The main goal of the present thesis was to study the effects of stress hormones on the retrieval of emotional memories in healthy humans. In addition, we were interested in the effects of stress hormones on post-retrieval processes like reconsolidation. That is, are there only acute and temporary effects of stress hormones on memory retrieval, or are there also long-term effects? Studying effects of stress hormones can be done in two ways; either by (experimentally) inducing stress in humans, or by exogenously administering doses of stress hormones. In the present thesis both ways were used. Furthermore, when investigating emotional memories, we can make use of memories that are created in a laboratory setting or those that derive from real life experiences, i.e. autobiographical memories. Again, both methods were investigated. In the introduction, we described current knowledge on the neurobiology of emotional memory retrieval and concluded that it is still unclear whether recent and remote memories are mediated by the same or different brain areas and therefore potentially differentially affected by stress. Therefore, we also studied the influence of stress and stress hormones on the retrieval of recent vs. remote memories. In the following section we will give an overview of the findings and conclusions from our studies as described in chapter 2 to 6. We will follow this discussion with some implications of our findings for memory models and clinical practice and conclude with some suggestions for future research.

Overview of findings

The effects of cortisol increase on long-term memory retrieval during and after psychosocial stress (chapter 2)

In chapter 2 we studied the effects of cortisol increases on memory retrieval during and after psychosocial stress in healthy young men. In this study we intended to induce endogenous cortisol increases in healthy young men by means of a psychosocial stress task (the Trier Social Stress Task; TSST), after which we studied memory retrieval of neutral and emotional word pairs that were learned 1 day earlier (recent memory) or were learned 5 weeks earlier (remote or long-term memory). We were interested in the interplay between cortisol and sympathetic arousal induced by the stress task. To study the effects of cortisol in an arousing condition, we tested memory retrieval for word pairs while the men were still taking part in the stress task, i.e. the committee that observed the participants was still present. To study the effects of cortisol increase in a non- (or less-) arousing situation, we studied memory retrieval after the stress task had finished, i.e. the committee had left, but while cortisol levels would still be high. Sympathetic arousal was measured by means of increased heart rate and blood pressure.

We were indeed able to induce significant increases in cortisol and sympathetic arousal in the men that underwent the stress task compared to the men who were in the control condition. Sympathetic arousal decreased directly after the stress task, while cortisol stayed high. Overall, we found that stress reduced recall of emotional words, which is in line with previous studies that found effects of stress mostly on emotional memory retrieval (Domes et al., 2004; Kuhlmann, Piel et al., 2005). However, only during the stress task, thus in a highly arousing situation, were
cortisol increases related to reduced memory retrieval. This was significant for the retrieval of both neutral and emotional words. This indicates that indeed a certain level of adrenergic arousal is necessary for cortisol to impair memory retrieval, as was indicated by animal models (Roozendaal et al., 2003, 2006). Our finding was recently confirmed by a study that blocked (nor)adrenergic arousal by means of a beta-blocker while administering cortisol (de Quervain et al., 2007). In that study, cortisol could only impair memory when adrenergic functioning was intact. Our study leaves open the question of which brain areas are involved in the impairing effects of cortisol in combination with adrenergic arousal. Future imaging studies involving induction of stress before or while being scanned could shed more light on this issue. We should note that these results are all based on the retrieval of remote memories. The retrieval of the word pairs that were learned 1 day before the stress and retrieval task was too easy and could not be analyzed properly due to a ceiling effect. The paradigm we used to create the word pairs, i.e. based upon personal associations to neutral and emotional words, might have caused this effect. It does indicate that remote memories are equally affected by stress as more recent memories (based upon previous studies using material learned a few hours or days earlier to test memory retrieval after stress).

**Long-term outcomes of memory retrieval under stress (chapter 3)**

In Chapter 2 we thus found that acute stress impairs the retrieval of emotional words and that cortisol increases are related to reductions in memory retrieval when arousal is high. We became interested as to whether these impairments were only temporary, or whether there are long-term effects of stress on memory. Long-term effects could be expected as stress and cortisol have been found to impair memory when either of them was administered during reactivation of fear or recognition memories in rodents (Cai et al., 2006; Maroun & Akirav, 2007; Yang et al., 2005). Furthermore, reduced retrieval due to stress might lead to less rehearsal of the learned information and hence to long-term impairments in memory. To study this question, we did a 6-months follow-up to the first study to assess memory retrieval half a year after learning the word pairs. Chapter 3 described and discussed the results.

In short, we found that the group that retrieved words during stress 5 weeks after learning remembered fewer words after 6 months than the control group. The stress group did not only recall fewer words, but even showed a further decrease in the retrieval of the reactivated words compared to the control group, indicating that both rehearsal and reconsolidation processes might have been affected. In contrast, when words were retrieved under stress 1 day after learning, at six months the retrieval of these words was slightly improved compared to the control group. This study thus indicates that stress does have a long-term effect on memory, even when memories are recalled only once under the influence of stress and high cortisol levels. The fact that the time between learning and recall under stress modulates this relation indicates that different processes might be involved in the retrieval, but also the post-retrieval processes, of recent and remote memories. Recent memories might still be consolidated into long-term memory, and as consolidation is found to be enhanced by stress hormones (Buchanan & Lovallo, 2001; Cahill et al., 2003; Cahill & Alkire, 2003), this might explain the improved long-term memory performance after
reactivation of 1 day old memories under stress. Remote (5 weeks old) memories might be fully stored in long-term memory, but when reactivated might have become labile again and prone to disruption by stress, explaining the impaired long-term memory retrieval. This study was the first to show long-term effects of stress on memory but could not clearly identify which hormones were involved as correlations between cortisol increases due to stress and memory retrieval during follow-up were not significant.

Immediate and prolonged effects of cortisol, but not propranolol, on memory retrieval in healthy young men (chapter 4)
We found that stress can have long-term effects on memory, but the specific role of cortisol in the long-term effects on memory remained unknown. Cortisol has not only been found to impair human memory retrieval (see chapter 2 and Het et al., 2005), but has also been found to impair long-term memory retrieval when administrated during or shortly after reactivation in rodents (Cai et al., 2006; Wang et al., 2008). Cortisol might thus impair post-retrieval processes like reconsolidation, which could lead to long-term impairments in memory. Another view is that it might boost extinction of learned associations and therefore attenuate memory on the long-term when administered during or after reactivation (Abrari et al., 2008; Yang et al., 2005). No human studies had yet examined whether exogenous cortisol administration could lead to prolonged impairments in memory retrieval. As this is of interest to clinical practice, where prolonged attenuations of emotional memory retrieval could be valuable, we decided to study both the immediate and prolonged effects of cortisol on memory retrieval. Chapter 4 described the results of this study. The second purpose of this study was to examine the immediate and prolonged effects of propranolol administration on memory retrieval. Like cortisol, propranolol is being studied in clinical practice, where diminishing emotional memory retrieval might enhance treatment. As propranolol is found to weaken encoding and consolidation of emotional memories by blocking the strengthening effect of adrenaline on memory formation, it is also thought to potentially weaken reconsolidation of emotional memories. Animal studies have indeed found evidence for such effects (Debiec & LeDoux, 2004; Przybyslawski et al., 1999). Thus, chapter 4 studied the immediate effects of cortisol and propranolol on memory retrieval of previously learned words as well as the potentially prolonged effects of this administration 1 week later.

We found cortisol to impair memory retrieval as was found before (de Quervain et al., 2000; Het et al., 2005) and was also indicated by our study on stress (Chapter 2). We also found long-term effects of cortisol on memory retrieval. That is, one week after the single dose of 35 mg cortisol, memory retrieval was still impaired compared to a placebo group. This is in line with our findings of a long-term impairing effect of stress on memory. However, while memory remained lower in the cortisol versus the placebo group, it had not further diminished over time. This indicates that it might solely be an effect of less rehearsal during reactivation in the cortisol group. No direct indications of lowered reconsolidation were found. Important was that the effects we found applied to the retrieval of both neutral and emotional words, similar again to chapter 3. Stress and cortisol administered during
retrieval thus seem to lead to long-term attenuation of both neutral and emotional memories.

Regarding the propranolol group, we found no immediate or prolonged effects of propranolol on memory retrieval. While we did not expect immediate memory retrieval to be affected by propranolol (in line with de Quervain et al., 2007), we did expect propranolol to impair memory retrieval one week later by lowering the reconsolidation of the reactivated words. One of the factors that may have contributed to the non-results is that the words did not elicit enough emotional arousal. Namely, animal studies have mainly studied fear conditioning paradigms and clinical studies in humans have investigated reactions to traumatic memories (Brunet et al., 2008). It might also be that propranolol does not affect declarative memory retrieval but rather only affects emotional reactions to memories. Chapter 6 will describe the results of a study that examined these emotional reactions to memories.

**Autobiographical memory after acute stress in healthy young men (chapter 5)**

While the effects of stress and cortisol increase on the retrieval of declarative memories has been well studied in recent years (Het et al., 2005; Kuhlmann, Kirschbaum et al., 2005; Kuhlmann, Piel et al., 2005; Kuhlmann et al., 2006b; Oei et al., 2007; Tollenaar et al., 2008a, 2009; Wolf, 2003; Wolf, Convit et al., 2001), all of these studies examined the retrieval of memories that were created in the lab and consisted mostly of words or word pairs. It is still to be elucidated whether these findings can be generalized to the retrieval of more realistic autobiographical memories. Different brain areas might be involved in the processing of such memories and overall they are more complex and are experienced more intensely and vividly. Therefore, we set out to study the effect of stress on autobiographical memory (AM) retrieval and the results are described in Chapter 5. A difficult part of studying AM retrieval is that it is virtually impossible to control the content of the memories, i.e. whether they are accurate and complete. However, AM retrieval can be measured by means of its specificity (Williams & Broadbent, 1986). Specific memories refer to single events that happened at a specific time and place, consisting of event specific knowledge. Retrieving a specific memory follows a hierarchical sequence (Conway & Pleydell-Pearce, 2000), starting with life time periods including general events, followed by the retrieval of event specific knowledge for one such event. If AM retrieval is blocked or less accessible, this will lead to memories that are less specific in time and place and remain over-general, or categorical, in nature. Categorical memories describe events that repeat themselves regularly (e.g. going to the gym every Monday evening instead of a specific event that happened during one gym class). We studied whether stress and its related cortisol increases led to such over-general memories.

We did not find any effects of stress on AM specificity, even though the stress task did evoke both cortisol and sympathetic responses. A small correlation was found between cortisol increases and a lower specificity of recent neutral memories, indicating there might be some relation between cortisol and AM specificity. This would be in line with an earlier finding by Buss et al. (2004) that cortisol administration can cause neutral memories to be less specific. We also did not find any effects of the stress task on the subjective emotional experience of the memories.
In chapter 5 we described several causes that may have led to these non-results, including a possible ceiling effect of the memory task. Furthermore, higher cortisol increases might be required to diminish AM retrieval. Another issue might be that memory specificity is not the right way of examining the retrieval of AM memories. Even though it gives insight into the depth of retrieving memories in a hierarchical model (Conway & Pleydell-Pearce, 2000; Williams et al., 2007), perhaps AM tasks that examine accuracy or retrieval speed might give more insight into the functioning of AM retrieval after stress. Several elegant neuro-imaging studies have recently been performed using tasks with pictures selected by family members (Cabeza et al., 2004), or that measured different stages of AM retrieval (Daselaar et al., 2008), which could potentially be of use in future studies.

While no effects were found of stress on AM specificity, we did find differences in specificity between recent and remote memories. That is, neutral memories that were relatively recent (from the last 2 years) were more specific than remote neutral memories (from the primary school time). On the other hand, emotional memories were equally specific whether they were recent or remote. This might indicate that remote emotional memories are remembered and potentially stored differently in the brain than remote neutral memories, potentially due to more frequent and more intense re-experiencing. Remembering specific knowledge on the source of past emotional events might also be important for survival.

**Psychophysiological responding to emotional memories in healthy young men after cortisol and propranolol administration (chapter 6)**

As chapters 2 to 5 have all described studies investigating the effects of stress hormones on retrieval of declarative memories, chapter 6 described a study examining the emotional reactions to memory retrieval. We studied the effect of cortisol and propranolol on both subjective emotional reactions and physiological responses to emotionally disturbing memories. Cortisol has been found to impair declarative memory retrieval, potentially through ways of affecting the hippocampus, while propranolol has been found to impair the reconsolidation of fear memories in animals, potentially by blocking the amygdala. Therefore, it is of interest to know in what respect both of these drugs can impair the experienced intensity of emotional memories. Het and Wolf (2007) found that cortisol administration reduced increases in mood due to a stress task in healthy women, indicating that cortisol may affect the emotional experience of negative events. This might also apply to negative memories. Also, shortly before completion of our investigation, Brunet et al. (2008) published a study in which they examined the effects of propranolol administration on the psychophysiological responding to traumatic memories in PTSD patients. They found that propranolol significantly reduced heart rate and skin conductance responding to script driven imagery of their trauma. Our study resembled their study but rather we examined the effects of propranolol and cortisol on script driven imagery of negative disturbing memories in healthy young men, in addition to the subjectively experienced emotions.

While we did find significant physiological responding to imagery of the emotional memories compared to imagery of a neutral story (as reflected in lowered heart rate and heightened skin conductance responses), no effects of either cortisol or
propranolol were found. This contradicts the findings of the study by Brunet and colleagues, at least for propranolol. We might conclude that the effects of propranolol on the retrieval of emotional memories in healthy men are not comparable to the effects of these drugs in a clinical population characterized by excessive retrieval of traumatic memories and a hyper-aroused state. However, differences in study designs may account for our conflicting findings, as our reactivation procedure was relatively short compared to the study by Brunet et al. and we gave the drugs before instead of after reactivation. Most likely though, the memories in our study did not elicit high enough arousing responses to find an attenuating effect. We also didn’t find any effects of the two drugs on the subjective experience of the memories, contradicting the earlier findings by Het and Wolf (2007). Again, differences in study design and population (women vs. men) might explain these divergent findings. Thus, whether cortisol can diminish psychophysiological responding in PTSD as propranolol does, remains to be investigated.

Additional conclusions
As discussed before, cortisol might only exert an impairing effect on memory retrieval when noradrenergic functioning is intact or heightened. In chapter 2 we indeed found evidence that cortisol was only related to decreased memory retrieval when participants were aroused by the stressor. However, in Chapter 3 we described the finding that cortisol administration by itself impaired memory retrieval without any arousing environmental factors. Even recall of neutral words was impaired, indicating no additional emotional arousal was necessary. This might indicate that at higher cortisol levels (i.e. administration led to an almost tenfold increase in salivary cortisol levels compared to the stress task) no additional arousal is necessary to impair memory retrieval. Only blocking baseline adrenergic arousal can then diminish the impairing effect (see de Quervain et al., 2007). The fact that propranolol can block the impairing effects of cortisol on memory retrieval is potentially of interest for situations where people are bothered by memory problems due to stress, e.g. during exams or job interviews. Beta-blockers have already for long been used to suppress extreme nerves, and this might indicate to another possible working mechanism.

When comparing the long-term effects of stress and cortisol on memory retrieval (chapter 3 and 4), it has become clear that while both can lead to long-term impairing effects, only stress diminished memory even further after its reactivation. During stress, not only are cortisol levels increased, but adrenergic systems are activated as well as a cascade of other hormones (e.g. Carlson, 1998; Lupien & LePage, 2001; Vander et al., 2001). It is therefore difficult to conclude as to which hormones or systems are responsible for the further decline in memory after retrieval under stress and more human studies on stress hormones and post-retrieval memory stages are warranted. We should note though that in chapter 3 memory was reactivated during stress 5 weeks after learning and retested after 6 months, while in chapter 4 memory was reactivated after cortisol administration 1 week after learning and retested another week later. Even though in both studies retrieval was measured well after encoding, differences in timing might also account for the different outcomes.