieved without relation to personal experiences. Persons suffering from semantic dementia, a disorder which causes severe loss of semantic knowledge, show atrophy in the anterior temporal lobe (Mayes & Montaldi, 2001). Finally, episodic memory is the memory of autobiographical events (times, places, associated emotions, and other contextual knowledge) that can be explicitly stated (Tulving, 1983). There is strong evidence that the medial temporal lobes (MTL) including hippocampus, but also the prefrontal cortex and medial and lateral parietal cortex are involved in normal episodic memory function (Aggleton & Brown, 2006; Ally, Simons, McKeever, Peers, & Budson, 2008; Burgess, Maguire, & O'Keefe, 2002; Burgess, Maguire, Spiers, & O'Keefe, 2001; Eichenbaum, 2000; Maguire, 2001a). An overview of long term memory systems together with brain structures thought to be especially important for each form of memory can be found in Figure 2-1.

Another distinction of declarative memory, which refers to the retrieval of previously stored information, depends on how the memory retrieval is tested (Schacter & Tulving, 1994).

During *Free Recall* a subject would be asked to study a list of words and then sometime later they will be asked to recall or write down as many words that they can remember.

In *Recognition* tasks subjects are asked to decide whether a given item was previously presented at a list of words or pictures. There are also items presented that were not presented in the original list.

Recognition memory is supposed to consist of two features: recollection and familiarity based recognition (Yonelinas, 2002). In the present thesis, those two processes were of special interest. Therefore, a description and discussion of different views that are concerned with the components of recognition memory will follow in the next section.
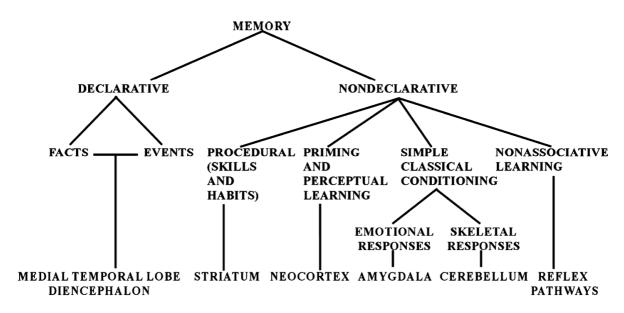
Figure 2-1: Long term memory systems in humans and their underlying brain structures. (Figure reprinted with permission from Squire, 2004).

2.1.2   Recognition Memory

Recognition memory performance can be described by two different retrieval processes: recollection and familiarity. The retrieval of studied items can be either accompanied by a feeling of familiarity or by the additional recollection of some aspects of the study event. Thus, the person is able to judge whether the recognition of a previously learned item is based on a feeling of familiarity that the item was seen before, or because he or she remembers additional details about the study event, such as when or where it occurred. Recollection sometimes is

referred to as contextual memory or relational based recognition, whereas familiarity is called non-contextual memory or item-based recognition. Although the terms recollection and familiarity will be applied in most parts of this thesis, the synonyms will be used if appropriate.

2.1.2.1 Dual Process Models of Recognition Memory – Recollection and Familiarity

There are different models that assume recognition memory judgments can be based on two distinct forms of memory (Yonelinas, 2002; Aggleton & Brown, 1999; Atkinson & Juola, 1974; Eichenbaum, Yonelinas, & Ranganath, 2007; Jacoby, 1991; Mandler, 1980; Tulving, 1985). Those models almost commonly assume that: 1. recollection and familiarity are independent processes at the stage of retrieval, 2. familiarity is faster than recollection, 3. familiarity is often described as reflecting a continuous index of memory strength, whereas recollection is thought to reflect the retrieval of specific information about a study event, and 4. recollection is dependent on the medial temporal lobes (MTL) and thus predict that amnesiacs should exhibit deficits in recollection but not in familiarity.

A prominent model, which combines all those features is termed dual-process signal-detection (DPSD) model (Yonelinas, 2001a). The model assumes that familiarity is well described by the classical signal-detection theory, whereas recollection is supposed to be a threshold retrieval process. In support for the DPSD model, there are several variables that influence recollection more than familiarity at the stage of encoding: deep vs. shallow encoding, generation vs. reading of a word, divided attention, and benzodiazepine administration. However, those results not necessarily support only dual process models. At the retrieval stage, there is evidence for a much more pronounced dissociation between recollection and familiarity by variables like speed, divided attention, change of the perceptual characteristics of a word between study and test, forgetting rates, manipulations of the processing fluency of test items, and the occurrence of false recognition (for a summary see Yonelinas, 2002).

Importantly, relaxing the response criterion in a recognition test leads to a large increase in the probability that items will be judged as familiar, but has very little effect on recollection (Strack & Forster, 1995), when no guess responses are

included in the test procedure (Gardiner, Richardson-Klavehn, & Ramponi, 1997). Moreover, familiarity, but not recollection, increases in a manner consistent with signal detection theory (Yonelinas, 2001b) supporting the core assumption of the DPSD model. Additional variables which affect recollection, but leave familiarity largely unaffected, include normal aging, selective hippocampal damage and to some extend frontal lobe damage (Yonelinas, 2002).

Alternative views on the recognition process state that single process theories are more viable than dual process theories of recognition memory. Thus, Squire, Wixted and Clark (2007) argue that the distinction between recollection and familiarity instead is a distinction between strong and weak memories. They do not assume that only familiarity could be described by a classical signal detection approach but rather that familiarity lies at the lower end of a continuum of confidence ratings, whereas recollection reflects the higher end. The UVSD model tries to combine the signal detection with a dual process approach and assumes that the distributions of targets and lures in strong memory conditions (i.e. recollection) show an unequal variance whereas weak memory conditions (i.e. familiarity) show an equal distribution (Squire, et al., 2007; Wixted, 2007a). For example, it has been suggested that because of encoding variability, the old item variance (targets) will be greater than the new item variance (lures). In contrast, the dual process models predict greater old than new item variance because they assume that new item responses rely on familiarity, whereas old item responses rely on familiarity and recollection (Yonelinas & Parks, 2007).

As with the DPSD approach, applying the UVSD allows an explanation of the differences in Receiver Operating Characteristic (ROC) curves found in recognition memory (see Figure 2-2, Figure 2-3). Squire et al. (2007) suggest that a symmetrical ROC curve (Plot of Hits vs. false alarms for different levels of confidence), which typically is plotted using only familiarity responses, reflects weak memory rather than the absence of recollection. Asymmetrical ROCs, which result from the plotting of both recollection and familiarity responses, only implies that the target and lure distributions have unequal variance, which is generally a sign of a strong memory.

According to Squire et al. (2007) and Wixted (2007a) those ROCs do not imply that recognition is supported by recollection, as supposed by Yonelinas (2002). There is scientific evidence for both models and the dispute has not yet been

solved (Parks & Yonelinas, 2007; Wixted, 2007a, 2007b). However, explanations of the dissociations between recollection and familiarity by a great amount of modulations during study and testing as well as by different samples are not provided by the representatives of one process models.

2.1.2.2 Measurement of Recollection and Familiarity

The most common tasks that are used to measure recollection and familiarity are the Process-Dissociation Procedure (PDP) (Jacoby, 1991), the Remember-Know procedure (R/K) (Tulving, 1985) and the Receiver Operator Characteristics (ROC) (Yonelinas, 2002). In the PDP, participants study a list of items in two different contexts, and are subsequently given two recognition tests. In the inclusion test, participants are asked to identify an item as old if they previously encountered it, regardless of the context in which it was presented. In the exclusion test, participants are asked to identify an item as old only if it was presented in one of the two study contexts. Thus, only the exclusion test is based on recollective memory. A potential limitation of the PDP is that it uses a rather strict measure of recollection — the ability to determine in which study list the item was presented. However, if they recollect some other aspect of the study event (e.g., "I remember coughing as the item was studied") that does not support the required discrimination this will not be measured as recollection. Another potential problem with the procedure is that it uses different test instructions in the inclusion and exclusion conditions, and this may influence the parameter estimates (Yonelinas, 2002).

a Recollection and familiarity

b Familiarity alone
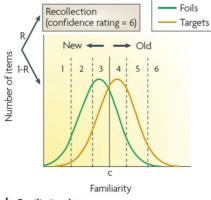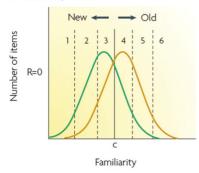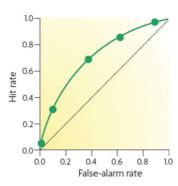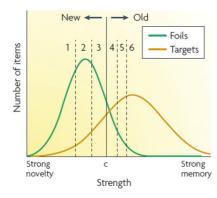
Figure 2-2: Signal-detection theory and receiver operator characteristic in Dual Process Signal Detection model of recognition memory (Figure reprinted with permission from Squire, et al., 2007a).

a Strong memory condition

b Weak memory condition
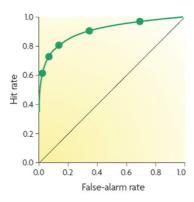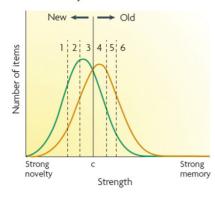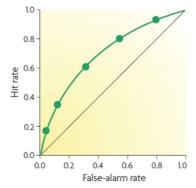
Figure 2-3: Signal-detection theory and receiver operator characteristic in the Unequal Variance Signal Detection model of recognition memory (Figure reprinted with permission from Squire, et al., 2007a).

In the ROC procedure participants are required to rate the confidence of their recognition memory responses – hence a varying response criterion. Then a curve is formed by plotting hits and false alarms against one another as a function of confidence. Familiarity and recollection estimates are then derived using mathematical algorithms that assume recollection is a threshold process, whereas familiarity reflects a signal-detection process. Limitations of the ROC procedure are that it is dependent on several assumptions and it needs a large number of responses from each subject (Yonelinas, 2002).

In the R/K procedure (Eldridge, Sarfatti, & Knowlton, 2002; Gardiner, Ramponi, & Richardson-Klavehn, 2002; Tulving, 1985), subjects are instructed to indicate when a recognition judgment is based on recollection and when it is based on familiarity in the absence of recollection. This method assumes that remember (recollection) and know (familiarity) are independent, and thus it is consistent with most dual-process models (Yonelinas, 2002). One advantage of this approach is that it provides a very inclusive measure of recollection in the sense that recollection is not limited to what a subject can recollect on PDP test.

However, relying on retrospective and introspective subjective reports may be problematic if subjects have no direct access to the processes that support recognition or if their reports are inaccurate. Studies that compared estimates of recollection and familiarity derived from R/K responses with those from other measures, though, suggest that subjects generally do have access to these memory processes (Yonelinas, 2001a).

2.1.2.3 Recollection and Familiarity and the Brain

2.1.2.3.1 *Neural Correlates of Recollection and Familiarity*

In line with the dual-process model neuroimaging and lesion data, as well as amnesia studies strongly support functionally distinct processes at the brain systems level underlying recollection and familiarity (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Aggleton, et al., 2005; Eichenbaum, et al., 2007; Parks & Yonelinas, 2007; Schofield, et al., 2008; Skinner & Fernandes, 2007). Although, this view is challenged by an alternative hypothesis (for a review see Squire, et al., 2007). Squire et al. (2007) suggest that Recollection and Familiarity simply reflect strong and weak memories, respectively. According to this, a study by

Shrager, Kirwan, & Squire (2008) found prefrontal and middle frontal, superior temporal, lateral and medial parietal, and precentral gyrus activity during encoding negatively correlated with subsequent memory strength. Some of these regions are also known to be related to recollection processes.

But, since Shrager et al. (2008) did not include a recollection vs. familiarity judgment in their procedure and therefore could not contrast confidence ratings to recollection-familiarity, the source of their findings remains unclear. A study by Yonelinas, Otten, Shaw, & Rugg (2005) did contrast recollection-familiarity judgments to confidence ratings of the recognized items. Thus they could separate high confidence familiarity responses (in other words a strong memory trace based on familiarity) to recollection responses, which are always supposed to be high confident memories. They found medial and middle frontal, precentral, cingulate, superior and middle temporal, as well as postcentral activation related to recollection responses and not merely to strong memories. In line with this, Vilberg and Rugg (2007) could not identify any brain region where recognition memory is only related to memory strength. Instead they showed specific regions associated with recollection (left parietal/occipital cortex, left anterior medial temporal cortex, left prefrontal cortex) and areas specifically related to familiarity (bilateral caudate nucleus, medial occipital/parietal cortex, left superior parietal cortex, left dorsolateral/anterior prefrontal cortex).

A recent review of neuroimaging and lesion data by Skinner and Fernandes (2007) also prefers the dual process view of recognition memory. They found strong activity in anterior and superior frontal regions, as well as in left inferior parietal lobe only during recollection. Intermediate agreement across studies regarding brain regions related to recollection was found in left anterior and posterior cingulate gyrus, superior parietal/precuneus areas, as well as in right inferior parietal lobe and bilateral BA 19. Left insula, left superior temporal gyrus, and bilateral inferior temporal gyrus were activated in 30-40% of the analyzed studies.

Skinner and Fernandes (2007) reported less concordance across the studies regarding the activation of brain regions during familiarity. Only left BA 19 was activated in 50 percent of the studies. Additionally, one third of the analyzed studies found right superior frontal gyrus and left precuneus activated during familiarity.

Overlapping activity during both recollection and familiarity responses was shown across most of the studies in the right dorsolateral prefrontal cortex (DLPFC) and left precuneus (BA 7).

Studies utilizing the Remember-Know dissociation in a word recognition task reveal that remember responses are related to a network of brain regions consisting of left dorsolateral prefrontal cortex, left middle and superior frontal gyrus, bilateral posterior cingulate gyrus, left inferior parietal gyrus, and right fusiform gyrus (Eldridge, Engel, Zeineh, Bookheimer, & Knowlton, 2005; Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000; Fenker, Schott, Richardson-Klavehn, Heinze, & Duzel, 2005; Henson, Rugg, Shallice, Josephs, & Dolan, 1999; Wheeler & Buckner, 2004; Woodruff, Johnson, Uncapher, & Rugg, 2005).

The association of the prefrontal cortex and the medial and lateral parietal cortex to recollection memory parallels the findings in other recognition tasks that implement the distinction between recollection and familiarity like PDP or ROC measures (Henson, Shallice, & Dolan, 1999; Montaldi, Spencer, Roberts, & Mayes, 2006; Rugg, Henson, & Robb, 2003; Yonelinas, et al., 2005) or R/K measures using different stimulus material (Sharot, Delgado, & Phelps, 2004).

Little congruence can be found regarding the neural circuitry serving Know responses. Henson et al. (1999) and Wheeler and Buckner (2004) highlight the role of dorsolateral prefrontal cortex, whereas both Eldridge et al. (2000) and Fenker et al. (2005) reported right superior frontal activation. Those differences maybe are caused by different measures of R/K. As mentioned above, the specific test procedure has great influence selectively on know responses (Eldridge, et al., 2002; Hicks & Marsh, 1999). In Eldridge et al. (2000) a two-step procedure with no guess response was used. In contrast, Henson et al. (1999) and Wheeler and Buckner (2004) used a one step procedure without and with guess category, respectively. Additionally, the delay between study and test strongly varied. Forgetting rates are very different between recollection and familiarity in intermediate test delays (Yonelinas, 2002). Thus, it is assumed that know responses do not provide an unbiased measure of familiarity (Gardiner & Richardson-Klavehn, 2000), which accounts for the differences in brain activations found in the literature.

2.1.2.3.2 Role of the parietal lobe

Recently, increasing interest in the role of the parietal lobe in recognition memory retrieval has developed. Although, medial and lateral parietal cortex are among the regions identified most consistently in studies of recognition memory. As mentioned above, several studies using different measures of recollection and familiarity found strong activation in lateral parietal cortex which was associated with recollection responses (Eldridge, et al., 2000; Fenker, et al., 2005; Henson, Rugg, et al., 1999; Sharot, et al., 2004; Skinner & Fernandes, 2007; Wheeler & Buckner, 2004; Woodruff, et al., 2005; Yonelinas, et al., 2005). A meta-analysis by Vilberg & Rugg (2008) identified a region concentrated around the intraparietal sulcus, the superior parietal cortex (BA 7/40), related to familiarity judgments, and an area localized in the posterior part of inferior parietal cortex (BA 39) consistently associated with recollection based responses. Left lateral parietal cortex often is found in studies that contrast hits vs. correct rejections (Kahn, Davachi, & Wagner, 2004; Konishi, Wheeler, Donaldson, & Buckner, 2000; Wheeler & Buckner, 2003) and reflects an old/new effect which also could be supported by Event Related Potentials (ERP) results (Rugg & Curran, 2007; Rugg, Otten, & Henson, 2002). Additionally, Vilberg and Rugg (2008) strongly support the idea that retrieval-related activity in inferior parietal cortex is not only related to correct recognition, but closely tied to successful recollection. The authors assume that the inferior parietal cortex supports the sustained focusing of attention on the contents of working memory, where recollected information is maintained (Ravizza, Delgado, Chein, Becker, & Fiez, 2004).

Another meta-analysis by Skinner and Fernandes (2007) supports inferior parietal lobe activations only for recollection based responses, whereas left precuneus (BA 7) was found to be active during both recollection and familiarity answers. There are conflicting results with respect to precuneus activations in recognition memory. It has been implicated to be a key component of a cortical network subserving episodic retrieval (Burgess, et al., 2001; Cavanna & Trimble, 2006). By contrast, Vilberg & Rugg (2008) could not report an association of precuneus activity with recollection based responses, the relatively few precuneus effects in their meta-analysis were rather related to familiarity-driven recognition.

Nonetheless, the authors suggest that the region may play some role in recollective processing, although what this role might be is currently ambiguous.

2.1.2.3.3 Role of the prefrontal cortex

In their meta-analysis of recollection and familiarity responses Skinner and Fernandes (2007) found activity in dorsolateral prefrontal (BA 46), anterior prefrontal (BA 10) and superior frontal regions (BAs 6, 8), as well as in anterior cingulate cortex (ACC) related to recollection. This has been interpreted to reflect successful retrieval of source information in the anterior prefrontal cortex (Dobbins & Wagner, 2005), as well as attentional control processes in right frontal lobe areas and ACC (Cabeza, Dolcos, et al., 2003). Another right dorsolateral prefrontal region (DLPFC, BA 9) was related to familiarity based judgments but also to overlapping activity during both recollection and familiarity responses which possibly reflects post-retrieval processing (Rugg, Fletcher, Frith, Frackowiak, & Dolan, 1996), retrieval mode (Lepage, Ghaffar, Nyberg, & Tulving, 2000), and monitoring and verification processes (Cabeza, Locantore, & Anderson, 2003; Henson, Shallice, et al., 1999). Right DLPFC involvement in familiarity is interpreted as an additional checking and verification behavior (Henson, Rugg, Shallice, & Dolan, 2000) or as an ongoing exhaustive search for details to accompany the feelings of familiarity with an item (Wheeler & Buckner, 2004).

2.1.2.3.4 Role of the Medial Temporal Lobe

A core assumption of dual process models of recognition memory is that recollection is dependent on the medial temporal lobes and thus they predict that amnesiacs should exhibit more deficits in recollection than familiarity (Yonelinas, 2002). More precisely, Aggleton and Brown (1999) suppose, that relatively selective hippocampal damage disrupts recollection, but not familiarity which instead is dependent on perirhinal cortex. Thus, extensive damage to the temporal lobe can reduce recall (recollection) and recognition (recollection and familiarity) so the same extent (Stark & Squire, 2000), whereas selective hippocampal lesions only reduce recollection (Holdstock, et al., 2002). However, the amnesia findings are less clear than it appears For instance, Skinner and Fernandes (2007) reviewed lesion studies investigating recognition memory and

conclude that both R- and F-based responses rely on the MTL, although recollection may have a greater reliance on this region than familiarity. Nevertheless there is no doubt that without the MTL no correct recollection responses are possible, whereas lesions to other regions of the brain do not offer such a clear causal relationship.

Excurse: The Anatomy of the Medial Temporal Lobe
Because of the undeniable importance of the MTL for episodic and recognition memory functions a closer look on those structures and its connections to other regions of the brain is taken.

The MTL comprises the hippocampus and surrounding interconnected structures which are combined to the hippocampal formation. Anatomically, the amygdala could be added to the MTL, too, but because of functional considerations this section will concentrate on the declarative memory system of the MTL to which the amygdala shows no critical contribution (Eichenbaum & Cohen, 2001). The hippocampal formation comprises the hippocampus proper, the entorhinal cortex, the perirhinal cortex and the parahippocampal cortex. The latter three are subsumed under the term 'parahippocampal region' (Witter, Groenewegen, Lopes da Silva, & Lohman, 1989). Entorhinal and perirhinal cortex are surrounding the rhinal sulcus, the parahippocampal cortex lies more lateral to it. The hippocampus proper is a folded structure of two thin sheets of neurons lying medial to the lateral ventricle and consists of the subiculum, the dentate gyrus, the Ammon's horn (Cornus Ammonis, CA1, CA2, CA3, CA4, and the fornix. A major input to the hippocampus is the entorhinal cortex by a bundle of axons called the perforant path (Amaral & Witter, 1989). Those axons synapse on neurons of the dentate gyrus, and those axons (Mossy fibers) form connections to the cells in CA3. CA 3 axons then branch, one branch leaves the hippocampus via the fornix, the other branch (Schaffer Collateral) connects with CA1 neurons (see Figure 2-5; Bear, et al., 2007).
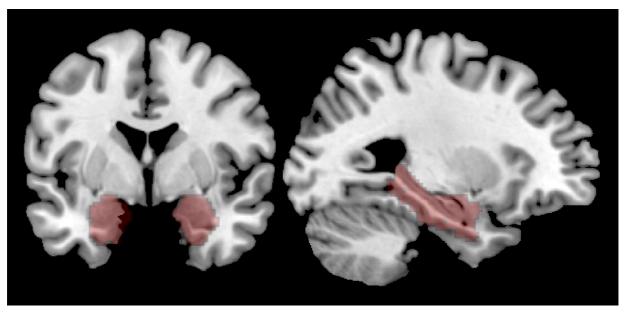
Figure 2-4: The medial temporal lobes (red) consists of the hippocampus proper, the parahippocampal gyrus, and the amygdala; coronar (left) and axial (right) slice of the brain.

Highly preprocessed sensory information from virtually all higher-order cortical areas, including the association areas, reaches the medial temporal lobe (for a summary see Eichenbaum & Cohen, 2001). Studies mostly using monkey, rat and mouse brains to search for hippocampal structural connectivity found that the hippocampus has widespread reciprocal connections to cortical areas including the insula, orbitofrontal, medial frontal, and dorsolateral prefrontal areas, to the temporal pole, the superior and inferior temporal gyrus, to anterior and posterior cingulate areas, retrosplenial cortex and BA 7 of the parietal cortex, as well as to BA 19 of the occipital cortex. Furthermore, hippocampal afferents and efferents were found with various subcortical areas including the anterior thalamic nuclei, mammillary bodies, amygdala, putamen, and caudate nucleus (for a summary see Nieuwenhuys, Voogd, & Huijzen, 2008).
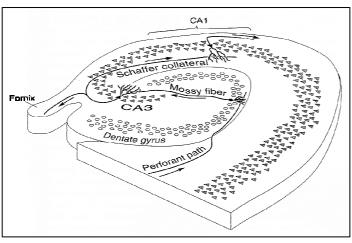
Figure 2-5: Schematic drawing of a coronar slice of the hippocampus (reprinted with permission from Bear, et al., 2007

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Returning to the involvement of the MTL in recognition memory, dual process views suppose that recollection and familiarity are related to different regions of the MTL (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Eichenbaum, et al., 2007; Schofield, et al., 2008). A recent review by Eichenbaum et al. (2007) states that the hippocampus is responsible for recollection processes, but the perirhinal cortex supports familiarity. This assumption is in accordance with previous theories about the involvement of MTL structures in recognition memory (Aggleton & Brown, 1999; Aggleton & Brown, 2006). Eichenbaum et al. (2007) hypothesize that neocortical input to the perirhinal cortex (PRC) and to the lateral entorhinal area (LEA) comes from association areas that process unimodal sensory information about qualities of objects ("what"). Supporting this assumption, studies that use electrophysiological recordings in monkeys showed that neurons in the anterior parahippocampal region, including the perirhinal cortex, respond strongly to pictures or objects that are new but only weakly when items have been seen previously (for a review see Schofield, et al., 2008; Xiang & Brown, 1998). Crucially, fMRI studies assessing non-contextual recognition in humans found decreased activation in the anterior parahippocampal region which contains the perirhinal cortex (e.g. Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006; Fernandez & Tendolkar, 2006; Henson, Cansino, Herron, Robb, & Rugg, 2003). Furthermore, Haskins, Yonelinas, Quamme, & Ranganath (2008) could show, that the perirhinal cortex supports encoding of novel associations in a unitized manner and subsequent associative recognition based on familiarity.

Eichenbaum et al. (2007) further describe that neocortical input to the parahippocampal cortex (PHC) and adjacent medial entorhinal areas (MEA) comes from regions that process polymodal spatial ("where") information. The "what" and "where" signals converge in the hippocampus together with the context in which the items were experienced. Back projections from hippocampus to the PRC-LEA (the "What" pathway) support recognition judgments of familiarity. Recovery of context and item associations ("what" and "where") are available in the hippocampus and through back projections to the PHC-MEA and constitute the experience of recollection. Eichenbaum et al. (2007) thereby relate fMRI activations in the anterior parahippocampal gyrus to activations in the perirhinal and lateral entorhinal areas and signal in the posterior parahippocampal region to

activation of the parahippocampal cortex with or without medial entorhinal area activation.

The reviewed results strongly support Eichenbaum et al.'s hypothesis about the functional organization of the MTL. Studies using ROC and R/K tasks could show that hippocampal and to some extent posterior parahippocampal activation during both encoding and retrieval is consistently higher for recollected than non-recollected items and is generally insensitive to changes in familiarity strength (e.g. Dolcos, LaBar, & Cabeza, 2005; Eldridge, et al., 2000; Montaldi, et al., 2006; Yonelinas, et al., 2005). Complementary, anterior parahippocampal activation is generally correlated with familiarity and rarely correlated with item recollection (e.g. Gonsalves, Kahn, Curran, Norman, & Wagner, 2005; e.g. Henson, Rugg, et al., 1999). However, the study by Gonsalve, et al. (2005) points to a memory strength function in posterior parahippocampal as well as perirhinal cortex.

In opposition to the dual process theories of recognition memory Squire et al. (2007) suppose that the distinction between recollection and familiarity effectively can be described by the distinction between strong and weak memories (see above). The authors relate strong memories to hippocampal activity, regardless of whether the item retrieval is accompanied by recollection of context or a feeling of familiarity. Thus, Squire et al. (2007) summarizes evidence, that selective hippocampal damage impairs recall (which is supposed to specifically reflect recollection) to the same extent than recognition (which comprises familiarity as well as recollection) (e.g. Manns, Hopkins, Reed, Kitchener, & Squire, 2003; Rutishauser, Mamelak, & Schuman, 2006). Additionally, a recent study by Bengner and Malina (2008) found fewer know responses in temporal lobe epilepsy patients with hippocampal sclerosis (HS) as compared with patients without HS. Additionally, there are findings disproving the assumption that in patients with hippocampal damage recollection, measured by associative or source recognition, is impaired while familiarity (single item recognition) is spared (Gold, et al., 2006; Stark, Bayley, & Squire, 2002).

However, other studies show sparing of recognition memory in relation to recall in patients with hippocampal damage (e.g. Holdstock, et al., 2002; Miyajima, et al., 2008; Vargha-Khadem, et al., 1997), or severe damage of the connections of hippocampus to diencephalon and other cortical regions (Gilboa, et al., 2006). Directly investigations of recollection and familiarity in patients with hippocampal

damage revealed pronounced deficits in recollection but not in familiarity (Turriziani, Fadda, Caltagirone, & Carlesimo, 2004; Turriziani, Serra, Fadda, Caltagirone, & Carlesimo, 2008).

Additionally, Skinner and Fernandes (2007) report that in their review of brain lesion data the estimate of recollection was significantly lower in the MTL compared to non-MTL patient group, though the estimate of familiarity-based responses did not differ across patient groups. So they support the assumption that both recollection and familiarity rely on the medial temporal lobe, but recollection may have a greater reliance on these structures.

Concerning recognition memory in healthy subjects, Shrager et al. (2008) found both hippocampus and perirhinal cortex activity during encoding positively correlated to the memory strength of subsequently recognized items as indicated by confidence ratings of the subjects. This is in line with one process models. One limitation of this finding is that the authors only could show this correlation in the high confidence ratings (4, 5 and 6) and not by investigating the whole memory strength continuum, as would be suggested by signal detection approaches. Additionally, the authors did not collect a recollection vs. familiarity judgment in this study to contrast those two approaches. Another study by Sperling et al. (2003) found that only high confidence recollection in relation to low confidence recollection was associated with anterior hippocampal activity at encoding. However, there was no activation within the hippocampal formation in incorrect, but high confidence memory encoding vs. incorrect but low confidence memory encoding. Therefore, Sperling et al. (2003) suggest that in associative memory formation the anterior hippocampus possibly is binding together items of information rather than creating a feeling of confidence. Montaldi et al. (2006) and Yonelinas et al. (2005) found that the hippocampus is only activated in recollection compared to high confidence familiarity judgments. Furthermore the hippocampal formation showed no increase in activity with increasing familiarity confidence. Those findings are more in line with the DPSD model.

Overall, the results point to a dual process view of recognition memory and its underlying functional correlates in the medial temporal lobe. Thus, in this thesis it is hypothesized that familiarity is related to perirhinal cortex (anterior parahippocampal cortex) and recollection is based on functioning of the hippocampus and posterior parahippocampal cortex.

2.1.2.3.5 Connectivity of brain regions in Recognition Memory

The results of the neuroimaging and lesion studies so far have provided support for some brain regions that are exclusively related to recollection and few brain regions that seem to be especially related to familiarity. Additionally, there is growing evidence for overlapping brain regions which are related to both processes. However, a description of how those brain regions are acting in concert to constitute a recollection or a familiarity judgment is still missing.

Functional connectivity of the hippocampus related to recognition memory is described by already mentioned models of the medial temporal lobes (e.g. Aggleton & Brown, 2006; Eichenbaum, et al., 2007). The model by Eichenbaum et al. (see above) supposes that perirhinal cortex receives information from association areas that process unimodal sensory information about qualities of objects ("what"), whereas the parahippocampal cortex receives input from areas that process polymodal spatial ("where") information. The "what" pathways support judgments of familiarity. "When a previously encountered stimulus is processed, perirhinal and lateral entorhinal areas can signal its match to a preexisting item representation, observed as overall suppressed activation. This match signal can be propagated back to neocortical areas, which may be sufficient to generate the sense of familiarity without the participation of the hippocampus." (Eichenbaum, et al., 2007, p. 142). The converging of "what" and "when", however, together with the context in which an item was experienced is supported by the hippocampus and constitutes the experience of recollection. Aggleton and Brown (1999, 2006) established an extended hippocampal system of episodic memory including a medial and a lateral part. The medial part comprises the subiculum, medial mammillary nucleus, anterior medial and anterior ventral thalamic nuclei, and the ventral tegmental nucleus. Via the thalamus it is linked to the prefrontal cortex, including the orbitofrontal, the medial and the dorsolateral part. The lateral part of the extended hippocampal system consists of the presubiculum, postsubiculum, lateral mammillary nucleus, anterior dorsal thalamic nucleus, and the dorsal tegmental nucleus. Both subsystems project over the thalamus to the posterior cingulate/retrosplenial cortex and support episodic memory encoding and retrieval, which is strongly impaired in amnesics with lesions comprising at least one of the parts of the system

(Aggleton, et al., 2000; Dusoir, Kapur, Byrnes, McKinstry, & Hoare, 1990; Harding, Halliday, Caine, & Kril, 2000; Maguire, 2001b; Spiers, Maguire, & Burgess, 2001; Van der Werf, et al., 2003). Further support for the Aggleton and Brown model of episodic memory comes from fMRI studies which consistently report activations in the hippocampus, the dorsolateral and anterior prefrontal cortex, and posterior cingulate/retrosplenial cortex (Fenker, et al., 2005; Henson, Rugg, et al., 1999; Jager, et al., 2009; for a review see Skinner & Fernandes, 2007; Wheeler & Buckner, 2004; Woodruff, et al., 2005). But those studies did not investigate the connectivity of those structures. In contrast, a study by Daselaar, Fleck, Dobbins, et al. (2006) showed recollection-related activity in a hippocampal-retrosplenial/parietotemporal network which is attenuated by aging. This deficit is compensated by shifting to a familiarity-related rhinal-prefrontal network, which has been suggested because older adults showed significantly greater correlations than younger adults between rhinal cortex and both left and right PFC regions. These results point to the existence of at least two different networks of interconnected brain regions in recognition memory and show that the recollection network can be disrupted by normal aging whereas the familiarity network function is maintained. A connectivity study by Habib, McIntosh, Wheeler, & Tulving (2003) investigated the correlations of the hippocampus with other brain regions during encoding of either situationally novel (encountered for the first time at encoding) or situationally familiar (encountered twice before earlier in the experiment) items. The authors could show large-scale neural networks that distinguish between the encoding of situationally novel and situationally familiar items but the same region of the hippocampus participated in those different networks. This would support an overlapping function of the hippocampus in novelty detection as well as context dependent familiarity during encoding.

The meta-analysis by Skinner and Fernandes (2007) points to the lack of connectivity studies concerning recollection vs. familiarity processes at the stage of memory retrieval. They propose that the connection between frontal, parietal areas and hippocampus may be stronger during recollection than during familiarity responses.

They consider it also possible that familiarity is associated with stronger connections between frontal, parietal and perirhinal regions. Further studies have to prove these assumptions.

An interesting study that may additionally lead to a hypothesis about a recollection network, could show that the hippocampal formation is functionally correlated with the inferior parietal lobule, retrosplenial cortex extending into posterior cingulate and precuneus, medial prefrontal cortex, superior frontal cortex, and lateral temporal cortex extending to the temporal pole in a resting state task (Vincent, et al., 2006). The authors then reanalyzed the data of two recollection vs. familiarity studies (Shannon & Buckner, 2004; Wheeler & Buckner, 2004). This analysis revealed that the resting state network reported above showed a strong relation to recollection responses. Thus, one could hypothesize that the brain activations which are independently related to recollection also work in concert in as a recollection-network.

In line with this, the so called Default Mode Network (DMN), a specific network of conjointly fluctuating brain regions in the resting stage, offers the chance to reveal some functional networks involved in recognition memory, because there is striking overlap between the DMN network and commonly recognition related structures (Vincent, Kahn, Snyder, Raichle, & Buckner, 2008; Vincent, et al., 2006). Thus, the presence of such a correlation in the DMN (as coherent spontaneous activity between brain regions) would establish a functional relationship which could be available in other functional states, too.

In line with this, Buckner, Andrews-Hanna, & Schacter (2008) have obtained results that suggest that the Precuneus/posterior Cingulate Cortex (pC/PCC), medial PFC and the bilateral intraparietal lobule (IPL), together constitute a "core hub" in the DMN. Additionally, the only interactions between the medial temporal lobes and the rest of the default mode network seem to be between the left MTL and the pC/pCC and the left temporal cortex, respectively (Fransson & Marrelec, 2008; Vincent, et al., 2006). Interestingly, a recent fMRI study has demonstrated reduced functional connectivity between the precuneus/PCC and the MTL in patients with amnesic mild cognitive impairment (Sorg, et al., 2007; see also Zhou, et al., 2008). The precuneus is a major association area and has wide-spread connections to other cortical and subcortical areas that may subserve a variety of behavioral functions including episodic memory retrieval (for a review see Cavanna & Trimble, 2006).

Overall, functional connectivity of brain regions during recognition memory remains unclear. Particularly, the question whether the different processes of

recollection and familiarity can be detected in different, possibly overlapping, functional brain networks has to be solved.

2.1.2.3.6 Summary

The recollection-familiarity debate is not solved until now. Brain activation studies using fMRI BOLD reaction alone cannot contribute to a concluding answer of this issue. I suppose that the analysis of functionally different networks of interconnected brain regions associated with either recollection or familiarity may shed further light into the question whether recollection and familiarity are based on different brain circuitries or not.

Additionally, several studies showed that recollection and familiarity can be systematically dissociated by other variables like level of processing, priming, age and the associated decline of executive functions, benzodiazepine administration and divided attention (Bugaiska, et al., 2007; Gardiner, et al., 2002; Yonelinas, 2002). In contrast, a study suggesting that recollection is not a threshold process, but rather shows different grades of confidence, showed that these different stages of recollection is also influenced by age (Simons, Dodson, Bell, & Schacter, 2004).

Thus, the search for more variables, which are assumed to highly influence hippocampal dependent processes and therefore have impact on episodic memory, could further contribute to the recollection-familiarity debate. It can be hypothesized that variables which influence memory consolidation in the hippocampus should differently contribute to recollection based processes as compared to familiarity. One of such variables is the Brain Derived Neurotrophic Factor (BDNF), which has already been linked to hippocampal function (Egan, et al., 2003). BDNF is involved in the development and maintenance of synaptic plasticity mainly in the hippocampus and is crucially involved in Long Term Potentiation LTP (Bramham & Messaoudi, 2005). Therefore in the next chapters, a closer look on those molecular processes will follow.

2.1.3   Molecular Mechanisms of Learning and Memory

During the last decades, intensive research has taken place to uncover the mechanisms involved in learning and memory processes. It is now clear that those essential features of human, as well as other vertebrate and invertebrate,

life result from experience-dependent alterations in synaptic transmission which is called synaptic plasticity. One of the basic principles that enable synaptic plasticity in the human brain is supposed to be the mechanism of Long Term Potentiation (LTP; Bear & Malenka, 1994; Lynch, 2004). Because this thesis is concerned with hippocampal-dependent memory, a closer look at synaptic plasticity in the hippocampus which is prototypically for LTP at almost every excitatory synapse in the brain (Malenka & Bear, 2004). Subsequently in chapter 2.2, we specifically address the role of the neurotrophin BDNF as a possible prominent modulating factor in LTP.

In 1973, Bliss and Lomo (1973) found that high-frequency electrical stimulation (HFS) of the perforant path fibers which lead to the dentate area of the hippocampus produced a long-lasting enhancement in the strength of the stimulated synapses. The authors suggested that a) an increase in the efficiency of synaptic transmission at the presynaptic perforant path terminals and b) an increase in the excitability of the postsynaptic granule cells in the dentate gyrus are responsible for this LTP. What we know today basically validates the findings of Bliss and Lomo (1973). The three characteristics of LTP, *cooperativity, associativity* and *input specificity*, described by Bliss and Collingridge (1993), strongly lead to the conclusion that LTP serves as neurobiological substrate for learning and memory. *Cooperativity* means that several synapses must be active simultaneously to cause spatial summation of EPSPs in the postsynaptic neuron. Thus, the postsynaptic neuron is sufficiently depolarized to induce LTP when sufficient stimulation of the presynaptic axon terminal supervenes (*association*). This sufficient postsynaptic depolarization additionally is achieved when the synapses are stimulated at frequencies high enough to cause temporal summation of EPSPs. Finally, LTP is *input specific* because other inputs that did not receive titanic stimulation and were not active at this time do not show a synaptic potentiation. There are several other features of LTP which are very similar to characteristics of memory (Lynch, 2004).

First, LTP most easily occurs in the hippocampus, an area of the brain known to be important in memory acquisition and retrieval (Eichenbaum, 2000; Eichenbaum, Otto, & Cohen, 1992; Scoville & Milner, 1957; Tulving, 2002) is taken. Second, particularly efficient protocols to induce LTP are "Theta-burst stimulations". In these procedures the HFS-bursts are given at an interburst

interval which mimics the naturally occurring theta rhythm (Bliss & Collingridge, 1993) recorded in the hippocampus and other brain regions during memory related behavior (Kirk & Mackay, 2003; Sato & Yamaguchi, 2003; Sauseng, et al., 2004; Sederberg, Kahana, Howard, Donner, & Madsen, 2003; Seidenbecher, Laxmi, Stork, & Pape, 2003; Wiebe & Staubli, 2001). This could be confirmed by studies showing that theta rhythm in the hippocampus is modulating LTP (Greenstein, Pavlides, & Winson, 1988; Maren, DeCola, Swain, Fanselow, & Thompson, 1994; Orr, Rao, Houston, McNaughton, & Barnes, 2001; Pavlides, Greenstein, Grudman, & Winson, 1988).

For the description of the molecular mechanisms of LTP it will be focused on the well established NMDA (*N*-methyl-D-aspartate) receptor-dependent LTP in the hippocampus. Briefly, LTP is supposed to consist of 3 stages (Raymond, 2007). The early phase of LTP (E-LTP, LTP 1; Blundon & Zakharenko, 2008; Lynch, 2004; Malenka & Bear, 2004; Raymond, 2007) - that lasts approximately 60 minutes – is realized by a NMDA-dependent postsynaptic Ca^{2+} rise and this activates protein kinase C (PKC) and calcium-calmodulin-dependent protein kinase II (CaMKII) (for a detailed description see Lynch, 2004). The activation of the protein kinases lead to a) phosphorylation of the AMPA receptor which results in an enhanced effectiveness of this receptor and/or b) the insertion of entirely new AMPA receptors into the postsynaptic membrane. It remains unclear whether presynaptic changes contribute to E-LTP (Malenka & Bear, 2004; Zakharenko, et al., 2003). There is some evidence that the neurotrophin BDNF (Brain Derived Neurotrophic Factor) may contribute to such a process as a retrograde messenger (Poo, 2001; but see Zakharenko, et al., 2003 for an opposing demonstration). Additionally, recent data have shown that LTP in the CA-region of the hippocampus consists of the rapidly developing postsynaptic component and a slowly developing presynaptic component (Bayazitov, Richardson, Fricke, & Zakharenko, 2007). Late-phase LTP is believed to mimic the processes involved in memory consolidation.

This phase of synaptic strengthening requires protein synthesis (LTP2) and a change in gene transcription (LTP3; Raymond, 2007). Protein translation in LTP2 is performed from pre-existing messenger RNA (mRNA) found in the dendrites of most neurons, whereas protein synthesis in LTP3 requires new gene transcription (Raymond, 2007). The process of gene expression is regulated by transcription

factors like cAMP response element binding protein (CREB), which is activated by protein kinase A (PKA), CaMKIV, and mitogen-activated protein kinase (MAPK). Morphological changes that have been reported to accompany late LTP include growth of new dendritic spines, enlargement of preexisting spines and their associated postsynaptic densities (PSDs), which possibly already occurs during E-LTP (Lynch, Rex, & Gall, 2007), and the splitting of single PSDs and spines into two functional synapses (for reviews see Lynch, 2004; Malenka & Bear, 2004). Those changes are supposed to account for the observed long-term strengthening of synapses and therefore may contribute to the consolidation of memories from short-term to long-term memory.

There are multiple modulators on molecular levels that are able to potentiate or impair LTP processes. Among the most prominent factors is the neurotrophin BDNF. The next chapter contains a description of BDNF and its genetic variation followed by an introduction of BDNF as a modulator of LTP.

2.2 The Brain Derived Neurotrophic Factor (BDNF)

2.2.1   BDNF is a member of the neurotrophin family

A possible candidate for an effect on recollection but not familiarity is the function of the Brain Derived Neurotrophic Factor (BDNF). BDNF belongs to the neurotrophin (NT) family, which also includes nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), neurotrophin-6 (NT-6), and neurotrophin-7 (NT-7). Neurotrophins are signaling molecules that are critical in the development and the function of the vertebrate nervous system by influencing the proliferation, differentiation, plasticity, and survival of neuronal cells (for a summary see Monk, et al., 2002). The mature active forms of NTs are very stable non-covalently associated homodimers with highly conserved residues. A dimer is a chemical or biological bond of two similar subunits, which are called monomers, when those subunits are identical they form a homodimer. The term non-covalently means that the two monomers do not share electrons with each other when they bond together. The residues enable the formation of heterodimers which seems to be an essential requisite for NT receptor activation (for reviews see Ibanez, 1998; Murer, Yan, & Raisman-Vozari, 2001).

The neurotrophins bind to two different receptors, the tropomyosin-related kinase (Trk) receptor type and the p75 neurotrophin receptor (p75^{NTR}). All neurotrophins bind to the low-affinity p75^{NTR} receptor, but there are three types of the high affinity Trk receptor (TrkA, TrkB, and TrkC). NGF binds specifically to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC (Murer, et al., 2001). The structure of Trk receptors includes a so called transmembrane region, which is an extracellular portion involved in NT binding, and an intracellular portion with protein-tyrosine kinase activity. Tyrosine kinase, similar to other protein kinases, is able to transfer a phosphate group (through splitting from Adenosine triphosphate, ATP) to a protein and thus modulates the function of that protein (phosphorylation). The extracellular region of Trk receptors includes two immunoglobulin-like domains (Schneider & Schweiger, 1991), one of which is essential for NT binding (Ultsch,

et al., 1999). Neurotrophins bind as dimers to Trk receptors, leading to activation of their catalytic tyrosine kinase domains.

The dimerized Trk receptors autophosphorylate several key intracellular tyrosine residues and rapidly initiate intracellular signaling cascades which could account for the multiple molecular functions of BDNF including the modulation of LTP (Bath & Lee, 2006; Murer, et al., 2001).

Among the known neurotrophins in humans BDNF is the most highly expressed in the cortex, the limbic structures, the hippocampus, and the cerebellum (Monk, et al., 2002; Murer, et al., 2001). BDNF mediated activation on Trk receptors influences cell survival, axonal outgrowth, dendritic growth, and BDNF is the only NT that leads to synaptic plasticity (Bath & Lee, 2006).

2.2.2   The BDNF Gene

2.2.2.1   Transcription

The human BDNF gene is located on chromosome 11 (Maisonpierre, et al., 1991). Liu et al. (2005) report at least seven noncoding and 1 coding exons. The non-coding exons each have at least one promoter region, resulting in 7 distinct transcript classes, which are formed when transcription is initiated at either exons I, II, III, IV, V, VI, or VII and the donor site of each of these exonic sequences is spliced to the major coding exon VIII acceptor site. There is evidence that the alternative transcripts are differentially distributed across the brain, in different cell types and even within different parts of the neuron. Thus, it is functionally important which transcripts are activated. For example, exon III transcripts are detected only in cell bodies, whereas exon IV transcripts are present in cell bodies and dendritic processes of visual cortex neurons in the rat (Pattabiraman, et al., 2005). Transcription through promoter III is suggested to be highly responsive to neuronal activity and is therefore implicated in synapse development as well as learning and memory (West, et al., 2001).

Thus, the gene displays a wealth of complexity due to (a) use of alternative promoters, (b) use of alternative splice donor and acceptor sites that produce between- and within-exon patterns of alternative splicing, and (c) use of alternative polyadenylation sites.

A frequent single nucleotide polymorphism (SNP) resulting from a replacement of the base Guanine by Adenine at nucleotide 196 (G196A, dbSNP number rs6265) has been identified in the human BDNF gene producing an amino acid substitution (valine to methionine) at codon 66 in the prodomain of the BDNF protein (val66met) (Egan, et al., 2003). This sequence variant is located in the 5' pro-BDNF sequence, which encodes the precursor peptide (pro-BDNF) that is proteolytically cleaved to form the mature BDNF protein (Seidah et al., 1996). Egan et al. (2003) could show that this SNP, though located in the 5' pro-BDNF sequence, and thus unlikely to alter the intrinsic biological activity of the mature protein, affects intracellular processing and secretion of BDNF, leading to impairments in hippocampal function in humans. The prodomain of the BDNF protein is controlling dendritic trafficking and synaptic localization in neurons. The Met substitution leads to substantial defects in cellular transport (trafficking): (1) decreased variant BDNF distribution into neuronal dendrites, (2) decreased variant BDNF targeting to secretory granules, and (3) subsequent impairment in regulated secretion (Chen et al., 2005; Chen et al., 2004; Egan et al., 2003). Additionally Met carriers are supposed to exhibit decreased dendritic complexity, fewer neuronal and supporting cells, and increased cell death or decreased neurogenesis during embryological development or over the lifespan (Bath & Lee, 2006). In line with this, studies of brain morphometry repeatedly report smaller hippocampal volumes in Val/Met individuals (Pezawas, et al. 2004; Szeszko, et al. 2005).

2.2.2.2 Trafficking and secretion

The BDNF transcripts are translated into proBDNF in the endoplasmic reticulum of the cell. ProBDNF is then folded in the trans-Golgi and packaged into secretory vesicles in the soma in direct proportion to the level of its mRNA (Murer, et al., 2001).

There it can be sorted into either the constitutive (spontaneous release) or the regulated (release in response to stimuli) secretory pathway, the latter occurring more frequently (Mowla, et al., 1999; Poo, 2001). BDNF-containing vesicles are trafficked to postsynaptic neuronal dendrites and spines, as well as to presynaptic axons and terminals. However, the synaptic level of the NT may also be regulated by local translation of BDNF mRNA (Poo, 2001). Dendritic trafficking and synaptic

localization are controlled by BDNF's pro-domain, particularly in the region including the Val66Met SNP ('box2/3'; Chen, et al., 2005; Egan, et al., 2003). Crucially, this region has a key role in activity-dependent BDNF secretion. Chen et al. (2005) demonstrate that the interaction of BDNF with sortilin, a newly identified neurotrophin receptor, is markedly reduced by the presence of the 66Met allele. From this data one could educe that the BDNF Val66Met polymorphism is able to modulate LTP processes in the hippocampus and other cortical areas and hence may contribute to individual differences in memory performance.

2.2.3   The role of BDNF in synaptic plasticity and hippocampal-dependent learning

BDNF is sorted to the regulated pathway of secretion in neurons (Poo, 2001). This means, besides the spontaneous secretion, the secretion of the NT can occur in response to external stimuli. Thus, BDNF release can be induced by depolarization with high potassium in a calcium-dependent manner (Goodman, et al., 1996). Other studies by Balkowiec and Katz (2000) and Blochl and Thoenen (1995) could show that depolarization induced by veratridine, a steroid-derived alkaloid that activates sodium ion channels, glutamate or patterned electrical stimulation results in an elevated level of secreted and/or surface-bound NTs in hippocampal slices or dissociated cell cultures.

Interestingly, the magnitude of BDNF release from cultured sensory neurons triggered by electrical stimulation was most effective with high-frequency bursts (Balkowiec & Katz, 2000). Finally, BDNF-induced secretion of BDNF and other NTs can occur (Berninger, Garcia, Inagaki, Hahnel, & Lindholm, 1993; Canossa, et al., 1997; Kafitz, Rose, Thoenen, & Konnerth, 1999; Kruttgen, Moller, Heymach, & Shooter, 1998; Stoop & Poo, 1996). This is mediated by an elevation of intracellular calcium concentration resulting from BDNF–TrkB signaling in the cell or direct membrane depolarization induced by BDNF which links BDNF function to LTP

The BDNF protein is highly involved in activity dependent synaptic plasticity in the hippocampus and therefore contributes to both early and late Long Term Potentiation (E-LTP, L-LTP; Lu, Christian, & Lu, 2008; Poo, 2001). It is known that BDNF and its tyrosine receptor kinase B (TrkB) receptor are widely distributed

across subregions of the hippocampus and the adult forebrain (Bramham & Messaoudi, 2005; Murer, et al., 2001).

NT expression is sensitive to electrical activity. Seizure activity induces a rapid increase in messenger RNA levels of BDNF and other NTs in the hippocampus and the cerebral cortex (Ernfors, Bengzon, Kokaia, Persson, & Lindvall, 1991; Zafra, Hengerer, Leibrock, Thoenen, & Lindholm, 1990). Normal physiological activity that is capable of inducing long-term potentiation (LTP) also increases the level of BDNF mRNA in the hippocampus (Castren, et al., 1993; Patterson, Grover, Schwartzkroin, & Bothwell, 1992). Thus, brief depolarization (or spiking) of the presynaptic neuron in the presence of low BDNF concentration resulted in a marked potentiation of spontaneous and evoked transmitter secretion mediated by an elevation of cAMP levels (Boulanger & Poo, 1999a; Boulanger & Poo, 1999b). Moreover, Du, Feng, Yang, & Lu (2000) could show that high-frequency neuronal activity and synaptic transmission elevate the number of the BDNF receptor TrkB on the surface of cultured hippocampal neurons.

Additionally, BDNF affects the continued survival and functional differentiation of the neurons. Thus, BDNF increases the synthesis of ACh and neuregulin in spinal cord neurons (Loeb & Fischbach, 1997) and the expression of neuropeptides (Nawa, Pelleymounter, & Carnahan, 1994) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) subtypes of glutamate receptors (Narisawa-Saito, et al., 2002) in the neocortex of rats. These effects of NTs on gene regulation and protein synthesis resulting in synapse development are called long-term trophic actions. Such trophic actions are also reflected by NT-induced changes in intrinsic neuronal excitability (Lesser, Sherwood, & Lo, 1997; Rudy, Kirschenbaum, Rukenstein, & Greene, 1987; Sharma, D'Arcangelo, Kleinlaus, Halegoua, & Trimmer, 1993).

Neurotrophins are known to participate on the acute synaptic modification in the nervous system, too. BDNF modifies the transmitter release by triggering an up to ten-fold increase in cytoplasmic calcium (Berninger, et al., 1993), suggesting the presence of functional receptors for BDNF in rat hippocampal neurons. Pozzo-Miller et al. (1999) found a BDNF induced enhancement of the efficacy of presynaptic vesicle exocytosis. Finally, NTs may also act as transmitters themselves (Kafitz, et al., 1999).

To summarize, BDNF influences the acute synaptic modification, promotes long-term potentiation (LTP) by a presynaptic enhancement of synaptic transmission during high-frequency stimulation (HFS), and participates in the survival and functional differentiation of the neurons and their synapses,

All together these functions may contribute to neuronal and synaptic plasticity, and therefore to learning and memory.

2.2.4   The BDNF Val66Met Polymorphism and Declarative Memory

There is strong evidence that human carriers of the Met allele show poorer hippocampal-dependent memory performance, because the Met substitution leads to substantial defects in cellular transport of BDNF and this might lead to less efficient LTP in the hippocampus (Bramham & Messaoudi, 2005; Egan, et al., 2003), hence deficient memory consolidation. Studies using the Wechsler Memory Scale (WMS-R) delayed and immediate recall find lower scores in Met/Met carriers compared with homozygote Val/Val subjects (Dempster, et al. 2005; Egan, et al. 2003). Similarly, in recognition memory paradigms BDNF seems to have an effect on performance, such that Val/Val carriers show higher correct identified old words, correct rejections (Hariri, et al., 2003), and a higher d' (Goldberg, et al. 2008). However, a study by Hashimoto et al. (2008) could not find an effect on the performance in the recognition of complex scenes. Additionally, recall of words as measured by the California Verbal Learning Test (CVLT), did not show an influence by BDNF polymorphism (Egan, et al. 2003).

At a brain level, an fMRI study by Egan et al. (2003) found an abnormal pattern of hippocampal deactivation during a working memory task in healthy Val/Met individuals. Two similar studies (Hariri, et al., 2003; Hashimoto, et al., 2008) found Val66Met polymorphism influences mainly on encoding activity in the hippocampal regions than on retrieval activity during a recognition task, such that memory related hippocampal activity was greater in Val/Val carriers. 30% of total variation in recognition memory performance was accounted by BDNF genotype modulation of hippocampal engagement during encoding. But these studies did not differ between recollection and familiarity based recognition. It can be hypothesized, if BDNF Val66Met Genotype modulates the encoding of items

through effects on E-LTP in the hippocampus, then recollection answers in a subsequent recognition test must be more influenced by the genotype than familiarity answers. This dissociation possibly can account for the mixed results of BDNF influences on recognition performance found in the literature (Hashimoto, et al., 2008). Furthermore, a dissociation between recollection and familiarity by a genetic influence may further support a dual process model of recognition memory.

2.3 The Neurotransmitter Serotonin and its Role for Learning and Memory

Serotonin (5-Hydroxytriptamine, 5-HT) is one of the most extensively investigated neurotransmitters to date. This results from its importance for multiple biological and behavioral systems in humans (e.g. Bockaert, Claeysen, Becamel, Dumuis, & Marin, 2006; Carver & Miller, 2006; Cavallaro, 2008; Chaouloff, 2000; Costedio, Hyman, & Mawe, 2007; Cote, Fligny, Fromes, Mallet, & Vodjdani, 2004) as well as from the finding, that some genetic variations, e.g. in the serotonin transporter gene, have great impact on cognitive and emotional processes which has given great insight in serotonin function (e.g. Canli & Lesch, 2007; Murphy & Lesch, 2008). Specifically, there is evidence for an association between serotonin function and memory processes (e.g. Cavallaro, 2008; Jeltsch-David, Koenig, & Cassel, 2008; Meneses, 2007).

Following this, and given that there is growing evidence for an epistasis between the BDNF Val66Met and a serotonin transporter polymorphism (5-HTTLPR; Kaufman, et al., 2006; Mossner, et al., 2000; Pezawas, et al., 2008; Savitz & Drevets, 2009), an analysis of serotonin function in interaction with BDNF function was included in the current thesis, too. In the following sections a closer look on serotonergic neurotransmission and on a genetic variation in the serotonin transporter is given together with a description of serotonin function and its modulation of memory processes with an emphasis on the serotonin transporter.

2.3.1 Serotonergic system and neurotransmission

In addition to its importance for the regulation of the adrenal medulla, the gastrointestinal tract, the cardiovascular system, thermoregulation, and respiration (Aleksandrin, Tarasova, & Tarakanov, 2005; Cote, et al., 2004; Kato, Fujiwara, & Yoshida, 1999; Zifa & Fillion, 1992), most importantly for the current thesis is the serotonergic activity in the central nervous system (CNS; Hensler, Ferry, Labow, Kovachich, & Frazer, 1994; Jacobs & Azmitia, 1992; Whitaker-Azmitia, 2001; Whitaker-Azmitia, Shemer, Caruso, Molino, & Azmitia, 1990; Zifa & Fillion, 1992).

Serotonin is an amine-neurotransmitter (more specifically an indoleamine), is derived from the amino acid tryptophan and produced in the raphe nuclei in the brain stem (Bear, et al., 2007).

Serotonergic neurons project to the thalamus, the basal ganglia, hypothalamus, neocortex, cingulate gyrus, hippocampus, and amygdala (see Figure 2-6; Hensler, et al., 1994; Rosenzweig, Breedlove, & Watson, 2005). This accounts for the numerous functions of serotonin in the human CNS. Serotonergic function comprise the maintenance of the circadian rhythm, sleep states, appetite, aggression, sensorimotor activity, sexual behavior, mood, cognition, learning and memory (Rosenzweig, et al., 2005; Vizi, 2008).

Neurotransmission of serotonin is regulated by seven 5-HT receptor families (5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7) and several 5-HT receptor subtypes which are engaged in pre- or post-synaptic complexes (Bockaert, et al., 2006; Hannon & Hoyer, 2008).
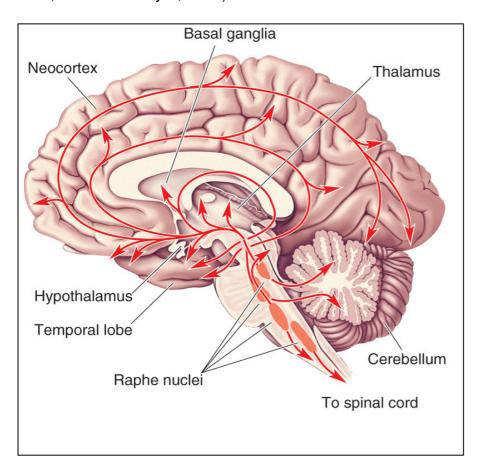
Figure 2-6: The serotonergic system in the brain. Distribution of serotonergic neurons (Figure reprinted with permission from Rosenzweig, Breedlove, & Watson, 2005).

Most 5-HT receptors are of the metabotropic, G-protein-coupled family, except the 5-HT3 receptor, which is a ligand-gated ion channel, ionotropic receptor. In contrast to the fast ionotropic receptor actions, the activation of G-protein-coupled receptors (GPCRs) leads to slower, long lasting and much more diverse synaptic outcome by triggering enzymes (e.g. protein kinases) that synthesize molecules (e.g. cAMP) which serve as so called second messengers. Second messengers can alter cellular metabolism and activate gene transcription which leads to the syntheses of new proteins (Bear, et al., 2007). The 5-HT1 receptor family can be characterized by its inhibitory effect on cellular cAMP levels (Vizi, 2008). 5-HT2A receptor subtype has been shown to activate second-messenger cascades responsible for the reduction of BDNF levels in the hippocampus (Vaidya, Marek, Aghajanian, & Duman, 1997), furthermore mutant mice with low levels of BDNF expression displayed a deficit in 5-HT2A receptor expression in the raphe nuclei and PFC (Rios, et al., 2006). The ionotropic receptor 5-HT3 is supposed to modulate the neurotransmission of various other neurotransmitters. For example, in the hippocampus, activation of the 5-HT3 receptor enhances, whereas 5-HT1A receptor inhibits 5-HT release (Martin, Hannon, Phillips, & Heal, 1992). The 5-HT4, 5-HT6 and 5-HT7 receptors are positively coupled to adenylate cyclase, an enzyme which is known to generate the second messenger cAMP, and enhances neuronal excitability (Vizi, 2008). In contrast, 5-HT5A receptor subtype may be negatively coupled to adenylate cyclase (Francken, Jurzak, Vanhauwe, Luyten, & Leysen, 1998; Hurley, et al., 1998).

After release of 5-HT, the serotonin transporter (5-HTT) presynaptically removes serotonin from the synaptic cleft (Rosenzweig, et al., 2005). By regulating the magnitude and duration of serotonergic responses, the 5-HT transporter is central to the modulation of brain serotonergic neurotransmission (Reith, 2002).

2.3.2 The 5-HT Transporter-linked Polymorphic Region (5-HTTLPR)

The gene of the serotonin transporter (SLC6A4) lies on chromosome 17q11.2 (Lesch, 2001; Lesch, Wolozin, Estler, Murphy, & Riederer, 1993). Transcriptional activity of the human 5-HT transporter gene is modulated by a polymorphic

repetitive element (5-HTT-linked polymorphic region, 5-HTTLPR) located upstream of the transcription start site.

At this position, Heils et al. (1996) found a length variation of a 43bp (base pair) insertion/deletion polymorphism consisting of two common variants. The deletion of the 43bp sequence results in 14 repeat elements (short allele, s), whereas the insertion results in 16 repeat elements. Carriers of the short allele (s-carriers) of the 5-HTTLPR display reduced functional capacity of serotonin transporters compared to homozygous carriers of the long allele (l/l; Lesch, et al., 1996). Most prominently, 5-HTTLPR short allele is related to anxiety related personality traits (Lesch, et al., 1996; Sen, Burmeister, & Ghosh, 2004) and depression (Brown & Harris, 2008; Caspi, et al., 2003; Savitz & Drevets, 2009). Additionally, there is growing evidence, that a gene-gene interaction with BDNF Val66Met is related to individual differences in vulnerability to depression and response to antidepressant treatment (Bocchio-Chiavetto, et al., 2008; Kaufman, et al., 2006; Kim, et al., 2007).

2.3.3 Serotonergic Neurotransmission and Memory Function

5-HT is an important regulator of brain development and plasticity (cell proliferation, migration, differentiation, and synaptogenesis; Frodl, et al., 2004; Frodl, et al., 2008; Gould, 1999; Sodhi & Sanders-Bush, 2004). Therefore, it can be assumed that 5-HT plays a major role in several brain functions which are dependent on synaptic plasticity, like learning and memory. Additionally, there is strong evidence that serotonin plays a critical role in the pathogenesis of several neuropsychiatric diseases, including depression and anxiety disorders (Leonardo & Hen, 2006; Lesch, 2001; Lesch, et al., 1996; Ressler & Nemeroff, 2000), which are also known to be accompanied by, possible stress related, deficits in learning and memory (Becker, Macqueen, & Wojtowicz, 2009; Brewin, 2001).

Although the relation between serotonin and memory is not as clear as with the BDNF, numerous studies have linked serotonergic neurotransmission to memory processes and morphological changes in brain structures known to be involved in

memory (Cavallaro, 2008; Frodl, et al., 2004; Frodl, et al., 2008; Meneses, 2007; Molodtsova, 2008; Perez-Garcia & Meneses, 2008).

First, 5-HT receptors occur in brain regions which are known to be involved in learning and memory, such as hippocampus (declarative memory), basal ganglia (procedural memory) and amygdala (emotional memory). For instance, the 5-HT2A, 5-HT4, 5-HT6 and 5-HT7 receptor subtypes are highly expressed in the hippocampus (Vizi, 2008). Second, subtypes of the 5-HT 1, 2, and 4 receptors were found to decrease in aging and Alzheimer's disease (AD), states which are related to hippocampal-dependent memory decline (for a summary see, (Meneses, 1999; Vizi, 2008). There is further evidence for an association of 5-HT1A receptor with learning and memory as receptor knock-out mice exhibited poorer performance in the Morris water maze and Y-maze than wild-types (Sarnyai, et al., 2000). More importantly, blockade of rat hippocampal 5-HT1A receptors produced a rapid increase in phosphorylated $Ca^{2+/}$calmodulin-dependent protein kinase II (CaMKII) and in Ca^{2+}-independent CaMKII and protein kinase A (PKA) enzyme activity. This increase was followed a few hours later by an enhanced membrane expression of AMPA receptor subunits. Those processes are critically involved in early LTP and the findings strongly suggest a relation between 5-HT1A receptor binding and LTP (Schiapparelli, Del Rio, & Frechilla, 2005). In line with this, Yasuno et al. (2003) found that the activation of 5-HT1A receptors in the hippocampal formation have a negative influence on explicit memory function as measured with the WMS-R.

Additionally, 5-HT2C receptor knock-out mice showed a significant impairment in the generation of perforant path - dentate gyrus LTP (Tecott, Logue, Wehner, & Kauer, 1998), whereas 5-HT3 receptor activation inhibits LTP in the rat hippocampus (Passani, Pugliese, Azzurrini, & Corradetti, 1994). 5-HT4 receptor may also enhance hippocampal LTP through a cAMP-dependent mechanism (Chapin, Haj-Dahmane, Torres, & Andrade, 2002) and 5-HT7 receptor is believed to increase neuronal activity in the hippocampus (Bacon & Beck, 2000; Tokarski, Zahorodna, Bobula, & Hess, 2003).

Studies that investigated expression of the 5-HT receptor genes in the rat hippocampus after learning in the Morris water maze and passive avoidance training demonstrated that 5-HT receptor subtypes showed significant changes in

gene expression (Cavallaro, D'Agata, Manickam, Dufour, & Alkon, 2002; D'Agata & Cavallaro, 2003).

Another group (Molodtsova, 2008) demonstrates that 5-HT is involved in the retrieval of a conditioned response rather than in its acquisition. The authors refer to a conditioning-related reduction in postsynaptic 5-HT receptor binding in the amygdala, periaqueductal gray matter, and striatum, whereas no changes have been seen in the hippocampus or prefrontal cortex. This is not surprising, as the neither the hippocampus nor the PFC are necessarily involved in classical conditioning. Nevertheless, Molodtsova's findings may suggest that emotional memory retrieval, which is modulated by amygdala activity, is crucially dependent on serotonin function. Additionally, an interesting study investigating avoidance training in rats suggests that down-regulation of the 5-HT in the limbic system, i.e., a reduction of the hippocampal 5-HT concentration and of amygdala 5-HT1A receptor expression, may be involved in the enhanced fear memory, possibly reflecting a blunted serotonergic inhibition in the brain limbic system (Chen, Lin, et al., 2008).

This may have implications for pathologies that are characterized by a disturbed emotional memory like Posttraumatic Stress Disorder (PTSD).

Studies in subjects using 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy"), which has been shown to damage brain serotonin (5-HT) neurons, more precisely the serotonin transporter in animals and in humans (McCann, Szabo, Scheffel, Dannals, & Ricaurte, 1998; Semple, Ebmeier, Glabus, O'Carroll, & Johnstone, 1999), point to long-lasting memory deficits, for example in the recall of verbal items, following extensive MDMA use (Reneman, Booij, Schmand, van den Brink, & Gunning, 2000; Reneman, et al., 2001; Reneman, et al., 2006).

With respect to the 5-HT transporter, some studies suggest a decrease in 5-HT uptake or 5-HT transporters mRNA in aging and Alzheimer's disease, but conversely 5-HT uptake inhibitors (SSRIs) have a facilitating effect in learning consolidation in patients with depression (Meneses, 1999). The 5-HTT shows high density in the hippocampus, as measured through SSRI [^{3}H] paroxetine yielded binding (Laruelle, Vanisberg, & Maloteaux, 1988), indicating an influence on hippocampal function.

In addition, 5-HT transporter (SERT) knockout rats (SERT(-/-) and SERT(+/-) showed impaired object memory, whereas SERT(+/+) rats showed intact object

memory in a recognition task (Olivier, et al., 2009). In accordance with animal studies, MDMA users show less 5-HT transporter in the hippocampus, the DLPFC, the parietal cortex, the posterior cingulate cortex, and the temporal cortex, and 5-HTT reduction in DLPFC and parietal cortex was associated with poorer performance on a variety of verbal memory tasks in the Wechsler Memory Scale-III, including recall (WMSIII) (McCann, et al., 2008).

Only a few studies that investigate the association between the 5-HTTLPR polymorphism and learning and memory function could be identified. In a fear conditioning paradigm with human healthy subjects, participants with 5-HTTLPR s allele displayed better acquisition, but not extinction, as measured by skin conductance response (Garpenstrand, Annas, Ekblom, Oreland, & Fredrikson, 2001). Given that fear conditioning in humans strongly depends on amygdala function, this is in accordance with studies that report an increased amygdala activity, as assessed by BOLD functional magnetic resonance imaging, in response to fearful stimuli in carriers of the 5-HTTLPR s-allele, (Hariri, et al., 2005; Hariri, et al., 2002; Munafo, Brown, & Hariri, 2008). Crucially, this effect has been confirmed by the finding that participants with reduced amygdala 5-HTT availability showed enhanced amygdala reactivity (Rhodes, et al., 2007). These results may be related to changes in amygdala morphology in carriers of the s allele, even though the direction of the reported changes remains unclear (Pezawas, et al., 2005; Scherk, et al., 2009). The amygdala is also discussed in terms of modulating hippocampal dependent memory (Roozendaal, McEwen, & Chattarji, 2009), resulting in, for instance, a better memory for emotional as compared to neutral events (Bohannon, 1988; Comblain, D'Argembeau, & Van der Linden, 2005). In line with this, Strange, Hurlemann, and Dolan (2003) and Strange, Kroes, Roiser, Tan, & Dolan (2008) showed that a retrograde amnesia caused by emotional modulation of memory is amygdala-dependent and is more pronounced in humans carrying the s/s allele of the 5-HTTLPR.

In his review Meneses (1999, p. 1120) summarizes "The finding that an increase in 5-HT levels provoking the multiple postsynaptic 5-HT receptors activation, as occurred with 5-HT uptake facilitators and inhibitors, enhances learning suggests that the 5-HT role in cognitive processes is more complex than that representing a simple imbalance."

It is known to date that 5-HT neurotransmission, as reflected by 5-HT receptor binding and gene expression, 5-HTT binding and variations in the 5-HTT gene, is involved in different kinds of learning and memory. Strong evidence has been found with respect to emotional memory and fear conditioning (Garpenstrand, et al., 2001; Hariri, et al., 2002; Marsh, et al., 2006; Rhodes, et al., 2007), evidence for a modulation of the 5-HTTLPR on declarative, more specifically hippocampal-dependent memory is still missing. However, 5-HTTLPR has been found to influence hippocampal morphology (Frodl, et al., 2004; Frodl, et al., 2008). In addition, in interaction with the BDNF Val66Met genotype, 5HTTLPR has been associated with amygdala structure and function (Pezawas, et al., 2008). This leads to the hypothesis that hippocampal morphology and function might also be influenced by an epistatic effect between the serotonin transporter and the BDNF polymorphism. Thus, as a first step the analysis of this interaction with regard to hippocampal volume was included in the current thesis.

2.4 Synopsis of the Theoretical Background

In summary, the findings of neural correlates of recollection and familiarity lead to the assumption that there are different brain regions activated in either process but there are, to the best of my knowledge, no studies assessing how these brain regions are working together in a recollection or a familiarity network, respectively. Additionally, there are almost no studies to date, which directly searched for overlapping regions. Most of the studies that report regions, which are activated in recollection as well as in familiarity, have not statistically substantiated their assumption, for instance by using a statistical masking procedure or a conjunction analysis. Therefore, in study I of the current thesis, brain regions associated with both recognition processes are searched by using a statistical procedure that inclusively masks two brain maps with using a statistical threshold of significance. Additionally a connectivity analysis will investigate functional correlated brain activations that either build a recollection or a familiarity network.

Undoubtedly, the BDNF is strongly involved in synaptic plasticity in the hippocampus and there is evidence that a genetic variant of this neurotrophin is related to poorer memory performance. Inconsistent results were found in studies that associate the BDNF Val66Met with recognition memory. This may be due to the fact that those studies did not dissociate between recollection and familiarity. Therefore, in study II of the current thesis, the effect of BDNF Val66Met on recollection and familiarity performance and related brain activations is investigated. It is strongly assumed that a specific deficit in recollection, hence contextual memory, in carriers of the BDNF 66Met allele may serve as a vulnerability factor for such memory distortions in clinical disorders like PTSD. This issue is not investigated in the current thesis, but it is suggested that future research should follow up the question, if there is a specific influence of BDNF on contextual memory.

Finally, one could summarize, that serotonin, like BDNF, is strongly involved in brain development and plasticity as well as in learning and memory processes. More precisely, there is evidence for alterations in the structure of brain regions, which are known to be involved in emotional memory formation and retrieval, like amygdala and hippocampus.

One study found a slight epistatic effect of BDNF and 5-HTTLPR on the grey matter volume of the amygdala. Therefore, in study III, it is investigated if such an interaction effect could be substantiated for the amygdala and additionally revealed for the hippocampus.

3 HYPOTHESES AND METHODS

3.1 Research Questions and Hypotheses

3.1.1 Study I

The aim of this study was to address the issue of functional connectivity of brain regions during recognition memory in humans. Prior to that, an investigation of uncorrelated brain activations during the two recognition processes was conducted.

3.1.1.1 Question 1: Brain regions related to recollection and familiarity

Can the findings of different brain areas contributing to recollection and familiarity based recognition be replicated?

3.1.1.1.1 *Hypotheses*

It is hypothesized that there are non-overlapping distinct brain regions which are activated either during recollection (correct Remember) based responses or during familiarity (correct Know) based responses.

It is additionally hypothesized that activation of the left lateral parietal lobe and the hippocampus is only related to recollection based responses. (see (Skinner & Fernandes, 2007)

3.1.1.2 Question 2: Overlapping brain regions

Are there overlapping brain regions that are activated to both recollection and familiarity processes?

3.1.1.2.1 *Hypotheses*

It is hypothesized that the right dorsolateral prefrontal cortex and the left precuneus are related to both processes.

3.1.1.3 Question 3: Networks of brain regions related to recollection and familiarity

Are there different, non-overlapping brain networks (brain regions that are functional connected) that contribute distinctively to either recollection or familiarity?

3.1.1.3.1 Hypotheses

There is no specific hypothesis about the brain regions that are part of a recollection or familiarity network, respectively, because of the innovative character of this question. However, it is hypothesized that only a network supporting recollection involves connectivity of the hippocampus.

3.1.2 Study II

In study II, it was aimed to investigate the impact of a genetic variant of the BDNF polymorphism on the recognition of words and on the brain activations underlying recognition based on recollection as compared to familiarity in a sample of healthy subjects.

3.1.2.1 Question 1: Effect of BDNF function on recognition performance

Is there an impact of the BDNF Val66Met polymorphism on the recognition performance?

3.1.2.1.1 Hypotheses

It is hypothesized that carriers of at least one Met allele in the BDNF polymorphism show a poorer performance in correct recognition of old words based on recollection.

It is hypothesized that the BDNF Val66Met polymorphism has no impact on the performance in correct recognition of old words based on familiarity judgments.

3.1.2.2 Question 2: Effect of BDNF function on brain activation

Is there a difference in the activation of brain areas related to recollection as compared to familiarity between carriers of the Met allele and homozygote Val carriers of the BDNF genotype?

3.1.2.2.1 Hypotheses

There is no specific hypothesis about the direction of the BDNF effect on the activation of brain regions which are related to recollection based recognition, because of the innovative character of this question. However, it is hypothesized that there is a higher activation of the hippocampus in carriers of the homozygote Val variant than in carriers of the Met allele during recollection as compared to familiarity.

3.1.3   Study III

In this study it was examined whether there is an interaction between the BDNF Val66Met and 5-HTTLPR polymorphism in respect to the grey matter (GM) volume of hippocampus and amygdala and whether the recently found epistatic effect of the two polymorphisms for the anterior cingulate cortex (ACC) can be replicated.

3.1.3.1   Question 1: BDNF effect on grey matter volume

Are there differences in GM volume between carriers of the BDNF 66Met allele and homozygote BDNF 66Val carriers?

3.1.3.1.1 Hypotheses

It is hypothesized that carriers of the 66Met allele show a reduced GM volume in the hippocampus and amygdala as compared to homozygote carriers of the 66Val allele.

3.1.3.2   Question 2: 5-HTTLPR effect on grey matter volume

Are there differences in GM volume between carriers of the 5-HTTLPR s allele and homozygote carriers of the 5-HTTLPR l allele?

3.1.3.2.1 Hypotheses

It is hypothesized that carriers of two l alleles show a reduced hippocampal volume as compared to carriers of the s allele.

It is hypothesized that carriers of the s allele show a reduced volume in amygdala and ACC GM as compared two carriers of two l alleles.

3.1.3.3 Question 3: Interaction effect between BDNF and 5-HTTLPR

Is there an epistatic effect of BDNF Val66Met and 5-HTTLPR polymorphism with respect to GM volume of amygdala, ACC and hippocampus?

3.1.3.3.1 Hypotheses

It is hypothesized that in carriers of the BDNF 66Met allele there is no difference in the GM volume of the ACC and the amygdala between 5-HTTLPR s and l genotype, whereas in the BDNF 66Val genotype s allele carriers show a reduced volume in those areas as compared to carriers of two l alleles .

It is hypothesized that there is also an interaction effect of BDNF Val66Met and 5-HTTLPR with respect to hippocampal GM volume but there is no hypothesis about the direction of this effect.

3.2 The Remember- Know Task

The Remember-Know (R/K) procedure is a recognition task that differs between subjective judgments of recollection (i.e. respond 'remember' if the item is recognized, because you recollect additional context details of the study event) or familiarity (i.e. respond `know' if the item is familiar and you know it was studied but you cannot recollect anything about the study event) accompanying a recognition process (Yonelinas, 2002). The original R/K recognition test by Tulving (1985) is a two-step procedure. Participants first indicate if an item is old or new and then label each item R or K. This procedure avoids that participants treat R/K judgments as measures of confidence (Hicks & Marsh, 1999) and assures that the K category is not used for guess responses only (Eldridge, et al., 2002). In conclusion, the R/K procedure offers a simple, understandable, and practical measure of recollection and familiarity which is applicable in different study environments. Therefore it has been decided to use this version of the R/K task in the current thesis. The specific procedure used in the current thesis was adapted from Eldridge et al. (2000) and is described in more detail in study I and II as well as in Figure 3-1 and Figure 3-2.
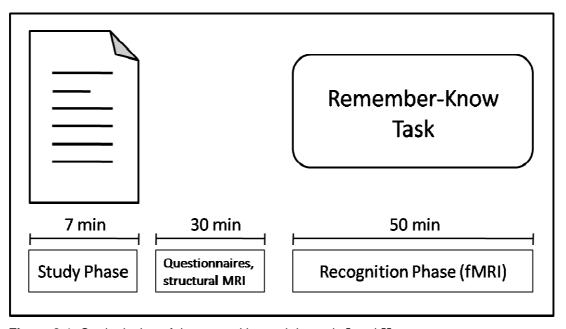
Figure 3-1: Study design of the recognition task in study I and II
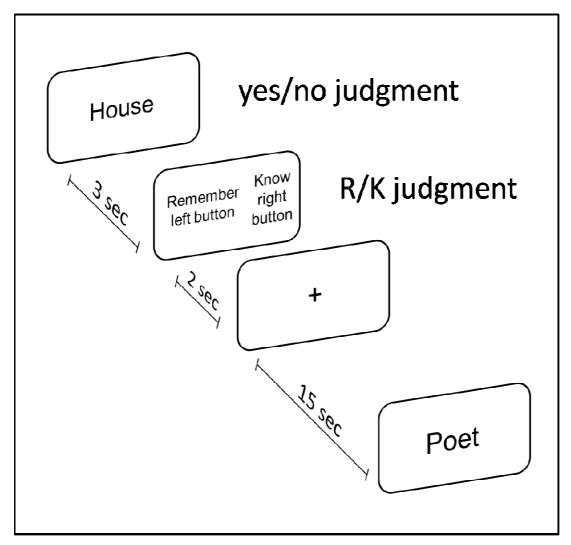
Figure 3-2: Procedure of the recognition phase in study I and II.

3.3 Structural and functional Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is used to visualize the structure and the function of the body, in the neurosciences primarily for measuring brain structure and function. Briefly, MRI uses a strong magnetic field (1.5 up to 9 Tesla) to align the nuclear magnetization of hydrogen atoms in the body. Additional radio frequency (RF) fields are used to systematically alter the alignment of this magnetization. When the RF fields are turned off, the magnetization is emitted back which is detectable by the scanner. This process is repeated for several times and the signal is manipulated by additional magnetic fields to build up enough information to construct an image of the body (e.g. to achieve the exact

coordinates of a signal in the 3 dimensional space). The MRI signal is then via an inverse spectrum analysis transformed into a structural image with different grey scales (Weishaupt, Köchli, & Marincek, 2006). Structural MR images are of very high resolution and are able to cover the whole brain, which is one of the great advantages of MRI over, for instance, Electroencephalography (EEG). Functional MRI (fMRI) measures signal changes in the brain that are due to changing neural activity. The brain is scanned at low resolution but at a rapid rate (typically once every 2–3 seconds). Increases in neural activity cause changes in the MRI signal, this mechanism is referred to as the BOLD (blood-oxygen-level dependent) effect (Thulborn, Waterton, Matthews, & Radda, 1982). Increased neural activity causes an increased demand for oxygen, and the vascular system actually overcompensates for this, increasing the amount of oxygenated hemoglobin relative to deoxygenated hemoglobin. Because deoxygenated hemoglobin attenuates the MRI signal, the vascular response leads to a signal increase that is related to the neural activity (Logothetis, 2003). In combination with a high resolution structural brain image, measuring the BOLD response with fMRI is an excellent tool for the noninvasive imaging of the human brain. Details about the preprocessing and statistical analyses of the BOLD data are described in the methods sections of study I and II. In study III an analysis of brain morphology was used which is called voxel based morphometry (Ashburner & Friston, 2000). Briefly, the structural MRI raw images were normalized to tissue probability maps and segmented into grey matter, white matter, and CSF. The resulting modulated grey and white matter images were then smoothed with a 12 mm Gaussian kernel. The resulting grey and white matter maps represent maps of grey or white matter volume and can be further used in a statistical analysis. All preprocessing and statistical steps for both structural and functional MR images were accomplished with Statistical Parametric Mapping (SPM 5 software package, Wellcome Department of Imaging Neuroscience, Institute of Cognitive Neurology, London).

3.4 Genotyping

Genotyping was achieved using the polymerase chain reaction (PCR). After extraction of the DNA out of buccal cells, PCR amplifies a single or few copies of a piece of DNA generating thousands to millions of copies.

The method consists of thermal cycling, including cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) that contain sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. As PCR progresses, the generated DNA itself is used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified (Hartl & Jones, 2008). The detailed procedure of genotyping with PCR is described in the method sections of study II and III. The genotyping was conducted at the Department of Neurobehavioral Genetics, Institute of Psychobiology, University of Trier.

4 STUDY I: DISTINCT BRAIN NETWORKS IN RECOGNITION MEMORY SHARE A DEFINED REGION IN THE PRECUNEUS

4.1 Abstract

Current models of recognition memory performance postulate that there are two fundamentally distinct retrieval processes: recollection and familiarity. This view has been challenged and little is known from human research about the functional connectivity of brain areas involved in these processes. In our study we used a Remember-Know procedure to assess the functional connectivity of brain regions under recognition memory in 30 healthy adults. Using functional Magnetic Resonance Imaging (fMRI), we analyzed the blood-oxygen-level dependent (BOLD) responses during correct remember, correct know, correct rejection and miss answers of the subjects during recognition of non-emotional nouns. One activation cluster was found in the left precuneus associated with both recollection and familiarity answers. To acquire information about the way in which activity in one brain region modulates activity in another brain region in response to the active task, we performed a psychophysiological interaction analysis (PPI) with the left precuneus as a seed region. This analysis revealed functionally distinct networks of brain areas underlying recollection and familiarity. Furthermore, we discuss the differential involvement of the hippocampus in a recollection network as compared to a familiarity network. In summary, our results further strengthen the assumptions of a dual process view of recognition memory (e.g., Eichenbaum, et al., 2007; Yonelinas, 2001a) and add empirical findings about the functional interconnectivity of brain regions supporting either recollection or familiarity.

4.2 Introduction

The retrieval of previously studied items can either be accompanied by a feeling of *familiarity* or by the additional recollection of some aspects of the study event, such as when or where it occurred (*recollection*, e.g., Gardiner, et al., 2002; Jacoby, 1991; Mandler, 1980; Montaldi, et al., 2006; Wheeler & Buckner, 2004;

Yonelinas, 2002; Yonelinas, et al., 2005). The dual-process signal-detection (DPSD) model by Yonelinas (2001a) assumes that these two processes are fundamentally distinct retrieval processes. Support for the dual process assumption (Yonelinas, 2002; Gardiner, et al., 2002) comes from neuroimaging studies, lesion data and amnesia studies which have identified distinct functional neural networks underlying recollection and familiarity (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Aggleton, et al., 2005; Eichenbaum, et al., 2007; Parks & Yonelinas, 2007; Skinner & Fernandes, 2007).

In a recent meta-analysis Skinner and Fernandes (2007) found a consistently reported network for recollection including left inferior parietal lobe (IPL), prefrontal and superior frontal regions. During familiarity left Brodmann Area (BA) 19 was activated. Despite the activation of distinct brain areas, overlapping activity during both recollection and familiarity responses is still under discussion. Skinner and Fernandes (2007) report activity in the right dorsolateral prefrontal cortex (DLPFC) and left precuneus (BA 7), whereas a meta-analysis by Vilberg and Rugg (2008) showed that the precuneus was mainly associated with familiarity-driven recognition.

Studies and current models of the involvement of the medial temporal lobes (MTL) also point to the dual process view of recognition memory by showing evidence that the hippocampus and the posterior parahippocampal cortex is responsible for recollection processes, but the anterior parahippocampal cortex, including perirhinal cortex supports familiarity (Aggleton & Brown, 1999; Aggleton & Brown, 2006; Aggleton, et al., 2005; Brown & Aggleton, 2001; Dolcos, et al., 2005; Eichenbaum, et al., 2007; Eldridge, et al., 2000; Fernandez & Tendolkar, 2006; Haskins, et al., 2008; Henson, et al., 2003; Montaldi, et al., 2006),

Alternative views on the recognition process state that the distinction between recollection and familiarity rather constitutes a distinction between strong and weak memories (Single Process Models; Squire, Wixted, & Clark, 2007b; Wixted, 2007a). In line with this, Shrager et al. (2008) found regions that are also known to be related to recollection processes which are negatively correlated with subsequent memory strength. However, studies directly contrasting recollection and familiarity to response confidence offer no support for the proposal that recollection merely reflects higher levels of memory strength or confidence than

familiarity driven recognition (Skinner & Fernandes, 2007; Vilberg & Rugg, 2007; Yonelinas, et al., 2005).

In summary, there is strong evidence that recollection and familiarity rely on different brain regions which cannot be explained in terms of differences in response confidence. However, the brain regions underlying familiarity are still under debate. Additionally, there is controversial evidence for overlapping brain areas modulating both recollection and familiarity processes (Skinner & Fernandes, 2007). Assuming the validity of Single Process Models, most of the areas found in recognition research should be activated in both recollection and familiarity processes and show a decrease or increase with response confidence (recollection > familiarity > new and vice versa). Following Dual Process Models, only a few overlapping areas should be found. And those regions which are specifically associated with recollection should be different from those associated with increased response confidence. The meta-analysis of Skinner and Fernandes (2007) strongly supports this assumption. Additionally, functional network analyses should show different maps of activation in recollection and familiarity, respectively. This pattern would support an independence view of recollection and familiarity (Jacoby, Toth, & Yonelinas, 1993) predicting that an item may be either recollected or familiar and only a subset are both recollected and familiar at the same time.

However, in functional neuroimaging research there is almost no consensus about the (inter)connectivity of cortical and subcortical structures supporting the different processes of recognition memory. Therefore, functional connectivity analyses of brain regions activated during recognition memory might help shed further light on the processes involved in recollection and familiarity.

Functional connectivity of brain regions during recognition memory has been described in models of the medial temporal lobes (Aggleton & Brown, 2006; Eichenbaum, et al., 2007). Eichenbaum et al. (2007) postulate that recollection is relying on connections between regions that process polymodal spatial ("where") information with the parahippocampal cortex (PHC), medial entorhinal areas (MEA) and the hippocampus. The feeling of familiarity is created by neocortical input from the association areas that process unimodal sensory information about qualities of objects ("what") to the perirhinal cortex (PRC) and to the lateral entorhinal area (LEA).

Aggleton and Brown (1999, 2006) established an extended hippocampal system of episodic memory including the hippocampus, the mammillary bodies, and the anterior thalamic nuclei which are linked to the prefrontal cortex, including the orbitofrontal, the medial and the dorsolateral part, as well as the posterior cingulate/retrosplenial cortex. A familiarity network is assumed to comprise the perirhinal and parahippocampal cortex which are linked to prefrontal cortex, medial and dorsal thalamus, and the association cortices (Aggleton & Brown, 1999).

Based on their meta-analysis, Skinner & Fernandes (2007) propose that the connection between frontal, parietal areas and hippocampus may be stronger during recollection than during familiarity responses. They suggest that it is also possible that familiarity is associated with stronger connections between frontal, parietal and perirhinal regions. To our knowledge, there is no study to date that directly assessed functionally different networks supporting recollection and familiarity by using functional or even effective connectivity analyses. Though there are some studies which have examined memory retrieval related functional connectivity of brain regions, the designs applied in these studies did not differentiate between recollection and familiarity. However, importantly, it can be suggested that recognition processes require functional connectivity between the medial parietal lobe (precuneus) and the MTL supporting relational memory as well as between the intraparietal sulcus and the middle temporal gyrus possibly indicating retrieval success (Takahashi, Ohki, & Kim, 2008). Additionally, there is evidence for functional connectivity of the lateral parietal cortex with ventro- and dorsolateral PFC and with the MTL, which is assumed to aid the retrieval of episodic memory (Kohler, McIntosh, Moscovitch, & Winocur, 1998; McIntosh, Nyberg, Bookstein, & Tulving, 1997; Takahashi, et al., 2008).

The aim of the present study is to address the issue of functional connectivity of brain regions during recognition memory in humans. In particular, we investigate whether the two different processes of recollection and familiarity are associated with distinct functional brain networks, and hypothesize that only a network supporting recollection involves connectivity of the hippocampus. We will also explore the question of whether overlapping areas, i.e. those activated during recollection and familiarity, show strong functional connectivity to both recognition systems.

4.3 Materials and Methods

4.3.1   Subjects

Thirty right-handed volunteers (19 female, mean 23.3 years, range 16 - 31 years of age) participated in this study. All participants were native German speakers with normal or corrected-to-normal vision. The subjects had been screened to exclude any participant with current or past neurological illness as well as current depressive or anxiety symptoms. Written informed consent was obtained from all subjects in accordance with institutional guidelines. All procedures were approved by the ethics committee of the German Psychological Association (DGP) and are therefore in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

4.3.2   Procedure

The Remember-Know (R/K) task was adapted from Eldridge et al. (2000). This procedure prevents participants from treating R/K judgments as measures of confidence (Hicks & Marsh, 1999) and assures that the Know category is not used for guess responses only (Eldridge, et al., 2002). The stimuli consisted of three similar lists of 177 non-emotional and non-arousing nouns each from a standardized wordlist by Hager and Hasselhorn (1994). The similar lists were randomly assigned to the subjects. In the study phase, 30 minutes prior to scanning, subjects had to learn a list of 150 nouns within 7 minutes. Following the procedure in Eldridge et al., the subjects were not explicitly instructed to use any specific strategy. In the recognition phase during fMRI scanning, the subjects were shown 108 old words and 27 new words. We used this relatively low number of items since we did not want the duration of the fMRI scanning to exceed one hour for ethical reasons. Each of the 9 functional runs contained 12 target words and 3 lures in a random order. Following the procedure of Eldridge et al. (2000), we used the low number of lures (20%) to increase the number of Know responses, which is the result of a relaxation of the response criterion as reported in a review by Yonelinas (2002). Thus, we could ensure that the remember and the know condition would offer an almost equal number of trials for fMRI analysis. In each five-second trial, subjects first saw the word for 3 seconds and within that

time period had to decide whether or not they recognized it (first response). Subsequently, for recognized items, they were prompted to decide whether they remembered or knew the item within 2 seconds (second response). The instructions for R/K distinction were clarified with examples before starting the fMRI scan. Responses were recorded via button presses. If the item was not recognized, the subject pressed either button at the second prompt. Between trials, subjects maintained fixation for 15 seconds. Subjects were instructed to disengage from the previous item during the fixation period. All reports of reaction times correspond to the first responses. The classification of the answers into different recognition types (correct Remember, correct Know, false Remember, false Know, correct Rejection and Miss) was obtained using the second responses. For the analysis of the fMRI BOLD response, the beginning of the word prompt paralleled the beginning of the hemodynamic response function. Behavioral responses were analyzed using SPSS 16.0.1 for Windows (SPSS.Inc©).

4.3.3   fMRI data acquisition

Images were acquired using a 1.5 T whole body scanner *Siemens Sonata,* running under *Syngo VA25A* (Siemens, Erlangen, Germany) and equipped with an 8-Array Head Coil. Participants wore earplugs for noise protection and laid on a padded scanner table in a dimly lit room. Foam padding minimized head movement. Stimuli were generated by *Presentation* (Version 0.71, Neurobehavioral Systems, Albany, CA), and were projected with a video projector onto a transparent plastic screen installed in front of the scanner. Participants viewed the stimuli through an angled mirror positioned immediately in front of their eyes. Two structural scans were recorded before the functional scans using a 3D T1 sequence (104 slices, TR = 6 ms, TE = 2,92 ms, matrix 512 x 512, orientation = sagittal, slice thickness = 2 mm, band width of 240 Hz/Pix) and a MPRAGE sequence (104 slices, TR = 2200 ms, TE = 4,39 ms, matrix 320 x 320, orientation = sagittal, slice thickness = 0,79 mm, band width of 130 Hz/Pix). Functional data were acquired using a T2*-weighted EPI sequence (30 slices, TR = 3000 ms; TE = 45 ms, FOV = 230 mm, Matrix = 64x64, orientation = axial, slice thickness = 3 mm, band width of 750 Hz/Pix) to measure blood-oxygen level dependent contrast (BOLD).

Functional data were collected in 9 runs, each run contained 104 volumes (scans) covering the whole brain.

4.3.4   fMRI data analysis

For the preprocessing and statistical analyses, the *Statistical Parametric Mapping software package* (SPM5, Wellcome Department of Imaging Neuroscience, Institute of Neurology) implemented in *Matlab 7.1* (Release 14, SP 3, Mathworks, Inc., Natick, MA, USA) was used. After slice timing, the functional data were realigned to the first volume of the time series (six-parameter, rigid-body-transformation) to correct for movement artifacts. This step resulted in a mean image containing all information about realignment parameters. After that, the T1 image was coregistered to the mean image of the realigned functional scans, and parameters for spatial normalization of the coregistered T1 to the standard space of the Montreal Neurological Institute brain (MNI Brain) were determined. The normalization parameters were then applied to both the structural T1 and the functional EPI images (4th degree B-spline interpolation). Smoothing was executed with a three-dimensional Gaussian filter with a full width at half maximum (FWHM) of 8 mm.

4.3.5   Event-related responses analysis

The first three functional scans were discarded from the analysis. Then, we classified the subjects' responses as 'correct Remember' (corrRem, old word correctly recognized and remembered), 'correct Know' (corrKnow, old word correctly recognized and known), 'correct Rejection' (corrRej, new word correctly rejected) or 'missed responses' (Miss, old word not recognized). Within the general linear model (GLM) framework, regressors of events, modeled by the canonical hemodynamic response function (hrf, no time derivation), were created for each trial type (corrRem, corrKnow, corrRej, Miss). As we were interested in the BOLD responses of the recognition processes, an event was defined as the beginning of the prompt of the word with no specifications about the duration. Because of the low false Remember rate, these trials, together with the false Know trials, were discarded from the model. A 128-s temporal highpass filter was applied to the data to exclude low-frequency artifacts such as scanner drift. At the first-level analysis voxel-wise statistical parametric maps (SPM) were calculated

for Remember trials, Know trials, Rejection trials and Miss trials for every subject. The results of these t-contrasts from each subject were then entered into a random-effects analysis at the group level (second-level analysis, within subjects repeated measures ANOVA with factor subject and within-factor recognition type). Then at the second level SPMs were created for the main effect of recognition (corrRem and corrKnow compared to corrRej), for the contrasts corrRem-Miss and corrRem-corrKnow (recollection), corrKnow-Miss and corrKnow-corrRem (familiarity). All voxel-wise statistics are corrected for multiple comparisons (Family Wise Error, FWE) at $p < .05$ with an extent threshold of $k = 5$. When the statistical correction leads to no suprathreshold voxels, the threshold was lowered to $p < .001$ with no correction for multiple comparisons (corrKnow > Miss and corrKnow > corrRem, familiarity based responses). These results should be used with caution. Regions were labeled with the *SPM toolboxes Automatic Anatomical Labeling* (AAL, Tzourio-Mazoyer, et al. 2002) and *Anatomy* (Version 1.5, Eickhoff, et al. 2005).

4.3.6   Psychophysiological Interaction Analysis

To assess the functional integration of a defined region of interest (ROI), we performed a psychophysiological interaction (PPI) analysis (Friston, et al. 1997). PPI analysis captures the interaction between brain regions in relation to the experimental paradigm. This method provides information about the way in which activity in one brain region modulates activity in another brain region specifically in response to the active task relative to the baseline or another task. In the case of the current study this refers to correct Remember relative to Miss and correct Know relative to Miss responses. To perform PPI analyses the individual first eigenvariate time series from a sphere of 5 mm radius (physiological variable), centered on the most significant voxels from the previous event related random-effects analysis (inclusive masking of the contrasts corrRem vs. Miss with corrKnow vs. Miss responses) were extracted. Then two new linear models were built, one for the functional connectivity of brain regions in relation to recollection, one for familiarity. The time series data of the ROI, the task, which represents the psychological variable (recollection [Remember vs. Miss contrast] or familiarity [Know vs. Miss contrast], respectively), and the interaction term of task with time series were then entered as regressors into the respective model.

The effect of the interaction term was then studied using the contrast [1 0 0] for positive interactions, where the first column represents the interaction term, the second column the psychological variable and the third column the physiological variable. The individual contrast images were then taken to the second level to perform a random-effects analysis. Because of the explorative character of this analysis, all voxel-wise statistics of the PPI are not corrected for multiple comparisons at p > .001, but we applied an extent threshold of k = 5.

4.4 Results

4.4.1 Behavioral data

The mean proportions and first response reaction times for each response type are shown in Table 4-1. Subjects accurately recognized 35% of the old items when they made a remember response and 37% of the old items when they gave a know judgment. The overall hit rate was 72%. CorrRem and corrKnow occurred significantly more than falseRem and falseKnow answers, respectively (T_{29} = 13.575, p < .001; T_{29} = 13.067, p < .001). The overall false alarm rate was 40%, with respective false alarm rates being 32% for Know and 8% for Remember responses. Importantly, the subjects gave significantly less falseRem than falseKnow answers (T_{29} = -7.486, p < .001).

Table 4-1: Proportions and reaction times (RT) of correct and false Remember, correct and false Know, correct Rejection and Miss responses

	corrRem	corrKnow	falseRem	falseKnow	corrRej	Miss
Proportion Mean (SE)	0.35 (0.03)	0.37 (0.02)	0.08 (0.02)	0.32 (0.03)	0.57 (0.03)	0.26 (0.02)
RT (ms) Mean (SE)	1230 (35)	1560 (54)	1244 (68)	1565 (57)	1682 (49)	1730 (58)

corr = correct; Rem = Remember; Rej = Rejection, SE = standard error of the mean; RT = reaction time of the first response (recognition judgment)

All conditions included in the fMRI analysis contained sufficient numbers of trials (at least 10 per subject), except corrRej (at least 9 per subject, due to the low number of new items). FalseRem and falseKnow had to be excluded from the

fMRI analysis, because of the low number of trials per subjects (false Rem min = 0, max = 7; false Know min = 1, max = 15).

4.4.2   Imaging data – BOLD activations during Remember and Know
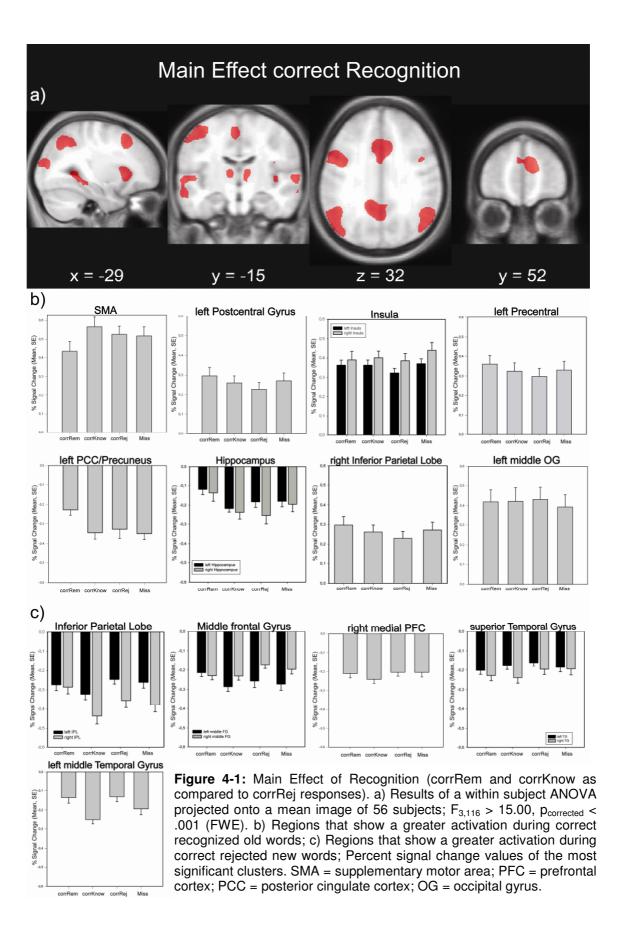
The main effect of correct recognition (F-contrast: corrRem and corrKnow compared to corrRej answers) together with the percent signal change plots of this regions of interest are shown in Figure 4-1. The supplementary motor area (SMA), left postcentral gyrus, inferior parietal lobe, middle frontal and medial orbitofrontal gyrus, right medial PFC, left posterior cingulate cortex and precuneus, left precentral gyrus, insula, left middle temporal gyrus, bilateral hippocampus and left middle occipital gyrus were the most significant voxels associated with correct recognition of items as compared to correct rejection responses (Threshold $F_{3,116} > 15.000$, $p_{corrected} < .001$, FWE). All significant activation clusters revealed by separate T-contrasts (corrRem>Miss, corrKnow>Miss, corrRem>corrKnow, corrKnow>corrRem) together with the specification of Brodmann Area, coordinates, T-value and cluster size are presented in Table 4-2. Left angular gyrus and left precuneus were more active during corrRem responses relative to Miss responses (Threshold $T_{116} > 5.30$, $p_{corrected} < .05$, FWE). During corrKnow responses, increased activity in the right postcentral gyrus, the right SMA, the left precentral gyrus and the left precuneus was found (Threshold $T_{116} > 3.00$, $p_{uncorrected} \leq .001$).

Direct comparison of Remember and Know trials revealed greater activity in the left angular gyrus, left middle cingulate gyrus, and left precuneus during corrRem (Threshold $T_{116} > 5.10$, $p_{corrected} < .05$, FWE). Compared to corrRem, corrKnow responses were associated with greater activity in right and left middle frontal gyrus, bilateral SMA, as well as left superior frontal gyrus (Threshold $T_{116} > 3.70$, $p_{uncorrected} < .001$).

A hypothesis-driven regions of interest (ROIs) approach was used to investigate the right and left hippocampus, parahippocampal cortex, and perirhinal cortex (BAs 35 and 36; (Witter, et al., 1989). For this an ROI created with the Wake Forest University PickAtlas (WFUPickAtlas; Maldjian et al., 2003) and the ROI tool

of the SPM5 Software Package (Wellcome Department of Imaging Neuroscience, Institute of Neurology London) was used.

We expected hippocampal and parahippocampal involvement only in remember responses (Aggleton & Brown, 2006; Eichenbaum, et al., 2007), whereas perirhinal cortex deactivation has been associated with familiarity (corrKnow responses) and novel objects seem to activate this structure (Eichenbaum, et al., 2007; Wan, Aggleton, & Brown, 1999; Xiang & Brown, 1998). The ROI analysis revealed activation clusters in the left parahippocampal gyrus and in the bilateral hippocampus only during remembering (corrRem>corrKnow; $T_{116} > 3.07$, $p_{uncorrected} \leq .001$; see Figure 4-2). No suprathreshold voxels could be found in the perirhinal cortex during all contrasts (corrRem>corrKnow, corrKnow>corrRem, corrRem>Miss, corrRem>corrRej, corrKnow>Miss, corrKnow>corrRej, corrRej>Rem, corrRej>Know; $T_{116} < 3.16$, $p_{uncorrected} > .001$).

Figure 4-1: Main Effect of Recognition (corrRem and corrKnow as compared to corrRej responses). a) Results of a within subject ANOVA projected onto a mean image of 56 subjects; $F_{3,116} > 15.00$, $p_{corrected} < .001$ (FWE). b) Regions that show a greater activation during correct recognized old words; c) Regions that show a greater activation during correct rejected new words; Percent signal change values of the most significant clusters. SMA = supplementary motor area; PFC = prefrontal cortex; PCC = posterior cingulate cortex; OG = occipital gyrus.

Figure 4-2: ROI analysis including left and right hippocampus and parahippocampal gyrus as well as the perirhinal cortex (BA 35 and 36, borders showed in green lines). BOLD responses in the left posterior parahippocampal gyrus and bilateral hippocampus are higher during corrRem responses compared to corrKnow. Threshold $T_{116} = 2.96$, $p_{corrected} < .05$ (FWE), k = 5.

Neither hippocampal nor parahippocampal activation could be found during Knowing. No activation could be found in the perirhinal cortex ($p_{uncorrected} > .001$).

In order to look for areas activated during both Remember and Know responses, the corrRem-Miss contrast was inclusively masked with the corrKnow-Miss contrast (mask $p_{uncorrected} = .001$, SPM $p_{uncorrected} = .001$). This means both contrasts were thresholded at the p<.001 uncorrected-level and then inclusively masked with each other using a procedure of the SPM software. The resulting SPM was not corrected for multiple comparisons at p<.001. One activation cluster was found in the left precuneus at coordinates -3, -60, 27 (MNI space, see Figure 4-3) which was associated with both Remember and Know answers. The precuneus region, as the most significant result in this overlap analysis, was entered into the following psychophysiological interaction analysis as the source region for both PPI analyses (one for the Remember condition, one for the Know condition).

Table 4-2: Brain Regions activated during remember and during know answers.

Brain Region	BA	Side	MNI-Coordinate x	y	z	T value	cluster size
corrRem > Miss							
Angular Gyrus (extending to inferior Parietal Gyrus)	39	L	-51	-66	33	6.20	48
	7	L	-39	-69	42	4.90	
Precuneus	23	L	-3	-60	30	5.31	9
corrKnow > Miss							
Postcentral Gyrus	1/2	R	39	-39	66	3.99	14
Supplementary Motor Area	6	R	9	-12	54	3.62	19
Precentral Gyrus	4	L	39	-21	60	3.15	9
Precuneus	23	L	-6	-60	27	3.05	7
(corrRem+corrKnow) > Miss							
Precuneus	23	L	-3	-60	27	5.31	24
corrRem > corrKnow							
Angular Gyrus	39	L	-54	-63	30	6.55	83
	7	L	-39	-69	45	5.05	
Middle Cingulate Gyrus	23	L	-3	-36	39	6.04	20
Precuneus (extending to Calcarine Gyrus)	17	L	-6	-57	12	6.35	13
		L	-15	-54	12	5.15	
corrKnow > corrRem							
Middle Frontal Gyrus	46	R	33	39	33	4.30	20
Supplementary Motor Area	6	B	0	-6	54	5.19	27
			0	6	51	3.46	
Superior Frontal Gyrus	6	L	-21	-12	54	3.98	22
Supplementary Motor Area	6	R	15	0	60	3.94	24
Middle Frontal Gyrus	9	R	42	18	45	3.81	18
Middle Frontal Gyrus	46	L	-30	36	27	3.70	12

Anatomical locations, Brodmann Areas (BA), hemispheres (side, L = left, R = right), coordinates in MNI space (Montreal Neurological Institute), T values, and cluster sizes are given for regions showing significant activity during recollection (corrRem > Miss) and familiarity (corrKnow > Miss) responses, as well as for a comparison of recollection and familiarity responses (corrRem > corrKnow, corrKnow > corrRem). Statistics for the contrasts corrRem > Miss and corrRem > corrKnow are Family Wise Error (FWE, p < .05; extent threshold k = 5) corrected. However, FWE correction leads to no suprathreshold voxels in corrKnow > Miss and corrKnow > corrRem analysis. Therefore, the results for these contrasts are uncorrected (p < .001; extent threshold k = 5). (corrRem+corrKnow) > Miss means inclusive masking of the contrasts corrRem > Miss and corrKnow > Miss to analyze for regions activated by both Remember and Know processes. This statistic is not corrected for multiple comparisons (p < .001).

4.4.3 Imaging data – functional connectivity of Remember and Know (Psychophysiological Interaction analysis)

In order to acquire information about the way in which activity in one brain region modulates activity in another brain region in response to the active task we performed a PPI analysis. A 5 mm (radius) sphere centered at the most significant voxel resulting from the inclusive masking of the contrasts corrRem>Miss and corrKnow>Miss was chosen as the region of interest (left precuneus at coordinates -3, -60, 27, see **Figure 4-3**a).
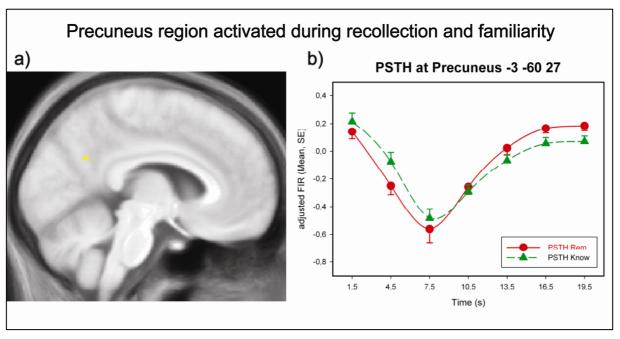
Figure 4-3: Region that is activated during recollection and familiarity

During remembering, the left middle temporal gyrus and the right superior temporal pole (extending to insula) were positively connected with the left precuneus (see Figure 4-4b, $T_{29} > 3.40$, $p_{uncorrected} < .001$). Based on the hypothesis that only a functional network supporting recollection includes the hippocampus, we conducted an ROI analysis including left and right hippocampus and parahippocampal cortex (see above). This leads to a positive connectivity of the left and right hippocampus with the left precuneus region during corrRem vs. Miss answers (see Figure 4-4, $T_{29} > 3.3$, $p_{uncorrected} < .001$). Please note that a positive relationship to the deactivation in the precuneus means a deactivation in the reported brain regions.

No negatively correlated regions ($T_{29} > 3.3$, $p_{uncorrected} < .001$) could be found. However, if the threshold is lowered to $p < .005$, an area in the left middle frontal gyrus shows an activation ($T_{29} > 2.7$, $p_{uncorrected} < .005$) which is correlated with the deactivation in the left precuneus. Connectivity with the left precuneus during know responses was found with the left insula and rolandic operculum (inferior frontal gyrus), as well as with a cluster extending from the right middle occipital gyrus to the middle temporal gyrus, (also see Figure 4-4a, $T_{29} > 3.50$, $p_{uncorrected} < .001$). The hippocampal/parahippocampal ROI analysis within the familiarity network resulted in no suprathreshold clusters in the hippocampus and parahippocampal cortex ($T > 2.3$, $p_{uncorrected} < .01$). Negatively correlated clusters during corrKnow responses were found in the right putamen and the left middle cingulate gyrus ($T_{29} > 3.39$, $p_{uncorrected} < .001$).All significant connectivity clusters together with the specification of Brodmann Area, coordinates, T-value, and cluster size are presented in **Table 4-3**.
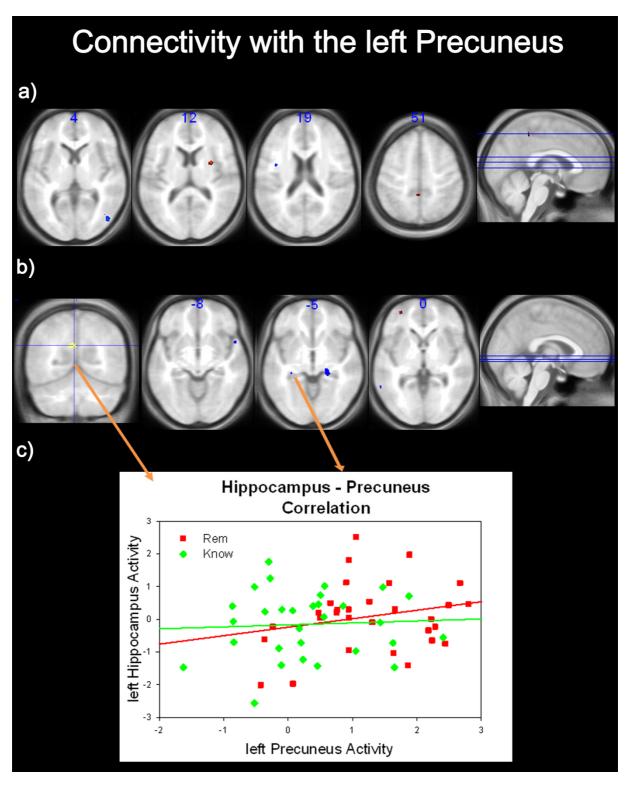
Figure 4-4: Regions that are functional connected to left Precuneus (b), yellow) during a) Knowing (corrKnow > Miss) and b) Remembering (corrRem > Miss). T > 3.40, $p_{uncorrected}$<.001, extent threshold k = 5. The threshold was lowered to p < .005 in the analysis of negatively connected areas during Remembering. The BOLD time series of these areas are positively (blue) or negatively (red) correlated with the time series of the precuneus during Remember and Know, respectively. c) Region of Interest analysis of the hippocampus: Scatterplots and regression lines of the hippocampus-precuneus correlation in Remembering and Knowing, respectively.

Table 4-3: Brain Regions positively connected with Precuneus at coordinates -3 -60 27 as revealed through Psychophysiological Interaction (PPI) Analysis.

Brain Region	BA	Side	MNI-Coordinate			T value	cluster size
			x	y	z		
positive Connectivity to left Precuneus (corrRem > Miss)							
Superior Temporal Pole	38	R	54	12	-9	3.84	6
Middle Temporal Gyrus	21	L	-60	-54	0	3.77	8
Hippocampus (ROI)		L	-30	-33	-3	3.56	5
Hippocampus (ROI)		R	36	-27	-6	3.39	6
negative Connectivity to left Precuneus (corrRem > Miss)							
Middle Frontal Gyrus*	10	L	-33	60	3	3.24	15
positive Connectivity to left Precuneus (corrKnow > Miss)							
Insula / Rolandic Operculum	48	L	-39	0	18	4.46	6
Middle Occipital Gyrus / middle Temporal Gyrus	19	R	45	-75	3	4.07	14
negative Connectivity to left Precuneus (corrKnow > Miss)							
Putamen		R	30	3	12	4.55	6
Middle Cingulate Gyrus	23/31	L	-3	-42	51	4.20	11

Anatomical locations, Brodmann Areas (BA), hemispheres (side, L = left, R = right), coordinates in MNI space (Montreal Neurological Institute), T values, and cluster sizes are given for regions showing significant connectivity with Precuneus during Remember (corrRem > Miss) and Know (corrKnow > Miss) responses. Statistics for the contrasts are uncorrected (p < .001; extent threshold k = 5). *The threshold was lowered to p < .005 in the analysis of negative connectivity during Remember (corrRem > Miss).

In Figure 4-4c) the correlations between the left precuneus and the left hippocampus during corrRem vs. Miss and corrKnow vs. Miss are compared. Descriptively there seems to be a difference between the slopes of the two regression lines. To obtain further information about the correlation between precuneus and hippocampus during recollection and familiarity, respectively, we analyzed the correlation coefficients of the contrast estimates (corrRem vs. Miss; corrKnow vs. Miss) between those two regions. Although the PPI reports significant correlations between hippocampus and precuneus in the Rem-Miss contrast, the correlation analysis results in only a small and non significant coefficient (r = 0.226, p = 0.231).

The correlation coefficient between the contrast estimates of precuneus and hippocampus during Know-Miss is much lower and far from significant ($r = 0.0741$, $p = 0.697$).

To analyze the difference between the two correlation coefficients we conducted a procedure recommended by (Steiger, 1980) for the comparison of correlation coefficients from the same sample. The results show that the correlation coefficients between the contrast estimates in precuneus and hippocampus is not significantly different between corrRem-Miss and corrKnow-Miss ($Z = 0.746$; $p = .456$). As a next step, we compared the two slopes of the regression lines computing the difference of the slopes divided through the standard error of the slopes (Rasch et al., 2008). This analysis revealed no significant difference between the slopes ($T_{29} = .783$, $p > .10$), too.

4.5 Discussion

We used a Remember-Know procedure to assess the different processes of recognition memory, namely recollection and familiarity, and the functional connectivity of brain regions during recognition memory. In summary, our results further support the assumptions of a dual process view of recognition memory (Eichenbaum, et al., 2007; Eldridge, et al., 2000; Parks & Yonelinas, 2007; Yonelinas, 2001a; Yonelinas, et al., 2005). Using psychophysiological interaction analysis (PPI), we identified two functionally distinct networks underlying recollection and familiarity. Activation of the hippocampal formation was only related to recollection-based responses. Furthermore, we found hippocampal connectivity to the left precuneus in a recollection network. However, the additional analysis of this connectivity revealed no significant difference of the hippocampal-precuneus correlation between recollection and familiarity. Nevertheless, the left precuneus appears to be a core brain structure which was associated with both recollection and familiarity and which showed strong connectivity to both specific recognition systems.

4.5.1 Behavioral evidence for distinct recognition processes

Already at a behavioral level we found a dissociation between recollection and familiarity based responses. The subjects gave more false know answers than false remember answers.

This is in accordance with other studies that have found a higher acceptance of incorrect items in familiarity judgments as opposed to recollection judgments (Yonelinas, 2002). We therefore conclude that the R/K task used in our experiment was efficient in detecting distinct recollection and familiarity processes. CorrRem and corrKnow occurred significantly more than falseRem and falseKnow answers, respectively. This indicates that the subjects were able to correctly discriminate between old and new items using the Remember response as well as the Know answer. However, we found a higher falseKnow rate as compared to other studies using the R/K distinction (Gardiner et al., 2002), except for the study of Eldridge et al., (2000) which found comparable false alarm rates. Both, our study and Eldridge et al. used an unusually low number of lures. This leads to an increase of Know responses as a result of a relaxation of the response criterion as reported in Yonelinas (2002). This increase not only seems to be limited on correct Know, but also on false Know responses. Additionally, in our study the number of old words that were shown in the recognition phase was lower than the number of learned items. In most of the R/K experiments all studied items were presented as old words in the test session (Woodruff et al., 2005; Yonelinas et al., 2005) and lower falseKnow rates were reported. Thus, we suggest that the high "memory load" and therefore the greater difficulty of our task led to a bias to classify more new words as old (e.g. a force to produce false alarms), but this bias only affects the Know process because of the lack of retrieved context details

4.5.2 Evidence for the activation of distinct brain regions from BOLD response analyses

fMRI analysis revealed different brain areas related to recollection and familiarity, respectively. *Familiarity* was strongly associated with activation of a right postcentral area (BA 1/2, extending at a less stringent statistical level to BA 3).

BA 2, 1 and 3 are known as the primary somatosensory cortex, which gets information from sensory thalamus areas (Nieuwenhuys, et al., 2008) and is primarily processing tactile information (Zhou & Fuster, 2000). Although, at this point, we can only speculate about this activation, one might suggest that associated visual stimuli may activate the primary somatosensory cortex through visuo-haptic associations. Those associations possibly were established by a special encoding strategy that preferentially leads to familiarity based retrieval.

In a direct comparison of familiarity and recollection answers, right dorsolateral prefrontal cortex (rDLPFC) was activated only during familiarity based responses. This is in line with evidence that right dorsolateral prefrontal areas support familiarity by means of a postretrieval monitoring process or by additional searching for details of the item (Henson, Rugg, et al., 1999; Wheeler & Buckner, 2004). Henson et al. (2000) showed that rDLPFC is more activated in low vs. high confidence recognition responses. Following the assumptions of the DPSD approach which always associated recollection responses with high confidence memory judgments (Yonelinas, 2001a), we conclude that the additional monitoring and checking performed by rDLPFC before a decision is made, is not necessary in recollection based responses. Thus our data are also in line with Henson et al. (1999, 2000) who suggested that rDLPFC activation reflects additional monitoring, and this is only necessary during the familiarity responses with lowest confidence ratings. Bilateral SMA was also associated only with familiarity answers. The left SMA is often discussed in connection with speech production (Alario, Chainay, Lehericy, & Cohen, 2006) and may therefore contribute to a subvocal rehearsal (Muller & Knight, 2006). Thus, its activation in this study indicates that the subjects are internally or even openly forming the word with the mouth to come to a judgment of familiarity of this mouth movement. For recollection this is not necessary because the additional retrieval of context information is sufficient to lead to a correct recognition answer.

Recollection based responses activated regions in a left anterior precuneus region and the left angular gyrus which is part of the inferior parietal lobule (IPL). One cluster of the activated IPL region (center at -39, -69, 42) corresponds to a cluster found by a meta-analysis of parietal contributions to recollection (Vilberg & Rugg, 2008).

The authors reported recollection-related activations in the parietal cortex, lateral and inferior to the intraparietal sulcus (IPS, center of mass -43, -66, 38), which overlaps the angular gyrus. Thus, our results further support the role of left IPL, more specifically the angular gyrus, in recollection based responses. Findings from both fMRI and ERP studies suppose the IPL activations to be a recollection success effect (Rugg & Curran, 2007; Vilberg & Rugg, 2008) and that they support the sustained focusing of attention on the contents of working memory (Ravizza, et al., 2004). Although we cannot proof the hypothesis of sustained attention with our data they strongly suggest the existence of an IPL recollection success function, as we only found IPL activation during hits that were based on recollection responses.

4.5.3 Evidence for differential involvement of the MTL from Region of Interest Analyses

Our data also point to the important role of the hippocampal and posterior parahippocampal activations during recollection memory. This is in accordance with models of episodic memory in the MTL (Aggleton and Brown 2006; Eichenbaum, et al. 2007). In a Remember/Know paradigm Fenker et al. (2005) were able to show, that the hippocampal activity during remembering showed no increase from a neutral to a fearful context. This finding is compatible to Dual Process Models of recognition memory, because recollection is assumed to be a threshold process. Thus, during recollection hippocampal activity is elevated above a threshold, but does not need to be further increased with more details about the study event.

However, in line with other fMRI studies on recognition memory (for a review see Skinner & Fernandes 2007), we could not find evidence for perirhinal activation or deactivation during familiarity based responses which would further strengthen the assumptions of Eichenbaum et al. (2007). Proof of this activation has rarely been found in pure BOLD activation analyses of recollection and familiarity because the association of perirhinal cortex and familiarity judgments seems to depend on response confidence (Daselaar, Fleck, & Cabeza, 2006a; Montaldi, et al., 2006).

We have discussed evidence for distinctive brain regions associated with recollection and familiarity in some detail and will elaborate on the multiple roles

the precuneus may play in recognition memory in the following section. We found evidence for its involvement in recollection processes alone and for a shared function in both recollection and familiarity.

4.5.4 Evidence for the Involvement of the Precuneus in Recollection

Previous fMRI studies have demonstrated that precuneus/posterior cingulate areas show greater activity during recollection based judgments (Henson, Rugg, et al., 1999; Wagner, Shannon, Kahn, & Buckner, 2005) or during episodic memory (Burgess, et al., 2001). We found that the posterior cingulate/anterior precuneus region was activated during recollection based answers (center at coordinates -6 -57, 12), which corresponds to some of the reported activations in the literature. Interestingly, a recent study by Peters, Daum, Gizewski, Forsting, & Suchan (2009) found a very similar active cluster in the left precuneus/posterior cingulate/retrosplenial region during associative as compared to feature-based encoding, pointing to the importance of this structure also in the encoding of contextual information. However, a study by Lundstrom, Ingvar, & Petersson (2005) linked a posterior precuneus region to source memory retrieval. This is not supported by our results. The activation cluster found in our study lies in the anterior part of the precuneus. It is possible that, in the R/K paradigm we utilized, subjects are not able to retrieve rich episodic details that would activate the posterior precuneus as is supposed by Lundstrom (Lundstrom, et al., 2005; Lundstrom, et al., 2003). Nevertheless one could assume that a defined area in the anterior precuneus plays a role in recollection processes, maybe in retrieving spatial or other contextual details, which would support findings by Burgess et al. (2001) and Takahashi et al. (2008).

4.5.5 Evidence for a shared function in both recollection and familiarity

We found a defined cluster of activation within the medial precuneus (center at -3, -60, 27) that proved to be the only region associated with both recollection and familiarity.

In contrast to findings that link precuneus activation to recollection and episodic memory (Fletcher, et al., 1995; Henson, Rugg, et al., 1999; Shallice, et al., 1994; Wagner, et al., 2005). Vilberg & Rugg (2008) could relate precuneus activations only to familiarity based responses, and the meta-analysis of Skinner and Fernandes (2007) found left precuneus (BA 7) to be associated with both recollection and familiarity. This leads to the conclusion that the precuneus plays an important role in both processes and, on closer inspection, possibly may be divided into different parts maintaining specific functions in recognition memory. Thus, the overlapping cluster which was found by the masking of a recollection with a familiarity contrast lies more inferior to the recollection cluster described above. A study by Wiesmann and Ishai (2008) reported activation in the precuneus which was associated with both correct remember and correct know comparable to the cluster found in our study. However, their cluster showed an earlier peak of activation in recollection. In contrast to this, the precuneus cluster we report here shows exactly the same time course in both recollection and familiarity.

The functional topography of the precuneus includes motor imagery in a posteromedial part, activations that are associated with attention orientation in the anterior and posterior medial precuneus, episodic memory functions in widespread parts of the medial part with memory-related imagery activations in the bilateral anterior region, an Old vs. New memory effect in posterior areas, as well as a self-reference related function in the anteromedial part of the precuneus (Cavanna & Trimble, 2006). Strikingly, an overlap was found between a memory-related imagery cluster (Fletcher, et al., 1995) and a self reference cluster (Kircher, et al., 2002) in the anteromedial precuneus. The precuneus cluster that is reported in the current study lies near those activations. Hence, it tentatively could be suggested that the function of the common recollection and familiarity cluster is related to those processes.

There is some evidence for more overlapping areas in recollection and familiarity (Henson, Rugg, et al., 1999; Wheeler & Buckner, 2004). However, fundamental methodological differences in the analysis of overlapping structures between those studies and ours may account for this difference.

Strikingly, the time course of the precuneus cluster found in our study shows a deactivation. There is strong evidence that the posterior cingulate cortex, and

medial and lateral parietal cortex show decreases during performance of attention-demanding cognitive tasks like memory processes (Mazoyer, et al., 2001; McKiernan, Kaufman, Kucera-Thompson, & Binder, 2003; Shulman, et al., 1997) as well as passive tasks (Binder, et al., 1999), and that these deactivation patterns already are represented intrinsically in the resting human brain (Fox, et al., 2005). Supporting this, Buckner, Raichle, Miezin, & Petersen (1996) could identify an anterior medial parietal area, near the overlapping precuneus region that was found in the current study, which decreased during memory recall. In contrast, a more posterior medial parietal area showed an increase during recall. This supports our assumption of functionally dissociable locations in the precuneus area. One possible explanation for the decrease in activity in one area of the brain is that it might reflect a decrease in the activity of the cells that are projecting to this area (Gusnard & Raichle, 2001). Therefore, we assume that this shutting down is correlated with functional deactivations in other regions, which might be specific to the respective cognitive process (recollection or familiarity). We therefore chose this precuneus region as a seed region for both functional connectivity (PPI) analyses of recollection and familiarity responses.

4.5.6 Evidence for two distinct recognition brain networks

The PPI analysis revealed functional connectivity between the medial precuneus and left middle temporal gyrus, right temporal pole, and the left and right hippocampus in the recollection condition. This is similar to a study by Takahashi et al. (2008) which found functional connectivity between the medial precuneus and the MTL in a recognition task maybe indicating the retrieval of relational memory. Alternatively, one could assume that the concertedly occurring deactivation of medial precuneus, middle temporal gyrus, superior temporal pole and MTL reflects the closing down of the default mode or resting state of the brain (Gusnard & Raichle, 2001), because there is evidence for a connectivity between those regions during spontaneous low frequency fluctuations in the resting state (Fransson, 2005). However, given that the deactivations found in our study are more pronounced in correct Remember than Misses, we assume that this reflects retrieval success rather than merely a shutting down because of the beginning of a cognitive task.

Takahashi et al. (2008) and Kohler et al. (1998) found the middle temporal gyrus was associated with lateral parietal areas whereas we found a functional connectivity with the medial precuneus. However, both studies did not differentiate between recollection and familiarity in their designs and Kohler et al. (1998) did not include medial parietal areas in their Structural Equation Model. As the lateral temporal gyrus has been implicated in nonrelational item-based memory (Konishi, Asari, Jimura, Chikazoe, & Miyashita, 2006), we suggest that at least some recollection trials are accompanied by a fast item-based retrieval process. This familiarity process possibly occurs first and then initiates an additional search for contextual, relational information to come to a correct recognition judgment based on recollection. A middle frontal area (BA 10) shows a negative connectivity with the medial precuneus indicating an increase of activation when precuneus decreases. This area is related to attention, object perception (for review see Cabeza & Nyberg, 2000) but also imagery processes resulting from recall attempts (Roland & Gulyas, 1995). McIntosh et al. (1997) could show a negative functional connectivity between the right BA 10 and hippocampal as well as posterior cingulate areas during recognition memory possibly reflecting retrieval mode. Additionally, Kahn et al. (2004) suggests that a similar left frontopolar region is sensitive to perceived familiarity, being engaged during recollection attempts only for items eliciting above criterion familiarity.

Interestingly, connectivity studies consider the posterior cingulate/precuneus area as a "core hub" in the so called Default Mode Network (DMN; Buckner, et al., 2008; Fransson & Marrelec, 2008). Vincent et al. (2006) describe the DMN as a network which is strongly related to recollection responses and comprises the hippocampal formation, retrosplenial cortex extending into the posterior cingulate gyrus/precuneus, inferior parietal lobule, medial prefrontal cortex, superior frontal cortex and lateral temporal cortex extending to the temporal pole. These regions overlap the structures found in our connectivity analysis. Attention should be paid to the connection between the left hippocampus and the medial precuneus in the recollection network.

This is in accordance with a DMN study by Fransson & Marrelec (2008) who found that the MTL is only connected to the precuneus/posterior cingulate cortex and the left temporal cortex. Additionally, studies of episodic memory (e.g., Burgess, et al., 2001; Ranganath, Heller, Cohen, Brozinsky, & Rissman, 2005)

have found a network of precuneus, retrosplenial, parahippocampal, and hippocampal areas during episodic retrieval as well as encoding. However, we could find no evidence suggesting that the connection between hippocampus and precuneus is actually greater in recollection than familiarity. Further studies using more hypothesis-based network analyzing methods like structural equation modeling (SEM) or dynamic causal modeling (DCM) may help to clarify this open question.

Taken together, the precuneus/posterior cingulate area not only seems to be a core structure in DMN and episodic memory but also the core connection to the lateral and medial temporal cortex in those systems. It is conceivable that recognition memory relies on the functional connections, partly via the precuneus area, between hippocampus and other cortical structures (e.g. prefrontal cortex, lateral parietal cortex, lateral temporal cortex). Some evidence for this comes from Fransson & Marrelec (2008) who showed that the precuneus/posterior cingulate cortex was the only area that directly interacted with all other brain structures of the DMN (inferior parietal lobe, temporal cortex, medial PFC and MTL) in a working memory task. Additionally, a review by Cavanna and Trimble (2006) summarizes that the precuneus is a major association area with widespread structural connections to both cortical and subcortical brain regions. It is also known that the hippocampus has direct and indirect reciprocal connections to the retrosplenial cortex and BA 23 and BA 7 of parietal cortex, including the precuneus (e.g., Nieuwenhuys, et al., 2008).

Our results do not support a stronger connectivity between perirhinal and parietal regions during familiarity, as supposed by Skinner and Fernandes (2007). Know responses rather were characterized by stronger positive connectivity of the left precuneus with the left insula, the right occipital gyrus (BA 18 and 19), and the right middle temporal gyrus as well as by negative connectivity to the middle cingulate gyrus and the putamen.

It is noticeable that we found more areas related to sensory processing, such as BA 18, and insula, during familiarity than recollection based responses (for a summary of sensory processing areas see Nieuwenhuys, et al. 2008). This fits well with the evidence that, compared with recollection, familiarity is more dependent on perceptual processes (Yonelinas, 2002). Additionally, Montaldi et al. (2006) and Yonelinas et al. (2005) also found that the insula is involved in

generating feelings of familiarity. However, the positively correlated regions also show deactivations when the precuneus region decreases, but this occurs in familiarity to a lesser extent than in unsuccessful recognition (Miss responses). Hence, we suggest that these differences in the decrease of the reported network contribute to a correct feeling of familiarity, which may be due to the conjoint activation of the middle cingulate gyrus. The specific function of the middle cingulate gyrus (BA 23/31) here is not clear but as the region is discussed in terms of verbal and spatial working memory as well as semantic memory retrieval (for a review see Cabeza & Nyberg, 2000) we suggest that this activation contributes to a correct recognition response.

Our data and previous findings (for a summary see Cavanna & Trimble, 2006) suggest that the precuneus seems to "decide" whether a recognition response can utilize context information about the item, which would involve hippocampus (recollection), or has to be based on mere perceptual features of the item (familiarity), possibly in cooperation with self-referential and memory-related imagery functions that are also located in the anteromedial precuneus. In line with this, our findings suggest that the anterior medial precuneus area is the region that facilitates the link between episodic memory (hippocampus), nonrelational item-based memory (middle temporal gyrus), the processing of stimuli from the environment coming from primary and secondary sensory areas (somatosensory cortex, insula, BA18) and attention related structures which are associated with retrieval mode (i.e. BA 10)." Support for this assumption comes from Naghavi and Nyberg (2005) who showed that BA 7 (including precuneus) is commonly activated across different functions like attention, episodic memory retrieval, working memory and conscious perception.

4.6 Conclusion

We consider the findings presented here to be in line with dual process models of recognition memory. However, these models are challenged by an alternative hypothesis (for a review see Squire, et al., 2007). Thus, Squire et al. (2007) and Wixted (2007a) suggest that Recollection and Familiarity simply reflect strong and weak memories, respectively. Although this view has support from empirical findings (Wiesmann & Ishai, 2008), at least for a memory strength component in

the MTL (e.g., Kirwan, Wixted, & Squire, 2008; Shrager, et al., 2008), it is often disproved by other findings directly contrasting memory strength and dual process models in both healthy human subjects (Yonelinas, et al., 2005) and amnesics (Turriziani, et al., 2008). We did not directly contrast memory strength and recollection/familiarity in our study. Nevertheless, we consider our data hardly compatible to one process models.

First of all, we chose a version of the Remember-Know-Task to measure recognition memory that prevents subjects to use the R/K distinction as a confidence rating (Hicks & Marsh, 1999), which would indicate memory strength, but rather leads to true recollection and familiarity judgments. Second, directly contrasting familiarity with recollection based responses revealed distinct brain activations, which is in line with previous findings of recognition memory studies supporting dual process models (e.g., Eichenbaum, et al., 2007; Eldridge, et al., 2000; Ranganath, et al., 2004; Skinner & Fernandes, 2007; Yonelinas, 2001a; Yonelinas, et al., 2005). Moreover, hippocampal and posterior parahippocampal activations could only be found during recollection, even at a low statistical correction level. This would not have been the case if high memory strength familiarity responses would activate hippocampal structures too, as is proposed by memory strength models. Furthermore, using psychophysiological interaction analysis, our results suggest that only a brain network supporting recollection involves connectivity of hippocampal formation with other cortical areas. Last but not least, we found functionally distinct networks of brain areas underlying recollection and familiarity.

In summary, our results strongly point to an independence relationship between recollection and familiarity (Skinner & Fernandes, 2007), but a redundancy model cannot be fully refused in light of our data. Given the fact that both recognition networks also have overlapping cortical areas, it can be concluded that there are brain areas associated with both processes which seem to maintain more global functions in recognition memory, such as attention and conscious perception. One of the cortical regions orchestrating these functions is the left precuneus.

5 STUDY II: BDNF VAL66MET IS RELATED TO INDIVIDUAL CONTEXTUAL MEMORY VARIATIONS – POSSIBLE IMPLICATIONS FOR INCREASED PTSD RISK AFTER TRAUMA?

5.1 Abstract

The Brain Derived Neurotrophic Factor (BDNF) is involved in long term potentiation (LTP), synaptic plasticity, and neurotransmission in the hippocampus, and therefore plays a crucial role in hippocampal dependent contextual memory. A variation in the human BDNF gene (66Met) has been related to poorer declarative memory performance and to altered fear learning. Therefore, it might play a crucial role in the development and maintenance of Posttraumatic Stress Disorder (PTSD), a condition that is characterized by specific alterations in emotional memory, i.e. a lack of fear extinction and explicit memory deficits. Hence, we investigated in an analogue sample of 28 students the effect of BDNF genotype on contextual recognition memory, which is crucial for fear extinction, and the underlying brain function especially in the hippocampus. We used the remember-know task and found that carriers of the Met allele show a selectively decreased performance only in the remember condition (i.e. contextual memory) which was related to deactivations in the left temporal cortex and left prefrontal cortex (PFC). Contrary to our hypothesis, hippocampal activation during the remember condition was higher in carriers of the Met allele than in the homozygote Val group. The findings suggest that the BDNF genotype has a specific effect on contextual memory, pointing to a deficit in left PFC activation but not in hippocampal functioning. Further studies may therefore consider the BDNF Val66Met polymorphism as a possible risk factor for the development of PTSD.

Keywords: BDNF Val66Met, Recognition, Familiarity, Recollection, Contextual Memory, fMRI, Hippocampus, PFC, Temporal Gyrus

5.2 Introduction

The role of the Brain Derived Neurotrophic Factor (BDNF) for brain functioning and associated behaviors has increasingly become of interest. The reason for this is twofold. *First*, as a protein involved in long term potentiation (LTP) in the hippocampus (Bramham & Messaoudi, 2005; Christianson & Lindholm, 1998; Lu, et al., 2008) and acting on widely distributed receptors across subregions of the hippocampus and the adult forebrain (Murer, et al., 2001), BDNF is crucial for synaptic plasticity (Bramham & Messaoudi, 2005), for the maintenance of synaptic connections (Huang & Reichardt, 2001) and for the neurotransmission (Poo, 2001) in key brain regions that are involved in episodic memory (Eichenbaum, 2001). A variation in the human BDNF gene, the Val66Met polymorphism, is located in the 5' pro-BDNF sequence, which encodes the precursor peptide (pro-BDNF) that is proteolytically cleaved to form the mature BDNF protein (Seidah, Benjannet, Pareek, Chretien, & Murphy, 1996). The variant results from a replacement of the base Guanine by Adenine at nucleotide 196 (G196A, frequent single nucleotide polymorphism (SNP); dbSNP number rs6265) producing an amino acid substitution (valine to methionine) at codon 66 (val66met). The Met substitution leads to substantial trafficking defects such as decreased BDNF distribution into neuronal dendrites, decreased BDNF targeting to secretory granules, and subsequent impairment in regulated secretion (Chen, et al., 2005; Chen, et al., 2004; Egan, et al., 2003). All these deficits are supposed to account for the poorer memory performance found in human carriers of the Met allele (Dempster, et al., 2005; Egan, et al., 2003; Goldberg, et al., 2008; Hariri, et al., 2003). However, a study by Hashimoto et al. (2008) failed in detecting a BDNF effect on recognition. Possibly, a dissociation between recollection and familiarity based recognition (Yonelinas, 2002) can account for the mixed results of BDNF influences on recognition performance.

Second, and more specifically, BDNF has been linked to the etiology of dysfunctional conditions such as depression and anxiety disorders (Castren, 2005; Chen, Bath, McEwen, Hempstead, & Lee, 2008; Chen, et al., 2006; Groves, 2007; Pezawas, et al., 2008). Following this and because of its role in memory function it can be assumed that BDNF may also play a role in the etiology of

Posttraumatic Stress Disorder (PTSD), a condition associated with a pattern of emotional memory alterations.

It is conceptualized as an impaired ability to recover from trauma, and memory of the traumatic event is disturbed in patients with PTSD in two ways: on one hand they experience stressful, intrusive recollections (flashbacks) and on the other hand their conscious recollections of details and the temporal order of the event is fragmented and disordered, i.e. there is impaired contextual memory (Brewin, 2001) which affects 'normal' extinction of fear memories (Charney, 2004; Shin & Handwerger, 2009).

Preliminary evidence for a potential role of BDNF as a risk factor of PTSD can be drawn from a number of findings. First, alterations in cortico-limbic circuitries including the hippocampus, the amygdala and frontal regions (Karl, et al., 2006; Rauch, Shin, & Phelps, 2006) have been linked with PTSD-related memory distortions. There is evidence that BDNF is crucial for structural and functional variations in this circuitry as will be pointed out in detail below. Second, fear extinction seems to be dependent on hippocampal dependent context modulation (Barnes & Thomas, 2008; Milad, Orr, Pitman, & Rauch, 2005) and animal research has shown that BDNF is associated with variations in fear extinction (Chen, et al., 2006). Third, it is unresolved if hippocampal and memory alterations are consequences or antecedences of the trauma. Even with severe trauma there is not a 100% prevalence rate for PTSD after trauma (Kessler, Berglund, et al., 2005; Kessler, Chiu, Demler, Merikangas, & Walters, 2005). This suggests that some people may be at risk to develop PTSD after a trauma while others are not. Research suggests that the amplification or extinction of conditioned fear responses over time and the PTSD-related memory alterations could depend on premorbid factors such as a genetic predisposition (Gilbertson, et al., 2002a; Gilbertson, et al., 2007; but also see Milad, et al., 2008). Support for a premorbid PTSD vulnerability is provided by twin research showing smaller hippocampal volume (Gilbertson, et al., 2002a) and deficits in contextual cue processing for which the hippocampus is crucial (Gilbertson, et al., 2007) in patients with PTSD. In addition, Parslow and Jorm (2007) found poorer pre-trauma neurocognitive functioning (immediate and delayed verbal recall) in subjects who developed PTSD symptoms after a major natural disaster.

We suggest therefore that one possible vulnerability factor for impairments in contextual memory, which seems to be crucial in PTSD, is the Met variant of the human BDNF gene as it has been shown to impact episodic memory encoding and retrieval (Dempster, et al., 2005) as well as recognition memory (Goldberg, et al., 2008) and associated brain activation (Hariri, et al., 2003; Hashimoto, et al., 2008). Additionally, recent animal research points towards an important role of BDNF in learning and retention of persistent fear responses (Chen, et al., 2006; Rasmusson, Shi, & Duman, 2002; Rattiner, Davis, & Ressler, 2005). Of special interest is BDNF's role in the consolidation and extinction of contextual fear memories (Barnes & Thomas, 2008; Monfils, Cowansage, & LeDoux, 2007; Ou & Gean, 2006, 2007) for which the hippocampus is a critical site (Corcoran & Maren, 2001; Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998; Maren, 2001; McDonald, Ko, & Hong, 2002; Phillips & LeDoux, 1992; Selden, Everitt, Jarrard, & Robbins, 1991). Impairments in the extinction of conditioned fear has been related to decreases in hippocampal BDNF (Heldt, Stanek, Chhatwal, & Ressler, 2007).

In the current study, we aim to investigate contextual memory retrieval of emotionally neutral words in a sample of healthy subjects and suggest that a genetic variant of BDNF accounts for individual variability in hippocampus-dependent contextual memory function. In order to test our assumption, we evaluated BDNF function through assessment of the BDNF Val66Met polymorphism in the human BDNF gene and used a recognition task that separates retrieval of items together with context details of the study event (recollection, contextual retrieval) from retrieval based on familiarity judgment (Remember-Know-Task; Eldridge, et al., 2000; Tulving, 2001; Yonelinas, 2002) to further clarify the specific role of BDNF in contextual memory. It is assumed, that recognition based on the recollection of context details of the study event involves hippocampal activation whereas familiarity based recognition does not (Dörfel, Werner, Schaefer, Von Kummer, & Karl, 2009; Eichenbaum, et al., 2007; Eldridge, et al., 2000; Yonelinas, et al., 2005). We hypothesize that the recollection performance is selectively more susceptible to influence of Val66Met genotype variants because it is particularly the hippocampal synaptic efficacy (immediate LTP effects, neuronal growth and sprouting) that has been affected by the genotype.

Thus, Met carriers should show a poorer performance only in retrieval based on contextual details (recollection).

5.3 Materials and Methods

5.3.1   Subjects

28 right-handed healthy volunteers (19 females mean 26.3 years, range 18 - 41 years of age) participated in this study. All participants were native German speakers with normal or corrected-to-normal vision. The subjects had been screened to exclude any participant with current or past neurological illness as well as current depressive or anxiety symptoms and history of trauma. Written informed consent was obtained from all subjects in accordance with institutional guidelines. All procedures were approved by the ethics committee of the German Psychological Association (DGP) and are therefore in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Due to technical problems, fMRI records of 2 subjects could not be used, therefore the final fMRI sample consisted of 26 participants (18 females mean 25.8 years). According to genotypes, these subjects were categorized into two groups: the Val/Val-BDNF group (15 subjects, 11 females) and the Val/Met-BDNF group (11 subjects, 7 females). There were no subjects in the homozygous Met/Met group in the sample for this fMRI study, due to the infrequent occurrence of this genotype in Caucasians. The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium (chi-square$_{24}$ = 1.8715, p = .788). The T-test revealed that there was no significant difference of age, (T$_{27}$ = .033, p = .974) between the two groups. The chi-square test revealed that there was no significant difference of distribution of gender ratio (chi-square$_1$ = 0.28, p = .597). All subjects had equal social and educational backgrounds (general qualification for university entrance or university degree).

5.3.2   Genotyping

DNA from buccal cells was collected with swabs (Catch-All™ Sample Collection Swabs, Epicentre) and extracted according to standardized protocols (BuccalAmp™ DNA Extraction Kit, Epicentre). The BDNF rs6265G/A single nucleotide polymorphism (SNP), coding for the Val66Met substitution, was

investigated by a restriction-fragment polymorphism-length analysis (RFLP). A 403bp BDNF exon 3 fragment PCR product was created by using specific primers (BDNF_for 5`-AAA GCC CTA ACC AGT TTT CTG; BDNF-rev 5`-TCC TCC AGC AGA AAG AGA AG) in a final volume of 50µl containing the following reaction mix: 100ng of DNA, 10pmol of each primer, 200µM of each dNTP, 2.0mM MgCl2, 50mM KCl, 10mM Tris-HCl, (pH 8.3 at 25°C), 0,0025mg/ml BSA, 0,025% Tween 20 and 1U of AmpliTaq Gold DNA Polymerase (Applied Biosystems). After initial denaturation for 5min at 95°C, 35 cycles of denaturation at 95°C for 30s, primer annealing at 56.6°C for 45s, and extension at 72°C for 30s were performed followed by a final extension step at 72°C for 5min. Ten µl of the PCR products were digested using 1U PmlI (NEB BioLabs) for 1h at 37°C. RFLP analysis was performed on a 1.5% agarose gel, stained with ethidium bromide visualized under UV illumination. Samples showing an undigested DNA band of 403bp derived from rs6262G alleles, whereas two bands of 262bp and 141bp revealed presence of the rs6265A allele.

5.3.3 Procedure

The Remember-Know (R/K) task was adapted from Eldridge et al. (2000). This procedure prevents participants from treating R/K judgments as measures of confidence (Hicks & Marsh, 1999) and assures that the Know category is not used for guess responses only (Eldridge, et al., 2002). The stimuli consisted of three similar lists of 177 non-emotional and non-arousing nouns each from a standardized wordlist by (Hager & Hasselhorn, 1994). The similar lists were randomly assigned to the subjects. In the study phase, 30 minutes prior to scanning, subjects had to learn a list of 150 nouns within 7 minutes. Following the procedure in Eldridge et al., the subjects were not explicitly instructed to use any specific strategy. In the recognition phase during fMRI scanning, the subjects were shown 108 old words and 27 new words. We used this relatively low number of items since we did not want the duration of the fMRI scanning to exceed one hour for ethical reasons. According to Eldridge et al.'s procedure, we used the low number of lures (20%) to increase the number of Know responses. Thus, we could ensure that the remember and the know condition would offer an almost equal number of trials for fMRI analysis. Each of the 9 functional runs contained 12 target words and 3 lures in a random order. In each five-second trial, subjects

first saw the word for 3 seconds and within that time period had to decide whether or not they recognized it (first response). Subsequently, for recognized items, they were prompted to decide whether they remembered or knew the item within 2 seconds (second response). The instructions for R/K distinction were clarified with examples before starting the fMRI scan. Responses were recorded via button presses. If the item was not recognized, the subject pressed either button at the second prompt. Between trials, subjects maintained fixation for 15 seconds. Subjects were instructed to disengage from the previous item during the fixation period.

5.3.4   fMRI data acquisition

Images were acquired using a 1.5 T whole body scanner *Siemens Sonata,* running under *Syngo VA25A* (Siemens, Erlangen, Germany) and equipped with an 8-Array Head Coil. Participants wore earplugs for noise protection and laid on a padded scanner table in a dimly lit room. Foam padding minimized head movement. Stimuli were generated by *Presentation* (Version 0.71, Neurobehavioral Systems, Albany, CA), and were projected with a video projector onto a transparent plastic screen installed in front of the scanner. Participants viewed the stimuli through an angled mirror positioned immediately in front of their eyes. Two structural scans were recorded before the functional scans using a 3D T1 sequence (104 slices, TR = 6 ms, TE = 2,92 ms, matrix 512 x 512, orientation = sagittal, slice thickness = 2 mm, band width of 240 Hz/Pix) and a MPRAGE sequence (104 slices, TR = 2200 ms, TE = 4,39 ms, matrix 320 x 320, orientation = sagittal, slice thickness = 0,79 mm, band width of 130 Hz/Pix). Functional data were acquired using a T2*-weighted EPI sequence (30 slices, TR = 3000 ms; TE = 45 ms, FOV = 230 mm, Matrix = 64x64, orientation = axial, slice thickness = 3 mm, band width of 750 Hz/Pix) to measure blood-oxygen level dependent contrast (BOLD). Functional data were collected in 9 runs, each run contained 104 volumes (scans) covering the whole brain.

5.3.5   fMRI data preprocessing

For the preprocessing and statistical analyses, the *Statistical Parametric Mapping software package* (*SPM5,* Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, Institute of Neurology, London) implemented in *Matlab*

7.1 (Release 14, SP 3, Mathworks, Inc., Natick, MA, USA) was used. After slice timing, the functional data were realigned to the first volume of the time series (six-parameter, rigid-body-transformation) to correct for movement artifacts. After that, the T1 image was coregistered to the mean image of the realigned functional scans, and parameters for spatial normalization of the coregistered T1 to the standard space of the Montreal Neurological Institute brain (MNI Brain) were determined. The normalization parameters were then applied to both the structural T1 and the functional EPI images (4th degree B-spline interpolation). Smoothing was executed with a three-dimensional Gaussian filter with a full width at half maximum (FWHM) of 8 mm.

5.3.6   fMRI event-related responses analysis

The classification of the answers into different recognition types was obtained using the second button presses of each trial. We classified the subjects' responses as 'correct Remember' (corrRem, old word correctly recognized and remembered), 'correct Know' (corrKnow, old word correctly recognized and known), 'correct Rejection' (corrRej, new word correctly rejected) or 'missed' responses (Miss, old word not recognized). There were too few false Remember and false Know trials for further analysis. The first three functional scans were discarded from the analysis. Within the general linear model (GLM) framework, regressors of events, modeled by the canonical hemodynamic response function (hrf), were created for each trial type (corrRem, corrKnow, corrRej, Miss). For the analysis of the fMRI BOLD response, the beginning of the word prompt paralleled the beginning of the hemodynamic response function. A 128-s temporal highpass filter was applied to the data to exclude low-frequency artifacts such as scanner drift. At the first-level analysis voxel-wise statistical parametric maps (SPM) were calculated for corrRem trials, corrKnow trials, corrRej trials and Miss trials for every subject. The results of these t-contrasts from each subject were then entered into a random-effects analysis at the group level (second-level analysis, full flexible design, repeated measures ANOVA with factor subject, group factor BDNF Genotype and within-factor Recognitiontype). Because of less than 5 males in each group, the non significant chi-square-test might be invalid and there could be a difference in the gender distribution in the two groups. Thus we included gender as a covariate in the ANOVA.

Then SPMs were created for the main effect of BDNF Genotype (Val/Met vs. Val/Val and vice versa), the main effect of Recognitiontype (corrRem, corrKnow, corrRej, and Miss), and for the interaction of BDNF Genotype with Recognitiontype. Additionally we searched for regions that were more activated in the Val/Met group or the Val/Val group, respectively, only during remember responses as compared to correct know responses using separate analyses for each group. For all analyses we used an uncorrected p-Threshold of .001 with an extent cluster threshold of k > 10 voxels. Regions were labeled with the *SPM toolboxes Automatic Anatomical Labeling* (AAL, Tzourio-Mazoyer, et al., 2002) and *Anatomy* (Version 1.5, Eickhoff, et al., 2005).

Contrast estimates (CE) and percent signal changes (PCC) were extracted, imported into and analyzed with *SPSS 16.0.1* using simple T-Tests (CE), a 2x2 (BDNF Genotype X Recognitiontype) repeated measures ANOVA, as well as two-tailed Pearson correlation analyses (PCC). Here we used p < .05 as a statistical threshold of significance.

5.3.7   Behavioral event-related responses analysis

Recognition performance, i.e. the proportion of correct responses to all old words for corrRem, corrKnow; proportion of correct responses to all new words for corrRej, and proportion of incorrect responses of all old words for Miss, was analyzed using *SPSS 16.0.1* for Windows (SPSS.Inc©) by using a repeated measures ANOVA (2 x 4) with factor BDNF genotype (Val/Met, Val/Val) as between and Recognitiontype (corrRem, corrKnow, corrRej, Miss) as within subject factor.

5.4 Results

5.4.1   Recognition Performance

The repeated measures ANOVA revealed a significant main effect of Recognitiontype ($F_{3,78} = 17.67$, $p < .001$, $Eta^2 = 0.41$, Figure 1) and a significant Genotype X Recognitiontype interaction (see Figure 5-1, $F_{3,78} = 4.03$, $p = .010$, $Eta^2 = 0.13$). In detail, the number of corrRem answers was significantly lower than the corrKnow responses in Val/Met carriers, whereas in the Val/Val group

there is no difference between corrRem and corrKnow ($F_{1,26}$ = 7.39, p = .012, Eta^2 = 0.22). The number of corrRem was also lower than the Miss responses only in the Val/Met group, whereas in the Val/Val group the subjects showed more corrRem than Miss responses ($F_{1,26}$ = 8.13, p = .008, Eta^2 = 0.24). Finally, the difference between the number of corrRem and corrRej responses was greater in the Val/Met than in the Val/Val group ($F_{1,26}$ = 8.86, p = .006, Eta^2 = 0.25). All these significant differences seem to depend on the lower number of corrRem responses in Val/Met carriers as compared to Val/Val carriers (T_{26} = -2.863, p = .008, Eta^2 = 0.24), while the performance of correct Know and correct Rejection was not different between the two BDNF genotypes (T_{26} = 1.654, p = .110, Eta^2 = 0.10; T_{26} = 1.546, p = .134, Eta^2 = 0.08, respectively), and Val/Met and Val/Val carriers only showed a trend towards a difference in Miss answers (T_{26} = 1.925, p = .065, Eta^2 = 0.12), as revealed by separate T-Tests.
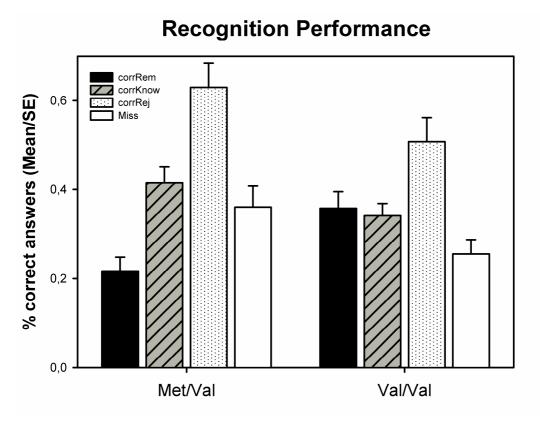
Figure 5-1: Behavioral recognition performance in the two BDNF groups.

5.4.2 Functional Brain Imaging Data

5.4.2.1 Whole Brain Analysis

The within subject ANOVA of the fMRI data revealed a significant main effect of BDNF Genotype (see supplementary Table S- 5-1, Table S- 5-2; $F_{1,72} > 39.266$, $p < .001$, Family Wise Error, FWE corrected). Please note that the statistical threshold in the analysis of this main effect was raised to FWE correction to emphasize on the most significantly activated clusters for the purpose of clarity. The middle occipital gyrus, the right inferior frontal gyrus, the inferior, superior and middle temporal gyrus, the left supramarginal gyrus, the left and right postcentral gyrus, and right parahippocampal cortex were significantly activated in Met carriers as compared to the Val/Val group across all conditions ($T_{72} > 6.10$, $p < .001$, FWE corrected, $k \geq 10$). On the other hand, the homozygote Val carriers showed significantly higher activation in the precuneus, the Brodmann Area (BA) 18 (cuneus, middle and superior occipital gyrus), in the superior medial frontal gyrus extending to left supplementary motor area (SMA) and anterior cingulate

gyrus, the superior and middle frontal gyrus, the precentral gyrus, the right insula, and the inferior parietal lobe ($T_{72} > 6.10$, $p < .001$, FWE corrected, $k \geq 10$). The main effect Recognitiontype showed activations in left medial precuneus and cuneus, left inferior parietal lobe including angular gyrus, and in left middle frontal gyrus ($F_{1,72} > 11.77$, $p_{uncorrected} < .001$, $k > 10$). The interaction BDNF Genotype X Recognitiontype revealed no significant results ($F_{1,72} < 11.77$, $p_{uncorrected} > .001$). In order to search for differences in the functional activation of brain areas between the Val/Met and the Val/Val group in contextual memory processing, we analyzed the functional activations in corrRem vs. corrKnow answers in the Val/Met and the Val/Val group, separately (see Table 5-1, Figure 5-2). In the Val/Met group, the contrast corrRem vs. corrKnow revealed significant activations of the left angular gyrus, the right calcarine gyrus, the left precuneus, the middle cingulate gyrus, the left middle temporal gyrus, right precuneus, the right hippocampus (see also Figure 5-4), and the medial orbitofrontal gyrus ($T_{72} > 3.21$, $p_{uncorrected} < .001$, $k \geq 10$). In the Val/Val group, the contrast corrRem vs. corrKnow revealed significant activations in the left middle cingulate gyrus, the left angular gyrus, the right pallidum, the left precuneus, and in the superior frontal gyrus ($T_{72} > 3.21$, $p < .001$, $k \geq 10$). The difference in the contrast estimates between the Met/Val and the Met/Met group shows a trend towards significance in the left middle temporal gyrus (Figure 5-3a; $T_{24} = 1.81$, $p = .083$, $Eta^2 = 0.12$), and is significant in the right calcarine gyrus ($T_{24} = 2.41$, $p = .024$, $Eta^2 = 0.19$) and the left superior frontal gyrus ($T_{24} = -2.73$, $p = .012$, $Eta^2 = 0.24$). A repeated measures ANOVA (2x2, BDNF as group factor; Recognitiontype corrRem, corrKnow as within subject factors) confirmed, that in the right calcarine gyrus the Val/Met carriers show a deactivation during corrKnow responses, whereas in the Val/Val group there is no difference between corrRem and corrKnow regarding the BOLD signal (percent signal changes, PCC; Figure 5-3b, $F_{1,24} = 4.93$, $p = .036$, $Eta^2 = 0.17$). Additionally, carriers of two Val alleles show significant less deactivation of the left superior frontal gyrus during corrRem as compared to corrKnow, whereas in the Val/Met group there is no difference between the two Recognitiontypes (Figure 5-3b, $F_{1,24} = 7.10$, $p = .014$, $Eta^2 = 0.23$).

Correlation analyses between the BOLD signal in those areas (PSC) and the recognition performance (corrRem, corrKnow responses) revealed negative

correlations between the recognition performance and the BOLD signal (PSC) in the left middle temporal gyrus during corrRem only in the Val/Met group (corrRem: $r = -0.75$, $p = .008$, $r^2 = 0.56$; corrKnow: $r = -0.80$, $p = .002$, $r^2 = 0.64$; corrRej: $r = -0.66$, $p = .028$, $r^2 = 0.44$). Additionally, we found a negative correlation between the BOLD signal in the left superior frontal gyrus during correct remember answers and the remember performance only in the Val/Met group, which almost reached significance ($r = -0.58$, $p = .061$, $r^2 = 0.34$).

Table 5-1: Regions more activated during recollection (contextual memory) as compared to familiarity based recognition in the Val/Met and the Val/Val genotype group.

Brain Region	BA	H	MNI-Coordinate			T value	cluster size
			x	y	z		
corrRem > corrKnow							
Val/Met group							
Inferior Parietal Cortex (Angular Gyrus, Inferior Parietal Lobe)	39	L	-54	-66	27	6.41	263
			-48	-72	33	5.12	
			-48	-63	42	4.86	
Lingual Gyrus/Thalamus	27	B	3	-30	-3	4.81	48
			-6	-30	-3	4.23	
Calcarine Gyrus	17	R	15	-54	12	4.55	29
Precuneus/Cuneus	30	B	-3	-57	15	4.64	100
	18		-18	-63	21	4.42	
	23		-3	-66	24	4.13	
Middle Cingulate Gyrus	23	B	-6	-36	42	4.42	50
			-9	-24	39	4.18	
			3	-42	42	3.46	
Middle Temporal Gyrus	21	L	-63	-21	-12	4.22	30
			-63	-33	-9	4.09	
Precuneus/ Hippocampus	27	R	9	-45	6	3.74	14
			15	-39	-3	3.61	
Hippocampus[1]		L	-30	-33	-12	4.03	6
Medial Orbitofrontal Gyrus	10	R	3	45	-6	3.51	13
Val/Val group							
Middle Cingulate Gyrus	23	L	-3	-36	39	4.97	43
			-9	-42	33	4.44	
Inferior Parietal Cortex (Angular Gyrus)	39	L	-48	-69	33	4.83	96
			-36	-69	33	4.27	
Caudate Nucleus	/	R	15	0	9	3.98	11
Precuneus	30	L	-6	-54	12	3.83	11
Superior Frontal Gyrus	8	L	-21	12	54	3.63	14
Hippocampus[1,2]		L	-21	-36	3	2.31	26

Anatomical locations, Brodmann Areas (BA), hemispheres (H, L = left, R = right), coordinates in MNI space (Montreal Neurological Institute), T values, and cluster sizes are given for regions showing significant activity during contextual memory (corrRem > corrKnow) in the Val/Met group and the Val/Val group, separately ($T_{72} > 3.21$, $p_{uncorrected} < .001$, $k \geq 10$). [1] ROI Analysis. [2] $T_{72} > 1.67$, $p_{uncorrected} < .05$, $k \geq 10$).
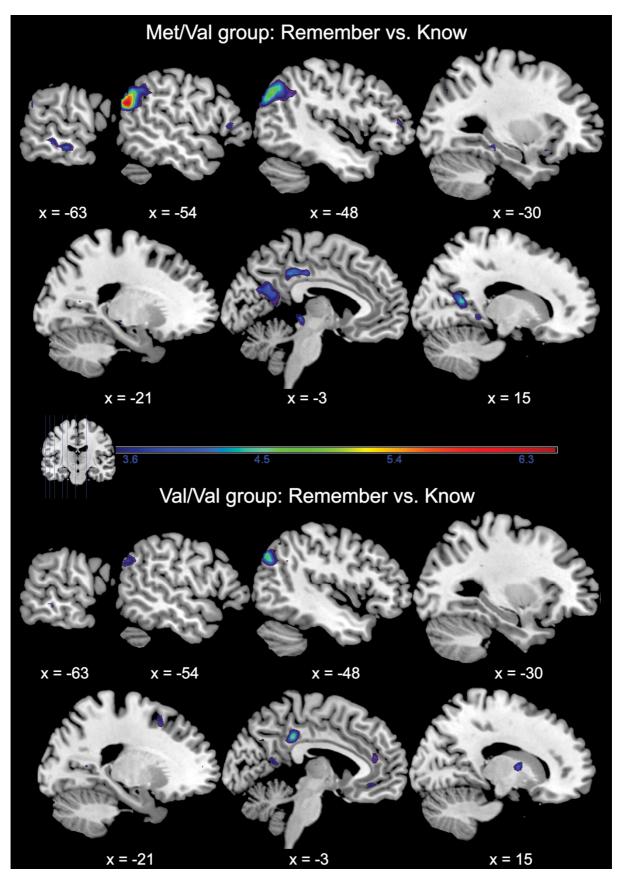
Figure 5-2: BOLD responses during contextual memory in the Met/Val as compared to the Val/Val group; contrast corrRem vs. corrKnow ($T_{72} > 3.21$, $p_{uncorrected} < .001$). Color bar represents T-Value.
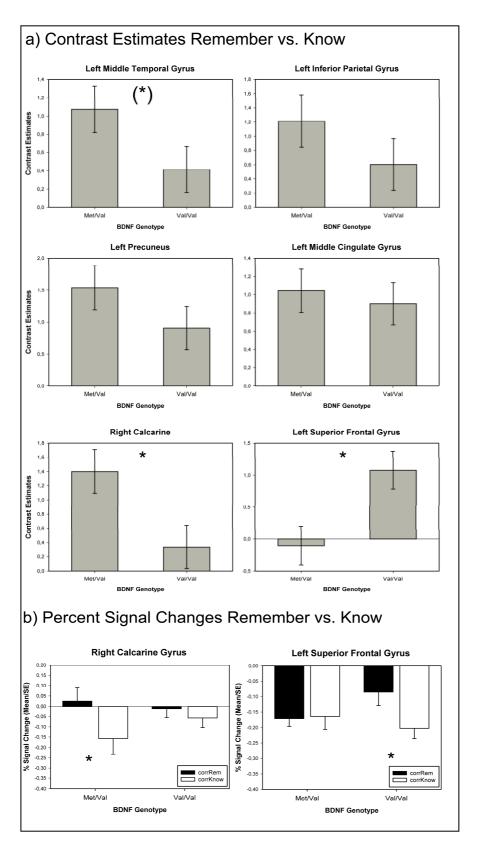
Figure 5-3: Contextual Memory in the Met/Val as compared to the Val/Val group: a) Contrast estimates of the contrast corrRem vs. corrKnow (Means, Standard Errors). b) Percent Signal Changes during corrRem and corrKnow (Means, Standard Errors). (p < .05; asterisk in parentheses p < .1).

5.4.2.2 Region of Interest Analysis

A hypothesis-driven regions of interest (ROIs) approach was used to investigate the right and left hippocampus and left and right parahippocampal cortex using a ROI created with the *Wake Forest University PickAtlas* (WFUPickAtlas; (Maldjian, Laurienti, Kraft, & Burdette, 2003) and the ROI tool of the *SPM5* Software Package (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London). In the hippocampal formation the ANOVA revealed a significant main effect of BDNF Genotype ($F_{1,72} > 11.77$, $p_{uncorrected} < .001$, $k > 10$). Regions in the left and right parahippocampal cortex as well as in the posterior hippocampus were significantly more activated in the Val/Met group as compared to the Val/Val group ($T_{72} > 3.20$, $p_{uncorrected} < .001$, $k > 10$). One cluster in the posterior hippocampus (at coordinates -30 -30 -15) revealed a significant difference in the contrast estimates of the contrast corrRem vs. corrKnow between the Val/Met and the Val/Val group (see Figure 5-5a; $T_{24} = 2.267$, $p = .033$, $Eta^2 = 0.18$). Additionally, the repeated measures ANOVA revealed a difference between the BOLD signals (Percent Signal Changes, PSC) of corrRem and corrKnow only in the Val/Met group (Figure 5-5b, $F_{1,24} = 5.55$, $p = .027$, $Eta^2 = 0.19$).

Furthermore, the main effect BDNF Genotype showed a region in the right anterior hippocampus, which was more activated across the recognition types in Val/Val as compared to Val/Met subjects ($T_{72} = 5.51$, $p_{uncorrected} < .001$, $k = 25$). Neither a significant main effect of Recognitiontype nor a significant interaction BDNF Genotype X Recognitiontype was detected ($F_{1,72} < 11.77$, $p_{uncorrected} > .001$).

Again, we wanted to search for differences in the functional activation of brain areas between the Val/Met and the Val/Val group in contextual memory. Hence, we analyzed the functional activations in corrRem vs. corrKnow answers in the Val/Met and the Val/Val group, separately (Table 1, Figure 4). In the Val/Met group, the contrast corrRem vs. corrKnow revealed significant differences in the activation of a left posterior hippocampal/parahippocampal region ($T_{72} = 4.03$, $p_{uncorrected} < .001$, but note $k = 6$). In fact, there was less deactivation during corrRem as compared to corrKnow. However, we could find no significant difference in the contrast estimates between the Val/Met and the Met/Met group in this region ($p > .05$).

In the Val/Val group, the contrast corrRem vs. corrKnow revealed no significant activations ($T_{72} < 3.21$, $p_{uncorrected} > .001$)
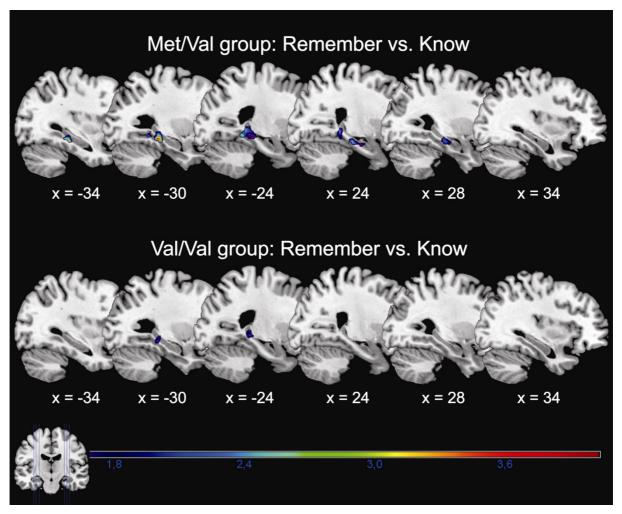
Figure 5-4: BOLD responses during contextual memory in the Met/Val as compared to the Val/Val group in the Hippocampus; contrast corrRem vs. corrKnow ($T_{72} > 1.6$, $p_{uncorrected} < .05$). Color bar represents T-Value.

5.5 Discussion

In the current study, we assessed hippocampal-dependent contextual memory retrieval and its modulation by BDNF function. We used a recognition task which can separate retrieval of items together with context details of the study event (recollection, contextual retrieval) from retrieval based on familiarity judgment (Remember-Know-Task, Eldridge, et al., 2000). We analyzed the modulation by variations in the BDNF Val66Met polymorphism (Val/Met and Val/Val allele carriers) on the performance and the brain activations during contextual as compared to familiarity based retrieval.

Remember vs. Know

a)   Contrast Estimates   b)   Percent Signal Change
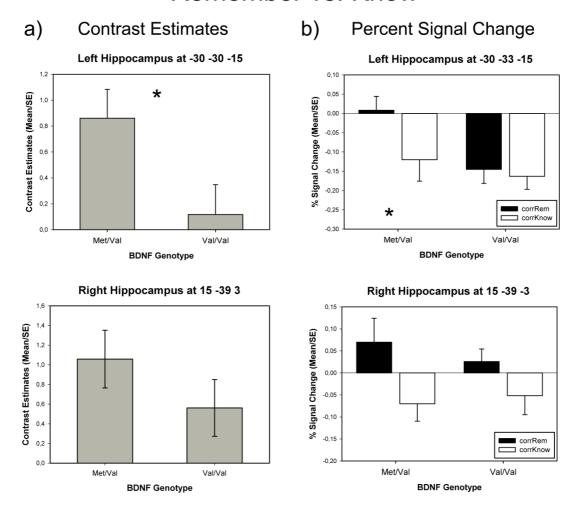
Figure 5-5: Contextual Memory in the Met/Val as compared to the Val/Val group in the hippocampus.

5.5.1   Evidence for differential effects of BDNF genotype on the recognition performance

The performance in retrieval based on contextual details (recollection) was significantly decreased in Val/Met carriers as compared to Val/Val carriers, while the performance of familiarity based retrieval and the correct rejection of new items did not vary according to BDNF genotype. This finding confirms our hypothesis that only recollection based retrieval should be related to BDNF genotype. BDNF is known to be involved in the LTP in the hippocampus (Lu, et al., 2008; Poo, 2001) and is acting on receptors across subregions of the hippocampus (Bramham & Messaoudi, 2005; Murer, et al., 2001). Egan et al. (2003) showed that hippocampal function is impaired in a variant of the BDNF

gene (carriers of the Met allele). Thus, it is hippocampus-dependent contextual memory that should be specifically influenced by BDNF.

Our results are in line with previous research (Hariri, et al., 2003) and add additional information about a specific role of the BDNF gene in contextual memory retrieval. Additionally, the specific effect of the BDNF genotype only on recollection supports Dual Process Models of recognition memory (Eichenbaum, et al., 2007; Yonelinas, 2002), which state that recollection and familiarity are functionally independent.

Based on the assumption that brain regions, which are involved in the correct retrieval of contextual information about a studied item are impaired in carriers of the Met allele, such as left inferior parietal lobe or left prefrontal cortex (PFC) we also analyzed the effect of the genotype on additional brain activations during contextual as compared to non-contextual (familiarity based) retrieval.

5.5.2 Evidence for differential effects of BDNF genotype on brain activations during contextual retrieval

We found differences in brain activations during contextual memory retrieval in the calcarine gyrus, the superior frontal gyrus, and with limitations in the left middle temporal gyrus between the Val/Met genotype and the homozygote Val carriers. Furthermore, the BOLD signal in the left middle temporal gyrus (BA 21) decreased, when the performance in contextual memory increased. Given that the association between the BOLD signal in this area and retrieval performance is only apparent in the Val/Met group, we suggest that an increase of activity in the left middle temporal gyrus contributes to the poor contextual memory performance in carriers of the Met allele. Furthermore, we suggest that the left middle temporal gyrus is not necessarily activated in the retrieval of episodic, contextual details of a studied item, because we did not find an association of this region's activation with the (very good) performance in recollection (contextual memory) in the Val/Val group. This is in accordance with findings by Konishi et al. (2006) who link the lateral temporal gyrus to non-relational (hence, non-contextual) item-based memory. The authors suggest that the lateral temporal region implements item-based recency judgments that emerge themselves when relational processing is dysfunctional. In the Met carriers, relational processing which is, for instance,

based on functional hippocampal involvement (Egan, et al., 2003) could be impaired. Hence, relational processes are activated to a lesser degree, and the item-based processes are enhanced instead. This might be reflected by the poorer performance in contextual retrieval and the higher activation in lateral temporal gyrus that we found in the carriers of the Met allele. However, in contrast to Konishi et al. (2006), we found a left lateralized temporal area instead of activations on the right hemisphere, which may be explained by the different stimulus material that was used in our study (words vs. Japanese characters in the Konishi et al. study).

Additionally, we found a difference in the activation of left superior frontal gyrus (BA 8) between recollection and familiarity only in the Val/Val group. Similar left-lateral prefrontal (PFC) activations were found in other studies which differentiate between recollection and familiarity based retrieval (Eldridge, et al., 2000; Henson, Rugg, et al., 1999; Wheeler & Buckner, 2004). A meta-analysis by (Skinner and Fernandes (2007) found that BAs 8, 6 and 10 showed high agreement in activation during recollection across different studies. Left PFC activity has been related to episodic retrieval (Cabeza, Dolcos, et al., 2003) as well as to context recognition as compared to mere item-recognition (Cabeza, Locantore, et al., 2003). Thus, the left PFC activity found in our and in previous studies may be related to the successful retrieval of contextual details. Furthermore, we suggest that this function is impaired in carriers of the BDNF Met allele, because we could not find a difference in the activity of the left PFC between recollection and familiarity in the Val/Met group. However, the activity of this left PFC region decreased with increasing performance in contextual memory only in carriers of the Met allele. This is contrary to the findings that describe a positive relationship between recollection and a left PFC activation (Skinner & Fernandes, 2007) and further supports the assumption of a Met-allele associated impairment in brain regions that support a recollection network. However, the significance of the correlation reported here is weak, maybe because our group of Met allele carriers is rather small (n = 11), and further investigations of the association of the BDNF Val66Met polymorphism with recognition processes have to confirm our result.

5.5.3 Evidence for differential effects of BDNF genotype on hippocampal activations during contextual retrieval

In the hippocampal ROI, we also found differences in the BOLD response during contextual memory retrieval between the Val/Met and the Val/Val group. A left posterior hippocampal region (at coordinates -30 -33 -15) was significantly differently activated during contextual retrieval in carriers of the Met allele as compared to the homozygote Val carriers. Whereas the Met carriers show no change in hippocampal activation in recollection responses, the Val/Val group showed a deactivation which is comparable to the deactivation during familiarity responses. Contrary to our hypothesis, we could only find significant differences between recollection based and familiarity based retrieval in the hippocampus in the Val/Met group. However, the Met substitution in the BDNF gene is supposed to lead to substantial defects in synaptic plasticity and neurotransmission specifically in the hippocampal formation (Chen, et al., 2005; Chen, et al., 2004; Egan, et al., 2003; Huang & Reichardt, 2001; Poo, 2001). Hence, we suggested that there should be a deficit in the involvement of the hippocampus in the Val/Met group as opposed to a functional response, i.e. higher activation, during contextual retrieval in the Val/Val carriers. In contrast, the Val/Val group showed no significant difference in the activation of the hippocampus between contextual (recollection) and non-contextual (familiarity) memory retrieval, which is a surprising result that needs further exploration.

If our results of differential efficiency and brain activation in contextual memory in those with at least one Met allele are confirmed in larger samples this may also have implications for explaining specific alterations in psychological disorders with a pronounced memory deficit. Future studies need to establish that the marked impairment in contextual memory performance in carriers of a known dysfunctional variant of the human BDNF gene (66Met), that we report in the current study, could be a vulnerability factor for these disorders such as PTSD. There is evidence that patients with PTSD and their twins show a premorbid smaller hippocampal volume (Gilbertson, et al., 2002b) and deficits in contextual cue processing for which the hippocampus is crucial (Gilbertson, et al., 2007). Additionally, a recent meta-analysis (Karl & Werner, 2009) of ^{1}H MR-

Spectroscopy studies in patients with PTSD found reduced N-acetylaspartate (NAA) in the left hippocampus of PTSD patients as compared to non-exposed healthy controls and trauma survivors who did not develop PTSD (non-PTSD), which may reflect decreased neuronal density and/or axonal density and viability. Given that the mechanism underlying a reduction in neuronal density can be downregulation of BDNF mRNA after traumatic stress (Kozlovsky, et al., 2007; Rasmusson, et al., 2002), an interaction of this downregulation with an existing BDNF trafficking deficit, as apparent in carriers of the Met allele, could lead to a vulnerability in developing smaller hippocampal volume after severe traumatic stress or even make an existing smaller hippocampus more vulnerable to severe stress and therefore more likely to develop a PTSD. However, reanalyzing the same twin sample of the Gilbertson et al. study, by using a different approach of measuring brain volume (VBM), Yamasue et al. (2008) could not confirm the hypotheses that a reduced hippocampal volume may serve as a risk factor for developing a PTSD. Additionally, a meta-analysis showed that trauma-exposed persons without PTSD also have significantly reduced bilateral hippocampal volumes as compare to non-traumatized healthy controls, pointing to an acquired deficit after severe traumatic stress (Karl, et al., 2006). In line with this, our data could not proof that the BDNF Met variant constitutes a vulnerability factor for hippocampal deficits which would explain the impairments in contextual (recollective) memory that we found in carriers of the Met allele, though similar impairments are discussed in terms of the development and maintenance of PTSD (Ehlers & Clark, 2000; Ehlers, Hackmann, & Michael, 2004; Rauch, et al., 2006) and reduced hippocampal activity is found in patients with PTSD during memory-related tasks (Francati, Vermetten, & Bremner, 2007). However, a study by Geuze, Vermetten, Ruf, de Kloet, & Westenberg (2008) could not relate the reduction of hippocampal activity in PTSD patients during associative memory retrieval with task performance. Future research should therefore apply the question whether hippocampal dependent memory of neutral content is altered in PTSD and how this relates to the brain function of the hippocampus and other cortical areas known to be involved in those processes. Additionally, we found alterations in brain activity that were associated with the reduction in contextual memory performance in carriers of the BDNF Met allele in left PFC and left lateral temporal cortex. Both regions proofed to be related to reduced cortical thickness

in patients with PTSD as compared to trauma-exposed healthy subjects (Geuze, Westenberg, et al., 2008). Additionally, there is growing evidence that left PFC and left lateral temporal activity is altered in patients with PTSD (Bremner, et al., 2003; Geuze, Vermetten, et al., 2008; Shaw, et al., 2002) and that left lateral temporal function is related to the performance in retrieval of neutral associative word pairs as well as to symptom severity in PTSD patients (Geuze, Vermetten, et al., 2008). We therefore suggest that our findings of altered brain function in left lateral prefrontal and temporal areas in carriers of the Met allele mimic those deficits found in PTSD patients. As a next step, future research should include Met and Val allele carriers in groups of PTSD patients, trauma-exposed controls, and non-exposed healthy controls to strengthen those results.

Until now, there is only one study that assessed an association of the BDNF Val66Met polymorphism with the development of PTSD (Zhang, et al., 2006). The authors found no association between the polymorphism and chronic PTSD. One limitation of this study was that they did not include trauma-exposed controls and thus could not clarify the role of BDNF as a vulnerability factor for developing PTSD after a traumatic event. However, it is also possible, that an epistatic effect of BDNF genotype with other polymorphisms is more applicable to explain why at least half of the trauma exposed persons do not develop a PTSD. Recently, such an effect was found in terms of subgenual ACC volume and, with limitations, amygdala volume (Pezawas, et al., 2008) in interaction with the 5-Hydroxytriptamine (Serotonin) Transporter-Linked Polymorphic Region (5-HTTLPR) insertion/deletion polymorphism which has implications for depression and anxiety disorders. Unpublished data from our working group partly confirms this finding in a sample of 45 healthy subjects and extends the effect to left hippocampal volume (Dörfel, et al., submitted).

Because we only investigated healthy subjects, we cannot directly proof our hypothesis that variants in the BDNF gene constitute a premorbid vulnerability factor for contextual memory deficits in PTSD, which are supposed to contribute to the development and maintaining of the disorder (Ehlers & Clark, 2000; Gilbertson, et al., 2002b; Gilbertson, et al., 2007). However, the specific role of BDNF in contextual memory function that we could show in our study and the known role of the neurotrophin in the consolidation and extinction of contextual fear memories (Barnes & Thomas, 2008; Monfils, et al., 2007; Ou & Gean, 2006,

2007), should be further investigated in different patient populations with known memory alterations.

5.5.4 Conclusion

In conclusion, we found preliminary evidence for less efficient contextual memory performance in conjunction with deactivations in the left lateral temporal cortex (BA 21) and the left PFC (BA8) in carriers of the Met allele in healthy volunteers. We could not confirm our hypothesis of a deficit in hippocampal processing in the carriers of the Met allele. A limitation of our study is that our sample is very homogenous in terms of age and no marked memory deficits are apparent in our healthy subjects. Thus, less variability in our sample might lead to difficulties in detecting a possible medium effect of BDNF genotype on hippocampal activation during contextual memory retrieval. Another explanation for the lack of differences in hippocampal function between Val/Val and Val/Met carriers could be that there is a dose dependent effect on memory related hippocampal activity (Hashimoto, et al., 2008) and impairments in hippocampal function are most pronounced in homozygote carriers of the Met allele. However, we could not find homozygote Met carriers for this sample, because this genotype very seldom occurs in Caucasians.

5.6 Supplementary Tables

Table S- 5-1: Main Effect BDNF Genotype: Brain Regions that are more activated in the Val/Met as compared to the Val/Val group in a Recognition Memory Paradigm

Brain Region	BA	H	x	y	z	T value	cluster size
Frontal Lobe							
Inferior Frontal Gyrus (P.Opercul.)	44/45	R	42	6	27	17.01	154
Inferior Frontal Gyrus (P.Opercul.)	44/45	L	-48	9	27	12.22	33
Rolandic Operculum	48	R	60	-18	21	12.16	49
Inferior Orbitofrontal Gyrus	47	L	-39	24	-3	10.57	16
Inferior Frontal Gyrus (P.Triangul.)	45/44	L	-48	33	18	7.75	14
Supplementary Motor Area	6	B	0	12	51	10.23	12
Middle Cingulate Gyrus	4/5/6	B	3	-42	42	10.91	174
Precentral Gyrus	44	L	-51	6	4	12.62	14
Precentral Gyrus	4/6	L	36	1	4	11.32	25
Precentral Gyrus	6	L	-33	-9	57	11.25	47
Precentral Gyrus	6/44	R	51	0	51	10.68	20
Temporal Lobe							
Parahippocampal Gyrus	37	R	3	-36	-15	13.11	19
Inferior Temporal Gyrus	21/37	R	60	-51	-9	13.04	41
Inferior Temporal Gyrus	37	L	-45	-60	-3	10.70	18
Middle Temporal Gyrus	22/48	L	-54	-15	-3	8.37	12
Superior Temporal Gyrus/Inferior Parietal Cortex	41/42 22/39	R	60	-45	21	16.06	206
Parietal Lobe							
Postcentral Gyrus	2/1	L	-27	-48	63	14.35	178
Postcentral Gyrus	2/1/3	R	60	-21	48	13.47	147
Postcentral Gyrus	3	L	-57	-9	27	11.79	119
Supramarginal Gyrus/Inferior Parietal Cortex	40	L	-66	-33	33	15.16	33
Superior Parietal Gyrus	7/5	R	18	-60	54	11.51	33
Superior Parietal Gyrus/Angular Gyrus	7	L	-24	-63	51	10.51	133
Superior Parietal Gyrus/Postcentral Gyrus	40	R	33	-36	42	8.41	25

Occipital Lobe

Fusiform Gyrus	37	L	-27	-45	-9	12.96	103
Fusiform Gyrus	37/19	R	27	-51	-6	10.76	18
Middle Occipital Gyrus	18/19	L	-36	-87	9	40.53	179
Middle Occipital Gyrus	19	R	42	-84	12	25.28	97
Superior Occipital Gyrus	19/7	R	27	-72	36	12.24	49
Caudate Nucleus	/	L	-9	-6	15	11.10	17
Cerebellum	/	B	0	-39	-9	9.25	14

Anatomical locations, Brodmann Areas (BA), hemispheres (H, L = left, R = right), coordinates in MNI space (Montreal Neurological Institute), T values, and cluster sizes are given for regions showing greater activity in the Met/Val group as compared to the Val/Val group (FWE, p < .001; extent threshold k = 10); P.Opercul. – Pars Opercularis, P.Triangul. – Pars Triangularis.

Table S- 5-2: Main Effect BDNF Genotype: Brain Regions that are more activated in the Val/Val as compared to Val/Met the group in a Recognition Memory Paradigm

Brain Region	BA	H	MNI-Coordinate			T value	cluster size
			x	y	z		
Frontal Lobe							
Superior Medial Frontal Gyrus	10	B	-3	60	30	11.58	46
Superior Medial Frontal Gyrus	10	R	9	63	9	10.09	216
Middle Frontal Gyrus	45/46	L	-39	36	30	16.21	193
Middle/Inferior Orbitofrontal Gyrus	47	L	-45	45	-12	8.95	11
Superior/Middle Frontal Gyrus	6	L	-24	-9	48	14.02	34
Superior/Middle Frontal Gyrus	46/45	R	36	36	24	13.67	143
Superior Frontal Gyrus	9/8	R	18	30	51	10.27	102
Insula	48	R	36	18	12	15.70	159
Precentral Gyrus	4/6/44	L	-42	-3	36	16.18	461
Precentral Gyrus	6/44	R	51	6	39	13.25	52
Precentral Gyrus	6	R	33	-15	51	10.94	133
Supplementary Motor Area	6	L	-9	6	54	16.49	337
Middle Cingulate Gyrus	23	L	-6	-6	48	10.74	12
Temporal Lobe							
Temporal Pole	21	R	42	3	-18	6.96	13
Amygdala	34/48	R	27	-9	-3	7.42	32
Superior/Middle Temporal Gyrus	41/42	L	-51	-39	18	8.87	21
Superior/Middle Temporal Gyrus	21	R	60	-15	-6	8.16	10
Superior Temporal Gyrus	22	R	66	-24	9	8.67	23
Superior Temporal Gyrus	21/37	R	57	-54	6	8.63	10
Parietal Lobe							
Postcentral Gyrus	1/3/4	L	-48	-18	51	9.23	12
Inferior Parietal Lobe	40/39	L	-42	-51	48	14.81	88
Inferior Parietal Lobe	40/39	R	45	-54	48	12.08	38
Supramarginal Gyrus/Angular Gyrus	39/22	L	-60	-57	27	9.04	13
Occipital Lobe							
Superior Occipital Gyrus/Precuneus	17/18/7	B	18	-96	12	29.90	824
Thalamus	/	L	-9	-18	-3	8.50	15
Putamen	/	L	-27	-12	6	8.20	15

Anatomical locations, Brodmann Areas (BA), hemispheres (H, L = left, R = right), coordinates in MNI space (Montreal Neurological Institute), T values, and cluster sizes are given for regions showing greater activity in the Met/Val group as compared to the Val/Val group (FWE, p < .001; extent threshold k = 10); P.Opercul. – Pars Opercularis, P.Triangul. – Pars Triangularis.

6 STUDY III: BDNF AND 5-HTT INTERACTION ASSOCIATED WITH LOWER GREY MATTER VOLUME IN EMOTIONAL MEMORY CIRCUITRY

6.1 Abstract

Background: Variations in the genes encoding the serotonin transporter (5-HTT) and brain-derived neurotrophic factor (BDNF) have been associated with altered cognitive-affective processing. Recently, it has been shown that the BDNF 66Met allele protects against 5-HTTLPR s allele related reductions in gray matter (GM) volume in the subgenual anterior cingulate cortex (ACC). The aim of this study was to explore if there is an interaction between BDNF and 5-HTT genetic variation and brain volume in structures underlying emotional memory as these are often altered in anxiety disorders such as PTSD.

Methods: 45 healthy subjects were genotyped and examined for differences in GM volume of ACC, hippocampus, and amygdala using voxel-based morphometry of structural brain images.

Results: We observed a genetic interaction effect in both amygdala and hippocampus indicating lower l/l related GM volume in BDNF Met carriers and higher l/l related GM volume in Val/Val carriers.

Conclusions: The observed genetic differences in hippocampus and amygdala volume do not support the hypothesis of a protective effect of the BDNF Met allele against 5-HTTLPR s allele related GM volume reductions, but rather point to a differential susceptibility of the l/l genotype depending on the BDNF genotype.

6.2 Introduction

Variations in the genes encoding the serotonin transporter (5-HTT, SLC6A4) and brain-derived neurotrophic factor (BDNF) have been associated with altered cognitive-affective processing possibly predisposing individuals to acquire clinically relevant depression or anxiety (Martinowich & Lu, 2008). The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) modulates the gene's transcriptional efficiency, and hence, the expression of 5-HTT (Lesch, et al., 1996). Carriers of the less efficient short (s) allele of 5-HTTLPR have been observed to exhibit higher levels of anxiety-related personality traits, affective and anxiety disorders, and functional as well as structural alterations in the amygdala, the anterior cingulate cortex (ACC) and the hippocampus (Caspi, et al., 2003; Frodl, et al., 2008; Lee, et al., 2005; Lesch, et al., 1996; Munafo, et al., 2008; Pezawas, et al., 2005).

The variation in the BDNF gene (BDNF 66Met) has been associated with abnormal hippocampal function and associated memory performance (Hariri, et al., 2003), reduced hippocampal and amygdala volume (Bueller, et al., 2006; Montag, Weber, Fliessbach, Elger, & Reuter, 2009) and higher amygdala activity in response to emotional stimuli (Montag, Reuter, Newport, Elger, & Weber, 2008). In contrast, the BDNF 66Met allele seems to be associated with *lower* scores in anxiety- and depression-related personality traits (Frustaci, Pozzi, Gianfagna, Manzoli, & Boccia, 2008). Additionally, a study in PTSD patients failed to show a diagnose x BDNF genotype interaction which may be due to the lack of trauma-exposed controls (Zhang, et al., 2006). Nevertheless, the above-mentioned findings indicate that both genes may interact in the etiology of psychopathology associated with altered emotional memory, such as posttraumatic stress disorder (PTSD).

Recently, Pezawas and colleagues (2008) observed that the BDNF 66Met allele protected against 5-HTTLPR s allele related reductions in gray matter (GM) volume in the subgenual anterior cingulate cortex (ACC) and, marginally significant, in the amygdala. In the present study, we examined whether the epistatic effect of the BDNF Val66Met polymorphism and 5-HTTLPR on GM

volume can be demonstrated in brain structures vital for emotional memory such as the hippocampus and amygdala and whether it can be replicated for the ACC.

6.3 Methods and Materials

6.3.1   Subjects

45 subjects (16 male; age mean: 25.8, age SD: 5.47) participated in the study. They were free of any current or past neurological illness as well as current depressive or anxiety symptoms, as screened prior to testing and examined by self report questionnaires, the Beck Depression Inventory (BDI; Hautzinger, Bailer, Worall, & Keller, 1995)) and the State Trait Anxiety Inventory (STAI-T; Laux, Glanzmann, Schaffner, & Spielberger, 1981)). All subjects gave written informed consent and the procedures were in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the ethics committee of the German Psychological Association (DGP).

6.3.2   Genotyping

Buccal samples were obtained and DNA was extracted using the BuccalAmp system (Epicentre Technologies, Madison, USA). Genotypes were determined for BDNF Val66Met Genotypes were determined for BDNF Val66Met, performing PCR and PmII digestion (BDNF specific primers, PCR and RFLP conditions are available on request) and as described earlier for 5-HTTLPR (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). BDNF genotype frequencies were 4.4% for Met/Met, 33.3% for Val/Met, and 62.2% for Val/Val genotypes. 5-HTTLPR genotype frequencies were 37.8% for l/l, 40.0% for l/s, and 22.2% for s/s. All genotypes were in Hardy-Weinberg-Equilibrium (chi-square-tests with $df = 1$, all $p > 0.50$). Following the approach used by Pezawas et al. (2008), individuals with the BDNF Met/Met and Val/Met genotypes were combined and compared to Val/Val genotype carriers. Likewise, 5-HTTLPR s allele carriers (s/s and s/l genotypes) were compared to l/l genotype.

6.3.3   Structural image processing

Images were acquired using a 1.5 T *Siemens Sonata*, running under *Syngo VA25A* (Siemens, Erlangen, Germany) and equipped with an 8-Array Head Coil.

Structural images were recorded using a 3D T1 sequence (104 slices, TR = 6 ms, TE = 2.92 ms, matrix 512 x 512, orientation = sagittal, slice thickness = 2 mm, band width of 240 Hz/Pix). Preprocessing of the data was conducted using a *Voxel Based Morphometry* protocol by Christian Gaser (*VBM 5.1*) implemented in *SPM 5* (Statistical Parametric Mapping software package, Wellcome Department of Cognitive Neurology, London) running on *Matlab 7.1* (Release 14, SP 3, Mathworks Inc., Natick, MA, USA).

6.3.4   Statistical image processing

We performed a two-way ANOVA with BDNF genotype (Met allele vs. Val/Val genotype carriers) and 5-HTTLPR genotype (s allele vs. l/l genotype carriers) as factors and age and gender as covariates. By the use of grey matter images, which were only modulated for non-linear warping during normalization in the VBM 5.1 preprocessing, the inclusion of grey matter total volume as a covariate is no longer needed. Based on the evidence outlined in the introduction, we conducted a region of interest (ROI) analysis of the amygdala, the hippocampus, and the ACC including its subgenual part. The amygdala and hippocampal ROIs were created using the cytoarchitectonic maps of the *Anatomy toolbox* (Version 1.5; Eickhoff, et al., 2005)). The ACC ROI was created with *MRIcroN* (Chris Rorden, University of South Carolina, Columbia, USA) based on the AAL atlas (*Automatic Anatomical Labeling;* Tzourio-Mazoyer, et al., 2002) and an atlas of Brodmann areas.

We employed a statistical uncorrected *p*-threshold of .005. To correct for multiple comparisons, an extent threshold (number of voxels in one cluster, if alpha < .05) according to the ROIs was estimated using MonteCarlo simulations with Alpha Sim (B. Douglas Ward, 2008). For visualization purposes, the *p*-threshold for all analyses was lowered to .05 (uncorrected level) in all Figures and in Table 6-1, where significant differences were labeled accordingly.

6.4 Results

First, we found significant main effects of 5-HTTLPR as well as BDNF genotype on amygdala and hippocampal morphology (Table 6-1). Second, the two-way ANOVA revealed an interaction effect in both the amygdala and the hippocampus

indicating *greater* s allele related GM volume in the BDNF Met group and *lower* s allele related volume in the Val/Val group (Table 6-1; Figure 6-1, top, and upper panels of Supplementary Figure S- 6-1 and Figure S- 6-2). Similarly, when contrasting the 5-HTTLPR s allele carriers and the l/l genotype carriers separately for BDNF genotypes, we observed that in the BDNF Met group, there were s allele related *increases* of GM volumes in bilateral amygdala and left hippocampus (Table 6-1; middle panels of Figure S- 6-1 and Figure S- 6-2), whereas in the BDNF Val/Val group were significant s-allele related *reductions* in bilateral amygdala and left hippocampal GM volume (Table 6-1; lower panels of Figure S- 6-1 and Figure S- 6-2). The reverse contrasts were not significant (see Table 1).

In the subgenual ACC, there were no interactions between BDNF and 5-HTTLPR genotype (Table 6-1 and Figure S- 6-3).

To scrutinize these results, we extracted the relative GM volumes of bilateral amygdala and hippocampal ROIs using the *Anatomy toolbox* and analyzed the mean values with *SPSS 16.0.1 for Windows* (SPSS Inc., Chicago, IL, USA) adopting a multivariate ANOVA with gender and age as covariate. We found highly significant interactions between BDNF and 5-HTTLPR genotype in both the left amygdala ($F_{1,39}$ = 9.569, p = .004, Eta^2 = 0.20) and the right amygdala ($F_{1,39}$ = 5.863, p = .020, Eta^2 = 0.13) as well as a trend towards an interaction between the two polymorphisms in left hippocampus ($F_{1,39}$ = 3.663, p = .063, Eta^2 = 0.09; see Figure 6-1). Figure 6-1 (bottom) reveals that the significant interaction between the two genotypes is driven by an l/l genotype related reduction of GM volume in the Met group and an increase in the Val/Val group, whereas the GM volume of s allele carriers shows no difference between the BDNF groups. The BDI and STAI-T scores did not significantly correlate with the GM volumes of amygdala and hippocampus (p > .05), but there is an interaction between BDNF and 5-HTTLPR in the BDI sum score indicating higher l-allele related score in the Met group and a lower l-allele related score in the Val/Val score ($F_{1,34}$ = 5.421, p = .026, Eta^2 = 0.14).

Table 6-1: Regions in the hippocampus, amygdala, and ACC ROIs which show different grey matter volumes with respect to Met vs. Val/Val BDNF genotype in interaction with s vs. l/l 5-HTTPLR genotype.

Region	H	BA	t	p[1]	x	y	z
Interaction Effects							
Met s > l/l × Val s < l/l							
Hipp (CA Region)/Parahipp. G.	L	20/37	3.01	.002*	-29	-34	-13
Hipp (CA Region)	L	20	2.84	.004*	-26	-13	-11
Hipp (CA Region)	R	20	2.74	.005*	35	-30	-15
Hipp (Subiculum)	L	27	2.26	.015	-16	-44	-3
Hipp (Entorhinal Cortex)	R	36	2.11	.021	27	-12	-32
Amyg (Basolateral)[2]	L	–	3.06	.002*	-24	-11	-12
Amyg (Superficial)[2]	R	–	2.71	.005*	23	-10	-11
ACC	B	32	2.44	.010	-4	49	17
Met s > l/l							
Hipp (CA Region)	L	20/30	3.42	.001*	-28	-30	-15
Hipp (CA Region)	R	20	2.95	.003*	24	-13	-12
Amyg (Basolateral)	R	–	3.34	.001*	23	-10	-13
Amyg (Basolateral)	L	–	3.33	.001**	-22	-12	-12
Val s < l/l							
Hipp (Entorhinal Cortex)	L	35/28	3.46	.001*	-17	-3	-31
Hipp (CA Region)	R	20	2.55	.005*	34	-28	-17
Hipp (CA Region, Subiculum)	L	20	2.20	.014	-29	-16	-23
Amyg (Basolateral)	R	–	3.31	.001**	24	-5	-5
Amyg (Superficial)	L	–	3.03	.002**	-25	-5	-4
ACC/Medial Orbitofrontal G.	B	10/32	2.66	.006	5	49	-4
ACC	B	24	2.61	.006	1	31	33
ACC	R	32	2.11	.020	4	46	20
Met s < l/l × Val s > l/l							
ACC/Middle Cingulate Gyrus	B	–	2.88	.003*	0	1	30
Met s < l/l							
–							
Val s > l/l							
Hipp (Subiculum)	L	27	2.40	.011	-19	-28	-6

Table is continued on the next page

Main Effects

5-HTTLPR

s < l/l

Hipp (Entorhinal Cortex)	L	28	3.59	.000*	-17	-1	-31
ACC/Middle Cingulate G.	B	24	2.73	.005*	0	30	34
ACC (subgenual, orbitofrontal G.)	B	11	2.31	.013	3	36	-9
ACC	L	10	2.13	.019	-4	45	3

s > l/l

Hipp (Subiculum)	L	20/27	3.43	.001*	-20	-30	-6
Hipp (CA Region)	R	27	2.65	.006	20	-21	-15
Amyg (Basolateral)	R	–	3.04	.002*	22	-11	-14
Amyg (Superficial)	L	–	2.73	.005*	-20	-12	-12

BDNF

Met > Val

Hipp (Entorhinal Cortex)	R	28	3.16	.002*	25	6	-30
ACC (subgenual)	L	11	2.49	.009	-10	39	-4

Met < Val

Hipp (CA Region, Subiculum)/ Amyg (Centromedial)[2]	L	20	3.78	<.000*	-26	-14	-12
Hipp (Subiculum)	R	36	3.11	.002*	25	-17	-27
Hipp (CA Region)	L	27	2.55	.007	-17	-39	5
Hipp (CA Region)	R	37	2.46	.009	32	-36	-3
Amyg (Centromedial)	R	–	3.49	.001**	26	-5	-7
ACC/Superior Medial G.	R	10	2.46	.009	12	48	7

Abbreviations: H = hemisphere, BA = Brodmann area, Hipp = hippocampus, Amyg = amygdala, ACC = anterior cingulate gyrus, G. = gyrus; [1] uncorrected level of significance; [2] Labels are based on the distinction by (Amunts, et al., 2005); x-, y-, and z-coordinates are MNI coordinates; T > 1.6, $p_{uncorrected} < .05$. * T > 2.7, $p_{uncorrected} < .005$; ** $\alpha \leq .05$ (extent threshold according to the applied ROIs, estimated by using MonteCarlo Simulations: Amygdala k ≥ 247, Hippocampus k ≥ 611, ACC k ≥ 696).
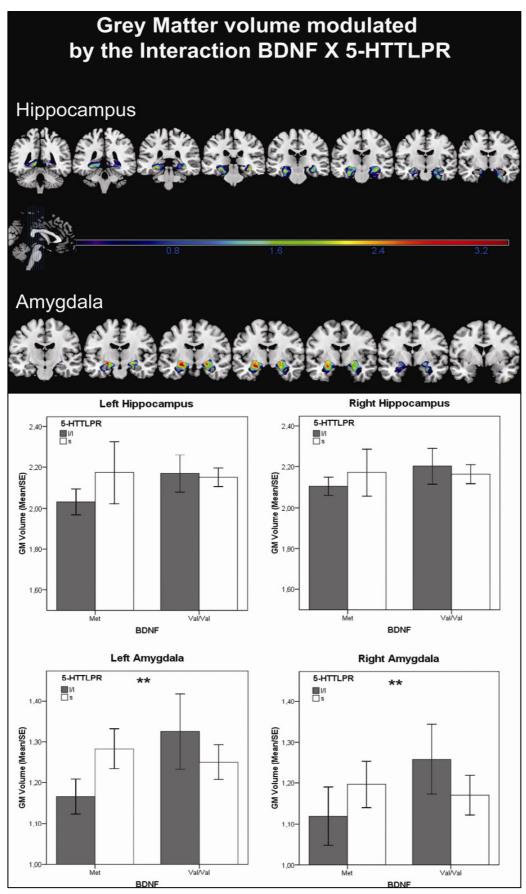
Figure 6-1: Interaction effect in both the amygdala and the hippocampus indicating greater s allele related GM volume in the BDNF Met group and lower s allele related volume in the Val/Val group (T > 1.6; $p_{uncorrected} \le .05$).

6.5 Discussion

The present data show statistical evidence for an interaction between the BDNF Val66Met polymorphism and 5-HTTLPR on grey matter volume in two key brain structures that are important for emotional memory, the amygdala and the hippocampus. Carriers of two long alleles of the 5-HTTLPR polymorphism show a reduction in hippocampal and amygdala volume when additionally carrying one Met allele of the BDNF polymorphism, whereas in the Val/Val group the l/l genotype shows an advantage over the s carriers with respect to the GM volume of those regions.

Our data did not replicate Pezawas et al's findings showing that the Met allele protected carriers of the s-allele from ACC volume reduction. However, our results point to an important interaction effect for the amygdala and the hippocampus, which have been affected in disorders of emotional memory such as PTSD (Karl & Werner, 2009; Shin, Rauch, & Pitman, 2006). Furthermore, the interaction was found for depressive symptoms in the same way, but independently from amygdala and hippocampal volumes. This suggests that, as discussed before, an interaction between BDNF and 5-HTT may modulate depressive symptoms (Martinowich & Lu, 2008), which often occur comorbid with PTSD.

Additionally, our findings add to the growing evidence suggesting that common genetic variants may rather act as plasticity factors than as risk or protective factors, respectively (Belsky, et al., 2009) by interacting with other genetic and environmental factors.

In conclusion, the present findings support the hypothesis that genetic interactions between BDNF and 5-HTT modulate the GM volume of structures implicated in emotional memory. Since changes in hippocampal and amygdala structure and function are discussed in association with PTSD (Shin, et al., 2006) our findings suggest that carriers of the l/l genotype may therefore have a vulnerability to develop this disorder, too, when carrying at least one BDNF Met allele. The observed interaction effect may also explain the scarce and inconclusive results with respect to the role of genetic polymorphisms in PTSD etiology (Broekman, Olff, & Boer, 2007).

6.6 Supplementary Figures
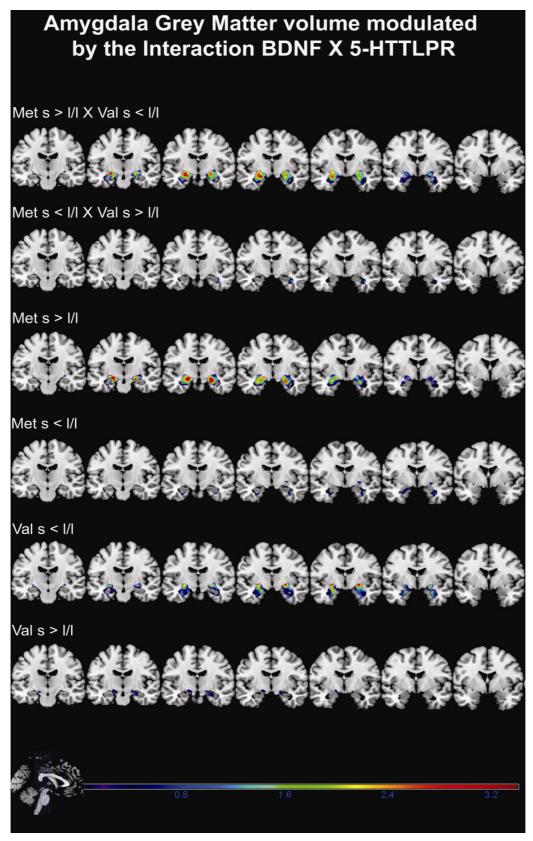
Figure S- 6-1: Projection of grey matter volume differences in the amygdala that show a modulation by an interaction of BDNF and 5-HTTLPR genotype. Colour scale represents t-value. (T > 1.6; $p_{uncorrected}$ ≤ .05).
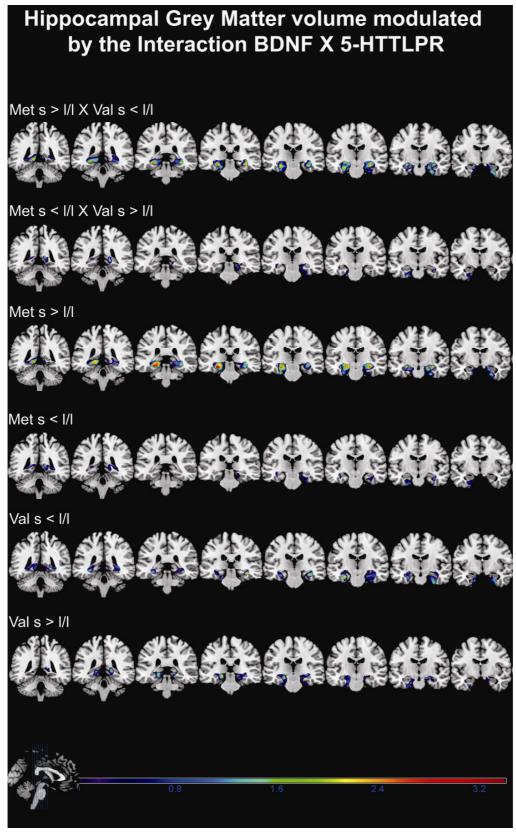
Figure S- 6-2: Projection of grey matter volume differences in the hippocampus that show a modulation by an interaction of BDNF and 5-HTTLPR genotype. Colour scale represents t-value. (T > 1.6; $p_{uncorrected} \leq .05$).
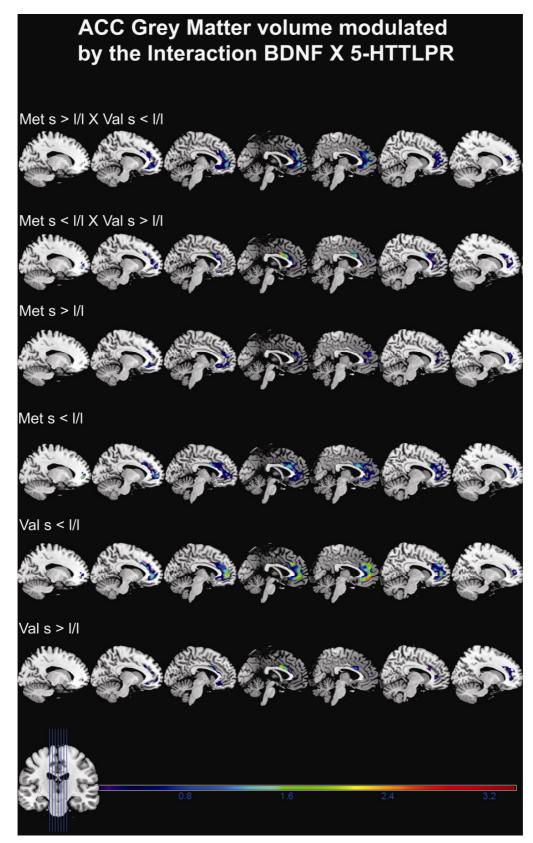
Figure S- 6-3: Projection of grey matter volume differences in the anterior cingulate gyrus that show a modulation by an interaction of BDNF and 5-HTTLPR genotype. Colour scale represents t-value. (T > 1.6; $p_{uncorrected}$ ≤ .05).

7 GENERAL DISCUSSION

7.1 Summary of the Results and Answers to the Research Questions

7.1.1　Neural Correlates of Recognition Memory

In study I, a Remember-Know procedure (Eldridge, et al., 2000) was used to assess functional activation and connectivity of brain regions during two different processes of recognition memory, namely recollection and familiarity. Following Dual Process models of recognition memory, it was suggested that these two distinct processes contribute to the recognition of previously presented words (old words) at both behavioral and brain systems level (see also chapter 4). Additionally, it was hypothesized that those processes can be dissociated at a behavioral as well as at a brain systems level, which would replicate previous findings. Beyond a pure replication of results, however, the study aimed at finding brain regions which are associated with both processes.

At brain level, correct recognition of old words that was accompanied by the recollection of context details was related to the activation of the inferior parietal lobe (IPL), the left medial precuneus and the left middle cingulate gyrus. The same areas also showed a greater activation in direct comparison to familiarity based correct recognition. These results further support the role of left IPL, more specifically the angular gyrus, in recollection based responses. Findings from both fMRI and event-related potentials studies suggest that the IPL activation indicates a recollection success effect, hence an effect resulting from correct item recognition that was accompanied by the additional recollection of details of the study context (Rugg & Curran, 2007; Vilberg & Rugg, 2008), and that it supports sustained focusing of attention on the contents of working memory (Ravizza, et al., 2004). One could suppose that during retrieval the working memory is reactivating the content of episodic memory that is related to the study event from the episodic buffer, as supposed by Baddeley (2000). Following this, attention on the contents of working memory has to be increased in recollection as compared to familiarity. In line with this assumption Vilberg and Rugg (2008) propose that the IPL may be a part of the brain network that supports the episodic buffer. The

posterior cingulate/anterior precuneus region that was also activated has been shown in previous fMRI studies in association with recollection based judgments (Henson, Rugg, et al., 1999; Wagner, et al., 2005). Additionally, a recent study by Peters et al. (2009) points to the importance of this structure also in the encoding of contextual information. Therefore, it can be assumed that this defined area in the anterior precuneus might play a role in retrieving spatial or other contextual details, which would support findings by Burgess et al. (2001) and Takahashi et al. (2008). In a virtual reality study Burgess et al. (2001) revealed large parahippocampal, retrosplenial and precuneus activations during the retrieval of contextual details as compared with a non-retrieval condition. Takahashi et al. (2008) showed functional connectivity of the precuneus region with the medial temporal lobe during recognition which indicates an involvement of this medial parietal structure in the retrieval of relational information.

Consistent with our hypothesis, our data also strengthen the important role of the hippocampal and posterior parahippocampal gyrus in recollection memory (Aggleton & Brown, 2006; Eichenbaum, et al., 2007). In a direct comparison of recollection to familiarity based responses, it could be shown that a region in the posterior hippocampus is significantly more activated when the subjects correctly recognized the item with additional retrieval of context details of the study event. In fact, the percent signal changes show less deactivation during recollection than familiarity in comparison to a baseline. A discussion of this result will follow below (chapter 7.2.5).

In line with previous research, the results are much less clear if one takes a look at the brain activations during familiarity based recognition. Activation differences between familiarity and other conditions are only observable when the statistical threshold is lowered to an uncorrected p-level of .001. Thus, among other findings, increased activity in the left precuneus and right dorsolateral prefrontal gyrus (DLPFC) was found. These results are in line with meta-analytic findings which reveal that familiarity is correlated with an involvement of those areas (Skinner & Fernandes, 2007). DLPFC is suggested to reflect a post-retrieval monitoring process or is possibly involved in additional searching for details of the item and additional monitoring of this search (Henson, et al., 2000; Henson, Rugg, et al., 1999; Skinner & Fernandes, 2007; Wheeler & Buckner, 2004).

However, to date it cannot be concluded that this area has an exclusive function in familiarity based recognition memory, because a right DLPFC activation was also linked previously to recollective processes (Skinner & Fernandes, 2007). With respect to the involvement of the MTL in familiarity, current models by Aggleton and Brown (1999, 2006) and Eichenbaum et al. (2007) could not be supported, because a perirhinal activation or deactivation during familiarity based responses was not found. However, this is not surprising, given that proof of this activation has rarely been found in pure BOLD activation analyses of recollection and familiarity, because the association of perirhinal cortex with familiarity judgments seems to depend on response confidence (Daselaar, Fleck, & Cabeza, 2006b; Montaldi, et al., 2006) which was not assessed in our study.

Regarding our research **question 1** for study I, findings could be replicated that link the activation of the hippocampus and the left IPL to recollection based recognition. Additionally, it could be confirmed that there are distinct brain regions involved either in recollection or familiarity based item recognition.

Previous work has pointed to the precuneus as a region involved in both recognition processes (Skinner & Fernandes, 2007; Wheeler & Buckner, 2004; Wiesmann & Ishai, 2008). In support of those findings and confirming our hypothesis to research **question 2,** a defined cluster of activation within the medial precuneus that proved to be the only region associated with both recollection and familiarity was found. This cluster lies more inferior to the recollection cluster in the precuneus described above and maybe is functionally separable from it. Similarly, other studies relate the precuneus to recollective processes and to episodic memory (Fletcher, et al., 1995; Henson, Rugg, et al., 1999; Shallice, et al., 1994; Wagner, et al., 2005). Following this, it could be assumed, that functionally different regions within the precuneus contribute to different features of declarative memory (see also chapter 7.2.2). Obviously, the anteromedial precuneus deactivates during correct recognition (see Figure 4-4). It is possible that this pattern reflects a deactivation in response to the beginning of the task as it is observed in regions of the so called default mode network (DMN; Raichle, et al., 2001). A detailed discussion of this deactivation will follow below referring to the connectivity results.

It was also hypothesized that the right DLPFC is associated with both recognition processes. This could not be confirmed by our results. One has to be careful in the interpretation of non-findings, but this may tentatively suggest a specific function of the DLPFC in familiarity processes alone in supporting post-retrieval processes which possibly are not necessary after recollection (Henson, et al., 2000).

7.1.2 Functional Networks of Recognition Memory

The second part of study I contains an analysis of functional connectivity between brain regions during recognition memory retrieval. Based on the finding that a region in the anteromedial precuneus is involved in both recollection and familiarity processes, a functional connectivity analysis was conducted using this cluster of activation as a seed region. Starting from this point, and separately for recollection and familiarity based responses, functionally correlated clusters were explored without specific hypotheses. The left middle temporal gyrus, the right superior temporal pole and the bilateral hippocampus were positively connected to the left precuneus in the recollection condition. This means, those regions are concertedly decreasing in activation when the retrieval of an item together with additional contextual information is occurring.

Those connectivity findings are in accordance with a study by Takahashi et al. (2008) that found functional connectivity between the medial precuneus and the MTL in a recognition task. Connectivity between precuneus and MTL maybe an indicator for the retrieval of relational memory (Aggleton & Brown, 2006; Eichenbaum, et al., 2007). Alternatively, it could indicate that the concertedly occurring deactivation of medial precuneus, middle temporal gyrus, superior temporal pole and MTL reflects the closing down of the default mode or resting state of the brain (Gusnard & Raichle, 2001). However, the deactivations found in our study are more pronounced during recollection as compared to the miss of a previously shown item. Therefore, it can be assumed that this reflects a more specific retrieval success rather than a non-specific task-independent shutting down of the DMN because of the beginning of a cognitive demand. The connection between the left hippocampus and the medial precuneus in the recollection network that was found in the current study is in accordance with a study by Fransson and Marrelec (2008) who found that in the DMN the MTL is

only connected to the precuneus/posterior cingulate cortex and the left temporal cortex. Additionally, studies of episodic memory (e.g., Burgess et al., 2001; Ranganath, et al., 2005) have found a network of precuneus, retrosplenial, parahippocampal, and hippocampal areas during episodic retrieval as well as encoding. This indicates that the precuneus could serve as central station in an episodic memory network that organizes input to and output from the MTL.

The functional connectivity analysis additionally revealed a left middle frontal area (BA 10) that is negatively correlated with the medial precuneus in recollection responses. This indicates an increase in the activation of BA 10 when the precuneus decreases. This area has been found activated in relation to attention, object perception (Burgess, et al., 2001; Cabeza & Nyberg, 2000) but also imagery processes resulting from recall attempts (Roland & Gulyas, 1995). As the precuneus region also is related to memory-related imagery processes (Fletcher, et al., 1995) an interaction between those two regions may lead to an imagery of past events and a correct recollection response.

Furthermore, McIntosh et al. (1997) could show a negative functional connectivity between the right BA 10 and hippocampal as well as posterior cingulate areas during recognition memory which possibly reflects retrieval mode. The concept of retrieval mode refers to a neurocognitive state, in which one mentally holds a segment of one's personal past, treats incoming and on-line information as "retrieval cues" for particular events in the past, refrains from task-irrelevant processing, and becomes consciously aware of the product of successful recovery of stored information, should it occur, as a remembered event (Lepage, et al., 2000). Hence, retrieval mode parallels the challenges that working memory has to encounter during retrieval of episodic information and maybe reflects the activation of the episodic buffer (Baddeley, 2000). Thus, a functional interaction of the precuneus with the left PFC (BA 10), additionally to the suggested regions by Vilberg and Rugg (2008), could be part of an episodic buffer network.

With regard to the connectivity of the medial precuneus to other brain regions in the familiarity condition, the analysis revealed strong positive connectivity of the precuneus with the left insula, the right occipital gyrus (BA 18 and 19), as well as negative connectivity to the middle cingulate gyrus and the putamen. It is noticeable that more areas related to sensory processing were found, such as BA 19, and insula, during familiarity than recollection based responses (for a

summary of sensory processing areas see Nieuwenhuys, et al., 2008).This fits well with the evidence that, compared with recollection, familiarity is more dependent on perceptual processes (Yonelinas, 2002). Additionally, Montaldi et al. (2006) and Yonelinas et al. (2005) also found that the insula is involved in generating feelings of familiarity. In contrast, another group (Karl, Rabe, & Dörfel, 2004) considers that the insula is strongly involved in imagery processes of positive emotional scenes (e.g. an island) which may be the result of a recollection of a positive episodic memory.

However, the current data do not support a stronger connectivity between perirhinal and parietal regions during familiarity, as supposed by Skinner and Fernandes (2007).

With respect to our research **question 3** for study I, it can be assumed that there are different brain networks either associated with recollection or familiarity, which at least overlap in the anteromedial precuneus. Furthermore, the fMRI data analysis revealed a functional connectivity between the precuneus and the hippocampus only in the recollection network. However, a direct comparison of the hippocampus-precuneus correlation between recollection and familiarity revealed no significant difference. Thus, our hypothesis that only a network supporting recollection involves connectivity of the hippocampus can not be fully confirmed.

7.1.3   Differential Effects of the BDNF Val66Met Polymorphism on Recollection and Familiarity

In study II recognition memory retrieval and its modulation by BDNF function was investigated. Again, the Remember-Know task (Eldridge, et al., 2000) was used to separate recollection from familiarity based retrieval. Then, the effect of variations in the BDNF Val66Met polymorphism (Val/Met and Val/Val allele carriers) on the performance and on brain activations with an emphasis on recollection based recognition memory was analyzed. As BDNF function has been shown to influence hippocampal dependent memory (Egan, et al., 2003; Hariri, et al., 2003) it was suggested that there is a relation to recollection based recognition and the activity of recollection related brain regions. With respect to the recognition performance, the initial hypothesis could be confirmed that the BDNF genotype is

only associated with recollection (research **question 1** of study II). The performance in recollection was significantly decreased in Val/Met carriers as compared to Val/Val carriers, whereas the performance of familiarity based retrieval and the correct rejection of new items did not vary according to BDNF genotype. Given that BDNF distribution is highest in the hippocampus (Murer, et al., 2001), that BDNF is known to be involved in LTP in the hippocampus (Lu, et al., 2008; Poo, 2001) and that previous studies could show an impairment in hippocampal function in the Met variant of the BDNF gene (Egan, et al., 2003) our finding supports Dual Process Models of recognition memory (Eichenbaum et al., 2007; Yonelinas, 2002) which state that only recollection processes depend on the hippocampal formation. Additionally, the dissociation of BDNF genotype between the performance in recollection and familiarity supports the assumption that those two processes are distinct at least at a behavioral level. Additionally, the result adds information about a specific role of the BDNF gene in recognition based on the retrieval of context details of the study event, which to our knowledge was not investigated before and which may have important clinical implications for disorders, which are known to be accompanied by specific distortions in contextual memory. For instance, PTSD is described by an inability to retrieve details of the traumatic event at all, or the patients have difficulties to retrieve details in the correct spatial and chronological order (Brewin, 2001; Ehlers & Clark, 2000). Thus, they have a deficit in episodic, relational memory which may be measured by recollection in a recognition memory task. It is suggested that the BDNF 66Met allele could serve as a vulnerability factor for contextual memory deficits that contribute to the development and the maintenance of PTSD, but future research has to proof this assumption.

Additional to the behavioral results, it was found that the brain activation of a region in the left middle temporal gyrus (BA 21) decreased, when the performance in recollection increased. Given that this negative correlation is only apparent in the Val/Met group, it may be suggested that an increase of activity in the left middle temporal gyrus contributes to the poor contextual memory performance in carriers of the Met allele.

This is in accordance with findings by Konishi et al. (2006) that link the lateral temporal gyrus to non-relational item-based memory. The authors suggest that

the lateral temporal region implements item-based recency judgments that emerge when relational processing (hence recollection) is dysfunctional.

The left superior frontal gyrus (BA 8) also shows a different activation in recollection between the Val/Val and the Val/Met group. Whereas the homozygote carriers of the Val allele show a difference in the hemodynamic response between recollection and familiarity based retrieval, no difference could be detected in the Val/Met carriers. Furthermore, the activity of this left PFC region decreased with increasing performance in contextual memory only in carriers of the Met allele. This is contrary to the findings that describe a positive relationship between recollection and a left PFC activation (Skinner & Fernandes, 2007) and further supports the assumption of a Met-allele associated impairment in brain regions that support a recollection network. Similar to study I, an ROI analysis of the hippocampal formation was conducted. The results show that a left posterior hippocampal region was significantly differently activated during recollection in carriers of the Met allele as compared to the homozygote Val carriers. Whereas the Met carriers showed no change in hippocampal activation during recollection, the Val/Val group showed a deactivation which is comparable to the deactivation during familiarity responses. This is a rather surprising result as it would have been suggested that there should be a deficit in the involvement of the hippocampus in the Val/Met group as opposed to a functional response, i.e. higher activation, during contextual retrieval in the Val/Val carriers (Egan, et al., 2003; Hariri, et al., 2003). In contrast, the Val/Val group showed no significant difference in the activation of the hippocampus between recollection and familiarity memory retrieval. This result should be interpreted with caution and needs to be replicated in further research. One possible explanation of this inconsistent result may be that an interaction between hippocampal activation and the BDNF Val66Met is more pronounced during encoding than during retrieval as shown previously (Hariri, et al., 2003; Hashimoto, et al., 2008).

In conclusion, it could be confirmed that there are differences in the activation of brain areas related to recollection, more precisely in the left middle temporal gyrus, the left superior frontal gyrus and the hippocampus, between the BDNF 66Met and the Val/Val genotype (research **question 2** of study II). Furthermore, the former two brain areas are correlated with the performance in recollection

based correct recognition. It could not be confirmed our hypothesis that the homozygote Val carriers show a higher activation in the hippocampus during recollection than subjects with one Met allele.

7.1.4 Individual Differences in the Grey Matter Volumes of Hippocampus and Amygdala are related to BDNF Val66Met and 5-HTTLPR Genotype and their Interaction

In study III, structural differences in the grey matter (GM) of hippocampus, amygdala and the ACC between carriers of the BDNF Met allele and the homozygote Val genotype in interaction with the 5-HTTLPR polymorphism were analyzed. Given that the BDNF Val66Met genotype has been shown to be related to reduced hippocampal and amygdala volume (Bueller, et al., 2006; Montag, et al., 2009) and the 5-HTTLPR is discussed with respect to the structure and function of those brain areas (Frodl, et al., 2008; Pezawas, et al., 2005) it can be suggested that an interaction between those genotypes is associated with hippocampal and amygdala morphology. Furthermore, such an interaction effect may contribute to the clarification of inconsistencies regarding the association between the BDNF polymorphism and amygdala volume (Frodl, et al., 2007; Montag, et al., 2009) and the 5-HTTLPR and the amygdala volume (Pezawas, et al., 2005; Scherk, et al., 2009). Additionally, investigating the involvement of the 5-HTT and BDNF in an emotional memory circuitry consisting of hippocampus and amygdala could shed further light on the etiology of clinical disorders like PTSD, which are known to be related to deficits in this brain network (Brewin, 2008).

First, looking at the impact of the BDNF genotype separately, a significantly higher volume in the CA region and the subiculum of the hippocampus as well as in the amygdala in homozygote Val carriers was found. Those findings confirm our hypothesis (research **question 1** of study III) and are in accordance with previous results on hippocampal and amygdala volume modulation by BDNF genotype (Bueller, et al., 2006; Frodl, et al., 2007; Montag, et al., 2009; Pezawas, et al., 2004; Szeszko, et al., 2005).

With respect to 5-HTTLPR genotype, the morphological analyses revealed a greater volume in hippocampus and amygdala in s-allele carriers. This confirms

the first hypothesis of our research **question 2,** regarding the hippocampal volume, but is in contrast to our second hypothesis and to a study by Pezawas et al. (2005) which suggested a reduced s-allele related amygdala volume. However, Scherk et al. (2009) also related the short allele of the 5-HTTLPR to an increased amygdala volume. With regard to the hippocampus volume, findings by Frodl et al. (2008) and Taylor et al. (2005) could be replicated. Furthermore, the data show a statistical interaction effect between the BDNF Val66Met polymorphism and 5-HTTLPR on grey matter volume in both amygdala and hippocampus: Carriers of two long alleles of the 5-HTTLPR polymorphism show a reduction in hippocampal and amygdala volume when additionally carrying one Met allele of the BDNF polymorphism, whereas in the Val/Val group the l/l genotype shows an advantage over the s carriers respective to the GM volume of those regions.

Those results point to a differential susceptibility of the l/l genotype in respect to the BDNF Val66Met. Hence, this finding adds to the growing evidence suggesting that common genetic variants may rather act as plasticity factors than as risk factors (Belsky, et al., 2009) by interacting with other genetic factors. It has been suggested that inhibition of 5-HTT, or less efficient 5-HTT, enhances serotonergic transmission through 5-HT4, 5-HT6, and 5-HT7 receptor subtypes, which are positively coupled to adenylate cyclase and PKA. The activation of those enzymes results in increases in CREB phosphorylation which positively regulates transcription of BDNF. In turn, BDNF promotes the development and function of serotonergic neurons (see review by Martinowich & Lu, 2008). Furthermore, both BDNF and 5-HT are related to intracellular cascades that modulate learning and memory by influencing neuronal development, synaptic plasticity and LTP which may be reflected in brain morphology (see Figure 7 1; Mattson, Maudsley, & Martin, 2004). Those findings can explain why there is an interaction effect between BDNF and 5-HTTLPR on grey matter volume in the hippocampus and the amygdala.

Answering research **question 3** of study III, it can be confirmed that there is an interaction between the BDNF Val66Met polymorphism and the 5-HTTLPR with respect to the hippocampus and amygdala volume, but not regarding the ACC GM volume. In addition, the results do not permit a full confirmation of our hypothesis regarding the epistatic effect in the amygdala, because a difference in

the volume between the s and the l-allele carriers in the BDNF Val/Val genotype was suggested, but no difference in the BDNF Met genotype.
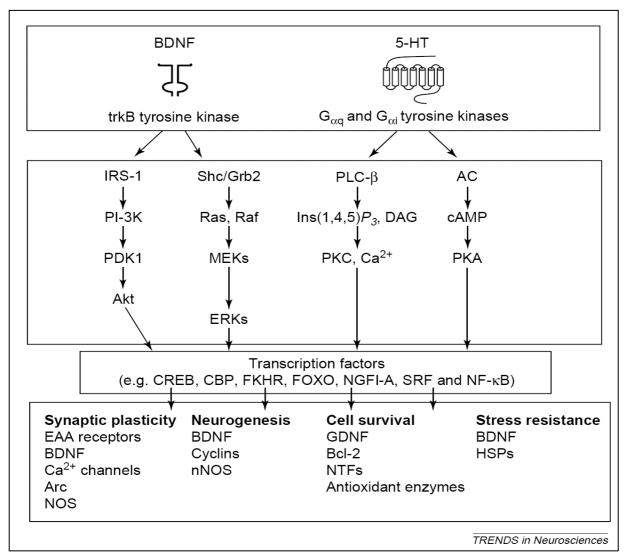
Figure 7-1: Signal transduction pathways by which brain-derived neurotrophic factor (BDNF) and serotonin (5-hydroxytryptamine, 5-HT) regulate neuronal plasticity and cell survival (Figure reprinted with permission from Mattson, et al., 2004).

7.2 Integration and Critical Discussion

7.2.1   Further evidence for Dual Process Models of Recognition Memory

In recognition memory research, two opposing views argue that their particular theory would most adequately describe the nature of recollection and familiarity. On the one hand, it has been assumed that recollection and familiarity form a continuum from high to low confidence in memory retrieval and simply reflect strong and weak memory traces (Squire, et al., 2007; Wixted, 2007a). On the other hand, there is support for a Dual Process Signal Detection model of recognition memory, which assumes that recollection and familiarity are distinct processes (Yonelinas, 2002). In this model, familiarity reflects the low vs. high confidence continuum and is well described by a signal detection approach, whereas recollection is supposed to be a high confidence recognition process that depends on additional retrieval of context details of the study event (Yonelinas, 2001b).

There is scientific evidence at a behavioral level for both models depending on the applied tasks, the independent variables and sometimes the type of data analyses (Dunn, 2004, 2008; Parks & Yonelinas, 2007; Wixted, 2007a, 2007b; Yonelinas, 2001b, 2002). However, at a brain systems level, it seems more viable to apply a dual process model to describe the processes leading to a correct recognition of an item (Aggleton, et al., 2005; Eichenbaum, et al., 2007; Eldridge, et al., 2000; Henson, et al., 2003; Henson, Rugg, et al., 1999; Ranganath, et al., 2004; Vann, et al., 2009; Wheeler & Buckner, 2004; Woodruff, Hayama, & Rugg, 2006; Yonelinas, et al., 2005).

However, studies that analyzed brain regions associated with high vs. low confidence ratings in recognition could show that activity in PFC, lateral and medial parietal cortex at encoding is negatively correlated with subsequent memory strength at retrieval, whereas activity in the hippocampus is positively correlated with subsequent memory strength, but only in the upper level of the confidence ratings (Shrager, et al., 2008). Another study showed that

hippocampal activation was related to memory strength even in unsuccessful source recollection (Kirwan, et al., 2008).

Summarized, these findings support the one process models of recognition memory. In contrast to this, a meta-analysis by Skinner and Fernandes (2007) concludes that brain regions sub-serving recollection are not simply those mediating highly confident memory decisions. Additionally, Kim and Cabeza (2009) could only relate hippocampal activation to high confidence recognition, which may reflect recollection processes (Yonelinas, et al., 2005).

The relationship between recollection and familiarity processes can also be depicted using Venn diagrams (see Figure 7-2). In the exclusivity model an item may be recollected or it may be familiar, but no one item can be both recollected and familiar at the same time (see Jones, 1987; Nelson, Schreiber, & McEnvoy, 1992). At a brain level, this model would imply that recollection and familiarity have different neural origins, and have no overlap in activation. The second relationship is that of redundancy, which states that all items that are successfully recognized are familiar, and that a subset of these can also be recollected (Joordens & Merikle, 1993). This model would predict that brain regions active during familiarity responses will completely overlap with those active during recollection responses and that recollection will produce neural activation additional to that of familiarity. The final model is that of independence (Jacoby, Toth, & Yonelinas, 1993) where an item may be either recollected or familiar, and only a subset is both recollected and familiar at the same time. This model suggests several possible patterns of brain activation: (a) there will be distinct brain regions of activation for recollection, (b) there will be distinct regions of activation for familiarity, and (c) there can be overlap in brain regions showing activation during recollection as well as familiarity responses.

The results reported in the current thesis strongly support the latter model, because it could be shown that there are brain areas which are uniquely activated during recollection and brain activations uniquely occurring during familiarity. Additionally, one brain area that is associated with both processes has been found. Furthermore, the brain areas that we relate to the familiarity process, only partly overlap with those regions that have previously been reported in terms of low confidence recognition (Kim & Cabeza, 2009) which indicates that familiarity

not only reflects low confidence ratings but a continuum of low to high memory strength as it is predicted by DPSD models (Yonelinas, 2002).

Additionally, the analysis of brain networks revealed distinctly interconnected brain areas associated with either recollection or familiarity which has been suggested by Skinner and Fernandes (2007). Finally, the finding that the BDNF polymorphism has an exclusive effect on recollection based recognition performance whereas familiarity remains unaffected adds BDNF to a group of behavioral and biological variables that are able to systematically dissociate between recollection and familiarity like level of processing, priming, age, benzodiazepine administration and divided attention (Gardiner, et al., 2002; Yonelinas, 2002).
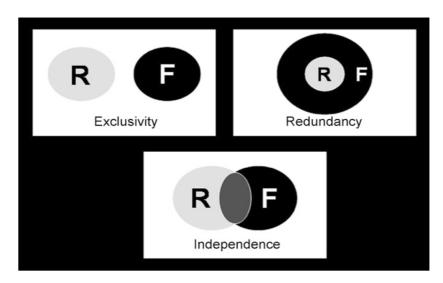
Figure 7-2: Possible relationships between recollection (R) and familiarity (F) (figure reprinted with permission from Skinner and Fernandes, 2007)

7.2.2   Precuneus

A main finding of the thesis is the involvement of the medial parietal cortex, more precisely the precuneus, in recognition memory. An anteromedial precuneus region is associated with both recollection and familiarity, which supports previous findings Skinner and Fernandes (2007). Another cluster of activation in the anteromedial precuneus seems to be only related to recollection based recognition. Those functional dissociations point to a complex role of this area in memory retrieval. The anteromedial part of the precuneus has been associated with self-reference related functions and memory-related imagery (for a review

see Cavanna & Trimble, 2006). Self-reference could be regarded as a common task-independent function which is turned off when an attention-demanding cognitive task starts.

This assumption is in line with our finding of a deactivation in the common recollection/familiarity cluster and with findings which relate the precuneus to the resting state of the brain (Fox, et al., 2005). In the resting state, at least two anticorrelated networks were found which parallel an activation/deactivation pattern routinely observed in response to attention-demanding tasks. There is a task-positive network which typically is activated during goal-directed task performance (Cabeza & Nyberg, 2000), and there is evidence for a task-negative network typically deactivated during such tasks and often referred to as the default mode network (DMN; Gusnard & Raichle, 2001). This routinely deactivating network comprises the medial parietal cortex including posterior cingulate cortex, retrosplenial cortex and the precuneus. A study by Fransson (2005) investigated the functional connectivity of this precuneus/posterior cingulate cortex region (precuneus/PCC) during rest and found strong positive correlations to the MTL. This precuneus/PCC region strongly overlaps the common recollection/familiarity cluster found in the analysis of recognition memory in study I. Therefore, it can be assumed that this shutting down is correlated with functional deactivations in other regions, which might be specific to the respective cognitive process (recollection or familiarity). According to this, activation in distinct brain regions, which are correlated with the precuneus activation in either a recollection or a familiarity network, were found. Thus, it is concluded that the precuneus serves as a core region in memory-related processes which is in accordance with a study by Vincent et al. (2006). The authors assign a central role to the precuneus both in the default mode network of brain regions and in a recollection related brain network (see Figure 7-3). Furthermore, a study by Fransson and Marrelec (2008) found that the MTL is only connected to the precuneus/posterior cingulate cortex and the left temporal cortex in an analysis of connectivity in the DMN.

Another function of the anterior precuneus seems to be memory-related imagery, occurring in episodic memory recall (Buckner, et al., 1996; Fletcher, et al., 1995; Fletcher, Shallice, Frith, Frackowiak, & Dolan, 1996). In accordance with this,

Henson et al. (1999) reported consistent activation of an anterior precuneus cluster for recollection judgments, which are supposed to be related to visual imagery that accompanies the correct recognition of previously learned words. This activation cluster in the precuneus overlaps the cluster that was found in study I of the current thesis.
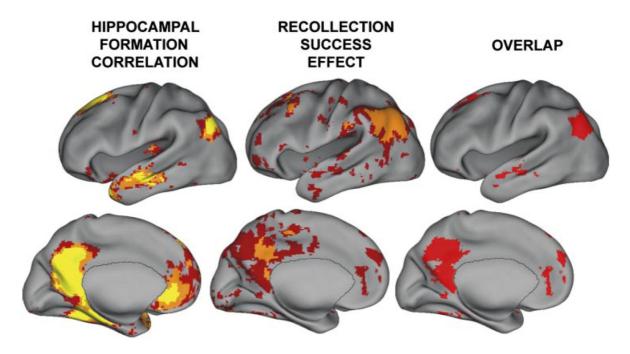
Figure 7-3: Overlap (right) between hippocampal formation correlations in a resting state analysis (left) and regions that show a recollection success effect (middle) (Vincent, et al., 2006, J Neurophysiol. Am Physiol Soc, with permission).

It could not be shown an effect of the BDNF polymorphism on precuneus activation in study II. This indicates that the neurotrophic factor may not be engaged in the precuneus functions described above. However, in an analysis of brain morphology which is not reported in the current thesis (Dörfel et al., unpublished data, Figure 7-4), we found a significant reduction of GM volume in the posterior as well as the anterior part of the precuneus in carriers of the BDNF 66Met allele. Therefore, an involvement of BDNF in the development or maintenance of neuronal density of the precuneus may be assumed. It is also possible that an existing effect of BDNF on precuneus function could not be detected due to lower statistical power, i.e. the lower number of subjects in the functional MRI study of BDNF influences on recognition memory. Therefore, it is strongly suggested that further studies should reinvestigate precuneus function in recognition memory and its modulation by BDNF function.
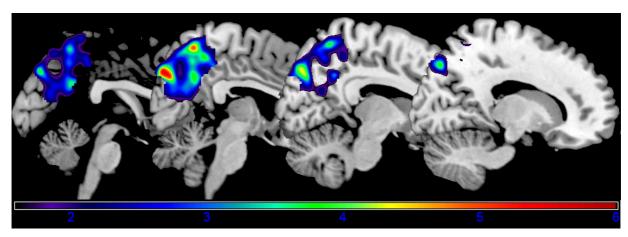
Figure 7-4: Projection of grey matter volume reductions in the right precuneus in carriers of the BDNF 66Met allele. Color scale represents t-value. (T > 1.6; $p_{uncorrected} \leq .05$).

7.2.3   Left Lateral Temporal Gyrus

As mentioned before, Fransson and Marrelec (2008) point to an exclusive connectivity between the precuneus/posterior cingulate cortex, the MTL and the left temporal cortex in an analysis of connectivity in the DMN. Additionally, Vincent et al. (2006) found an overlap of the DMN with a recollection network which also comprises parts of the left lateral temporal cortex (Figure 7-3). This is in accordance with our results which identified this region as part of a recollection network. The lateral temporal gyrus has been implicated in non-relational item-based memory (Konishi et al., 2006). Therefore, it is suggested that at least some recollection trials are accompanied by a fast item-based familiarity process, which would support both an independence and redundancy model describing the relationship of recollection and familiarity (Skinner & Fernandes, 2007). Furthermore, Konishi et al. (2006) suggest that an increase of activity in the left middle temporal gyrus may reflect a switch from recollection processes to item-based recency judgments (familiarity) when relational (recollection based) processing is dysfunctional. This is supported by the result of study II that in carriers of the BDNF 66Met allele the BOLD signal in the left middle temporal gyrus increased, when the performance in recollection decreased whereas the Val/Val group shows no correlation between recollection performance and left lateral temporal activation. Thus, the shift to more lateral temporal processing may

indicate that recollection is impaired in the Met group and this contributes to the poor performance.

In general the lateral temporal gyrus has been implicated in the encoding and recognition of recent verbal items (Ojemann, Schoenfield-McNeill, & Corina, 2009; Ojemann, Schoenfield-McNeill, & Corina, 2002) as well as in semantic memory (Martin & Chao, 2001). However, semantic memory processing has been associated with the anterior lateral temporal cortex, whereas the activation cluster found in study I and study II of this thesis lie more posterior in the middle temporal gyrus. Thus, it is unlikely, that this involvement of the lateral temporal gyrus in recognition memory is related to semantic processing.

7.2.4   Left Prefrontal Cortex

In line with current models and previous findings, the results of the current thesis implicate that activation of the right dorsolateral prefrontal gyrus (DLPFC) is associated with familiarity. It was further hypothesized that left prefrontal regions are related to recollection processes. When applying a very strict threshold, the statistical analysis of brain regions that are activated during recollection-based responses revealed no frontal regions. However, if the threshold is lowered to $p_{uncorrected} < .001$, several left PFC regions were activated (BAs 6, 9, 47, 10, see Figure 7-5). Even though it was decided to follow a more conservative approach when reporting recollection results in study I, this more lenient threshold is commonly used in memory (For a critical discussion of the thresholding procedure see chapter 7.3) Thus, the results of this thesis are in line with previous findings of prefrontal involvement in recognition (for reviews see Skinner & Fernandes, 2007; Spaniol, et al., 2009).

As an extension to previous research, functional connectivity analysis was used in this thesis (study I) and demonstrated that the left BA 10, an anterior prefrontal region, showed increased activity when the anteromedial precuneus activity decreased, but only in the recollection condition. Additionally, a left prefrontal region (BA 8) was negatively correlated with recollection performance but only in a subgroup of subjects with a variant of the BDNF gene (study II) that is known to be associated with poorer memory performance and less efficient BDNF function in the hippocampus (Egan, et al., 2003; Hariri, et al., 2003).
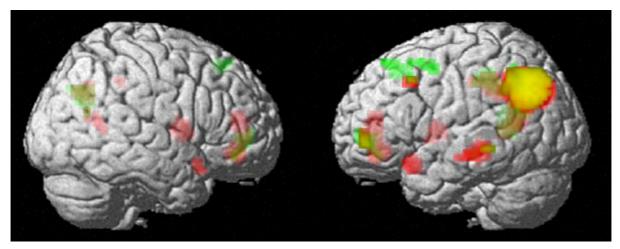
Figure 7-5: Projection of the brain activations during recollection reponses as compared to familiarity responses (red) and to miss responses (green) onto a render brain. Overlaps in brain activations between the two comparisons are shown in yellow; $T_{116} > 3.16$, $p_{uncorrected} < .001$; data from study I.

Neuroimaging studies have yielded that source memory retrieval, i.e., retrieval of additional context information about the study event, requires controlled cue specification and monitoring processes associated with left-lateralized PFC activation (Achim & Lepage, 2005; Dobbins, Foley, Schacter, & Wagner, 2002; Gilboa, 2004; Lundstrom, Ingvar, & Petersson, 2005; Lundstrom, et al., 2003). Dobbins et al. (2002) found a left frontopolar region that was selectively associated to source memory retrieval as compared to item recognition. This frontopolar source memory region is the same area that was found negatively correlated with left precuneus in study I of the current thesis. This would indicate that the decrease in anteromedial precuneus activity (associated with shutting down of self-reference processes, see discussion above; Cavanna & Trimble, 2006), was accompanied by an activation of memory processes that guide the monitoring or evaluation of episodic recollections ascribed to different stimuli.

This is in contrast to a study by Lundstrom et al. (2003) who found that BA 10 and anteromedial precuneus were not selectively associated with source memory retrieval. Instead, in a direct comparison with item recognition, Lundstrom et al. (2003) found a posterior precuneus region and BAs 44, 45 to be more activated during source memory retrieval, indicating an exclusive role of those areas in episodic memory. The authors could replicate their findings in an additional study (Lundstrom et al., 2005).

Thus, it remains unsolved whether BA 10 of the anterior prefrontal cortex is specifically related to the recollection of context details of a previously encountered item.

The left DLPFC has been consistently reported in studies contrasting associative (recollection based) to item recognition (familiarity based) (Achim and Lepage, 2005a; 2005b; Lepage, Brodeur, and Bourgouin, 2003; Rugg, Fletcher, Chua, and Dolan, 1999; Rugg et al., 2003) and it is suggested that this activation reflects a post-retrieval monitoring process (Achim and Lepage, 2005a; Rugg et al., 2003). Post-retrieval monitoring operates on the product of retrieval attempts and/or success. When information is elicited by a retrieval cue (i.e. an old word in a recognition task) it will be maintained in working memory while its relevance to the task is evaluated and leads to a behavioral response (i.e. an 'old' response). In item recognition tests old items are more likely to trigger post-retrieval monitoring than new items, because post-retrieval monitoring occurs principally when there is some retrieved information to process, or at least a feeling of knowing (Koriat, 2000). It can be assumed, that post-retrieval monitoring is even more involved in recollection as compared to familiarity, because more information (i.e. contextual information about the study event) has to be managed in working memory to yield a recollection judgment (Achim and Lepage, 2005a; 2005b). In study II of the current thesis, statistically significant activation of a left DLPFC region could only be observed in carriers of two Val alleles of the BDNF gene. Additionally, activity of this PFC region increased when recollection performance decreased, but only in subjects who carry one Met allele of the BDNF gene. First, this may implicate that an involvement of the left DLPFC is specifically necessary for correct item recognition which is based on recollection of contextual information and secondly, that a malfunction in DLPFC and hence in post-retrieval monitoring may be related to the BDNF Met allele. Impaired DLPFC function in Met carriers may be related to decreased dendritic complexity, or decreased neurogenesis during embryological development or over the lifespan (Bath and Lee, 2006), which is supported by findings that show reduced grey matter volume in DLPFC in carriers of at least one BDNF Met allele (Pezawas et al., 2004). In contrast, Egan et al. (2003) could not show an effect of BDNF Val66Met on working memory function

in general, which may lead to the suggestion that BDNF has specifically affects working memory function in relation to recollective memory processes.

7.2.5   Hippocampus

Consistent with several studies and models investigating recollection and familiarity in recognition memory, it could be shown that hippocampal involvement is only apparent in recollection based responses (Aggleton & Brown, 2006; Eichenbaum, Yonelinas, & Ranganath, 2007; Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000; Skinner & Fernandes, 2007; Yonelinas, Otten, Shaw, & Rugg, 2005). Furthermore, there is support for a hippocampal participation in a recollection network, whereas connectivity of the hippocampus in a familiarity network could not be found.

Interestingly, we only found an effect of BDNF genetic variation for recollection-based recognition performance. The higher susceptibility for impairment in this specific memory function as compared to familiarity-based recognition can be explained in the light of the hippocampus' selective role in recollection and its functional dependence on BDNF-related plasticity (i.e. E-LTP and L-LTP; Lu et al., 2008; Poo, 2001). The Met variant of the BDNF protein exhibits poorer BDNF trafficking and therefore a reduced regulated secretion in response to synaptic activity (Bath & Lee, 2006), maybe resulting in less efficient LTP, hence less efficient hippocampal function. Following the assumptions of dual process models of recognition memory (Aggleton & Brown, 2006; Eichenbaum, et al., 2007; Yonelinas, 2002) that only recollection is related to hippocampal function whereas familiarity is not, a BDNF effect on memory performance should be more pronounced in recollection as compared to familiarity, which is supported by the data of study II. Additionally, this finding points to a possible vulnerability of Met allele carriers for memory related deficits which may contribute to the development and maintenance of a PTSD. Those patients show a clear deficit in contextual memory (recollection, episodic memory; Brewin, 2001) which may be apparent already before the experience of the traumatic event (Gilbertson, et al., 2007). The findings revealed by study III of the present thesis and by other studies (Bueller, et al., 2006; Pezawas, et al., 2004) that hippocampal morphology is strongly modulated by BDNF function and patients with PTSD show a

pronounced reduction in hippocampal volume (Karl, et al., 2006) underscore this relationship.

However, study III of the current thesis emphasizes the importance of interactions between two or more predisposing factors for hippocampal structure and potentially for memory function. It could be shown that there is an epistatic effect between the BDNF Val66Met and the 5-HTTLPR polymorphism on hippocampal morphology. In carriers of the BDNF 66Met allele the left and the right hippocampus show an l-allele related reduction in GM volume, whereas in the Val/Val genotype there is no difference between carriers of the 5-HTTLPR short and long allele. This result points to a susceptibility of the l-allele carriers to variations in the BDNF gene (Belsky, et al., 2009). Given that this effect was additionally observed in the amygdala (see section 7.2.6) and those two limbic structures constitute an emotional memory circuitry which is disturbed under highly stressful experiences (Kim & Diamond, 2002), it may be suggested that an interaction between those two polymorphisms play an important role in stress related clinical disorders with known memory deficits.

7.2.6   Amygdala

In study III, an interaction effect between the BDNF Val66Met and the 5-HTTLPR was found with respect to the grey matter volume of the amygdala. The amygdala is strongly involved in emotional memory encoding and retrieval (Roozendaal, McEwen, & Chattarji, 2009) and modulates stress-related enhancement or attenuation of LTP in the hippocampus (Kim & Diamond, 2002). Amygdala activity is related to the processing of fear stimuli (Adolphs, 2008; LeDoux, 2003; Ohman, 2005) and altered amygdala function has been found in clinical disorders that are characterized by disturbed fear processing (Anand & Shekhar, 2003; Protopopescu, et al., 2005; Rauch, Shin, & Wright, 2003; Rauch, et al., 2000; Shin, Rauch, & Pitman, 2006). In search for predisposing factors that may alter amygdala function and structure, and therefore may be related to clinical disorders, it was found that the two genetic polymorphisms, 5-HTTLPR and BDNF Val66Met, became of interest. First, the 5-HTTLPR s-allele is related to exaggerated amygdala response to negative or fearful stimuli (Munafo, Brown, & Hariri, 2008) and to greater neuroticism scores (Sen, Burmeister, & Ghosh, 2004).

Second, a variant in the BDNF gene (66Met) has been associated with stronger activation of the amygdala (Montag, Reuter, Newport, Elger, & Weber, 2008), and an animal study by Chen et al. (2006) could show that the homozygote 66Met variant is associated with an altered BDNF secretion and higher anxious behavior. Association studies using self-report-measures for trait anxiety revealed associations between the 66Val but also the 66Met allele with higher trait anxiety (Jiang, et al., 2005; Lang, et al., 2005; Sen, et al., 2003). However, a recent meta-analysis revealed that Met individuals, as compared to Val/Val, showed a statistically significant *lower* neuroticism score, but no significant association between BDNF Val66Met polymorphism and anxiety disorders could be found (Frustaci, Pozzi, Gianfagna, Manzoli, & Boccia, 2008). This is in accordance with a study that could observe no association between BDNF Val66Met and PTSD diagnose (Zhang, et al., 2006), though the authors did not include trauma-exposed controls in their sample and therefore are not able to evaluate a possible vulnerability factor for developing a PTSD after a traumatic event. Additionally, studies assessing the amygdala morphology could not find a clear relationship between the BDNF Val66Met or the 5-HTTLPR on amygdala volume (Frodl, et al., 2007; Montag, Weber, Fliessbach, Elger, & Reuter, 2009; Pezawas, et al., 2005; Scherk, et al., 2009). The reported inconsistencies may lead to the suggestion, that there is no relationship between amygdala morphology and the reported polymorphisms or that more than one genetic factor may contribute to amygdala function and structure and therefore to anxiety related behavior and emotional memory processes. Support for this line of reasoning comes from a recent study that reports an interaction of BDNF Val66Met and 5-HTTLPR on ACC, and marginally significant, on amygdala volume (Pezawas, et al., 2008). The results of study III revealed an interaction effect on the amygdala volume but do not support the notion that the BDNF Met allele is serving as a protective factor for the 5-HTTLPR s-allele, i.e., preventing amygdala volume reduction as it was suggested by Pezawas et al. (2008). In contrast to the Pezawas et al. study, the l/l carriers in the BDNF Met group showed a reduced GM volume in the amygdala as compared to the s-carriers, whereas in the Val/Val group there was an l/l allele related increase in GM volume.

Results on the epistasis between BDNF and 5-HTTLPR and its association with brain morphology imply that amygdale volume, like hippocampal size, is underlying individual differences which might represent vulnerability or protective factors. To date it is unresolved if the morphology itself presents a risk factor or if the genetic polymorphisms present different predispositions for brain plasticity. Findings for the latter argument are controversial because studies on amygdala volume and psychopathology have revealed both smaller (Matsuoka, Yamawaki, Inagaki, Akechi, & Uchitomi, 2003; Wignall, et al., 2004) and larger (Lange & Irle, 2004) amygdalae when comparing patients to control. A recent meta-analysis even failed to find altered amygdala volume in PTSD (Woon & Hedges, 2009).

7.3 Reflection of Methods

In the present thesis, several methods were applied to investigate recognition memory and associated function of the hippocampus and other brain regions as well as the structure of the hippocampus. First of all, using functional MRI, the brain activations that occur during the different processes of the recognition task were measured. fMRI is an imaging method that is characterized by a high spatial resolution but a poor temporal resolution (Logothetis, 2003; Weishaupt, et al., 2006). To separate the BOLD responses on each word trial from each other, a very long interstimulus interval was included in the design of the recognition phase. This leads to a long testing time in the scanner (approximately 50 minutes) which may have caused tiredness in the subjects, mainly in the last 2 or 3 runs. As the words were randomized, and all responses of the runs were averaged over all 9 runs, it can be assumed that order effects have not compromised the results, but there may be some differences in motivation, recognition performance and reaction times in comparison to studies, that used other designs and realized shorter experiments.

With respect to the Remember-Know task, study I and II used an unusually low number of lures (new words), which was similar to Eldridge et al. (2000). However, this leads to an increase of Know responses as a result of a relaxation of the response criterion as reported in Yonelinas (2002).

Thus, it could be ensured that there is a sufficient number of know trials to include in the statistical fMRI data analysis and that the number of know trials was similar

to the number of remember trials, but also may have caused a response bias towards more false alarms. Additionally, in our study the number of old words that were shown in the recognition phase was lower than the number of learned items, what possibly has produced the higher number of falseKnow responses in studies I and II. In most of the R/K experiments all studied items were presented as old words in the test session (Woodruff et al., 2005; Yonelinas et al., 2005) and lower falseKnow rates were reported. However, as it was not of primary interest to investigate the false alarms, this issue has no impact on the results that are reported in study I and II.

Another important issue is related to the fMRI data analysis. In study I, the results of the analysis of brain activations during corrRem and corrKnow responses initially were corrected for multiple comparisons with a procedure called Family Wise Error (FWE). This correction step led to highly significant results with respect to the activations during corrRem responses but to no significant results in the analysis of the corrKnow responses. Therefore, the statistical threshold was lowered for this analysis to $p > .001$, without correction for multiple comparisons. Although it is common to report uncorrected results in neuroimaging-based memory research because only minor or medium effects are observed, this more lenient method leads potentially to an α-error inflation and therefore an artifact may be misinterpreted as a real effect. In the results and discussion section of chapter 4 (study I), it was noted that these results need to be interpreted with caution. In the connectivity analyses of study I and in study II, uncorrected results are also reported because of the exploratory character of these studies, but nevertheless the same limitation applies. Further studies are needed to replicate the results of the connectivity study and the BDNF study.

Regarding the number of subjects in study II, one could assume, that although there was a sufficient number of subjects in both groups (n = 11 for Val/Met, n = 15 for Val/Val; Friston, Holmes, & Worsley, 1999) more subjects in the Val/Met group would have revealed a possible existing, but rather minor effect in the hippocampus. However, insufficient sample size can most likely not explain, why there was no substantial recollection effect in the hippocampus in the Val/Val group, because 15 subjects constitute a sufficient sample size for an fMRI random effects analysis (Friston, Holmes, & Worsley, 1999).

The main and interaction effects of BDNF and 5-HTTLPR in study III are observed at a rather lenient statistical threshold of $p < .005$, uncorrected for multiple corrections. As already discussed above, the accumulation of the α-error may lead to the classification of an accidental effect as a real effect. This lenient threshold was applied because of the rather small sample, the effect sizes were in a minor range (in comparison with other morphology studies, see Pezawas, et al., 2008; Pezawas, et al., 2004) and because we had clear hypotheses about the effects in the ACC and the amygdala. In order to correct for multiple comparisons, however, an extent cluster threshold was applied (see chapter 6.3.4) which substantiated the interaction effects as well as the BDNF main effects in the amygdala. A replication of the less statistically significant effects with a greater sample has to proof the findings of study III.

7.4 Implications for Future Research

First of all, the finding that there are different functional networks of recollection and familiarity, respectively, needs to be replicated with the same and also with different methodologies. For instance, a similar study, using the ROC or the PDP procedure to measure recognition memory (for a summary see Yonelinas, 2002), should lead to similar results regarding the brain areas that are part of those functional networks, to support the results of study I.

Additionally, future studies should conduct a hypothesis-guided effective connectivity analysis based on the reported structures in study I. This would allow to measure the effective contribution of each target brain area to either a recollection or a familiarity network, for instance with structural equation modeling (Buchel & Friston, 1997).

The studies that are included in the present thesis only investigated recognition memory processes, hippocampal function and brain morphology of hippocampus and amygdala in healthy subjects and were focused on individual differences rather than pathological processes. Nevertheless, it can be suggested that the reported results have implications for stress related disorders characterized by episodic memory deficits, in particular PTSD.

Future studies should firstly measure recollective memory performance in a recognition task with neutral stimuli, like the Remember-Know task that was

introduced in the current thesis, which is scarcely reported in the PTSD literature to date (Geuze, Vermetten, et al., 2008). Thus, it is possible to evaluate the episodic memory performance of non-trauma-related content and to investigate hippocampal function in general, not only with respect to trauma memories (Brewin, 2008). Second, more emphasis should be placed on genetic vulnerabilities for PTSD. Given that a specific effect of BDNF on recollection memory performance is reported in the current thesis, one could assume that persons with the BDNF Met allele may be more vulnerable to develop deficits in recollection memory, when stressful experiences challenge their abilities to cope with it. The integration of a traumatic event in the autobiography of oneself is supposed to be of great importance for staying healthy. Both Brewin's and Ehlers & Clark's model assume that an important part of successful recovery from trauma or successful PTSD therapy involves the integration of the traumatic event in one's autobiography (Brewin, 2001, 2008; Ehlers & Clark, 2000). Recently it has been shown that a hippocampal-prefrontal cortical circuit plays an important role in spatial working memory in rats and that a disruption of this connection impairs spatial memory (Wang & Cai, 2006). Similar to this, pretraumatic deficits in cognitive processing (prefrontal, working memory component) and in the consolidation of the traumatic event (hippocampal episodic memory component) may interfere with the ability to integrate a traumatic event into the autobiographical memory of a human being, appropriately. This disability may be related to a working memory and/or DLPFC deficit, which was found in carriers of the Met allele in study II of this thesis, but has to be proofed in studies using working memory tasks which activate the DLPFC. Thus, it should be investigated whether carriers of the BDNF Met allele are more susceptible to impaired recovery from a traumatic event, and in turn whether carriers of two Val alleles are more protected because they show better premorbid episodic memory.

The results of study III strongly suggest that gene X gene interactions may account for individual differences with respect to brain morphology and possibly regarding brain function.

First, future studies should investigate whether an interaction between the BDNF Val66Met polymorphism and the 5-HTTLPR is also relevant for differences in amygdala and hippocampal functioning, for instance by using emotional memory

paradigms. Second, it is of interest that the epistatic effect on two brain structures involved in an emotional memory circuitry found in study III may have implications for PTSD which has been related to alterations in this circuitry (Brewin, 2008). Therefore, it is suggested that future studies interested in risk factors for the development of PTSD, collect a sufficiently sized sample of trauma-exposed individuals with different symptom severities to measure gene X gene interactions on emotional memory and the underlying brain function. The BDNF Val66Met and the 5-HTTLPR show great promise for such an interaction analysis, but a genetic polymorphism of the 5-HT1A receptor may also be interesting, because it has been shown that 5-HT1A receptor binding is associated with LTP (Schiapparelli, et al., 2005) and the activation of 5-HT1A receptors in the hippocampal formation have a negative influence on explicit memory function (Yasuno, et al., 2003).

7.5 Summary and Conclusion

In the current thesis, it was shown that there are different functional networks related to either recollection or familiarity, supporting dual process models of recognition memory. Additionally, the role of the precuneus in memory related processes is further substantiated and it has been shown for the first time that this structure is functionally connected to the hippocampus in a recognition memory paradigm. With respect to an association of BDNF function with recognition memory, it was found that there is a selective influence of BDNF function on recollection and that the relatively inferior performance is related to an altered function in left lateral temporal and left lateral prefrontal areas. To the author's knowledge, this is the first description of the effect of BDNF function on recollection and familiarity in relation to the brain function that underlies these processes. It could not be confirmed, that there is a different hippocampal activation in BDNF Val/Met as compared to Val/Val carriers, as was hypothesized based on previous findings (Egan, et al., 2003).

However, a BDNF effect on hippocampal morphology could be detected. Furthermore, a significant interaction between BDNF and 5-HTTLPR was found.

The results of the current thesis allow further comprehension of recollection, hence episodic memory, and point to a special role of the BDNF in temporal and

prefrontal brain regions. Additionally, the finding of an epistatic effect between BDNF and serotonin transporter function point to the need of analyzing interactions between genes and also between genes and environmental factors which reveals more information than the study of main effects alone. Moreover, the finding that the so called *risk factor* 5-HTTLPR s-allele (Caspi, et al., 2003; Lesch, et al., 1996) may also show an advantage over l/l allele carriers, when those persons are additionally carrying the BDNF Met allele, is in line with previous assumptions, that some genetic traits actually do not function merely as risk factors, but rather as plasticity or susceptibility factors. Thus, the s-allele could have an advantage or a disadvantage depending on other factors, like other genes or environmental variables (Belsky, et al., 2009). In turn, the Met allele which previously has been related to poor memory and reduced hippocampal volume, shows an equal brain morphology as compared to the Val/Val allele when the subjects additionally carry one 5-HTTLPR s-allele.

In conclusion, analyzing behavioral and neural correlates of episodic memory reveal allowed insights in brain functions that may serve as guideline for future studies in clinical populations with memory deficits, including susceptibility factors such as good or bad environment, as well as promising gene variants that influence episodic memory.

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STATEMENT

Erklärung gemäß § 5 Anlage 1 der Promotionsordnung der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden.

Versicherung

Hiermit versichere ich, dass ich die vorliegende Arbeit

"FUNCTIONAL INVESTIGATIONS INTO THE RECOGNITION MEMORY NETWORK, ITS ASSOCIATION WITH GENETIC POLYMORPHISMS AND IMPLICATIONS FOR DISORDERS OF EMOTIONAL MEMORY"

ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Dresden, den 26.10.2009

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Denise Dörfel