Memories in context: On the role of cortisol

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Chapter 8

Summary and general discussion

Background

The ability to store declarative memories into their original encoding context (i.e., memory contextualization) is highly adaptive as it can help to retrieve memories that are likely to be appropriate in a specific context, while at the same time preventing the retrieval of irrelevant or even undesired memories in that context. Several independent lines of evidence have suggested that stress, and more specifically, cortisol, can generally alter memory processes by exerting effects on local memory circuitry in the brain. However, the complicated relationships that exist between cortisol and the ability to integrate a central memory into its surrounding context have not yet been uncovered. The central aim of the present dissertation was to reveal mechanisms by which cortisol may alter memory contextualization. We will commence this general discussion with a summary of the aims and major findings of each individual study presented in this thesis. Then, we will focus on the overarching story that the present series of studies can tell on the role of cortisol in contextualization. Hereafter, via considerations on the hippocampus, clinical implications, and open questions for future research, we will put this thesis itself in context by some concluding remarks.

Summary of findings

A first important step of the present thesis was to assess whether cortisol could explain the relationship between stress and altered context dependent memory (**chapter 2**). In this first experiment we did not define the direction of the relationship between cortisol and memory contextualization, since two different predictions circulated in the existing literature. On the one hand, alterations in memory have been shown to positively relate to stress-induced cortisol levels (e.g., Cornelisse et al., 2011b; Smeets et al., 2008). On the other hand, the 'arousal-impairs-binding' theory (Payne et al., 2003), posed that stress enhances memory for item information from an arousing event at the cost of contextual binding. In line with both predictions we showed that cortisol uniquely mediated the effects of psychosocial stress on memory contextualization of both neutral and negative memories. Other important psychophysiological and psychological indices of stress did not appear to mediate stress effects on contextualization. In contrast with the 'arousal-impairs-binding' hypothesis (Payne et al., 2003), stress-induced cortisol enhanced memory contextualization. Thus, there seems to be a specific role for cortisol in the integration of a central memory into its surrounding context. This finding advances our insights of the mechanisms by which stress, through cortisol, affects human memory processes. On a broader level, these results suggest that stress-induced cortisol responses serve a protective function against memory generalization.

Having established a unique role for cortisol in the relationship between stress and memory contextualization, a further key question was under what circumstances cortisol may exert protecting as opposed to detrimental effects on memory contextualization (**chapter 3**). We tested the hypothesis that rapid cortisol effects impair the contextual dependency specifically of emotional memories and that delayed effects of cortisol enhance the contextual dependency of subsequent emotional memories. To probe these two time-domains (Joëls et al., 2012), cortisol elevations were induced directly, or some hours prior to memory encoding. In agreement with our hypothesis, cortisol's rapid effects impaired emotional memory contextualization, while cortisol's slow effects enhanced the contextualization of emotional memory. In contrast, the contextualization of neutral memory remained unaltered by cortisol irrespective of the timing of the drug. Thus, one and the same hormone can induce disparate effects on behavior: the *delay* between elevations in cortisol levels and memory encoding is a crucial factor in determining the direction of cortisol-induced alterations in subsequent memory contextualization.

In order to understand how abnormalities in memory contextualization contribute to development and/or maintenance of PTSD, an important question is furthermore whether altered memory contextualization is a trait in vulnerable individuals for PTSD. For this reason, delineating which factors in addition to cortisol alter memory contextualization is vital for understanding how psychological dysfunction characterized by reduced memory contextualization may develop. The strongest finding of this **chapter 4** was that trait anxiety was related to weakened specific contextualization, but at the same time to inflated non-specific memory contextualization. In other words, though higher trait anxiety was associated with enhanced memory when the cue was paired with an old context (non-specific), it was associated with reduced memory performance with regard to the specific and unique original face-context combination. The main conclusion of the chapter was that trait anxiety, depression and the experience of chronic stress all have important, but partly opposing effects on the way central memories are integrated into their original encoding contexts. These results shed a more refined view on which aspects of memory are enhanced versus impaired in certain individuals at risk for psychopathology. Overall, the findings support a model in which alterations in memory contextualization form an endophenotype that is related to (subsyndromal) psychological dysfunction.

In the previous studies we assessed the role of cortisol on the contextualization of declarative memories. But alterations in declarative memories by cortisol do not necessarily coincide with alterations in the affective (that is, psychophysiological) expression of memories (Hamm and Weike, 2005). In **chapter 5** a new paradigm was designed that incorporated context as a modulator of fear expression. We in-

vestigated the effect of cortisol on the contextualization of fear expression in men and women. The present results indicated that cortisol reduces the contextualization of fear in women resulting in enhanced fear generalization not only to threatening cues in different contexts, but also to safe cues within the threatening context. An opposite pattern was found for men: cortisol enhanced differential contextual processing, thus enhancing contextualization of affective fear expression. Importantly, these effects were found solely on the fear-potentiated startle, but not on other dependent measures assessing contextualization, such as online shock expectancy. The results are in line with models suggesting heightened vulnerability in women for developing anxiety disorders after stressful events. However, in order to construct a more valid experimental model for the etiology of anxiety disorders, retention of long-term fear expression should be tested as well. Therefore, we conducted another fear-conditioning experiment (**Chapter 6**) in which we tested how time-dependent effects of cortisol affect fear memory of delay and trace conditioning the following day. Though this paradigm did not directly manipulate contextualization, the hippocampus is thought to underly trace conditioning as well. In contrast, delay conditioning is mainly subserved by the amygdala. We reasoned that the subsequent retention of these two types of conditioning may be differently affected by the time-dependent cortisol manipulation. Similar to our previous study (**chapter 5**) the experimental manipulation revealed on the fear-potentiated startle, not on US-expectancy or skin conductance responses. The fear-potentiated startle data showed that cortisol intake 240 minutes before fear acquisition - focusing on genomic actions - uniquely bolstered trace, but not delay, fear memory the next day. Though we did not reveal any rapid effects of cortisol, the slow effect is in line with our previous finding where slow cortisol enhanced contextualization of emotional memories specifically (**chapter 3**). Together, these findings suggest that slow effects of cortisol do not merely restore baseline functioning, but may actually lead to a redistribution of neural resources towards superior executive functioning that can strengthen subsequent fear memory as well.

Finally, we concluded the experimental chapters with a small review (**chapter 7**). We intended to draw attention to a number of important modulatory factors on cortisol effects on memory and their underlying mechanisms that were not previously emphasized in the literature. We commenced with describing findings on the role of the GR and MR in emotional memory and cortisol reactivity that point to a role of these receptors and their genetic variations in emotional memory processes. Importantly, even with the same set of genes, epigenetic modification of expression of a variety of cortisol-related genes may take place. Findings especially point to early life stress as a plausible mechanism through which corticosteroid's effects on

learning are altered. We also discussed which brain structures are generally involved in altered encoding due to cortisol, as well as important situational factors that may alter cortisol effects on memory. The most well-known and extensively studied situational factor that can determine the direction of cortisol effects on memory is emotional arousal of the to-be remembered material. In line with observations in **chapter 2, 3** and **6**, we further discussed that context and the timing between cortisol elevations may be important determinants of how cortisol affects memory formation. Finally, we mentioned that memory traces are not permanent after they have initially been established, as was assumed in the classical view of memory. Instead, when a memory is retrieved, the underlying memory trace is labile and can be modified. Cortisol is one (among many others) of the agents able to modulate reactivated memories. We concluded that taking into account (some of) these factors in future studies may help to reduce variability in study findings of corticosteroid effects on memory.

Memories in context: on cortisol

By using a variety of cortisol manipulations (i.e., pharmacological or stress manipulation) and various dependent variables (e.g., fear-potentiated startle, recognition) **chapter 2**, **3** and **6** all converge on the idea that cortisol can pronouncedly alter the way memories are integrated in their original encoding contexts. In addition, these observations are reinforced by using an alternative paradigm that does not make use of a contextual manipulation, but that is nevertheless thought to be hippocampal dependent as well (trace-conditioning, Burman et al., 2006; Knight et al., 2004) in **chapter 6**. In the introduction of this thesis we indicated that one of our aims was to more closely delineate the means by which cortisol may alter the contextualization of memories. After having established an important mediating role for cortisol between stress and contextualization (**chapter 2**) one of the first questions that came to mind was into what direction cortisol would alter contextualization. Did we succeed in answering this important question? At first sight we did not. After all, we have found that stress-induced cortisol enhances contextualization (**chapter 2**), that exogenously manipulated cortisol levels by hydrocortisone can either impair or enhance contextualization (**chapter 3**), or impair contextual control over fear in women while enhancing it in men (**chapter 5**), or enhance trace-conditioned memories (**chapter 6**), or worst of all, having no effect at all (baseline cortisol, **chapter 4**). How can we reconcile these observations, and gain in the end a deeper understanding of these mechanisms?

The most conflicting finding seems to be that stress-induced cortisol in **chapter 2** enhanced contextualization, while exogenously cortisol (the rapid group in **chapter**

3) impaired it. The paradigms used in these chapters were very alike, and cortisol peaks fell approximately 10 minutes before memory encoding. One particularly important factor underlying the discrepancy in findings may be variation in cortisol concentrations (Abercrombie et al., 2003): the hydrocortisone manipulation resulted in cortisol levels over 50 times as high as the cortisol levels by the stress manipulation. Other studies investigating cortisol effects on memory by either using a hydrocortisone manipulation (e.g., Rimmele et al., 2003) or stress manipulation (e.g., Cornelisse et al., 2011b) were characterized by conflicting findings too, underlining the notion that these measures cannot straightforwardly be compared. Also, the hydrocortisone manipulation uniquely impacted emotional memories, which we explained by interacting cortisol and arousal-evoked central adrenergic release caused by the emotional words (Kensinger, 2004; Roozendaal et al., 2006a). Stress-induced cortisol on the other hand affected both neutral and emotional memories. We believe that stress-induced adrenergic activation acting on both emotional and neutral memories may have obscured possible differential effects of cortisol on these memories. The idea that emotional arousal by the stimuli themselves or by a stress induction enables stress or corticosteroid effects on memory consolidation is not new (de Quervain et al., 2009; Wolf, 2009). Thus, here we show that both emotional arousal and absolute cortisol levels are an important factor in determining how acute cortisol elevations affect memory contextualization, too.

In **Chapter 3** and **6** we investigated time-dependent effects on memory formation, focusing on the contextualization of declarative memories and classical fear conditioning, respectively. The experimental manipulation was designed to test the theory of Joëls and colleagues (de Kloet et al., 2005; Joëls et al., 2011), predicting differential outcomes of rapid non-genomic effects versus delayed genomic effects of corticosteroids. Notably, other theories such as the temporal dynamics model (Diamond et al., 2007) and the emotional tagging hypothesis (Richter-Levin and Akirav, 2003) designate an important role for cortisol timing as well, but these predictions additionally involve emotional or stressful experiences, which can be modeled by a stress or other adrenergic system manipulation, but not by mere cortisol. In contrast to the prediction that amygdala-dependent delay conditioning would be affected by rapid cortisol, we did not observe rapid effects of cortisol on any measure of fear memory. One possible explanation for this lack of effect is that participants were subjected to fear acquisition 60 minutes after cortisol administration. This delay of one hour might have been too long to exclusively examine non-genomic corticosteroid actions (Sapolsky et al., 2000), and genomic actions might have started to develop during testing. This idea is supported by the fact that we did observe rapid effects in **chapter 3**, where encoding already took place after 30 minutes. It must be

mentioned though, that rapid effects of cortisol in **chapter 5** are not entirely in line with these findings: contextual control over fear expression was enhanced in men, and impaired in women. In this study though we did not test long-term memory for the conditioned cues, and the dose of hydrocortisone was higher than the one used in **chapters 3** and **6**. Thus, it is difficult to directly compare these studies. Interestingly, the slow cortisol manipulation in both **chapters 3** and **6** enhanced memory contextualization and trace conditioned memory, respectively. Even though these two chapters used a declarative memory and fear conditioning paradigm, they have in common that both memory contextualization and trace conditioning are thought to rely upon the hippocampus (Knight et al., 2004; Phillips and Ledoux, 1992), thereby reinforcing these mutual findings. Together, these chapters underscore the relatively new notion that not only the absolute levels of cortisol per se may play an important role in modulating certain memory processes (Abercrombie et al., 2003), but also the time lag that exists between cortisol level enhancement and encoding. Slow cortisol effects of both studies are in line with the idea that slow corticosteroid effects activate a PFC-hippocampal dependent network (Henckens et al., 2011; Joëls et al., 2012). By activation of a PFC-hippocampal dependent network, slow corticosteroid actions might exclusively have enhanced the consolidation of memory contextualization and trace conditioning. This suggests that slow effects of cortisol do not merely restore baseline functioning (McEwen, 2007), but may actually lead to a redistribution of neural resources towards superior executive functioning (Henckens et al., 2012a; 2011; 2010; Joëls et al., 2012) that can enhance subsequent fear memory and contextualization.

In **chapter 4** we did not find any relations with memory contextualization measures and baseline cortisol. One explanation could be that the baseline measure could have been too crude, since the use of morning cortisol awakening curves are highly recommended for baseline cortisol assessment, as opposed to a single measurement in time (Pruessner et al., 1997). But we did find a positive relation with chronic stress and memory contextualization, suggesting that those individuals who reported more daily hassles in the last month were better at contextualizing memories. We speculatively suggested that one underlying mechanism may be that the chronic stress may have activated similar processes as the slow cortisol manipulation in **chapters 3** and **6**. That is, slower long-lasting genomic corticosteroid actions may have developed due to the continuing daily hassles (de Kloet et al., 2005; Wiegert et al., 2005).

Causal conclusions on delayed stress effects for the study presented in **chapter 4** are clearly not possible since the design was correlational in nature. For future studies it remains therefore an open question whether current reports of chronic

stress have differential (or even opposing) effects on memory contextualization. However, even though we experimentally manipulated cortisol in **chapters 3** and **6**, a general limitation is that we cannot say for certain which underlying mechanisms caused these effects. We cannot claim that the observed rapid effects were caused by binding of cortisol to their receptors, neither can we claim that the slower cortisol effects were achieved by a gene-mediated mechanism. A possible avenue to explore these mechanisms is by blocking certain corticosteroids receptors. For instance, Mifeprestone is a GR antagonist, but is also known to cause many unwanted side effects (Pecci et al., 2009).

In conclusion, by endogenously or exogenously manipulating cortisol levels previous studies have shown that single item memory can be altered by cortisol (e.g., Buchanan and Lovallo, 2001; Het et al., 2005; Lupien et al., 1997; Smeets et al., 2009). Since the majority of these studies did not manipulate context, these findings are comparable with our 'intact context' condition (though involving a broader definition of context than our local context manipulation). **Chapters 2**, **3**, and **6** (and to some extent **chapter 4** as well) add to this existing body of research by showing that cortisol does not only directly alters single item memory but, through cortisol, also alters the process of contextualization. Together, this set of experiments additionally suggests that timing relative to encoding, emotional arousal (either of the stimulus material or stress-induced) and absolute levels of cortisol likely play important roles in determining the direction and strength of the way cortisol affects contextualization.

Memories in context: on the hippocampus

In this thesis, the hippocampus has been ever-present, but it also shined by its de facto absence. We have not only formulated hypotheses based on a vast literature on hippocampal function, we have likewise drawn our conclusions. But no chapter in this thesis has provided direct evidence that the hippocampus was actually involved in any of the effects. Nevertheless, there is some circumstantial evidence that points to involvement of the hippocampus, as well as evidence that argues against it, that we will further discuss below.

In the introduction of this thesis we defined memory contextualization quite narrowly, but in our studies we have operationalized the concept rather broadly by utilizing fear conditioning to declarative memory paradigms. Even within the studies on declarative memories (**chapter 2**, **3** and **4**), the tasks ranged from cued retrieval to associative recognition tasks, though the way we manipulated context in these paradigms was rather consistent (i.e., by using background images). In those studies, apart from testing for objective alterations in memory function such

as memory accuracy, we exploratory analyzed whether possible changes in the subjective quality of memories were affected as well. In retrospect, the results on the different task versions were remarkably consistent: We did not find any effects of cortisol, or stress for that matter, on overall memory performance, cued retrieval or associative recognition. Instead, without exception, effects were observed on the contextualization of recognition memory, whether the cue to respond to were words or faces. In addition, the explorative analyses revealed that the effects on recognition rather consistently operated via alterations in familiarity, while leaving recollection estimates unaffected.

Such observations prompt us to speculate which specific memory processes may be most sensitive to stress and concomitant stress hormones, and what the underlying neural mechanisms may be. Considerable MR and GR expression is found in hippocampal pyramidal cell fields as well as the dentate gyrus, the amygdaloid and lateral septal nuclei, and some cortical areas (de Kloet et al., 2005). Interestingly, high-resolution MRI has indicated a specific reduction in volume of the dentate gyrus and CA3 subfields in PTSD, with sparing of other hippocampal subregions (Wang et al., 2010). It seems that especially these regions are selectively sensitive to stress and concomitant stress hormones, which is consistent with results in animal models of chronic stress (Mcewen, 2001). Computational models of hippocampal function have suggested that the dentate gyrus (and CA3, the DG-CA3 network) mediate a dynamic competition between two complementary processes of an associative memory system: pattern completion (or generalization) and pattern separation (Guzowski et al., 2004; Leutgeb, 2004; Vazdarjanova, 2004). Pattern completion enables to respond to a degraded input pattern with the entire previously stored output pattern. Pattern separation on the other hand makes the stored representations of two similar input patterns more dissimilar (Guzowski et al., 2004). Displaying a very similar approach as our context manipulations, in animals, responses in DG-CA3 are typically measured while exposing rats sequentially to two similar environments in which certain cues are altered. If the neural response to the second environment is different from the first, pattern separation is the stronger process. If, on the other hand, the two responses are very similar, pattern completion is said to dominate. In this case, context will be a less potent way to differentiate memories, leading to generalization of stored associations across contexts. Thus arguably, it may very well be that alterations in the DG-CA3 network were responsible for the alterations in memory contextualization we have observed by our various cortisol manipulations. Indeed, it has been proposed that impairments in pattern separation may underlie overgeneralization of fear in anxiety disorders and may be an endophenotype of anxiety disorders such as PTSD and panic disorder (Kheirbek et al., 2012a).

Then the question remains, why did we only find our effects on contextualization of recognition? It has been suggested that there may be two types of memory that contribute to recognition performance, only one of which is affected by hippocampal damage (Brown and Aggleton, 2001). That is, recognition of some items may be based on a detailed vivid feeling of re-experience (recollection), whereas other items may be recognized on the basis of a sense that the item has been previously encountered (a sense of familiarity). When the ability to recollect items is impaired, the task may nevertheless be accomplished by familiarity (Yonelinas, 2002). Thus, in contrast with the process of contextualization that arguably may be subserved by pattern separation/completion, single item encoding, and the feeling of familiarity that single items can generate, have been proposed to be subserved by the perirhinal cortex (Brown and Aggleton, 2001), a region that has typically not been implicated to be sensitive to stress and concomitant hormones. Our results generally showed that cortisol manipulations altered contextualization of familiarity responses, which would link effects of cortisol to an area not sensitive to it. One way to reconcile these opposing findings would be to assume that brain regions adjacent to the hippocampus may be sensitive to cortisol as well. Another way is to assume that the hippocampus is in fact involved in familiarity. It has been suggested that weak hippocampal memories are most likely to express themselves through a familiarity profile (Kirwan et al., 2010; Squire et al., 2007). Perhaps cortisol in this dissertation may have primarily affected such weak memories, leaving stronger memories untouched. This would also explain why cortisol emerged as a significant mediator of memory contextualization only on recognition and familiarity, but not on cued retrieval and recollection, which are supposedly subserved by solider memory traces (e.g., Kirwan et al., 2010; Squire et al., 2007). Taken together, the present set of studies suggests that the contextualization of recognition judgments are exquisitely sensitive to cortisol, effects that are perhaps mediated by alterations in familiarity profiles. In contrast, context effects on other measures of declarative memory seemed to be less sensitive to cortisol. Future studies will have to further determine to what extent these types of memory tasks relate to each other, and how they vary in their sensitivity to stress and stress hormones. Based on the present findings we cannot claim which particular brain regions may have been most pronouncedly affected by our cortisol manipulations. More in-depth investigations are worthwhile and needed, and neuroimaging may be the perfect tool to do so.

Clinical implications

The basic insights derived from this thesis have some implications for understanding and treatment of anxiety disorders. Especially our findings that cortisol

time-dependently affects the formation of fear memory and the contextualization of emotional memories (**chapters 3** and **6**) may shed light on the question how cortisol can sometimes have detrimental and sometimes protective effects on the formation of traumatic memories (Kaouane et al., 2012; Rao et al., 2012). In terms of contextualization, cortisol may serve a protective function against the generalization of memories across contexts. For instance, in anticipation of a potentially aversive event cortisol could be administered several hours in advance. Or, in the case of a moderate stressful situation like the one in **chapter 2**, stress-induced cortisol itself may serve to protect against the generalization of memories. On the other hand, cortisol may serve a detrimental function by decontextualizing subsequent memories when administered shortly before, or during, the experience. Acute, direct, effects may also immediately impair contextual control over fear expression (in women; **chapter 5**). In line with the rapid findings of **chapter 3**, one study showed that the number of traumatic memories from the intensive care unit correlated positively with the amount of cortisol administered to patients that underwent cardiac surgery (Schelling et al., 2004). This suggests that blocking glucocorticoid signaling right before or immediately after a traumatic event might be useful, in line with our predictions in terms of contextualization. But reduced cortisol excretion in response to a traumatic event has been associated with an increased risk of developing PTSD (McFarlane et al., 1997; Delahanty et al., 2000), and prolonged administration of cortisol during and after intensive care treatment seems to reduce later development of PTSD (Schelling et al., 2004, 2006). These observations are in line with our finding that higher endogenous cortisol responses and chronic stress help to integrate a central memory in its surrounding context (**chapter 2** and **chapter 4,** respectively).

In this thesis we manipulated cortisol levels around the time they were initially established. Therefore, cortisol likely affected encoding and consolidation processes. But the findings on the interactions between cortisol and context may have implications for processes that take place after memory establishment as well. Extinction processes have been found to be sensitive to the context in which memories are learned and extinguished (Bouton et al., 2006). The context-dependent expression of fear after extinction (that is, when the meaning of the CS has become ambiguous) appears to involve a 'gating' of CS–US and CS–no-US associations that are encoded in the amygdala. Considerable work implicates the hippocampus in regulating the context-dependence of extinction memories. Cortisol has been suggested as a facilitating agent in exposure therapy (Soravia et al., 2006; de Quervain and Margraf, 2008) – a process for which extinction of conditioned responses is an experimental model. Our results emphasize that it is crucial to carefully consider the time lag between cortisol administration and an (aversive) event, but these insights may ap-

ply too when considering cortisol as treatment for anxiety disorders in therapeutic session. If genomic effects of cortisol indeed enhance the contextual dependency of memories, clinicians should take care to administer cortisol in close proximity of exposure or extinction learning, in order to realize maximum generalizability of extinction learning. This, of course, would need to be tested experimentally.

In the introduction of this thesis we suggested that dysregulation of contextualization processes (for instance due to cortisol) might play a key role in the generation of PTSD symptoms such as memory intrusions (Acheson et al., 2012; Ehlers et al., 2004; Liberzon and Sripada, 2008). Other theories rather emphasize alterations in the ability to retain extinction memory in other contexts as the crucial abnormality in explaining the etiology of pathological anxiety (Kalisch et al., 2006; Milad et al., 2009). Both lines of theory emphasize the role of context, but note that these theories in fact make contrasting predictions; an impairment in contextualization during fear acquisition is thought to have detrimental consequences, while the same inability during extinction would have favorable consequences (i.e., safety memory is generalized). A strong asymmetry in the context-specificity of fear acquisition and extinction exists: as a rule, extinction is more context specific than acquisition (Bouton, 2002). This appears to be the case because extinction per definition is the second thing learned about the CS (Nelson, 2002). It is as if the learning and memory system encodes the second thing learned about a stimulus as a conditional, context-specific exception to the rule (Bouton, 2002). Therefore, context dependency of acquisition and extinction cannot easily be compared, and the contrasting predictions on context effects on these processes promise to be an interesting avenue for future studies (see for some initial studies already: Hamacher-Dang et al., 2013).

Finally, it is noteworthy that patients suffering from PTSD typically describe their intrusions as consisting of mainly very short sensory, mostly visual perceptually, experiences (Ehlers et al., 2004). In addition, intrusive memories are highly distressing, and are accompanied by physical sensations. In contrast, narrative, more elaborative, memories of the traumatic event typically develop more slowly, with trauma victims initially struggling to put their experience into words (Brewin et al., 2010). Therefore, it is generally assumed that perceptual trauma representations play a central role in PTSD, whereas conceptual representations are thought to be poorly developed (Brewin and Holmes, 2003). In addition, Brewin and colleagues (2010) have suggested there are distinct neural bases to abstract, flexible, contextualized representations and to inflexible, sensory-bound representations. This idea has been corroborated by studies showing that an increase in conceptual processing is positively related to a favorable treatment outcome in PTSD (Kindt et al., 2007). Our recurring observations that cortisol mostly affected recognition and familiarity

but not retrieval, recollection and/or associative memory, fit this model. Though admittedly speculative, it may be that especially paradigms that probe contextual binding of recognition are an appropriate way to assess memory processes that are dependent upon more sensory-bound representations.

Future directions

As already may have become clear from the previous paragraphs, several observations presented in this thesis plead for further investigation. The first suggestion for future research relates to the question how exactly the brain enables cortisol effects on memory contextualization processes. Will the hippocampus reveal to be as imperative as this thesis suggested? It is one of the most extensively studied areas of the brain, but at the same time the hippocampus seems to be most enigmatic. By communicating with different areas in the cortex hippocampal context representations modulate activity in distinct systems that guide different aspects of behavior (Hsieh et al., 2014). Zooming in on the hippocampus, computational neurobiological models and emerging rodent data suggest that the DG-CA3 network mediates a dynamic competition between pattern completion (or generalization) and pattern separation (Guzowski et al., 2004; Leutgeb, 2004; Vazdarjanova, 2004). It already is possible to segment human hippocampus into the subiculum, CA1, and a combined subregion consisting of the dentate gyrus, CA2, and CA3 (these subfields are difficult to unambiguously segment at current functional MR resolutions). Also, the anterior extent of the parahippocampal gyrus can be divided into entorhinal cortex (ERC) and perirhinal cortex (PRC) and differentiated from parahippocampal cortex (PHC) in the posterior portion of the gyrus using high resolution fMRI (Carr et al., 2010). In a first step, using such a technique, it would be tremendously interesting to establish the systems neuroscience mechanisms subserving memory contextualization. Then, in a next step, it could be tested whether alterations in the DG-CA3 network are indeed responsible for the alterations in memory contextualization we have observed by our various cortisol manipulations.

Elaborating on the thoughts described above, the hippocampus seems to be essential for expressing memories soon after encoding, while expression of the same (or at least equivalent) memory may become independent of the hippocampus at later time points (Frankland and Bontempi, 2005). It has been proposed that the transition from a hippocampus-dependent to hippocampus-independent form reflects a transformation of the memory from a precise (or detailed and contextually rich) form to a less precise (or generic and context-free) form in extra-hippocampal regions. In other words, such a process would lead to a decontextualization of memories over time, while sparing extra-hippocampally coded event components,

such as individual objects. This idea has been supported by a recent finding using an associative and object memory paradigm (Talamini and Gorree, 2012; but see, Wang et al., 2009). Interestingly, one of the targets of cognitive behavioral therapy is to contextualize trauma memory (Ehlers and Clark, 2000). The decontextualization of memories due to a mere passage of time may surge the generalization of trauma memories, and may therefore require occasional follow-up sessions.

An important shortcoming of virtual all studies in this thesis is that all cortisol and/or stress manipulations took place before encoding, thereby thwarting a possible distinction between stress effects on encoding and initial consolidation. Cognitive processes underlying encoding such as working memory (Blumenfeld and Ranganath, 2006) and attention (Miller and Cohen, 2001) have typically been shown being impaired by stress (Elzinga and Roelofs, 2005; Schoofs et al., 2008; Qin et al., 2009; Henckens et al., 2011). For example, selectively attending to aspects of our external environment will give rise to selective long-term memory for attended information. Likewise, memory retrieval may be thought of as selective attention to internal representations of past experience. In short, attention and memory cannot operate without each other (Chun and Turk-Browne, 2007). Thus, in theory, the rapid cortisol manipulation that impaired contextualization may have been accomplished by impairments in attention. Countering this hypothesis, one study has already shown that the emotion-modulated differences in memory were not resulting from differences in overt attention as assessed by eye-tracking (Riggs et al., 2011). Positive relationships between cortisol and contextualization such as the one in **chapter 2** are also unlikely to be explained by reduced attention. Nevertheless, for future studies it would be worthwhile to complement the study design with eye-tracking (for spatial attention) or a working memory task.

Furthermore, despite several suggestions in this thesis (as well as by others; Acheson et al., 2012; Kheirbek et al., 2012a; Maren et al., 2013), a pressing question remains whether it is indeed the case that abnormalities in memory contextualization are a vulnerability factor for the development of PTSD. Given that most human (neuroimaging) studies have investigated PTSD following trauma, it has been impossible to determine whether observations in brain differences are cause or consequence of the disorder. By regarding the traumatic encounter as a reference point for disease onset, prospective longitudinal (neuroimaging) studies of PTSD can potentially assign abnormalities in contextualization-associated processes to either predisposing (pre-exposure) or acquired (post-exposure) factors (Admon et al., 2013) in for instance populations at risk for trauma such as the police. Such studies can additionally take measures of hair cortisol (Steudte et al., 2013) to assess whether cumulative cortisol exposure are related to alterations in memory contextualization.

Furthermore, the last article (**chapter 7**) presented in this thesis already emphasized that further elucidation of the factors that modulate (or alter) cortisol's effects on memory is required to allow reconciliation of seemingly inconsistent findings in the basic and applied literature. A similar argument pertains to (cortisol effects on) memory contextualization. That is, it is likely that for instance the presence of corticosteroid receptors, genetic profiles, and dispositional characteristics all play a role in determining cortisol's effects on memory contextualization. Taking into account such factors in future study designs, one could answer questions like: 'Are individuals who have suffered from childhood trauma more prone to store emotional memories in a decontextualized fashion due to cortisol?' and 'Is the interplay between cortisol, genetic susceptibility and certain personal characteristics associated with fMRI signal changes in the hippocampus when storing context dependent memory?' In order to answer such a wealth of complex questions, future research may profit from analysis techniques that can take into account several levels of variables, instead of focusing on just a few at the same time. For instance, multilevel approaches provide a powerful way to simultaneously assess the contribution of genetic background, individual differences in trait characteristics, hippocampal reactivity and childhood experience to predict memory. Further knowledge regarding ways in which genetics, lasting characteristics, and other variables moderate cortisol's effects on memory consolidation and/or reconsolidation can serve as a foundation for treatment research, which could further elucidate the potential therapeutic benefits of manipulating neural signaling of corticosteroids.

Thesis in context: concluding remarks

This thesis commenced with the observation that memories of past experiences can guide our thoughts and behavior in profound ways, and that the remembrance of these bygone events is to a large extent dependent upon the specific context in which we are located. This mechanism is adaptive as it can help to retrieve memories that are likely to be appropriate in a specific context, while at the same time preventing the retrieval of unrelated or undesired memories in that context. However, patients suffering from anxiety are often confronted with memories that intrude completely out of context into consciousness, indicating that these emotional memories have not been properly contextualized. Several lines of theory have suggested that stress and concomitant stress hormones such as cortisol may underlie altered contextual dependency of memories, but whether this is indeed the case in healthy humans was unknown at the start of this thesis. Therefore, the central aim of the present dissertation was to reveal mechanisms by which cortisol may alter memory contextualization. With this thesis, we have corroborated the notion that memories are

highly context dependent. More importantly, we demonstrated that cortisol plays an important role in binding memories, whether it was sometimes in, and sometimes out, of context. Though the experimental models employed in this thesis are an oversimplification of the complexity of real life memories and trauma, these findings nevertheless provide further clues on the etiology and treatment of pathology characterized by disturbances in memory contextualization.