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Fading memories : the impact of stress hormones on the retrieval of emotional memories

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Fading Memories

The impact of stress hormones on the retrieval of emotional memories

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Chapter 1

General Introduction

We probably all know the feeling of being under pressure or a lot of stress and not being able to recall relevant information (e.g. during an exam) or to give examples of events that happened in the past (e.g. during a job interview). For several decades scientists have studied how it is possible for stress to affect memory in healthy people, but also in people that are diagnosed with stress related disorders like depression or acute and post-traumatic stress disorder. One of the recent findings in healthy populations is that the retrieval of information, particularly emotional information, is affected by stress. This outcome confirmed earlier findings in animal studies. However, the underlying mechanisms of and specific conditions in which these effects appear in humans, as well as the long-term effects on memory, are still unclear. These aspects will be topic of the present thesis.

To understand how it is possible for stress to affect memory, it is first important to precisely state what we mean by stress and by the retrieval of (emotional) memories. In the next paragraphs these issues will be discussed. We will start with a description of our conceptualization of the retrieval of emotional memories and its relevance to daily life. This will be followed by a more general overview of memory theories and the underlying mechanisms in the brain (i.e. the neurobiology) that make memory possible, in particular memory retrieval. We will then turn to the models that may explain how stress can affect memory, with specific details on stress hormones that are involved in this process. An overview will follow of previous research into the effects of stress on memory retrieval, also discussing gaps in the present knowledge regarding this issue.

While impairments in memory can be seen as a negative consequence of stress and stress hormones, a more positive use of this knowledge has been applied to clinical practice. The last part of this introduction will describe how the deliberate suppression of emotional memory retrieval by stress hormones could be useful in disorders characterized by excessive retrieval of emotional memories. However, these studies are still in an early stage and more insight into the effects of stress hormones on different aspects of memory is needed before a safe use of these drugs can be established. Therefore, the present thesis hopes to further elucidate the effects of stress and stress hormones on emotional memory retrieval.

Retrieving emotional memories

What do we mean?

When we reflect upon our life, we usually think of events and social interactions that were important in influencing our life and our sense of self. At the time these events happened, they probably elicited emotions as happiness, sadness, anger or fear. When thinking back of past events it might be that these memories still elicit emotions. For example, when thinking back of a terrible accident that you experienced or witnessed, intense feelings of fear or sadness might still be experienced, including physical responses like an increased heart rate. However, sometimes one can think of an event in the past that was very emotional at the time, but when thinking back of it is only thought of as a negative experience without actually feeling any arousing emotions. For example, you could have a memory of a break up after a short infatuation in high

school or of being embarrassed in front of a group of people. Memories of these experiences can still be important to our sense of who we were and are, but may have changed in the emotional appraisal we give them today. We might even laugh about such events years later on, although they were very distressing at the time. The meaning of memories can thus change over time as well as the associated emotions, or memories can just loose their emotional value over time. So we can think back of emotional events and retrieve these memories either without any of the previously associated feelings, or re-experience (part of) the emotions associated with the event. In the present thesis we will refer to both as emotional memory retrieval, but the distinction will be important in the light of clinical studies, as in certain disorders the reliving of emotional memories and associated emotional experience and responses is excessive and disabling.

On the other hand, a person might also remember an event that was not emotional at all, e.g. taking the train to work yesterday. We will refer to this as neutral memory retrieval. A difference between neutral and emotional memories is that the event(s) that formed an emotional memory elicited an emotional response at the time of formation. Research has shown that an emotional response to an event leads to a favorable position of these events in memory (Cahill & McGaugh, 1998), as they are better stored into long-term memory (LaBar & Cabeza, 2006) and can be remembered for decades. Also, memories of these events usually elicit more vivid and intense re-experiencing. These memories consist of both contextual and factual information and associated emotional responses. It is important to make a distinction between the contents and the emotional experience of a memory, given that different brain areas are thought to be involved in their processing.

A view on memory

Memory for events or for information that was learned in the past is often referred to as declarative, or explicit, memory. People can consciously recall declarative memories. When memories are related to a certain place and time, e.g. “the job interview at the University last week”, these declarative memories are called episodic memories. On the other hand, declarative memories can also consist of mere facts about the world and oneself, like names of friends and family members or the name of one’s high school. This is called semantic memory (Tulving, 1972, 2002). Even though we might still remember meeting a friend and hearing his or her name for the first time, the name belonging to the person is stored as a semantic fact, while the event of meeting is an episodic memory. The present thesis will be about such declarative memories, specifically episodic memories. In most of the chapters, we measured episodic memory retrieval with memories that were created in a laboratory setting (e.g. word pairs or word lists that were learned in the lab on an earlier occasion). But we also studied memories of participants’ personal past, which is called autobiographical memory. Autobiographical memories are usually rich memories with vivid images of contextual details and feelings associated with a certain time and place. Autobiographical memories contribute to an individual’s sense of self and make us able to keep track of our personal past and goals. Autobiographical memory can be viewed as a form of episodic memory, but also

consists of many semantic elements and general knowledge on life time periods (Conway & Pleydell-Pearce, 2000; Levine, 2004; Williams et al., 2007).

The formation and storage of declarative memories can be divided in several stages (see Figure 1.1). First of all, an event or new information has to be encoded before it can be consolidated, or formed into a long-term memory. However, not all new information will be selected to be consolidated to long-term memory after encoding. Attentional processes and current emotional state might play a role in the (automatic) selection of information to be remembered. Once new knowledge is being consolidated, a memory of this information or event can be retrieved, either intentionally or spontaneously. Retrieval can be repeated many times, as long as the memory is available. The present thesis will investigate the effects of stress specifically on this retrieval stage. However, recently it has become clear that when memories are reactivated by retrieval, they might get into a labile state in which these memories are prone to change. While newly formed memories undergo the process of consolidation to become stable, reactivation might make them labile again (Nader et al., 2000; Przybylawski & Sara, 1997). After reactivation, these memories are then 'reconsolidated' back into long-term memory, potentially affected by the context in which they were retrieved. This is still a controversial matter, but animal and human studies have shown that memories can indeed be affected upon reactivation (Debiec et al., 2002; Hupbach et al., 2007; Nader et al., 2000; Przybylawski & Sara, 1997; Walker et al., 2003). The present thesis will also explore the impact of stress on this post-retrieval stage.

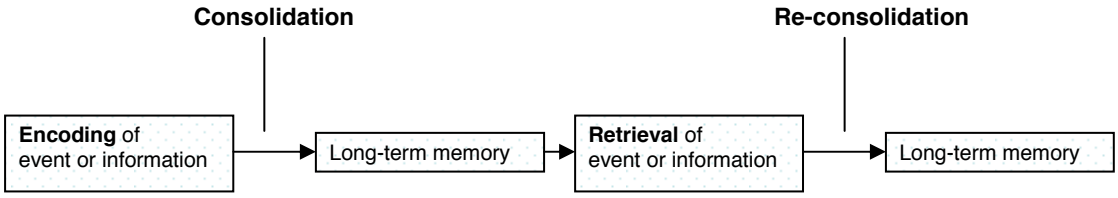

Figure 1.1. Schematic overview of memory stages.

Besides conscious, declarative memory, there is also a more un-conscious, implicit, form of memory. For example, there is memory for learned motor skills (procedural memory), like riding a bike or driving a car. These abilities were learned by repetition but can not easily be verbally described, although a specific driving lesson might be stored as an episodic memory. There is also a more temporary, active memory, referred to as working (or short-term) memory. While declarative memories are stored (consolidated) into long-term memory, which means that they are available long after an event or learning experience occurred, a working memory system is needed to integrate current perceptions and thoughts with knowledge from long-term memory. Information in working memory is temporarily maintained and can be updated and manipulated. This information will sometimes be consolidated into long-term memory, but will often be forgotten again (imagine a phone number you have to remember to dial and forget again as soon have you have used it).

Neurobiology of emotional memory retrieval

Up till now it is still not fully understood how the brain brings about the retrieval of (emotional) memories, but in the next paragraphs a brief overview of recent findings will be given. Knowledge on which brain areas are involved in (episodic) memory retrieval comes from studies in patients, characterized by specific lesions and memory problems, but more recently also from neuro-imaging studies with Electro Encephalography (EEG) and functional Magnetic Resonance Imaging (fMRI).

There are several key structures involved in both the formation and retrieval of memories. The hippocampus and amygdala (both within the medial temporal lobe and limbic system of the brain) and the prefrontal cortex are such key structures (see Figure 1.2). Especially the formation and retrieval of explicit, declarative memories are found to be mediated by the hippocampus as became clear from early studies on patients with lesions in this area (Corkin, 2002; Scoville & Milner, 1957; Steinvorth et al., 2005). Patient studies have further shown that hippocampal and amygdalar structures are involved in the formation of emotional memories (e.g. Phelps, 2004). While the hippocampus seems mostly involved in the formation of declarative (e.g. contextual) knowledge, the amygdala seems involved with implicit, conditioned emotional responses and the vivid recollection of emotional memories (Bechara, 1995; Buchanan et al., 2005; Labar & Cabeza, 2006).

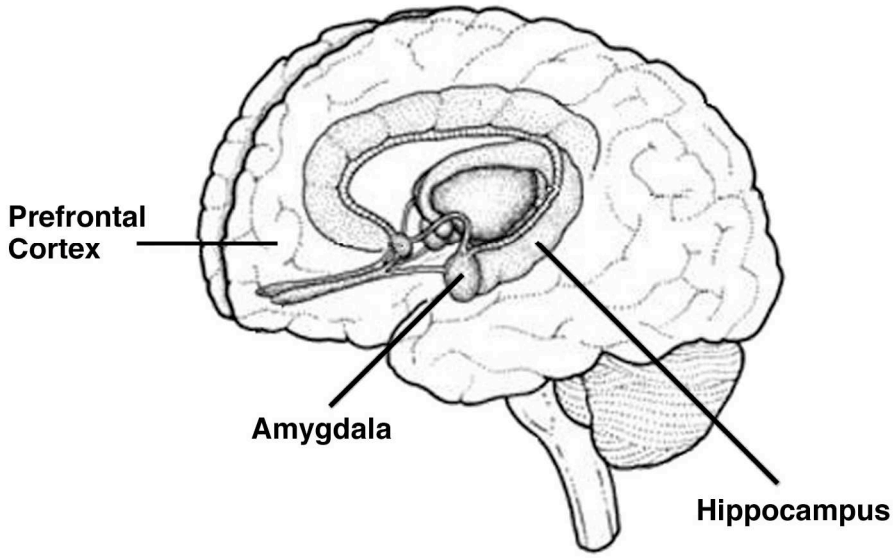

Figure 1.2. Brain structures involved in memory processing

Knowledge on the role of these two structures in memory *retrieval* comes mostly from neuro-imaging studies. Recent studies have shown that retrieving memories elicits activation in both the hippocampus and amygdala, with activity of the amygdala most pronounced in emotional memory retrieval (Dolcos et al., 2005; Smith et al., 2004). Furthermore, the connectivity between the hippocampus and amygdala gets stronger when relevant emotional memories are retrieved (Smith et al., 2006). Emotional memories are generally rated as more vivid, and high in re-experiencing and intensity. These subjective ratings are related to higher activity levels in

hippocampus and amygdala and to stronger feelings of remembering (Addis et al., 2004; Labar, 2007; Sharot et al., 2004). The emotionally driven activity in the amygdala, caused by attempts to retrieve memories, can also help to select memories or facilitate the retrieval of associated contextual information belonging to the memory (Buchanan, 2007; Labar, 2007). This may then lead to a full emotional experience (LeDoux, 2000; Tsuchiya & Adolphs, 2007)

The prefrontal cortex is also implicated in the successful retrieval of memories (Greenberg et al., 2005; Svoboda et al., 2006) and is thought to be involved in the initiation and control of the retrieval process (Buchanan, 2007; Simons & Spiers, 2003), as well as the maintenance of the retrieved information (a function related to working memory). In addition, the prefrontal cortex seems to be involved in the storage and retrieval of semantic information, even about emotions. That is, the prefrontal cortex is implicated in memories that are negatively or positively valenced, but do not elicit emotional arousal (Kensinger & Corkin, 2004). Therefore, patients with damage to the amygdala may still show enhanced memory for emotional information, but based more upon semantic knowledge of emotions, as generated by the prefrontal cortex.

The prefrontal cortex mainly has a controlling function, necessary for the correct retrieval of past events, especially remote events (Rudy et al., 2005). The prefrontal cortex might even inhibit the role of the hippocampus over time (Frankland & Bontempi, 2005; Takashima et al., 2006). That is to say, the role of the hippocampus in the retrieval of remote memories is not without controversy. For long, it was the norm to think that the medial temporal lobe (MTL; including the hippocampus) was only temporarily involved in the formation of memories. It would become unnecessary after the consolidation of information into long-term memory (Squire, 1992; Squire & Alvarez, 1995). This is called the Standard Model of Consolidation. The role of the MTL would be to activate brain regions in the neocortex that contain the information that was experienced, creating strong interconnections between these cortical sites, leading to memories independent of and no longer activating the hippocampus. According to this model, retrieval of both semantic memories and episodic memories would only be temporarily mediated by the hippocampus. Studies in patients with MTL damage have shown that remote memories are usually preserved, while recent memories are affected (e.g. Bayley et al., 2003), indicating the standard consolidation theory might be correct. A model that challenged the consolidation model is the Multiple Trace Theory (MTT; Moscovitch et al., 2005; Nadel & Moscovitch, 1997). This model proposes that the MTL remains necessary for the retrieval of episodic memories, while semantic memories become independent of the MTL over time. To store episodic memories, the hippocampus will bind representations in neocortical neurons via memory traces in the hippocampus, and every time a memory is reactivated, new traces are formed that bind together these representations. Older memories will thus have more memory traces in the hippocampus, making them less vulnerable to (partial) lesions of the MTL. In line with this model, recent findings have shown that the retrieval of remote episodic memories is less detailed in patients with extensive medial temporal lobe damage (Moscovitsch et al., 2006; Steinvorth et al., 2005). Imaging studies have furthermore revealed that remote memories can indeed show activation of the MTL, so long as the

memories are still vivid and rich in quality (Addis et al., 2004; Gilboa et al., 2004). It might well be that especially remote emotional memories, which are more vivid and re-experienced more intensely during retrieval, still need hippocampal structures to be retrieved, so long as they elicit emotional reactions. Once memories do no longer elicit emotional responses or vivid images, they might become independent of the hippocampus (like semantic memories), although this still needs to be elucidated.

To summarize, the hippocampus is mostly found to be involved in the formation of declarative memories and is active during retrieval. The amygdala is involved in the emotional strengthening of memories and is more active and in concordance with the hippocampus during emotional memory retrieval. The prefrontal cortex mainly has a controlling function and is necessary for the correct retrieval of past events. The prefrontal cortex might even inhibit or take over the role of the hippocampus over time, although there is still a considerable debate on the temporary role of the hippocampus in the retrieval of remote events.

Stress and stress hormone effects on memory retrieval

Stress hormones

When a person experiences stress, either physically or mentally, the body reacts in diverse ways to cope with the situation at hand. Here, we will focus on hormonal responses that are initiated by stress. When stress is experienced we can differentiate between a fast and a slow hormonal response. The fast response is mediated by the autonomic (sympathetic) nervous system (ANS) and the slower response by the hypothalamic pituitary adrenal (HPA) axis (Joels et al., 2006). The ANS system leads to the release of adrenaline and noradrenaline (or epinephrine and norepinephrine) in the body by the adrenal medulla, which leads to increases in e.g. heart rate, blood pressure and sweat production. (Nor)adrenaline is also released as a neurotransmitter in the brain and leads to a state of alertness and modulates the processing of emotional information via the amygdala (van Stegeren, 2008). We are often aware of this stressed ANS reaction as we can feel our body prepare to take action. On the other hand, through a cascade of hormones, the HPA axis leads to the release of cortisol into the blood stream by the adrenal cortex. The release of cortisol is not consciously noticeable and is involved in the re-mobilization of energy after the start of a stressful experience (Sapolsky, 2003; Sapolsky et al., 2000). It is also involved in the negative feedback regulation of the HPA axis, and cortisol levels will therefore decrease again after the end of the stressor (Lupien & LePage, 2001). Like (nor)adrenaline, cortisol is active both in the body and the brain, as it is able to cross the blood-brain barrier (BBB). Within the brain 2 types of cortisol receptors have been found that are differentially spread over brain areas, the minoralocorticoid (MR) and glucocorticoid (GR) receptors. Both receptors are abundantly present in the hippocampus and can hence influence cognitive processes mediated by the hippocampus like memory (De Kloet et al., 1999; Lupien & Lapage, 2001). In reaction to acute stressors, these hormonal effects might be adaptive to the present state, but when prolonged or chronic can lead to health problems in the long run (McEwen, 1998).

The amount of stress that is experienced by a person is dependent on several individual differences, like the way a person evaluates a stressor and available coping strategies. Furthermore, the stressor itself can have different properties that can lead to a strong or relatively mild stress reaction. In a meta-analysis, Dickerson and Kemeny (2004) investigated variables that can lead to stress. Situations that are new or unpredictable, uncontrollable or (socially) threatening will lead to the highest cortisol responses. A paradigm that includes all of these processes is the Trier Social Stress Task (Kirschbaum et al., 1993). This is a laboratory stressor that can be repeatedly used and is shown to lead to significant increases in cortisol in the majority of people over time. It includes a public speaking task preceded by an anticipation phase and followed by a cognitive task while being socially evaluated. In the present thesis, we have made use of this stress protocol.

One difficulty that arises when studying cortisol reactions to stress and its effects on memory is the fact that women and men respond with different physiological and cognitive responses to stress. For example, cortisol reactivity is mediated by the use of oral contraceptives and female hormones in different phases of the menstrual cycle (Kirschbaum et al., 1995, 1999), and memory functions are also differently affected by stress in females and males (Wolf, 2003; Wolf, Schommer et al., 2001). To avoid this variance, in the present thesis we have decided to only examine the effects of stress on memory retrieval in men. We acknowledge this as a shortcoming, since most stress related disorders (like PTSD and depression) are more prevalent in women than men. However, studying these effects in a homogenous group is a first step in further understanding the relation between cortisol and memory.

Research on the effects of stress and stress hormones on memory retrieval

Both cortisol and (nor)adrenaline are thus active in the brain after stress. They are able to strengthen the consolidation of memories into long-term memory by their effects on the hippocampus and amygdala (McGaugh, 2000), especially when stress is experienced in the context of or around the time of the events to be remembered (Joels et al., 2006; Smeets et al., 2007). However, the effects of stress and stress hormones on the retrieval of memories seem to be in the opposite direction. Memory retrieval has mostly been found to be impaired by acute psychosocial stressors (Domes et al., 2004; Kuhlmann, Piel et al., 2005; Smeets et al., 2008). Interestingly, especially emotional memories have been found to be sensitive to the effects of stress (Domes et al., 2004; Kuhlmann, Piel et al., 2005; Smeets et al., 2008). Stress thus seems to impair memory retrieval and this is thought to be due to increases in cortisol levels. Although stress elicits endogenous cortisol increases, and the individual differences in these increases could in some cases be related to memory retrieval (Domes et al., 2004; Smeets et al., 2008), the idea that cortisol may affect memory retrieval is also tested more directly by exogenous administration of cortisol. Recent studies have found that cortisol administration indeed impairs memory retrieval in humans (first study: de Quervain et al., 2000; for an overview see: Het et al., 2005), and again this effect is in some studies found to be most pronounced for the retrieval of (moderate) emotional information (Buchanan, Tranel et al., 2006; Kuhlmann, Kirschbaum et al., 2005; for an overview see Wolf, 2008).

The finding that mostly emotional memory retrieval is affected by stress and cortisol is in line with an animal model proposed by Roozendaal et al. (2003, 2006), in which noradrenergic activity is a prerequisite for cortisol effects on memory. That is, when noradrenergic signaling from the basolateral amygdala is blocked in rodents, cortisol no longer affects memory retrieval. Similar preventive effects on the impairing effects of cortisol on memory retrieval by a beta-adrenergic blocker (propranolol) are found in humans (de Quervain et al., 2007). As mentioned earlier, emotional memory retrieval is found to elicit amygdala activity and might thus supply the necessary noradrenergic activity that is needed for cortisol to impair memory retrieval. However, previous animal studies (Okuda et al., 2004) and preliminary studies in humans (Elzinga & Roelofs, 2005; Kuhlmann & Wolf, 2006b) have also shown that memories don't need to be emotional for cortisol to impair retrieval, so long as the environment elicits enough arousal. More research is needed to clarify the circumstances in which cortisol can impair memory retrieval.

We should note that when studying emotional memory retrieval, both negatively and positively valenced memories can be investigated. In the present thesis however, we will focus merely on the retrieval of negative memories, since these are most relevant in the field of clinical psychology, where problems are usually related to negative memories (e.g. depression and PTSD).

During stress, not only cortisol levels increase, but also (nor)adrenergic signaling is increased. The effects of stress might thus also be partly mediated by this hormone / neurotransmitter. While there is strong evidence that noradrenergic signaling is a prerequisite for cortisol effects on memory, the direct effects of (nor)adrenaline on memory retrieval in humans are basically unknown (Chamberlain et al., 2006). Increases in noradrenergic signaling might lead to a higher level of attention and vigilance, potentially increasing memory retrieval (Sara, 2000), but this will have to be specifically tested. On the other hand, blocking adrenergic activity by a drug called propranolol has only been done once in the context of a memory retrieval task, but did not show any effects (de Quervain et al., 2007). Animal research does show a role for (nor)adrenaline in extinction or reconsolidation processes (Debiec & LeDoux, 2004; Przybyslawski et al., 1999). That is, blocking of adrenergic activation with propranolol directly after reactivation was found to disrupt spatial and conditioned fear memories in rodents. Not much is known yet on the effects of blocking or enhancing (nor)adrenergic activity on human memory retrieval and reconsolidation. Therefore, more research in this area is needed.

Another issue that remains unclear is whether recent and remote memories are equally affected by stress. That is, retrieval of recent and remote memories is possibly mediated by different brain areas, and may hence be differentially affected by stress. If remote memories become less dependent on the hippocampus over time (as the consolidation theory states), they might also become less vulnerable to the effects of cortisol. However, animal studies have shown that GR receptors are also present in prefrontal and neocortex areas (Sanchez et al., 2000), the place where remote memories are stored according to consolidation theory. In that respect, even remote memories might be vulnerable to cortisol influences, as would be predicted from the Multiple Trace Theory as well. The only study till now that investigated retrieval of remote memories was by Wolf et al. (2002). He did not find any effects of stress on

the retrieval of these remote memories, but the learned information was all neutral, implicating there might not have been enough arousal for cortisol to affect memory. Studies on the effects of stress hormones on memory retrieval should try to make more distinction between recent and remote memories.

Furthermore, almost all studies on the effects of stress hormones on memory retrieval have used word list or short stories as experimental memory material instead of real autobiographical memories. Although memories created in the lab are better controlled than real life memories, conclusions might not fully generalize to autobiographical memory processes. And as the retrieval of autobiographical memories might be mediated by slightly different brain areas than simpler information about e.g. word pairs or pictures that were learned before (Gilboa, 2004), effects of stress might differ. A single study on the effects of cortisol on autobiographic memory retrieval did show a retrieval impairment, but this was most pronounced for neutral memories (Buss et al., 2004), contrasting studies using neutral and emotional words. When studying autobiographical memory retrieval, memories are usually not evaluated on accuracy, but on the level of specificity they reach. The model of Conway and Pleydell-Pearce (2000) may help to elucidate the mechanisms which explain why autobiographical memories can become over-general instead of specific. This model assumes that autobiographical memories are reconstructed in a dynamic and iterative process from stored autobiographical knowledge and semantic knowledge. Autobiographical knowledge is stored and accessed hierarchically, with upper layers containing general life-time information, narrowing down to lower layers, where detailed sensory and perceptual situation-specific information is found. According to this model, deliberate retrieval of autobiographical memories follows a hierarchical process starting with retrieval of life time periods involving general events, followed by the retrieval of event specific knowledge of one event. In the process of reconstructing autobiographical memories patients with stress-related disorders may get stuck in the intermediate level of this model and are unable to retrieve specific details that concern self-relevant information. Using a specificity measure (e.g. the Autobiographical Memory task (AMT), first described by Williams & Broadbent, 1986), the effects of stress on autobiographical memory can be studied.

The bright side of attenuating emotional memory retrieval

Clinical implications of fundamental knowledge

As described in the previous paragraph, emotional memory retrieval seems to be impaired by stress hormones like cortisol. While this might seem a negative consequence, in some situations it can be adaptive. That is, in some situations it might be desirable to block emotional memory retrieval. As described in the first paragraph, in some disorders, emotional memories elicit very strong emotions and sometimes so strong that they can disable normal life. For example, patients with post-traumatic stress disorder (PTSD) experience flashbacks and intrusions of the trauma, a sense of reliving the trauma and hyper reactivity to traumatic cues. But also depressed patients can have a bias in memory for the retrieval of negative memories (Leppanen, 2006). If

it is possible to impair the retrieval of emotional memories, or make memories less emotionally intense, this could enhance the efficacy of treating these disorders.

Pitman and Delahanty (2005) have proposed a model that might explain the pathogenesis of PTSD. During a traumatic experience, a strong stress response can lead to the release of an excessive amount of stress hormones like adrenaline and cortisol. This can lead to strong memories that are often and vividly relived. Every time the memory is relived stress hormones are released again and might strengthen the memory, leading to a cycle of over-consolidated memories. As described before, this process of memories being affected by their retrieval is also called reconsolidation. If we can attenuate either the excessive retrieval, or the strong reconsolidation of these memories, it might be possible to break this cycle. As cortisol is found to impair emotional memory retrieval, and preliminary studies in animals and humans have shown that propranolol can reduce reconsolidation of emotional memories (Brunet et al., 2008; Debiec & LeDoux, 2004; Przybyslawaki et al., 1999), these drugs are of interest to clinical practice. At this moment several studies with cortisol and propranolol in clinical populations have been performed or are being conducted, showing promising results (Aerni et al., 2004; Pitman et al., 2002; Soravia et al., 2006; Vaiva et al., 2003; Weis et al., 2006). As the mechanisms through which these drugs work are still largely unclear, the present thesis will explore the impact of both cortisol and propranolol on memory retrieval and reconsolidation in healthy people. While these results might not be directly applicable to clinical groups (with far stronger emotional, traumatic memories), it is a start to understand the working mechanisms of these drugs.

We can hypothesize on the mechanisms that are affected by cortisol and propranolol. Neuro-imaging studies have shown that the impairing effects of cortisol on memory retrieval might be largely mediated by the hippocampus (de Quervain et al., 2003; Oei et al., 2007). Cortisol might thus diminish the recall of emotional memories, but can possibly also reduce the emotional impact of the memory as the emotional reactions belonging to the memory are not fully accessed. However, unconscious automatic reactions to memory cues might possibly still elicit emotional responses (for a debate on conscious versus unconscious memories see Mitchell et al., *in press*). Whether cortisol can reduce the emotional impact of memories thus remains to be elucidated. Furthermore, if memories are not or not fully reactivated due to cortisol, less reconsolidation might take place, potentially leading to a less strong memory trace. Propranolol on the other hand is thought to mostly affect the amygdala (Strange & Dolan, 2004; van Stegeren, Goekoop et al., 2005). During the retrieval of emotional memories, propranolol might block the access to the emotional responses normally elicited by the amygdala in response to a memory, and could thus lead to a memory of an emotional event without the emotional experience. As the memory is remembered less intense, it might also be reconsolidated to a lesser extent, which could potentially also impact the conscious recall of the declarative components on the long-term.

In summary, while cortisol might block more declarative recall of memories mediated by the hippocampus, there is a possibility that it also affects the emotional experience of these memories. Propranolol on the other hand might block the retrieval of emotions associated with memories mediated by the amygdala, but possibly also

leads to less strong memories. In the present thesis we will explore the immediate and long-term impact of cortisol and propranolol administration both on declarative memories and on the emotional reactions to memories to examine these hypotheses. Understanding the effects of cortisol and propranolol on memory is important to assess both the potential positive effects of these drugs, but also possible side-effects and ethical consideration of the use of these drugs. That is, will factual memories actually be forgotten, or only become less emotional, and how long will those effects last? It might very well be that patients are better helped by a good processing of the memories instead of forgetting those memories (McCleery & Harvey, 2004). Before these considerations and implications can be fully discussed, more knowledge is needed on the effects of cortisol and propranolol on memory retrieval and reconsolidation. The present thesis will try to unravel some of these processes.

Outline of this thesis

Main aims

The main aim of the present thesis is to study the effects of stress hormones, in specific cortisol, 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. Previous research has shown that the retrieval of emotional information is negatively affected by stress. However, the specific conditions in which these effects appear, as well as the long-term effects, are still unclear. Several of these issues are investigated in the present thesis. More knowledge on the effects of cortisol, as well as propranolol, on the retrieval of declarative memories and emotional reactions to memories, could be useful in clinical practice that is involved with disorders characterized by excessive retrieval of emotional memories.

Chapters

In this introduction, background information was given on the field of emotional memory retrieval and the possible influences of stress and stress hormones on this process, as well as the potential useful implications for clinical practice. In the next five chapters, data from three studies that were conducted between September 2004 and 2008 will be described.

In chapter 2, the effects of a psychosocial stress task on memory retrieval of neutral and emotional words is described, in which the effects of cortisol increases during and after the stress task are related to memory performance. This was done to further explore the different effects of cortisol on the retrieval of neutral and emotional memories in both an arousing and a non-arousing setting. Furthermore, the memories were created in the lab either 1 day or 5 weeks before retrieval to asses whether retrieval of recent and remote memories is differentially affected by stress.

In chapter 3 a follow-up of the study from chapter 2 is described, for which all participants were contacted again 6 months after the start of the original study. As it might be possible to affect the retrieval of emotional memories by stress, it might also be possible that these memory traces are affected in their reconsolidation. It is thus

interesting to see whether retrieval impairments due to stress and stress hormones are temporary or have long-term effects.

Chapter 4 describes the immediate and prolonged effects of exogenous cortisol and propranolol administration on declarative memory retrieval of neutral and emotional words, to investigate the effect of these drugs both on retrieval and post-retrieval processes.

Chapter 5 describes a study on the effects of stress on the specificity of autobiographical memories, to test whether findings on laboratory memory tasks can be generalized to real life memories.

Chapter 6 is also directed to autobiographical memory and investigates the immediate and prolonged effects of cortisol and propranolol administration on the subjective experience of personal memories, as well as on the physiological reactivity to these memories. Chapter 6 is based on the same study as chapter 4, but chapter 4 measured effects on declarative memory retrieval with a different task.

Finally, chapter 7 will give an overview of the findings in chapters 2 to 6 and discuss the implications of these findings for memory models and clinical practice. The chapter will conclude with some suggestions for future research.

Chapter 2

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

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Summary

In this study the effects of stress-induced cortisol increases on long-term memory retrieval during and after acute psychosocial stress were examined. Seventy male students were exposed to either a psychosocial stress task or to a non stressful control task. During and after this task, retrieval was tested for idiosyncratic emotionally negative and neutral word pair associations that were learned 1 day or 5 weeks earlier. Within the stress condition, retrieval of negative words, 5 weeks after learning, was impaired both during and after the stress task compared to the control group. Further, during the stress task, when sympathetic activity was enhanced, impaired retrieval of both neutral and emotional words was significantly related to enhanced cortisol response. In contrast, after the stress task, when cortisol levels were still increased but sympathetic activity was low again, no association was found between cortisol increase and retrieval of either neutral or emotional material. These results are in line with previous animal research showing that when arousal is high, cortisol increase can impair memory retrieval.

Introduction

Glucocorticoid (GC) hormones and catecholamines are secreted by adrenal glands during stressful or emotional experiences. Besides regulating the bodily response to a challenging environment (Sapolsky et al., 2000), these hormones also influence cognitive functions (de Kloet et al., 1999; Roozendaal, 2002). One of the cognitive functions that is sensitive to stress hormones is memory, due to a high number of mineralocorticoid (MR) and glucocorticoid (GR) receptors in brain structures that play an important role in memory functioning, including the hippocampus, amygdala and prefrontal cortex (see Kirschbaum et al., 1996; de Kloet et al., 1999; Lupien & Lepage, 2001; Roozendaal, 2002; Wolf, 2008).

While learning seems to be facilitated by increased levels of stress hormones (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill et al., 2003; Kuhlmann & Wolf, 2006a), retrieval of previously learned material has repeatedly been found to deteriorate with increased levels of GCs. That is, placebo controlled studies administering exogenous doses of cortisol to humans have consistently found impaired memory retrieval (Buss et al.., 2004; de Quervain et al., 2000; Domes at al., 2005; Het et al., 2005; Kuhlmann, Kirschbaum et al., 2005; Wolf, Convit et al., 2001). Other studies have used a psychosocial stress task like the Trier Social Stress Task (TSST: Kirschbaum et al., 1993) to study the effects of endogenous cortisol increases on memory retrieval. Result are similar to, but less consistent than the pharmacological studies (Domes et al., 2004; Kuhlmann, Piel et al., 2005; Oei et al., 2006; Wolf et al., 2002). While Kuhlmann, Piel, et al. (2005) found impairing effects of stress on memory retrieval of both negatively and positively valenced (or arousing) material, Domes et al. (2004) found this effect only on the recognition of positive material. Oei et al. (2006) found a relation between increasing cortisol levels and impaired retrieval of only moderately and not highly arousing material, while Wolf et al. (2002) did not find any effect of stress or cortisol increase on the retrieval of neutral material.

The discrepancy between findings of pharmacological and psychosocial stress studies may be related to the level of cortisol, as cortisol levels obtained in stress studies are generally much lower than after exogenous administration of cortisol. However, the effects of endogenous cortisol levels on memory retrieval may also depend on several other modulating variables, e.g. the arousing properties of the material, concurrent activation of the noradrenergic system, and the time interval between learning and retrieval. Each of these variables will be discussed briefly.

First of all, stress induced cortisol increases are found to affect retrieval of emotionally arousing material more than neutral material (Domes et al., 2004; Kuhlmann, Piel, et al., 2005), possibly explaining the non-results of Wolf et al. (2002) using only neutral material. Recent animal studies have pointed to the role of the noradrenergic system in mediating the cortisol effects on retrieval. A number of such studies have shown that noradrenergic activation of the basolateral amygdala is necessary for effects of cortisol to occur on memory functioning in general, including memory retrieval (Roozendaal, de Quervain et al., 2004; Roozendaal et al., 2003; Roozendaal, Hahn et al., 2004). This adrenergic activity could be elicited either by intrinsic arousing properties of the learned material (explaining the effects on

emotional versus neutral material), or by the level of arousal induced by the environment, such as novelty stress (Okuda et al., 2004). In fact, a study by Elzinga and Roelofs (2005) has shown that in humans, cortisol-induced working memory impairments are only found under acute stress conditions, when sympathetic activation (as a measure of adrenergic activity) is elevated. They differentiated between a situation of acute psychosocial stress, during which participants had to perform in front of an audience (when both sympathetic activation and cortisol levels were high), and a situation where cortisol levels were high, while sympathetic activation was back to basal levels, that is, after the stress task. High cortisol responders showed impaired working memory compared to low cortisol responders only during, but not after the stress task. Testing after the stress task, when the audience has left and participants have been able to recover is the usual approach in studies investigating the effects of psychosocial stress (and related cortisol increase) on memory functioning. Conflicting reports regarding the role of endogenous cortisol increases on memory retrieval might thus be due to the level of arousal that participants experience at the time of memory testing.

Two human studies have looked into the effects of arousal elicited by the testing situation in combination with cortisol increases during memory retrieval. Buchanan, Tranel et al. (2006) measured skin conductance (as a measure of sympathetic activity) and cortisol levels in response to a cold pressure test, after which memory retrieval was tested. They found that increased cortisol levels, but not the skin conductance levels, were related to impaired memory retrieval. From this study, however, we can not conclude whether sympathetic arousal is necessary for the impairing effects of cortisol increases on memory retrieval to occur. Recently however, Kuhlmann and Wolf (2006b) reported a comparison of studies in which arousal related to the testing environment was manipulated while testing the effect of exogenous cortisol on retrieval. They compared two of their previous studies (Kuhlmann, Kirschbaum, et al., 2005; Kuhlmann & Wolf, 2005) that were conducted in a standard formal testing situation, with a highly similar study in which they had changed the testing situation into a more relaxing, non-arousing, environment. The impairing effect of administered cortisol on retrieval that was found earlier in the standard formal testing situations did not occur in the more relaxed setting. While these results may suggest that in humans, adrenergic activation is also necessary for the effect of cortisol to occur on memory retrieval, they did not assess sympathetic activity (or a more direct measure of adrenergic activity) in their participants, and hence it remains undecided whether the different findings are indeed related to differences in sympathetic arousal levels.

Another factor that could influence the effect of cortisol on memory retrieval is the time frame between learning and recall. The usual paradigm in retrieval studies is to test recall of material that has been learned a few hours to a day before, not always allowing a clear separation between consolidation and retrieval processes. Whether memory retrieval remains sensitive to the effects of stress long time after learning, is a topic that has not been well studied. To date, only the study of Wolf et al. (2002) examined the effects of a social stress task on the retrieval of material learned 4 weeks earlier. They did not find any effects of stress or cortisol increase on long-term memory retrieval, but this could also have been due to the nature of

material that was learned (e.g., not emotionally arousing) and the testing situation (e.g. the arousing stressor was no longer present at the time of retrieval testing). Another issue might have been a floor effect, with only few words remembered after 4 weeks.

In summary, further work is clearly required taking into account the factors described above. The study we describe here examines the effects of stress induced cortisol on the retrieval of neutral and emotionally arousing words, learned either 24 hours or more than a month before testing. Moreover, to test whether sympathetic arousal enables the effects of cortisol on memory, retrieval was tested both during an acute psychosocial stress task, with elevated cortisol levels and increased sympathetic activation, and after that stress task while cortisol levels are still high, but sympathetic activity is low again. During the acute stressor, we anticipated memory retrieval to be affected by cortisol increases, while after the stress task (the standard testing moment) this effect should be less evident. We expected that this distinction would be most prominent for the retrieval of neutral material, which elicits no intrinsic emotional arousal. In line with previous studies, we expected that retrieval of (negative) moderately arousing material might still be affected after the stress task, when sympathetic activation due to the stress task is low again.

Methods

Participants

Seventy healthy male Dutch students participated in the study. Their mean age was 21.34 ± 2.9 years (SD) with a minimum of 18 and maximum of 30. Their average body mass index (BMI) was 22.04 ± 3 kg/m^2 (SD). A male population was chosen to rule out potential effects of gender and endogenous estradiol on cortisol reactivity in response to stress (Kirschbaum et al., 1995). Participants were included in the study if they were free of any medication and reported no serious illnesses, substance abuse, or mental problems (on AXIS 1 of the DSM-IV) in the last year. Participants were randomly assigned to one of the four experimental conditions (see below). The four groups did not differ in age or BMI (all ps > 0.10).

All participants gave written informed consent and the study was approved by the ethics committee of the Leiden University Medical Center (LUMC). Participants received financial rewards or course credits for participation.

To minimize influences on baseline cortisol levels, cigarette smokers (n = 14) were instructed not to smoke at least 2 h before the start of both test sessions. Participants were also instructed to refrain from any heavy meals, sweets and coffee in the morning and not allowed to eat or drink anything but water in the hour previous to both testing sessions. In addition, participants were asked to minimize physical exercise or psychological distress in the hours prior to testing.

Procedures and Tasks

The study consisted of two experimental sessions both starting at either 11.30 am or 1.30 pm, and lasting 1.5 h each. Participants were instructed to awake at least 3 h prior to both sessions to avoid the morning rise in cortisol. The second session was

either one day after the first (1-day group) or 5 weeks later (5-week group). Participants were randomly assigned to the 1-day or 5-week group, so that both groups consisted of 35 participants. Within these two groups, 20 participants were assigned to the stress task and 15 to the control task in a random fashion. More participants were assigned to the stress groups to account for possible non-responders and to be able to perform within stress group correlation analyses. Figure 2.1 shows a schematic representation of the random assignment of the participants.

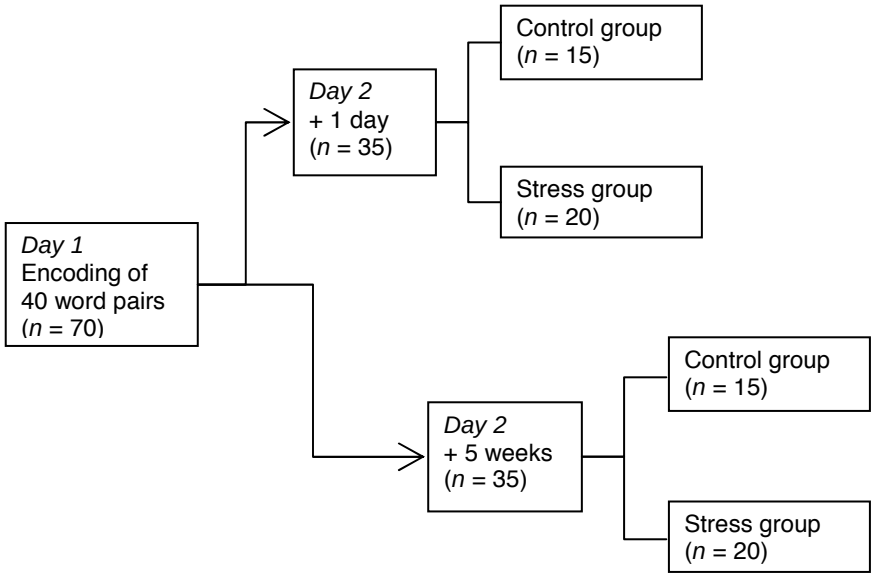

Figure 2.1. Randomization scheme of the groups.

Stress task

The Trier Social Stress Task (TSST) is a well established laboratory stress task that has been shown to consistently induce significant endocrine and cardiovascular responses in a large part of participants (Kirschbaum et al., 1993; see also Dickerson & Kemeny, 2004). The TSST consists of a short preparation period of 5 minutes, in which the participant is instructed to prepare for a 5-minute speech in front of an audience. Participants were told this audience consisted of a psychologist with 2 assistants, while in fact these were testing-assistants wearing white coats. Participants were told that the speech would mimic a job interview for a fictitious job in which they had to present themselves and convince the audience of their adequacy. In addition they were videotaped and voice-recorded and were told that the psychologists were trained to monitor nonverbal behavior. They were also told the speech would be critiqued on content and presentation style. Following preparation time, the audience entered the room and switched on the camera and microphone in view of the participant. Participants were instructed to stand in front of a table with the audience sitting at the other side, while the chairman led the interview. After the interview the chairman asked the participant to do a mental arithmetic task in which they had to serially subtract 13 from 1687. The audience responded to any mistakes by instructing participants to start over. This lasted for another 3 min before the

experimenter came into the room to perform physiological measures and to administer the first part of the memory word task, while sitting between the audiences who attentively watched the participant. After this task, the audience left the room. The control condition consisted of a reading period of 15 min, comparable to the timing of the TSST.

Memory task

For the present study an idiosyncratic word pair memory task was developed, which was tested in a pilot study for feasibility (n = 9). Idiosyncratic word pairs were used in order to increase emotionality and self relevance of the learning material, thereby increasing the generalization of the findings to autobiographical memories and to prevent a potential floor effect after 5 weeks.

On the first testing day participants were randomly given a list of 40 cue words, consisting of 20 neutral and 20 negative (emotion) words, similar in word length and frequency. Participants were asked to generate 2 associations to each word while having a clear image in mind of those associations (e.g. a participant named the words 'sport' and then 'water' in response to the cue word 'row'). After this was done for all words, the experimenter coupled the cue words with the second association words that participants had generated, forming word pairs (i.e. 'row' and 'water'). The cue word was coupled to the second word association to reduce mere implicit associative recall. The word pairs were read aloud twice. After the first repetition, recall was tested by asking the subject to name the coupled (second) word to each cue word. During the second repetition participants were asked to rate each word pair on two standardized, 9-point Likert scales on emotionality and valence from the Self-Assessment Manikin (SAM: Bradley & Lang, 1994). After this was done for all word pairs, recall was tested again.

On the second testing day, recall of word pairs was tested twice, once during (at t = 15) and once 20 minutes after the stress or control task (at t = 40) (see Figure 2.2). Cue words were randomly divided over the two trials, with the restraint that half would be neutral and half would be negative in valence, and that length and frequency of the two lists would be comparable. The cue words were read to the participant and they were asked to recall their second association to that word. Instructions were given to think back of the moment they associated these words. Recall performance was measured as the percentage of words remembered in relation to the number correctly recalled on the last recall trial of the learning day. This was done to account for possible between- and within-participant differences in initial learning on the two trials.

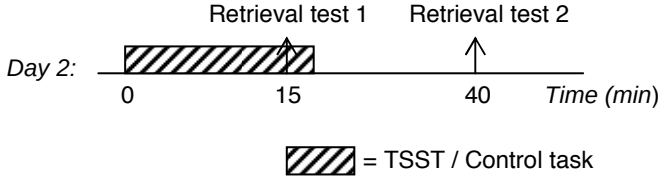

Figure 2.2. Scheme of the protocol on day 2 for all groups.

Stress measures

All physiological and subjective stress measures were taken at -10, 0, 15, 25, 40 and 60 minutes with reference to the stress task.

Cortisol assessment

Cortisol samples were obtained with Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Saliva samples were stored at -20 °C before assay. Biochemical analysis of free cortisol in saliva was performed with a competitive electrochemiluminescence immunoassay (Elecsys 2010: Roche Diagnostics, Laval, Quebec, Canada), as described elsewhere (van Aken et al., 2003).

Sympathetic activity

We used heart rate and blood pressure as measures of sympathetic activation. Heart rate was recorded continuously by an ambulatory monitoring system (Version 3.6: Vrije Universiteit Amsterdam), a small battery-powered device for ambulatory recording. It was measured with three Ag–AgCl disposable electrodes (ConMed, Utica, NY), placed just above the sternum, at the left side of the chest and at the bottom right side of the chest. For each participant, heart rate was averaged over a period of 2 min after markers given at each of the 6 assessment points. Systolic and diastolic blood pressure were measured from the non dominant arm with an automatic blood pressure monitor (Model Omron R5-I). Measures were taken after each saliva sampling.

Subjective measures

Tension, anxiety, insecurity, mood and tiredness were assessed on a visual analogue scale ranging from 0 to 100 mm during each saliva sampling.

Statistical Analyses

The effects of the stress task on both stress reactivity and memory were analyzed with repeated measure ANOVAs. Greenhouse-Geisser corrected p values were used when indicated by violated Sphericity, and follow-up analyses were done using two-tailed Holm-adjusted t-tests (Aickin & Gensler, 1996). To examine whether the levels of absolute cortisol increase were associated with impaired memory retrieval, follow-up analyses within the stress group were done using one-tailed Pearson's correlations. Analyses were performed with SPSS 14.0 (SPSS, Chicago, IL). The criterion for statistical significance was $p < 0.05$.

Results

Stress induction

Cortisol

Figure 2.3a shows mean (± SEM) free salivary cortisol (nmol/L) before, during, and after the stress or control task in the 2 stress groups and 2 control groups. Five participants (three from the 1-day stress group and two from the 5-week stress group)

had missing values of cortisol during the stress task, due to low saliva levels at that time, and 1 participant in the 1-day stress group missed the first baseline value, and were therefore left out of the next analyses. An ANOVA with repeated measures for mean cortisol levels showed significant increases in cortisol over time in the stress conditions compared to the control conditions (time x condition; $F(2, 128) = 32.9$, $p < .001$). There was no effect of retrieval period (1 day vs. 5 weeks) ($F(2, 128) = 1.63$, *ns*), nor an effect of the starting time of the experiment (at 11.30 am or 1.30 pm) ($F(2, 121) = 0.64$, *ns*).

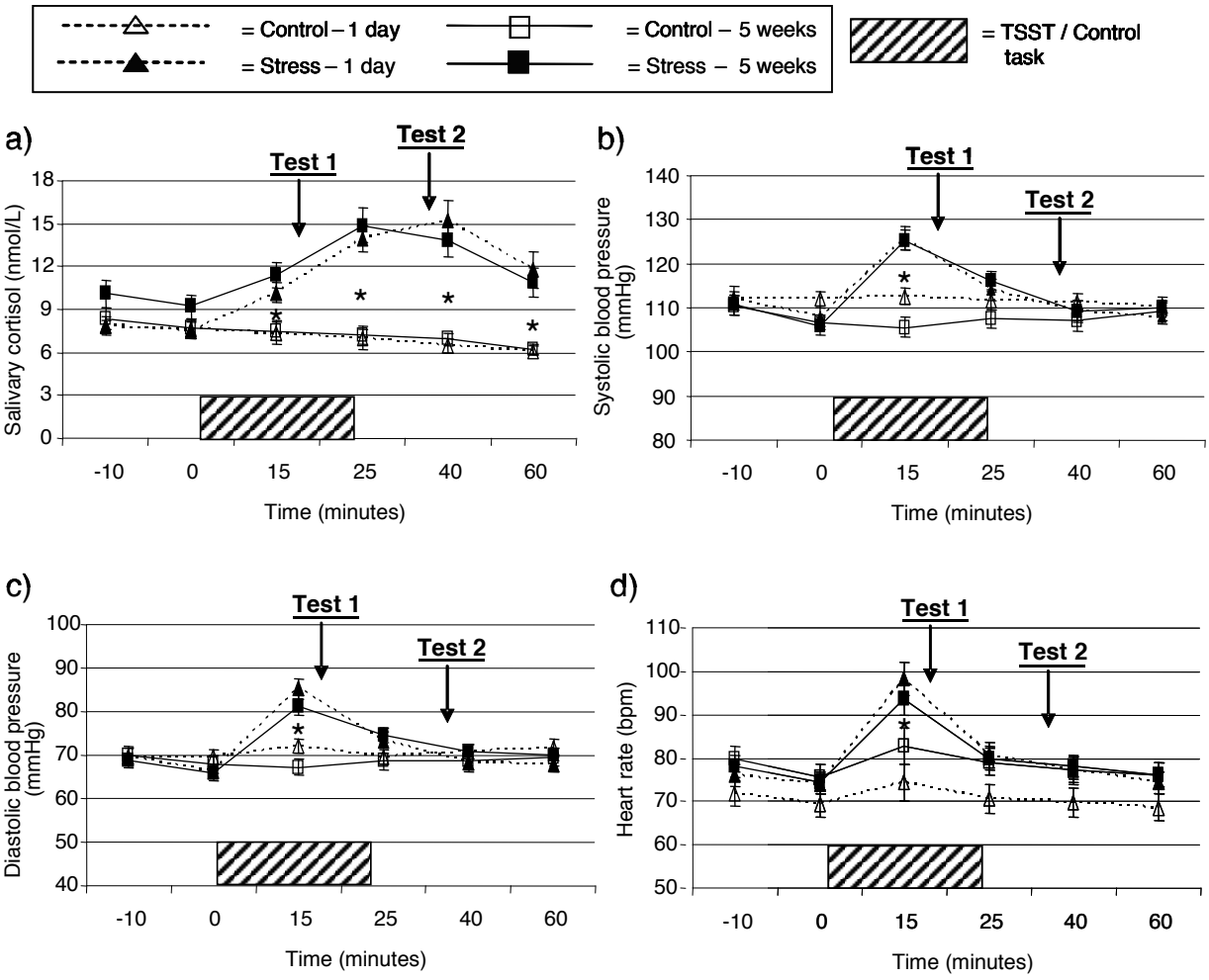

Figure 2.3. Mean physiological measures before, during, and after the stress or control task in the 2 stress groups and 2 control groups. a) Mean (± SEM) free salivary cortisol in nmol/L. b) Mean (± SEM) systolic blood pressure in mmHg. c) Mean (± SEM) diastolic blood pressure in mmHg. d) Mean (± SEM) heart rate in bpm.

Notes: Test 1 *= Retrieval testing during stress / control task;* Test 2 *= Retrieval testing after stress / control task;* * *= significant differences between control and stress conditions at p < 0.01.*

Post hoc (Holm-adjusted) paired sample t-tests within the stress conditions showed that cortisol levels were higher during the TSST (t = +15), at the time of the first retrieval task (10.81 ± 3.42 nmol/L) compared to baseline, right before the TSST (8.45 ± 3.24 nmol/L, $t(34) = 10.20$, $p < 0.001$), as well as after the TSST (t = +40), at the time of the second retrieval task (13.62 ± 5.02 nmol/L, $t(34) = 6.83$, $p < 0.001$). Consistent with diurnal rhythm, in the control conditions there was a significant decrease between cortisol levels right before (7.70 ± 2.37 nmol/L) and after the reading phase, at the time of the second retrieval task, (6.69 ± 1.79, $t(29) = 3.26$, $p < 0.01$), but not at the time of the first retrieval task (7.45 ± 2.72, $t(29) = 1.04$, *ns*).

Heart rate and Blood pressure

Figures 2.3b-d show respectively mean (± SEM) systolic blood pressure (mmHg), diastolic blood pressure (mmHg) and heart rate (bpm) before, during, and after the stress or control task in the 2 stress groups and 2 control groups. Repeated measure ANOVAs revealed significant condition by time interactions, due to increases in systolic blood pressure (SBP: $F(4, 276) = 36.46$, $p < 0.001$), diastolic blood pressure (DBP: $F(4, 267) = 39.21$, $p < 0.001$) and heart rate (HR: $F(2, 126) = 17.50$, $p < 0.001$) in the stress conditions compared to the control conditions. There was no effect of retrieval period (SBP: $F(4, 276) = 1.18$, *ns;* DBP: $F(4, 267) = 1.29$, *ns*; HR: $F(2, 126) = 0.97$, *ns*) or starting time of the experiment (SBP: $F(4, 253) = 0.98$, *ns;* DBP: $F(4, 242) = 1.30$, *ns*; HR: $F(2, 119) = 0.19$, *ns*).

Post hoc, Holm-adjusted, paired sample t-test within the stress conditions show that blood pressure and heart rate were higher during the TSST (t = +15), at the time of the first retrieval task compared to baseline, right before the TSST (SBP: $t(39) = 12.47$, $p < 0.01$; DBP: $t(39) = 10.87$, $p < 0.001$; HR: $t(39) = 9.69$, $p < 0.01$). After the stress task, at the time of the second retrieval task (t = +40), all three measures were significant lower than during the stress task (SBP: $t(39) = 14.73$, $p < 0.01$; DBP: $t(39) = 14.86$, $p < 0.01$; HR: $t(39) = 9.51$, $p < 0.01$), but were still slightly elevated compared to baseline (SBP: $t(39) = 2.04$, $p < 0.05$; DBP: $t(39) = 3.87$, $p < 0.01$; HR: $t(39) = 3.80$, $p < 0.01$). In the control conditions there were no changes in blood pressure from baseline over time. Heart rate was slightly elevated at the time of the first retrieval task ($t(29) = 2.81$. $p < 0.05$), but returned to baseline after the reading phase ($t(29) = 0.53$, *ns*).

Subjective stress measures

Participants tested under the stress condition showed a significant increase over time in tension, insecurity and anxiety compared to those tested under the control condition (all time by condition interactions in de repeated measure ANOVAs had p-values < 0.001, with no effect of retrieval period). Increases in these subjective stress measures during the stress task were still slightly elevated after the stress task (all paired samples t-tests; $p < .05$). Even though mood seemed to be decreased during the stress tasks, this effect was not significantly different from the control conditions, which was the same for tiredness (for both measures the interactions of time by condition; $p > 0.10$).

Memory task

Arousal and valence ratings

On day 1 (which was the same for all groups) word pairs were rated on level of arousal and valence. Both scales were rated on a 9 point scale ranging from 1 (low arousal) to 9 (high arousal), and 1 (very positive) to 9 (very negative). As expected, the word pairs we classified as negative were rated more negative in valence (6.6 ± 0.11) than the neutral word pairs (4.2 ± 0.07, $t(69) = 20.7$, $p < .001$) and elicited more arousal (4.6 ± 0.2) than the neutral words pairs (2.6 ± 0.16, $t(69) = 13.9$, $p < .001$). No group differences were found in the rating of the word pairs ($F(3, 66) = 0.32$, *ns*).

Memory retrieval

Data for all four groups on the retrieval task during and after the stress or control condition are shown in Figure 2.4.

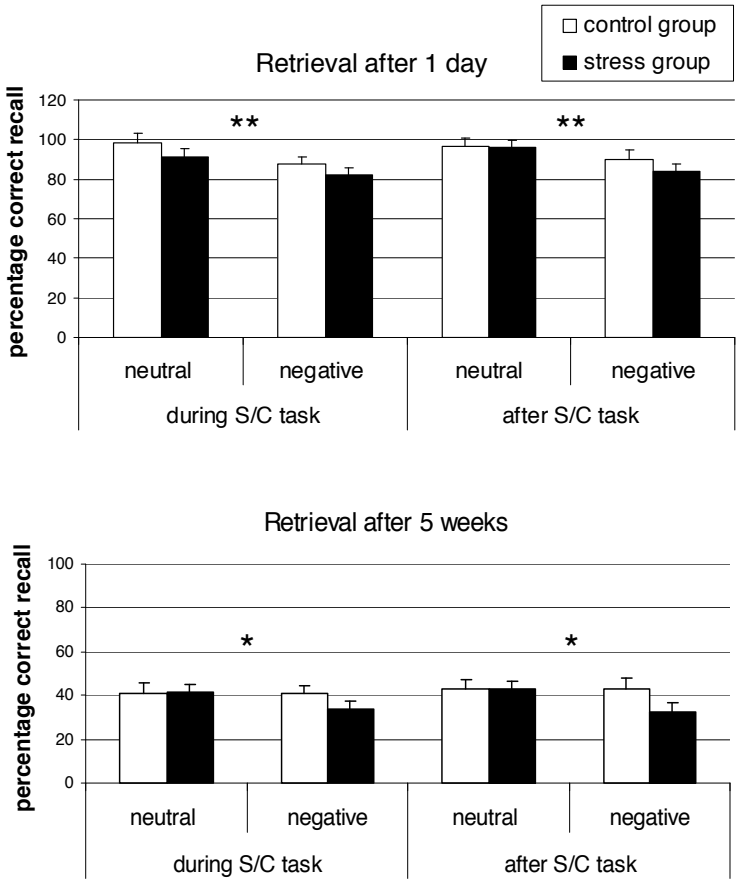

Figure 2.4. Performance (mean ± SEM) on the word pair retrieval task, both during and after the stress vs. control (S/C) task, in the groups with a retrieval period of 1 day vs. 5 weeks. Results are expressed as percentage recall of the last learning trial, for neutral and negative word pairs.

*Notes: ** = significant difference between neutral and negative words at p < 0.001; * = significant difference between neutral and negative words in the stress group at p < 0.01.*

In the groups with a retrieval period of 1 day, both the stress and the control condition retrieved significantly less negative words than neutral words ($F(1, 33) = 49.32$, $p < .001$). Overall, participants in the stress condition recalled fewer word pairs than the control condition, but this was only a trend ($F(1, 33) = 3.02$, $p = .09$). No interactions were found with valence or moment of testing (during vs. after the stress task). However, these results should be interpreted with caution, as more than 50% of the participants scored a 100% correct on the retrieval of neutral words and 87% or more correct on the retrieval of negative words. This indicates a ceiling effect after 1 day, and the variance in this data is most likely not enough for reliable statistical analyses.

In the groups with a retrieval period of 5 weeks, no main effect of condition on memory retrieval was found. Also, no main effect of effect of valence or moment was indicated, but the repeated measures ANOVA did show a trend for an interaction of condition by valence ($F(1, 33) = 3.23$, $p = 0.08$). Explorative follow-up analyses showed that within the stress condition, significantly fewer negative words were retrieved than neutral words ($F(1, 19) = 8.49$, $p < 0.01$), an effect not present in the control condition ($F(1, 14) = 0.00$, *ns*). Moreover, participants in the stress condition tended to recall fewer negative words compared to the control condition ($t(33) = 1.77$, $p = 0.09$), with no effect of moment of testing. Contrary to expectations, no effect of the stress condition was found on neutral words during the stress task.

Cortisol increase and retrieval performance

To investigate the relation between absolute cortisol increase and retrieval performance within the stress condition, one-tailed Pearson's correlations were calculated between absolute cortisol increases (test moments minus baseline level) and memory retrieval, both during and after the stress task (at $t = 15$ and $t = 40$). Because of the ceiling effect in the group with a retrieval period of 1 day, we analyzed these effects only in the 5 week stress group. In this group, two participants missed cortisol data at the moment during or after the stressor and were therefore removed from the analyses ($n = 18$).

Table 2.1 shows the results for the correlation analyses between retrieval performance and cortisol. Correlations to baseline cortisol levels and to increases in cortisol from baseline to $t = 15$ (during the stress task) and to $t = 40$ (after the stress task) are shown. During the stress task, cortisol increase (at $t = 15$) was significantly associated with impaired performance on the retrieval task ($r = -0.58$, $p < 0.01$). Correlations were significant for the retrieval of both neutral ($r = -0.48$, $p < 0.05$)[1] and negative words ($r = -0.45$, $p < 0.05$). No associations were found between cortisol increase during the stress task and performance afterwards (for neutral words: $r = 0.00$, *ns*; negative words: $r = -0.18$, *ns*).

[1] It may seem puzzling that even though there is no group effect of stress on the retrieval of neutral words, we do find a correlation between retrieval and cortisol. This can be explained by the fact that a small increase in cortisol may actually increase memory retrieval, as has been found before (inverted-U function relationship; Domes et al., 2005; Lupien & McEwen, 1997). We did not find a significant quadratic association between cortisol increase and retrieval ($F(2, 30) = 2.06$, *ns*), but when we perform a median split on the cortisol response, low cortisol responders do score higher on retrieval than controls ($M = 52.6$, *SD = 30* vs. $M = 41.1$, *SD = 17*) and high cortisol responders perform worst of all groups ($M = 28.8$, *SD = 14*).

Table 2.1. Pearson correlations between memory retrieval, baseline cortisol levels (t = 0) and increases in cortisol from baseline, during (t = 15) and after (t =40) the stress or control task (n = 18).

	Retrieval test 1 (t = 15)		Retrieval test 2 (t = 40)	
	neutral words	negative words	neutral words	negative words
Baseline cortisol (t = 0)	-0.17	-0.10	0.15	0.17
Increase in cortisol (t = 15 - 0)	-0.48*	-0.45*	0.00	-0.18
Increase in cortisol (t = 40 - 0)	-0.12	0.16	-0.05	-0.02

*Note: * = $p < 0.05$*

After the stress task, no significant associations were found between retrieval performance and cortisol increase (at t = 40) (for neutral words: r = -0.05, *ns*; negative words: r = -0.02, *ns*). No associations were found between cortisol increase after the stress task and performance during the stress task either (for neutral words: r = -0.12, *ns*; negative words: r = 0.16, *ns*). No significant associations were found between retrieval performance at any time point and absolute baseline cortisol levels.
We hypothesized that only when arousal is high, cortisol can impair memory retrieval. During the stress task sympathetic activity was indeed significantly elevated, and only then associations between cortisol increase and memory retrieval were found. However, not all subjects in the stress group responded with a similar heightened sympathetic arousal. Therefore we separately analyzed the correlation between cortisol increase and memory retrieval during the stress task, excluding subjects that responded with an increase in heart rate less than 10 bpm and an increase in systolic blood pressure less than 10 mmHg (n = 5). Including only subjects that responded with heightened arousal during the stress task (n = 13), correlations between cortisol increase and memory retrieval were even stronger (total: r = -.83, $p < 0.001$; for neutral words: r = -0.66, $p < 0.01$; for negative words: r = -0.76, $p = 0.001$).

Discussion

The present study examined the role of cortisol increases on long-term memory retrieval both during and after acute psychosocial stress. In the groups with a retrieval period of 5 weeks, the retrieval of negative, moderately arousing word pairs was affected compared to the retrieval of neutral words, both during and after acute stress. This is in line with previous research showing an impairing effect of psychosocial stress on the retrieval of emotional memory (Domes et al. 2004; Kuhlmann, Piel, et al., 2005), but not of neutral memory (Wolf et al. 2002). Interestingly, increase in cortisol was significantly associated with impaired memory retrieval only during and not after the stress task (the standard testing time for stress studies, when the audience of the TSST has exited). This effect was found for the retrieval of both negative and neutral words. Thus, even though no overall effect of stress was found on the retrieval

of neutral words during the stress task, within the stress group a significant association between increase in cortisol and impaired retrieval of neutral words was revealed. Apparently, only during acute stress, at a time of heightened sympathetic and subjective arousal, and in the presence of an audience, cortisol increases are associated with impaired retrieval of neutral and emotionally arousing material learned 5 weeks before. From the results in the groups with a retrieval period of 1 day, little can be concluded due to a ceiling effect leading to only slight variance in performance for appropriate statistical testing. Overall, there seemed to be a negative effect of stress on memory retrieval of material learned 1 day before, but no different effects for neutral and negative word pairs or for moment of testing could be discerned.

Several explanations can be put forward for the specific relation between cortisol and memory during, but not after the TSST (after 5 weeks). First of all, these findings are consistent with findings in animal studies that adrenergic arousal is needed for cortisol effects to occur on memory retrieval (Roozendaal, et al., 2003; Roozendaal, de Quervain, et al., 2004; Roozendaal, Hahn, et al., 2004). Moreover, these findings are also in line with the study of Kuhlmann and Wolf (2006b), who found indications in humans that an arousing environment is necessary for the impairing effects of exogenous cortisol on memory retrieval to occur. Indeed, in our study cortisol increases no longer influenced memory retrieval after the stress task, when the social evaluative stressor was gone. On group level, however, retrieval of emotional words 5 weeks after learning was still affected *after* the stress task. At that time in the stress group, sympathetic and subjective arousal due to the stress task was again comparable to the control group, but not completely back to baseline. It is possible that in combination with noradrenergic activation in the amygdala elicited by the retrieval of the emotionally arousing words, cortisol may still have had an impairing effect on retrieval performance. This would be consistent with and increasing number of studies showing that the amygdala is activated during emotional memory retrieval (Dolcos et al., 2005; Sharot et al., 2004; Smith et al., 2004; Sterpenich et al. 2006). No correlations were found, however, between retrieval of emotional words and cortisol increases after the stress task. Altogether, the effects of cortisol on memory retrieval in interaction with adrenergic activation in the amygdala, due to either emotionally arousing material or environmentally evoked arousal, should be further explored. An interesting approach would be to block the adrenergic system while testing memory retrieval under high cortisol levels [as was recently done by de Quervain et al., 2007]. This would be even more informative in combination with functional MRI. Functional MRI studies could also shed more light on which brain areas are specifically involved in long-term memory retrieval (for a discussion see: Moscovitch et al., 2006).

There is another possible explanation, however, for the finding of impaired retrieval in relation to cortisol increases during stress. During the stress task, participants perform the retrieval task in the presence of an audience. Performing a memory task while being socially evaluated asks of the participant to inhibit the processing of environmental cues and to focus on the memory task. Animal data has shown that whereas cortisol may facilitate the encoding of relevant stimuli (i.e. the stressful context), it may at the same time impair cognitive functions unrelated to the

stressor (i.e. the memory task) (see de Kloet et al., 1999). The amount to which a subject is able to inhibit thoughts and feelings related to the audience could depend on cortisol levels, therewith indirectly affecting memory retrieval. However, on group level there was no performance difference during and after the stress task, so it is questionable whether this is happening. To evaluate this hypothesis, measures of distraction and/or memory for the psychosocial task itself should be taken into account in future research using comparable designs.

One should also keep in mind that performance was tested at two moments, with the condition of high cortisol and high arousal always before the condition with high cortisol and low arousal. Time effects could thus have played a role, for example, fast cortisol responders might differ in their cognitive functioning from late cortisol responders. However, increase in cortisol during the stress task did not correlate to performance after the stress task or vice versa, so cortisol reactivity of participants can not explain their overall performance.

Although a large sample of 70 participants was recruited for the present study, when divided over treatment and retrieval period, the groups were rather small. Despite this, our results do confirm our expectations and could therefore be considered as evidence for impairing effects of stress induced cortisol elevations on long-term memory during stress. For stronger conclusions, additional research is necessary in larger samples. Further, earlier studies have found differences in the effects of stress on memory between men and women (Wolf, Schommer et al., 2001). Since only males were included in the present study, it is still to be investigated whether similar findings will be found in a female population.

The new paradigm we developed for idiosyncratic word pair generating proved effective as a sensitive memory task after 5 weeks. Delayed recall rates might have been a problem in the study of Wolf et al. (2002), where a floor effect could potentially have explained the non-results. However, the effectiveness on long-term memory retrieval in our study was at the cost of a low sensitivity of this task after 1 day, where we ran into a ceiling effect. Since idiosyncratic words were used, this task is more autobiographic than a standard word pair task and it makes a clear distinction in the valence and arousal ratings of the negative and neutral word pairs. We intended to measure episodic memory with the task, but subjects might possibly have been guessing on parts of the memory task if they did not directly recall their second association. This might have led to a more semantic type of memory testing which we did not control for but should be done with future use of the task. It is also interesting to note that this task shows opposite results of what is usually found in retrieval of neutral and emotional material. Usually emotionally arousing material is remembered better than neutral material (Cahill, 1999), which might be due to amygdala-related arousal effects on consolidation (van Stegeren, Goekoop et al., 2005; Strange & Dolan, 2004), while in our study recall of emotional words was lower than recall of neutral words. This might be related to a higher semantic cohesion in emotion words (Buchanan, Etzel et al., 2006; Dillon et al., 2006), making the emotional word pairs more difficult to keep apart.

To summarize, while exogenously induced cortisol levels seem to be able to impair memory retrieval as long as testing is performed in a formal research setting (Kuhlmann & Wolf, 2006b), stress induced cortisol levels may only have an

impairing effect on emotional memory retrieval or on memories retrieved during acute stress, with heightened sympathetic activation or a distracting evaluative component. Since psychological stress is a common real life condition, the effects of cortisol on memory retrieval may have implications in different fields. The results of our study suggest for a way to pharmacologically treat stress related memory problems like blackouts during stressful situations as exams or job interviews. If stress induced cortisol is blocking memory only when adrenergic arousal is high, administration of beta-adrenergic blockers like propranolol before a stressful experience may be able to reduce the impairing effects of cortisol, while leaving cortisol levels intact, which might be of importance for cognitive functioning (Lupien & McEwen, 1997). Whereas beta-blockers are already frequently in use by people with anxiety problems, the independent effects on memory retrieval have never been thoroughly studied before (Chamberlain et al., 2006). Another area of interest is the field of psychiatric disorders like post traumatic stress disorder (PTSD) and depression. Patients troubled with these disorders show disturbed patterns of basal cortisol levels or cortisol reactivity (Burke et al., 2005; Nemeroff & Vale, 2005; Raison & Miller, 2003; Yehuda, 2001) and also show problems in cognitive functions related to memory (Barnhofer et al., 2005; Elzinga & Bremner, 2002; McNally, 1998; Raes et al., 2006). If increases in cortisol can block the retrieval of emotionally arousing memories, administration of cortisol might be a useful treatment in patients that are bothered by involuntary recall of (emotional) memories. Recent pharmacological studies involving the administration of exogenous cortisol to PTSD patients (Aerni et al., 2004) and phobic patients (Soravia et al., 2006) have shown promising results.

In conclusion, the present study is the first to measure sympathetic activity in combination with cortisol elevations while studying memory retrieval during and after acute stress. It confirmed our hypothesis that acute stress and/or emotional arousal is necessary for endogenous cortisol effects on memory retrieval to occur, and is consistent with the study by Kuhlmann and Wolf (2006b) using exogenous cortisol elevations, and animal studies measuring and manipulating the adrenergic activity of the amygdala under cortisol administration (Roozendaal, de Quervain, et al., 2004; Roozendaal, Hahn, et al., 2004). The effects of stress hormones on memory in patient studies should shed more light on the use of pharmacologic stress hormones either in blocking unwanted memories or blocking the impairing effects of elevated stress hormones on memory retrieval. Furthermore, long-term outcomes of retrieving memories under stress hormones are still unknown and should therefore also be subject of future investigations.

Chapter 3

Long-term outcomes of memory retrieval under stress

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Summary

Previous studies have found impairing effects of stress hormones on memory retrieval. So far, it is unknown whether these impairments are temporary, persistent throughout time, or whether the strength of the memory trace changes after retrieval because of the effects of stress hormones on memory processes during retrieval. In the present study, delayed cued recall (6 months after initial learning) was compared between male participants who had retrieved previously learned word pairs during stress or a control condition. Retrieval (with / without stress) had taken place either 1 day or 5 weeks after initial encoding. The group that had retrieved words under stress 5 weeks after encoding performed worse on long-term recall than the comparable control group. However, when words were retrieved under stress 1 day after encoding, no long-term effect was found, although performance at 6 months with relation to performance under stress was slightly increased compared to the control group. These results support previous findings in animals that stress may affect memory during reactivation. It further suggests that time intervals between encoding and reactivation may play an important role.

Introduction

Previous studies have found impairing effects of (stress related) elevated cortisol levels on memory retrieval in humans (de Quervain, Roozendaal et al., 2000; Kuhlmann, Piel et al., 2005; for a review see: Het et al., 2005). This impairing effect is mainly found for emotionally arousing memories or under arousing conditions (Buchanan, Tranel et al., 2006; Kuhlmann, Kirschbaum et al., 2005; Kuhlmann & Wolf, 2006b; Tollenaar et al, 2008a / Chapter 2). Two studies suggest that the effects of cortisol may be mediated by reduced medial temporal lobe (MTL) activation during retrieval (de Quervain, et al., 2003; Oei, et al., 2007). The impairing effects of cortisol on memory retrieval contrast with the enhancing effects of cortisol on memory consolidation (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill, et al., 2003; Kuhlmann & Wolf, 2006a).

The long-term consequences of memory impairments due to cortisol have never been studied before in humans. Therefore, it is unknown whether these impairments during retrieval are temporary or may lead to permanent changes in the memory trace. Longer lasting changes might be related to diminished rehearsal and hence re-encoding under the influence of cortisol, thereby weakening the strength of the memory traces that have not been retrieved. Another possibility is that memory traces are affected by stress during reactivation. Animal studies have shown that certain drugs can affect memory (i.e., a conditioned fear response in most studies) even after its reactivation. Previously consolidated memories seem to become labile again during reactivation and hence susceptible to impairment or facilitation for a distinct time period, a process often referred to as reconsolidation (e.g. Debiec et al., 2006; Nader et al., 2000; Przybyslawski & Sara, 1997). A study by Tronel and Alberini (2007) has recently shown that reconsolidation might de dependent on the glucocorticoid system, as they found that a glucocorticoid receptor antagonist can disrupt conditioned fear in rats after reactivation of an inhibitory avoidance memory. In line with that, Maroun and Akirav (2007) have found an impairing effect of stress on reconsolidation in rats, which was reversed by a glucocorticoid receptor antagonist. Both increases and decreases in basal cortisol levels thus seem to affect the process of reconsolidation. In the last years, several studies have shown that human procedural and declarative memories become labile after reactivation too (Forgato et al., 2007; Gallucio 2005; Hupbach et al., 2007; Walker et al., 2003). It is thus possible that increases in cortisol levels during reactivation of declarative memories might affect reconsolidation, and hence long-term recall, in humans.

If the strength of memories that have been encoded and consolidated long before can be influenced by stress hormones, this might have important clinical implications. The treatment of psychiatric disorders that are related to memory, like post-traumatic stress disorder (PTSD), could potentially be aided by drugs that can influence the strength of (traumatic) memories. Some clinical studies are consistent with the idea that stress hormones can affect the strength of traumatic memories after consolidation has taken place (Aerni et al., 2004; Weis et al., 2006). For example, in the study by Aerni et al., chronic PTSD patients who suffered from emotional flashbacks and nightmares were administered a low dose of cortisol for a month. All three patients in the study showed reduced symptoms of re-experiencing and intensity

of the traumatic memories compared to placebo. The authors argue that this effect could be due to the inhibiting effect of cortisol on excessive retrieval of traumatic memories. However, memory for the traumatic events itself was not explicitly assessed. Furthermore, in the study by Weis et al., memories for experienced traumatic events were not reduced after cortisol administration, despite reduced chronic stress symptoms in subjects. Hence, it remains to be investigated whether a reduction in PTSD symptoms is indeed mediated by a blocking effect of cortisol on emotional memory. Even though traumatic memories as present in PTSD patients don't compare with the (relatively mild) emotional stimuli used in many laboratory studies, understanding the basic mechanisms through which cortisol can affect the strength of emotional memories will be very helpful in directing further research on improving the treatment of these disorders.

To our knowledge, no study has yet reported whether increases in cortisol levels during reactivation of emotional memories have long-term effects on human memory. The present paper describes the 6 month follow-up to a study in which the effects of psychosocial stress exposure, and subsequent endogenous cortisol increases, on retrieval of previously encoded material was examined (see Tollenaar et al., 2008a / Chapter 2). Performance at follow-up was related both to initial encoding and to retrieval performance during stress to test whether impairments that were found on memory retrieval were not only temporary or permanent, but were potentially even further increased.

Methods

Participants

In the original study, which was approved by the ethics committee of the Leiden University Medical Center (LUMC), all 70 participants had agreed they could be contacted again for future research. For the follow-up, all participants were contacted again by the experimenter (M.S.T.) via a surprise telephone interview. In this interview, participants were first asked if they were willing to be questioned for 10 min. All subjects who were reached agreed. Sixty-five out of the 70 male students who had participated in the original study were included in the follow-up (five students could not be reached by phone or email). Thirty-one of them had the second session 1 day after initial learning (of whom 15 were in the control and 16 in the stress condition, missing four people in the stress condition) and 34 had the second session 5 weeks after learning (of whom 14 were in the control and 20 in the stress condition, missing one person in the control condition) (see Figure 3.1 for an overview of the test sessions). All participants were free of any medications and physical or psychological problems at the time of encoding and all sessions had taken place after 11.30 am to ensure low baseline cortisol levels in all participants.

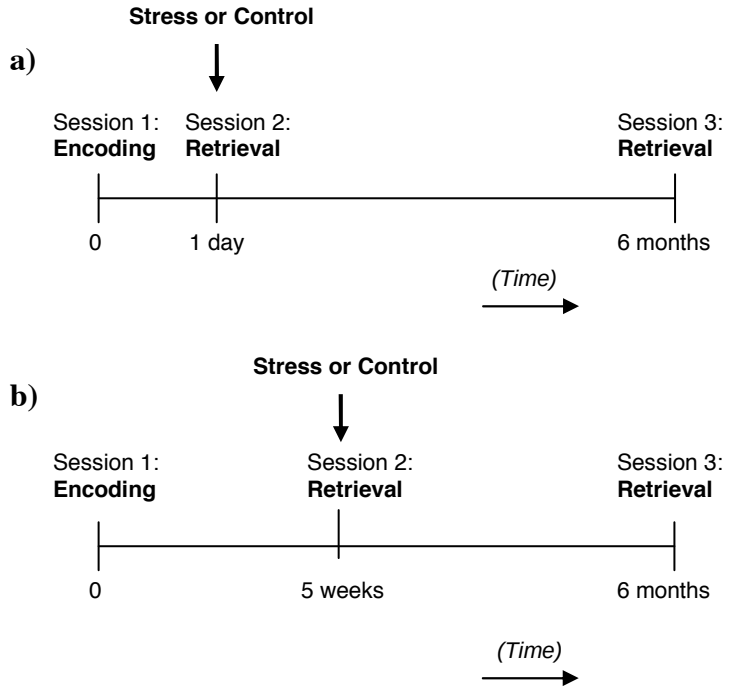

Figure 3.1. An overview of the testing sessions and retrieval tasks is presented (a) in the groups for whom the second session took place 1 day after encoding and (b) in the groups for whom the second session took place 5 weeks after encoding. On the first session, neutral and negative word pairs were encoded. On the second session, participants were exposed to either a stress or a control task after which retrieval of the word pairs was tested with a cued recall task. On the third session, retrieval was tested again with the cued recall task.

Memory task

A cued recall task was used, in which participants had to recall words that were coupled with cue words on the first day of the study, 6 months before the follow-up telephone interview. These words were the second, personal, associates to the cue words. On the first testing day participants were randomly given a list of 40 cue words, consisting of 20 neutral (e.g. "row") and 20 negative (emotion) words (e.g. "cry"), similar in word length and frequency. Participants were asked to generate 2 associations to each word while having a clear image in mind of those associations (e.g. a participant named the words "sport" and then "water" in response to the cue word "row"). After this was done for all words, the experimenter coupled the cue words with the second association words that participants had generated, forming word pairs (i.e. "row" and "water"). The cue word was coupled to the second word association to reduce mere implicit associative recall. The word pairs were read aloud twice and recalled twice to complete initial learning. There was no mention that recall would be tested again on the subsequent session. Either 1 day or 5 weeks after initial encoding of the memorized material, cued recall was tested, as well as 6 months later. During the second session, retrieval was tested under either a stress or a control condition. No feedback was given on any of the retrieval occasions.

Psychological stress protocol

Psychosocial stress was induced using the Trier Social Stress Task, which is well known for inducing hypothalamic-pituitary-adrenal (HPA) axis responses, and hence cortisol increases (Kirschbaum et al., 1993). Cortisol was significantly elevated in the stress groups compared to the control groups after the start of the stress task (interaction of group by time; $F(2, 118) = 27.8$, $p < .001$). The cortisol responses between the groups that came back after 1 day or 5 weeks did not differ ($F(2, 54) = 1.76$, $p = .19$). The average increase from baseline ($M = 8.37$, $SE = 0.59$) until 25 min after onset of the stressor ($M = 13.86$, $SE = 0.98$) was 79 % (see Figure 3.2).

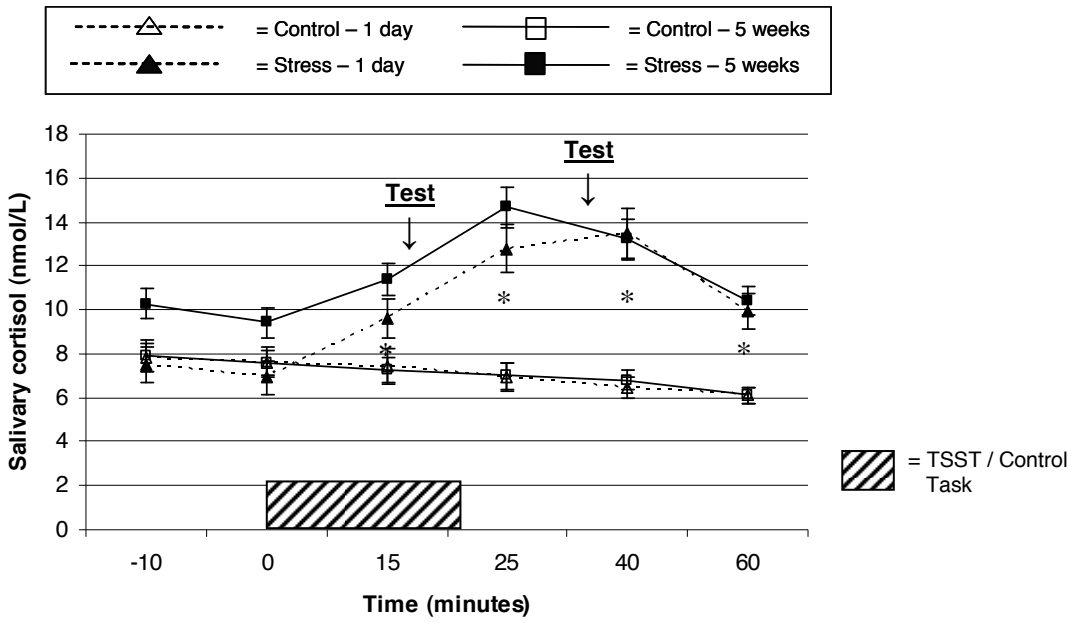

Figure 3.2. Mean (± SEM) free salivary cortisol (nmol/L) before, during, and after the stress or control task in the 2 stress groups and 2 control groups on the second session.

Notes: Test = *Retrieval testing during and after stress or control task; * = significant differences between control and stress conditions at p < 0.01.*

Statistical analyses

Delayed recall at 6 months follow-up (Session 3) was calculated as the percentage correct recall with respect to initial encoding (Session 1) and secondly with respect to retrieval performance 1 day or 5 weeks after encoding (Session 2). Valence of the words (neutral or negative) was treated as a within-subject variable and condition (stress or control) and the time intervals between the first and the second session (1 day or 5 weeks) were treated as between-subject variables in repeated measures ANOVA. In the original study a difference was made between memory retrieval tested *during* the actual stress task and memory retrieval tested *after* the stress task (when cortisol levels were still high), and we therefore added the factor "moment" as another within-subject variable in the above described analyses. Preliminary analyses revealed, however, that the moment factor did not have any significant main or interaction effects in the repeated measures ANOVAs (all *p*s > 0.05) and therefore all

data was collapsed on this factor and further analyses were performed in its absence. Areas under the curve (AUC) were calculated for the increases in cortisol between baseline and the end of the second session, and correlated to memory performance at 6 months in the two groups that received the stress task using Pearson's correlations. Analyses were performed with SPSS 14.0 (SPSS, Chicago, IL). The criterion for statistical significance was $p < 0.05$.

Results

Retrieval performance under stress versus control (on the second session) is described in detail in Tollenaar et al. (2008a) / Chapter 2. In short, retrieval performance 1 day after encoding was only slightly impaired by stress, on both neutral and negative memory. Retrieval performance after 5 weeks was affected by stress, but this effect was only seen in the recall of negative words. There were no effects of moment of testing (during or after the stress task) in the analyses in both groups.

Figure 3.3 shows memory performance at 6 months after encoding, with respect to initial learning, in the groups with the second session after 1 day and after 5 weeks (respectively, Figures 3.3a and b). The repeated measures ANOVA showed no significant main effects of condition ($F(1, 61) = 0.824$, $p = 0.37$) or time interval ($F(1, 61) = 3.09$, $p = 0.08$), but there was a significant condition by time interval interaction ($F(1, 61) = 6.57$, $p < 0.05$).

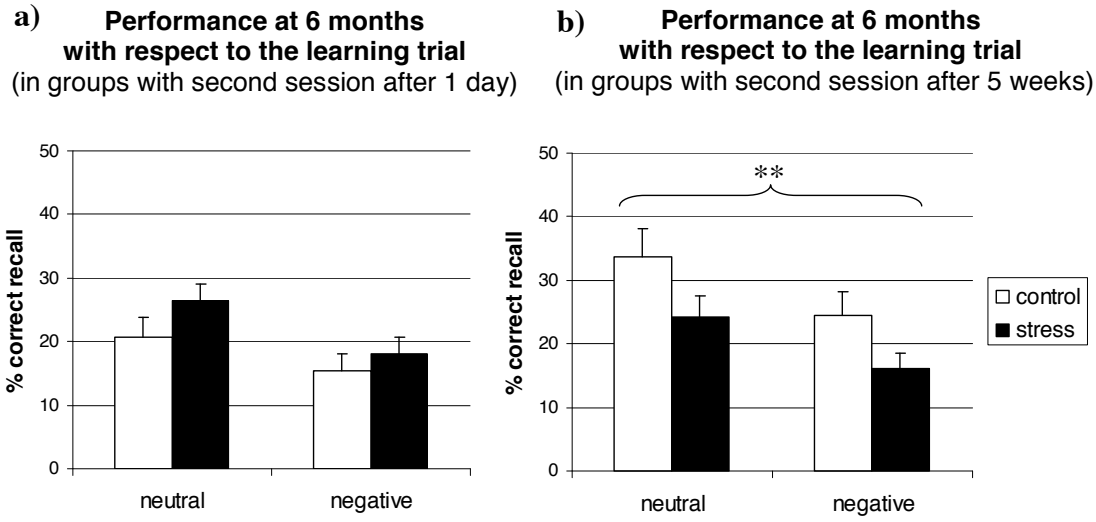

Figure 3.3. Recall of neutral and negative words at 6 months (Session 3), as a percentage of the last learning trial on the encoding day (Session 1) is presented (a) in the groups for whom the second session took place 1 day after encoding and (b) in the groups for whom the second session took place 5 weeks after encoding (for a description of the design, see Figure 3.1).

*Note: ** = significant difference between the stress and control group at p < 0.05.*

Post hoc analyses showed that the group that had recalled words under stress 5 weeks after learning retrieved significantly less of the words at 6 months than the comparable control group ($F(1, 32 = 4.82$, $p < 0.05$) (see Figure 3.3b), whereas the group that retrieved the words under stress 1 day after initial learning did not differ on 6 month recall from the comparable control group ($F(1, 29) = 1.96$, $p = 0.17$) (see Figure 3.3a). In addition, the control group that had its second session after 1 day performed worse at 6 months follow up than the control group that had its second session after 5 weeks ($F(1, 27) = 7.42$, $p < 0.05$), whereas the stress groups did not differ ($F(1, 34) = 041$, $p = 0.53$). Further, there was a main effect of valence ($F(1, 61) = 18.65$, $p < 0.01$), with more neutral words correctly recalled than negative words, but no interaction between condition and valence was found ($F(1, 61) = 0.08$, $p = 0.78$).

To investigate whether a *further* decline had occurred in memory performance after retrieval under stress, performance at 6 months was compared between the stress and control groups with respect to the second session, that is, performance at 6 months was calculated as a percentage of retrieval performance on session 2 (see Figure 3.4).

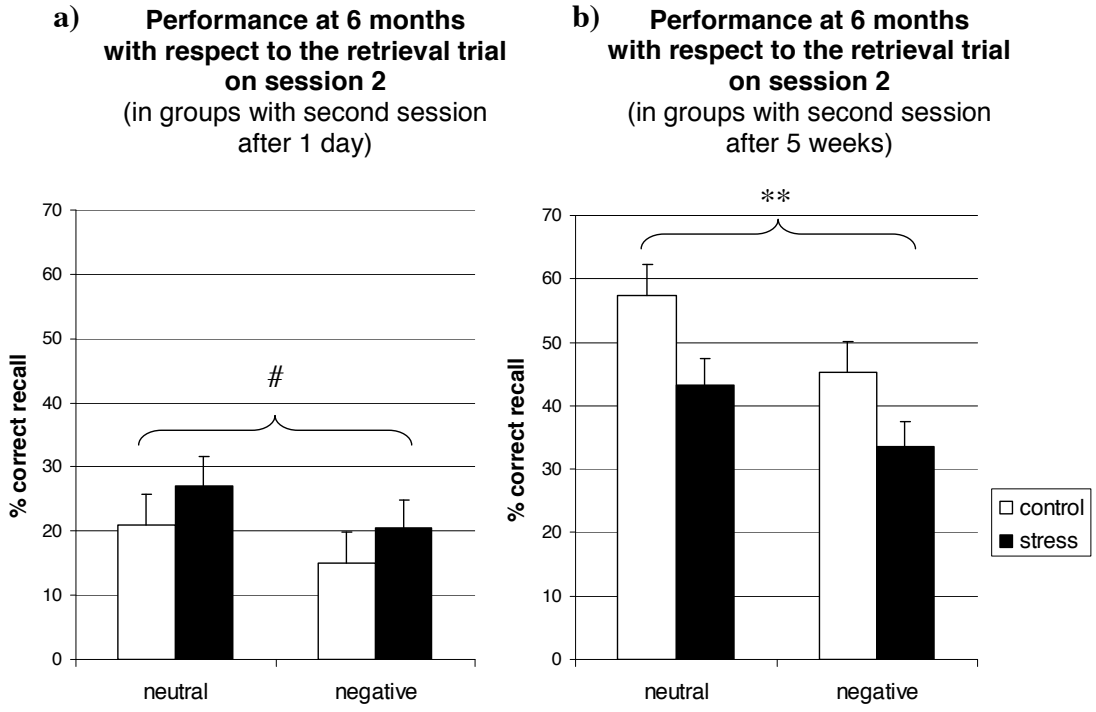

Figure 3.4. Recall of neutral and negative words at 6 months (session 3), as a percentage of the recall trial on the second session is presented (a) in the groups for whom the second session took place 1 day after encoding and (b) in the groups for whom the second session took place 5 weeks after encoding (for a description of the design, see Figure 3.1).

*Notes: The difference in recall percentages between Figure 3.4a and 3.4b is due to the differences in recall on session 2. ** = significant difference between the stress and control group at p < 0.05; # = difference between the stress and control group at p < 0.10.*

The repeated measures ANOVA showed no significant main effect of condition ($F(1, 61) = 1.10$, $p = 0.30$), but there was a significant main effect of time interval ($F(1, 61) = 46.75$, $p < 0.01$) and a significant condition by time interval interaction ($F(1, 61) = 7.07$, $p = 0.01$). Post hoc analyses showed that with the second session after 5 weeks, at 6 months the stress group also remembered significantly fewer words from the second session than the control group ($F(1, 32) = 4.47$, $p < 0.05$), see Figure 3.4b.

However, with the second session after 1 day, at 6 months the stress group tended to remember slightly more words from the second session than the control group ($F(1, 29) = 3.58$, $p = 0.07$), see Figure 3.4a. Since recall performance was calculated as a percentage of the second session, the main effect of time interval could not be interpreted clearly. That is, recall performance on the second session already differed significantly between the groups that came back after 1 day and after 5 weeks ($F(1, 66) = 328.68$, $p < 0.01$). Recall after 5 weeks was lower than recall after 1 day, leading to differences in recall performance at 6 months related to these baseline differences. Again, there was a main effect of valence ($F(1, 61) = 8.85$, $p < 0.01$), with more neutral words correctly recalled than negative words, but no interaction between condition and valence was found ($F(1, 61) = 0.02$, $p = 0.89$).

No significant correlations were found between total cortisol increase during the second session (with the stress task), and neutral or negative memory retrieval at 6 months, although correlations followed the trend with respect to group differences and even tended to be significant for the retrieval of negative words in the group with the second session after 1 day (1 day interval, neutral: $r = 0.28$, $p = 0.34$, negative: $r = 0.48$, $p = 0.08$; 5 week interval, neutral: $r = -0.06$, $p = 0.82$, negative: $r = -0.11$ $p = 0.65$). When performance at 6 months was related to performance on the second session, no significant correlations were found with cortisol increase either (1 day interval, neutral: $r = 0.24$, $p = 0.40$, negative: $r = 0.42$, $p = 0.14$; 5 week interval, neutral: $r = 0.02$, $p = 0.95$, negative: $r = -0.14$ $p = 0.58$).

Discussion

The present study found impairments in memory retrieval up to 6 months after initial encoding, when memories were recalled under stress 5 weeks after encoding. This effect was found for the retrieval of both neutral and negative words. Moreover, memory performance at 6 months was even further impaired with respect to performance under stress. These results thus show that retrieval during stress exposure does affect long-term memory.

The long-term memory impairments after retrieval under stress might partly be due to the fact that participants in the stress group retrieved less words under stress than the control group, leading to differences in amount of rehearsal and hence to differences in re-encoding of the learned material. However, besides impairments in the retrieval of emotional words (which was already present 5 weeks after learning under the influence of stress), after 6 months a further decrease was found in the retrieval of emotional words with respect to retrieval under stress compared to the control group, as well as a decrease in the retrieval of neutral words (which was not present 5 weeks after learning under the influence of stress). Taken together, this

suggests that stress affected memory also during or after reactivation and that more processes are involved than reduced rehearsal. These results are in line with the study by Maroun and Akirav (2007) who found an impairing effect of stress on reconsolidation in rats. A third process that may have been involved in the long-term memory impairments is enhanced extinction (Suzuki et al., 2004; Tronson & Taylor, 2007). Animal research has shown that cortisol can enhance extinction of learned associations after reactivation of the memory trace (Abrari et al., 2008; Cai et al., 2006; Yang et al., 2005). However, the word-pair learning paradigm that was used in the present study does not bear strong resemblance with fear conditioning or extinction, and hence extinction does not seem a very likely explanation for the present findings. Which processes are involved in the present findings can not be concluded from the current design.

No long-term impairing effects on retrieval were found for word pairs that were recalled under stress 1 day after encoding. In contrast, even a slight, borderline significant increase in memory was found for words retrieved under stress 1 day after encoding, associated with a moderate positive correlation between cortisol increase during the stress task and the retrieval of negative words at 6 months. The interval between encoding and reactivation thus seems to play a mediating role in the long-term outcomes of retrieval under stress. However, caution should be taken in interpreting differences between the 1 day and 5 week groups, because stress did not have an equally impairing effect on memory 1 day after learning as it did on memory 5 weeks after learning, potentially leading to the long-term differences. In addition, 1 day after learning, consolidation processes might still have played an important role. As cortisol has been found to increase memory consolidation in humans in some studies (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill et al., 2003; Kuhlmann & Wolf, 2006a) this may also partly explain the slightly enhanced long-term recall in the stress condition.

The effects of stress on long-term memory, when subjects were exposed to stress during retrieval 5 weeks after learning, are interesting in the light of two studies in rodents, showing that only recent memories can undergo reconsolidation at the time of memory reactivation (Milekic & Alberini, 2002; Suzuki et al. 2004). Our results suggest that in humans a greater time window for affecting consolidated memories may exist, even though the mechanisms through which these effects are mediated are still unclear. This is promising for clinical practice that would benefit from a long time span to affect well consolidated emotional memories. Furthermore, while Cai et al. (2006) only found effects of cortisol on long-term memory in mice when cortisol was administered during multiple retrieval trials, we found a long-term effect with only a single retrieval trial during stress.

Interestingly, the long-term effects of stress seemed to be equal for both neutral and negative memory. This is in contrast with studies showing that stress affects memory consolidation and retrieval mostly of emotional information (Cahill, et al., 2003; Kuhlmann, Piel, et al., 2005), but an animal study investigating the effects of a beta-blocker on reconsolidation also showed effects on both emotional and non-emotional material (Przybyslawski et al., 1999). It is to be noted that in this study, recall of negative material is lower than recall of neutral material. This contradicts common findings of enhanced recall of emotional material (Cahill, 1999),

and might be explained by the task that was used in this study (i.e., cued recall of negative versus neutral word pairs). Words associated with negative cue words may have been more difficult to keep apart because of a higher semantic cohesion between emotional words (Buchanan, Etzel et al., 2006; Dillon et al., 2006).

With regard to cortisol, we did not find clear results. Although the correlations between cortisol increase during stress and memory performance after 6 months did follow expected trends based on the group differences, the correlations themselves were not very strong (as the group sizes were rather small, power might be an issue). Future research will likely benefit from a greater focus on specific stress hormones through exogenous stress hormone administration in humans. Besides cortisol, (nor)adrenaline may play an important role in reconsolidation as well, as was found in animal studies (Debiec & Ledoux, 2004; Diergaarde et al., 2006; Przybyslawski et al., 1999) and in preliminary experimental studies in humans (e.g. Miller et al., 2004).

Another remarkable result from the present study is the fact that the control group that had its second session after 5 weeks performed significantly better on 6 month recall than the control group that had its second session after 1 day. An explanation could be that the time between the second and third session is shorter in the first group (21 vs. 26 weeks), but it is debatable whether this will have an impact on such a long time span. Another explanation could be that this group benefited from spaced learning (see Greene, 1989), with a longer time span of 5 weeks between the learning and first retrieval session compared to the group with only 1 day between these sessions. Interestingly, stress seems to have abolished the positive effect of delayed retrieval on long-term memory observed in the 5 week group.

Some limitations to the present study should be noted. In the present study, even though cortisol was still increased after the retrieval tasks, the effect of stress on memory retrieval confounds with the effects that stress hormones have *after* retrieval. Therefore, as was discussed above, we can not tell exactly whether the long-term memory effects are due to reconsolidation (or possibly extinction) mechanisms besides a rehearsal effect. To study whether stress hormones can affect memory *after* reactivation, treatments (or stress exposure) should be administered after the act of retrieval itself and should also be compared to a group that receives stress without retrieval to test whether reactivation of memory traces is necessary for the effects of stress hormones on long-term memory. Furthermore, since the group that retrieved words after 1 day responded differently to stress than the group that retrieved words after 5 weeks, comparison between these groups on the long-term is wary. Giving stress *after* memory retrieval could sort out these differences as well. In addition, even if effects of stress hormones are found on memory after reactivation, it still needs to be investigated whether these reconsolidation processes differ from consolidation processes (Walker et al., 2003).

To our knowledge, these results are the first to show that memory retrieval under stress has long-term effects on both neutral and emotional memory in humans. Further research is needed to elucidate the specific stress hormones and cognitive mechanisms that are involved in this process, as well as the specific time windows to affect memory. To this end, future research could benefit from adapting research designs from animal studies on extinction and reconsolidation. Such studies might be of importance to clinical practice, when more evidence indicates that cortisol or beta

blockers may moderate stress and excessive emotional memories (Aerni et al. 2004; Weis et al. 2006), or even reduce phobic fears (Soravia et al., 2006). However, the precise effects and timing of these interventions on memory should be discerned before deciding whether these therapies should become clinical practice.

Chapter 4

Immediate and prolonged effects of cortisol, but not propranolol, on memory retrieval in healthy young men

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Summary

While acute cortisol administration has been found to impair retrieval of emotional memories in healthy subjects, the duration of this memory impairment is still unknown. Propranolol, on the other hand, may impair the reconsolidation of emotional memories during reactivation, although human studies examining such effects are scarce. The present investigation was therefore undertaken to examine the immediate and prolonged effects of a single administered dose of cortisol or propranolol on memory retrieval in a double-blind placebo controlled design. Eighty-five healthy male participants were asked to retrieve previously learned emotional and neutral information after ingestion of 35 mg cortisol, 80 mg propranolol or placebo. After a washout period of one week, recall was again tested. Memory retrieval of neutral and emotional information was impaired by a single dose of cortisol compared to placebo. The memory impairment due to cortisol remained, even after a washout period of 1 week. No immediate or prolonged effects of propranolol on memory retrieval were found, despite significant reductions in sympathetic arousal. These results lend support to the hypothesis that cortisol is able to attenuate (emotional) memory recall in men over longer time spans and may therefore augment the treatment of disorders like post-traumatic stress disorder and phobias, but do not clarify the mechanism(s) through which propranolol exerts its therapeutic effects.

Introduction

Stress hormones like (nor)adrenaline (NA) and cortisol have since long been found to influence memory processes (Cahill et al., 1994; Lupien & McEwen, 1997; Wolf, 2008). Brain areas that are thought to mediate memory processes, like the hippocampus, the prefrontal cortex and for emotional memory, the amygdala, are highly occupied with both adrenergic and glucocorticoid receptors (de Kloet et al., 1998; Ramos & Arnsten, 2007). Cortisol is released by the adrenal cortex and can cross the blood-brain barrier (BBB) while NA is released both peripherally by the adrenal medulla and within the brain as a neurotransmitter (van Stegeren, et al., 2007; Wolf, 2008). The interaction between memory processes and stress hormones has been an area of interest in the last two decades (Cahill et al., 2003; de Kloet et al., 1999; Joels et al., 2006; Lupien & Lepage, 2001; Wolf, 2008).

It has been found that the effects of human stress hormones on memory are dependent on the memory stage that is studied (Roozendaal, 2002). Encoding and consolidation phases of memory in humans are found to be enhanced by increased cortisol and NA levels (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill & Alkire, 2003; O'Carroll et al., 1999) and impaired by beta-adrenergic blockers like propranolol that cross the BBB (Cahill et al., 1994; van Stegeren et al., 1998). Retrieval, on the other hand, is found to be impaired by increased cortisol levels (de Quervain et al., 2000; Het, Ramlow & Wolf, 2005). Furthermore, this impairment seems to be dependent on the activity of the adrenergic system (Kuhlmann & Wolf, 2006b; Roozendaal, Hahn et al., 2004; Tollenaar et al., 2008a / Chapter 2). There are no reports of increased levels of NA leading to impairment in memory retrieval in humans (see Chamberlain et al., 2006, for an overview of studies on NA and memory). A single human study, whereby NA levels were manipulated by blockade with propranolol (40 mg) and memory retrieval was measured, has been reported and did not find an effect on memory retrieval (de Quervain et al., 2007). More detailed studies investigating the effects of different doses of propranolol on memory retrieval are needed to clarify this relation.

Recent animal studies have suggested that when memories are retrieved, they are consolidated again after a labile period during which the reactivated memories are prone to change. This process is often referred to as reconsolidation (Debiec et al., 2006; Nader et al., 2000; Przybyslawski & Sara, 1997). Post-retrieval administration of propranolol has been found to disrupt spatial memory and inhibitory avoidance learning in rodents (Przybyslawski et al., 1999), as well as auditory fear conditioning (Debiec & Ledoux, 2004), and both findings have been explained in terms of impaired reconsolidation processes. Tronel and Alberini (2007) have recently shown that reconsolidation might also be dependent on the glucocorticoid system, as they found that a glucocorticoid receptor antagonist can disrupt conditioned fear in rats after reactivation of an inhibitory avoidance memory. In line with that, Maroun and Akirav (2007) have found an impairing effect of stress on reconsolidation in rats, which was reversed by a glucocorticoid receptor antagonist. However, cortisol may also impair memory after reactivation by enhancing extinction rather than reducing reconsolidation (Abrari et al., 2008; Cai et al., 2006). In the present study we will

therefore merely refer to reconsolidation as the post-retrieval stage during which memories might be prone to change.

While reconsolidation of fear related memories has most often been studied in animals, human declarative memories may also become labile during reactivation (Hupbach et al., 2007; Walker et al., 2003). Human studies on reconsolidation and the effects of cortisol and NA on this process are scarce. In a previously reported study, we examined the effects of elevated stress hormones on post-retrieval processes in humans (Tollenaar et al., 2008b / Chapter 3). In line with animal studies (Maroun & Akirav, 2007), a post-retrieval decline in memory performance was observed when memories were reactivated during stress (5 weeks after encoding). However, whether cortisol or other stress hormones were active in this process remains unclear. The effect of blocking adrenergic activity during memory reactivation has recently been studied in humans by Miller et al. (2004) and Brunet et al. (2008). Miller and colleagues reported that fear conditioning was reduced when a conditioned cue was reactivated and followed by NA beta-blockade. In addition, Brunet and colleagues found that post-retrieval propranolol reduced psycho-physiological responding to mental imagery of a past traumatic event in post-traumatic stress disorder (PTSD). However, the effects of propranolol on human declarative memory reconsolidation still remain to be elucidated.

Knowledge on the impact of (stress) hormones on human memory retrieval and reconsolidation is of therapeutic interest, since reducing the recall and/or experience of (intrusive) emotional memories might be of use in augmenting treatments for stress-related disorders like PTSD. Several studies have examined the utility of cortisol and a beta-adrenergic blocker (propranolol) in the treatment of PTSD. These studies have shown promising results, with reductions in re-experiencing and chronic stress symptoms after cortisol administration (Aerni, et al., 2004; Weis, et al., 2006) and reduced physiological reactivity after propranolol treatment (Brunet et al., 2008; Pitman, et al., 2002; Vaiva, et al., 2003). Phobic fears and mood responses to stress also seem to be reduced by cortisol administration (Het & Wolf, 2007; Soravia, et al., 2006).

To gain more insight into the effects of cortisol and propranolol on memory retrieval and reconsolidation, the present study investigated the effects of 35 mg hydrocortisone and 80 mg propranolol on memory retrieval and post-retrieval processes in healthy young men. By testing memory retrieval both during elevated cortisol or lowered NA levels and 1 week later (after clearance of the drug), the immediate treatment effects of cortisol and propranolol on memory retrieval were investigated, as well as whether these effects were prolonged up to 1 week later. We expected impairing effects of cortisol on memory retrieval, both immediate and prolonged. We had no expectations on the immediate effects of propranolol on memory retrieval, but did expect an impairing effect on reconsolidation, reflected in retrieval impairments one week after treatment.

Methods

Participants

Eighty-five Dutch male students were recruited through advertisements at colleges and the University of Leiden. Only men were selected because of possible confounding effects of menstrual cycle and contraceptive pills on the relation of cortisol and propranolol treatment with memory (Cahill & van Stegeren, 2003; Kuhlmann & Wolf, 2005). Participants were screened before inclusion. Inclusion criteria were: no reported history of disease or psychiatric problems, no current use of prescribed medication including corticosteroid containing ointments, no chronic disease requiring medical attention including diabetes, allergies and asthma, no use of psychotropic drugs, alcohol intake under 20 glasses per week, smoking less than 10 cigarettes per day, age between 18 and 35 years, an estimated Body Mass Index (BMI) between 19 and 26 and blood pressure levels over 100/70 mmHg. Before participation, written informed consent was obtained and after participation participants were rewarded with either course credits or a monetary compensation (40 Euros). The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre.

To minimize influences on baseline cortisol levels, participants were instructed to refrain from drinking any sweet or caffeinated drinks and eating heavy meals on the morning of the second (treatment) session. Furthermore, they were instructed not to eat or drink anything but water, and not to smoke an hour before the second session would start.

Of the 85 recruited participants, 2 men were excluded after the first session due to low blood pressure. Two participants were ill during one of the sessions and one person dropped out after the first session. We excluded one more participant due to problems with his Dutch written language. Hence, 79 participants completed the study. Participants were randomly assigned to one of three experimental groups in a double blind between subjects design (placebo: N = 27, cortisol: N = 26, propranolol: N= 26). Dependent on group, 35 mg hydrocortisone, 80 mg propranolol or a placebo was administered orally, in identical capsules.

Table 4.1 shows the demographic variables of the participants per group. No differences between groups were found for BMI, anxiety (STAI-trait) and general psychopathology (Symptoms Checklist, SCL-90). Age was significantly lower in the placebo group compared to the cortisol group ($t(32) = 2.42$, $p < .05$) and depression scores on the Beck Depression Inventory (BDI-II) were marginally higher in the control group compared to both the cortisol ($t(46) = 1.83$, $p = .07$) and propranolol group ($t(50) = 1.95$, $p = .06$).

Table 4.1. Demographic variables (mean ± SD).

	Placebo (N = 27)	Cortisol (N = 26)	Propranolol (N = 26)
Age	19.51 (1.37)[a]	21.35 (3.61)[a]	20.62 (2.16)
BMI	22.07 (2.35)	22.40 (1.98)	21.69 (2.07)
Depression (BDI-II)	6.59 (4.39)[b]	4.69 (3.04)[b]	4.44 (3.48)[b]
Anxiety (STAI trait)	33.74 (9.08)	33.73 (9.08)	31.38 (6.97)
Psychopathology (SCL-90)	28.19 (24.83)	28.73 (23.23)	28.00 (20.25)

Notes: BMI = Body Mass Index; BDI-II = Beck Depression Inventory II; SCL-90 = Symptom Checklist-90; [a] Significant difference in age between the placebo and cortisol group ($p < .05$); [b] Marginally significant difference in depression scores between the placebo group and the cortisol and propranolol group ($p < .10$)

Memory and attention tasks

To measure memory retrieval a word task adapted from Smeets et al. (2006) and Hermans and De Houwer (1994) was employed. Thirty emotional and 30 neutral words were selected that were matched on familiarity and word length. Fifteen words from each category were used for the retrieval task and the other words for a recognition task. During encoding of the words in the first session, words were randomly presented on a 17 inch computer screen for 4 seconds (word height: 13 mm, distance to screen: 60 cm). After presentation of each word, participants rated the word on two standardized, 5-point Likert scales on arousal (emotionality) and valence from the Self-Assessment Manikin (SAM: Bradley & Lang, 1994). A higher score on the arousal scale indicates higher emotionality and on the valence scale more negative emotions. After presentation of the words, a surprise memory task was given in which participants had to write down as many words as they could remember within 4 min (free recall). Then the same words were presented a second time, but with the deliberate instruction to remember as many words as possible. Words were presented for 5 s with 2 s intervals in between. A free recall test was again administered afterwards. These two trials served as the encoding/learning trials. During the second session, free recall of the words was again tested (in written form) with a maximum time of 4 min, followed by a cued recall task in which the first letters of each word were given and participants were asked to write down as many words as they could remember in 5 min. The third session consisted of a last free recall task, followed by a recognition task in which the old words were mixed with (15 neutral and 15 negative) new words and displayed on a computer screen. Participants were required to make a forced classification of words as old or new.

To obtain an estimate of verbal working memory, the digit span forward and backward from the WAIS were administered (WAIS, 1970; WAIS-III, 1997). Two versions of each task were randomly varied between the first and second session. In the forward condition, participants had to recall strings of numbers ranging from 4 to 8 in length. In the backward condition, participants had recall strings of numbers in a backward fashion.

To get an estimate of attention, the Sustained Attention to Response Task (SART) was administered, measuring vigilance (Manly, Robertson, Galloway & Hawkins, 1999). In this task, digits between 1 and 9 were presented for 250 ms in one of five randomly assigned font sizes with an inter-stimulus interval of 900 ms. Participants were asked to press a key (as fast as possible) in response to the digits except for the number 3. Misses and errors of commission were added to calculate an overall error score. Additional tasks were administered during the study that will be described in future reports.

Physiological and subjective measures

Saliva samples were obtained using Salivettes (Sarstedt, Germany) to measure unbound cortisol and alpha-amylase levels. Alpha-amylase has been shown to be an estimate of adrenergic activity (Nater et al., 2006; Rohleder et al., 2004) and is sensitive to beta-blockage by propranolol (van Stegeren et al., 2005). Saliva samples were stored at -20 °C prior to analyses. The saliva samples were analyzed by the Kirschbaum lab, Technical University of Dresden (see Rohleder et al., 2006).

Heart rate and blood pressure were measured to asses adrenergic functioning using an automatic upper arm blood pressure monitor (OMRON, M6). In addition to each physiological recording, participants were given a questionnaire with 7 questions on subjective experiences like anxiety, mood and motivation. Answers were given on Visual Analogue Scales (VAS) of 100 mm in length, leading to a score from 0 to 100 on each scale.

Questionnaires

The BDI-II (Beck, Steer, & Brown, 1996; van der Does, 2002) was administered to assess depressive feelings in the past 2 weeks, a Dutch version of the STAI-trait (Spielberger, 1983) to measure the level of generalized anxiety and the SCL-90 (Arrindell & Ettema, 1986) to assess psychological symptoms and psychopathology during the last week.

Procedure

Participants came to a lab at the Faculty of Social and Behavioral Sciences in Leiden for 3 sessions. The interval between each session was 1 week (see Figure 4.1a for an overview of the 3 test sessions). On the first session screening measurements of blood pressure and heart rate were taken after 3 rest periods of 4 min. During this first session, words were encoded for the retrieval task and baseline working memory performance was measured. At the start of the second session, after a 4 min rest period (given before each physiological measurement), baseline measurements of heart rate and blood pressure were assessed and baseline saliva samples obtained. Participant then ingested a capsule containing placebo, 35 mg hydrocortisone or 80 mg propranolol. During the next 75 min, participants completed several computer based questionnaires and were instructed to remain in the lab and read (reading material was provided). At t = 75 min after ingestion, participants heart rate and blood pressure were again assessed and saliva measurements obtained. Memory was then tested, including working memory. Physiology was measured again at 110 min after treatment after which an attention task was given. At 135 min after treatment, the last

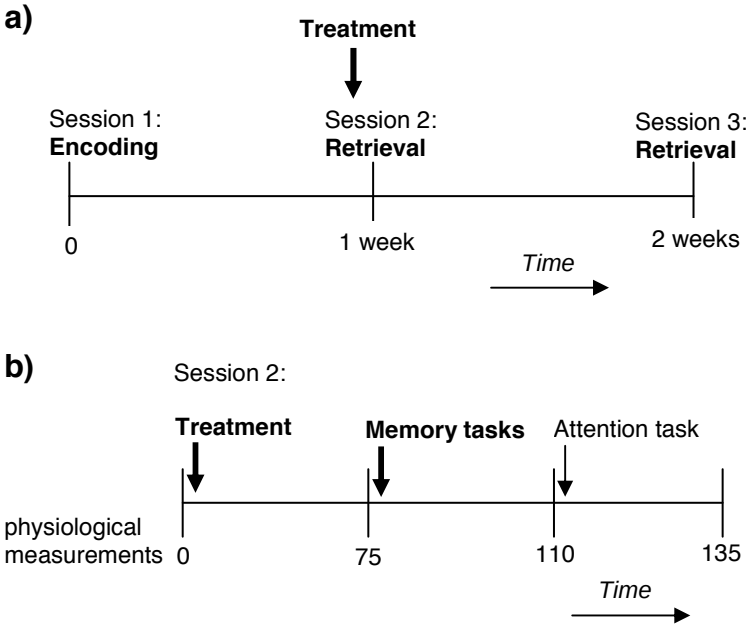


Figure 4.1. a) Schematic overview of the 3 sessions. b) Schematic overview of the second session. Treatment consisted of either cortisol (35 mg), propranolol (80 mg) or a placebo. The memory tasks were a free recall, cued recall and working memory task.

physiological measurements were taken as well as an interview on side effects and expectations of the memory task (see Figure 4.1b for an overview of session 2). In the third session, memory was tested again followed by an exit interview (including expectancies of the last memory task and an awareness check for treatment) as well as a debriefing concerning the goals of the study.

Data analysis

The effects of the treatment (placebo vs. cortisol vs. propranolol) on physiological and subjective measures were analyzed using repeated measure (RM-) ANOVAs with time as within subject and group as between subject variable, followed by Student Newman Keuls (SNK) *post hoc* tests. A χ^2 test was used to analyze side effects and treatments awareness in the three groups. Memory retrieval over the three sessions was analyzed using a RM-ANOVA with session and emotion as within subject and group as between subject variable. The percentages correct recall on session 2 and 3 were also analyzed using RM-ANOVAs with emotion as within subject and group as between subject factor. Additional analyses were conducted using univariate ANOVAs or simple *t*-tests. Greenhouse-Geisser corrected *p* values were used when indicated by violated Sphericity. Analyses were performed with SPSS 14.0 (SPSS, Chicago, IL). The criterion for statistical significance was $p < 0.05$.

Results

Effect of treatments on physiology

Cortisol measures

Table 4.2 shows the salivary cortisol levels in the three groups in nmol/L. For the RM-ANOVA, log values of cortisol were calculated to account for non-normality. One participant in the propranolol group was excluded from the analyses due to a missing sample. A significant group by time interaction was found ($F(3, 107) = 30.16$, $p < .001$). As expected, Student Newman Keuls (SNK) *post hoc* analyses revealed that cortisol levels were significantly increased in the cortisol group compared to both the placebo and propranolol group at t = 75, 110 and 135 min (all *p*s < .01), while not differing from the other 2 groups at baseline, t = 0 min ($p > .50$). In addition, a group by time interaction was also found between the placebo and propranolol group ($F(2, 87) = 10.26$, $p < .001$). The propranolol group showed increased cortisol levels compared to placebo at t = 110 and t = 135 (both *p*s < .01).

Table 4.2. Free salivary cortisol in nmol/L (± SEM) in each treatment group.

Group	Time			
	t = 0	*t* = 75	*t* = 110	*t* = 135
Placebo	9.01 (0.69)	5.02 (17.20)	4.57 (8.85)	4.95 (5.45)
Cortisol	7.47 (0.71)	206.61 (17.53) [a]	134.79 (9.01) [a]	99.37 (5.55) [a]
Propranolol	7.98 (0.72)	5.84 (17.88)	8.26 (9.19) [b]	9.81 (5.66) [b]

Notes: [a] Significant increase in cortisol levels in the cortisol group vs. the placebo and propranolol group (p < .001). [b] Significant increase in cortisol levels in the propranolol group vs. the placebo group (p < .001).

Adrenergic measures

Figure 4.2a-d shows the changes in alpha-amylase (AA), heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP) in all groups before (*t* = 0) and at three time points after treatment (*t* = 75, 110 and 135 min) on session 2.

For the RM-ANOVA, log values of AA were calculated to account for non-normality. Four participants were excluded from the analyses due to missing AA samples ($n = 2$ from the propranolol, $n = 1$ from the cortisol and $n = 1$ from the placebo group). A significant interaction between group and time was found for AA levels ($F(4, 160) = 5.33$, $p < .001$). SNK *post hoc* tests revealed that AA levels were marginally lower in the propranolol group compared to both the placebo and cortisol group at *t* = 75 ($p = .09$) and 110 min ($p = .06$) and significantly lower at 135 min ($p < .02$), while not differing from the other 2 groups at baseline, *t* = 0 min ($p > .20$).

A significant group by time interaction was also found for HR ($F(4, 134) = 6.03$, $p < .001$). SNK *post hoc* analyses revealed that HR levels were significantly decreased in the propranolol group compared to both the placebo and cortisol group at t = 75, 110 and 135 min (all *ps* < .01), while not differing from the other 2 groups at baseline, t = 0 min ($p > .80$).

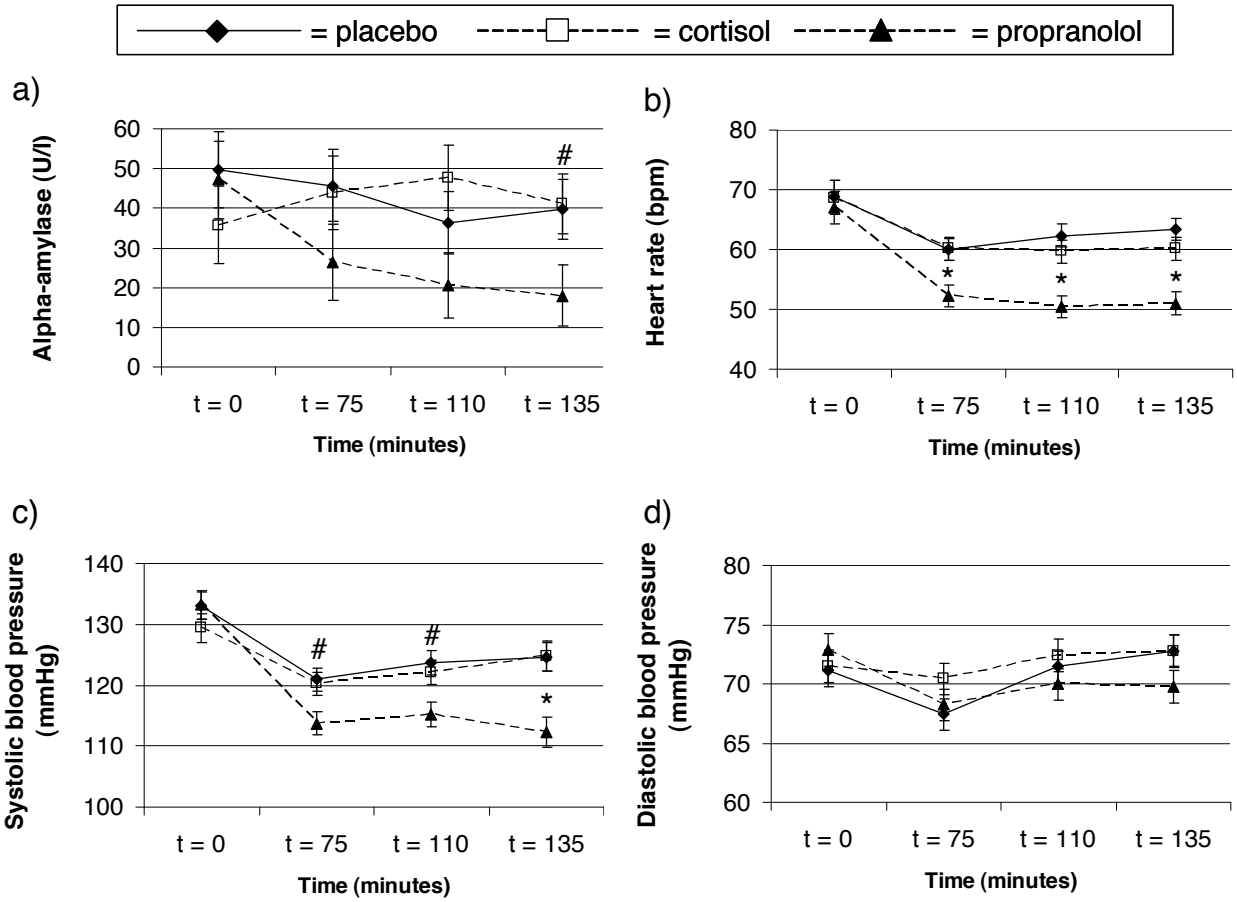


Figure 4.2. Physiological measures of adrenergic activation on session 2, before treatment (*t* = 0) and after treatment (*t* = 75, 110 and 135 min). a) Alpha-amylase. b) Heart rate. c) Systolic blood pressure. d) Diastolic blood pressure. Mean levels ± SEM are displayed.

Notes: U/L = Units per Liter; bpm = beats per minute; mmHg = millimeter of Mercury * *= significant difference in the propranolol versus the placebo and cortisol group (p < .01)* # *= significant difference in the propranolol versus the placebo and cortisol group (p < .05).*

Similar results were found for SBP. The group by time interaction was significant ($F(4, 171) = 9.41$, $p < .001$) and SNK *post hoc* analyses revealed that SBP levels were significantly decreased in the propranolol group compared to both the placebo and cortisol group at t = 75 ($p < .03$), 110 ($p < .02$) and 135 min ($p < .01$), while not differing from the other 2 groups at baseline, $t = 0$ min ($p > .40$). Even though an interaction was found for group by time for DBP as well ($F(5, 180) = 2.91$, $p < .05$), SNK *post hoc* tests revealed no significantly lower DBP at any of the time point in the propranolol group versus the other groups (all *p*s > .10).

Subjective measures

No effects of treatment over time were found on subjective feelings of tension, insecurity, irritation, motivation, mood and tiredness (all *p*s > .10). We did find an

interaction effect of group with time on anxiety ($F(5, 186) = 2.30$, $p < .05$). The propranolol group showed a trend towards lower anxiety at the end of the 2nd session compared to the placebo group ($t(46) = 1.74$, $p = .09$).

Side-effects and awareness check

After the treatment session participants were asked to report any side-effects or strange feelings. Feelings that were reported included: tiredness, tense feeling, cold hands, headache, light nausea and concentration problems. However, each of these reported feelings were evenly distributed across the 3 treatment groups (Pearson's χ^2 (6) = 5.35, $p = .50$). Furthermore, in the exit interview, participants were asked to speculate which treatment they received to check for awareness of treatment. Answers were categorized as placebo, cortisol, propranolol, no idea or simply a medicine. Participants did not guess which treatment they received (Pearson χ^2 (8) = 6.96, $p = .54$).

Memory performance

Arousal and valence ratings

On average, negative words were rated as significantly more emotional (mean = 2.76, SD = 0.74) than neutral words (mean = 1.70, SD = 0.55, $t(78) = 16.24$, $p < .001$). Negative words were also rated as significantly more negatively valenced (mean = 3.79, SD = 0.53) than neutral words (mean = 2.72, SD = 0.38, $t(78) = 22.25$, $p < .001$). No differences in ratings were found between the three groups (all ps > .10).

Memory performance

Table 4.3 shows performance on the memory tasks on sessions 1, 2 and 3. For session 1 recall performance on the last learning trial is shown. For session 2 data on the free recall and the cued recall task are shown and for session 3 performance on the free recall and the recognition task are shown.

Table 4.3. Memory performance (Mean ± SD) on session 1, 2 and 3 in number of words correctly recalled.

Group	Word valence	Session 1	Session 2		Session 3	
		recall	recall	cued recall	recall	recognition[a]
Placebo	Neu	8.63 (2.17)	4.70 (1.56)	6.11 (2.33)	5.22 (2.08)	10.52 (2.23)
	Emo	10.11 (2.31)	6.07 (2.73)	7.15 (2.43)	6.37 (2.39)	10.67 (2.63)
Cortisol	Neu	9.27 (1.85)	4.27 (2.18)	5.69 (2.29)	4.77 (1.80)	10.42 (2.89)
	Emol	10.42 (2.14)	5.46 (2.10)	6.58 (2.27)	5.42 (1.88)	9.31 (2.94)
Propranolol	Neu	9.35 (2.12)	5.54 (2.20)	6.42 (2.69)	5.85 (2.41)	11.73 (2.16)
	Emo	11.04 (1.64)	6.81 (2.12)	7.88 (1.75)	6.85 (1.99)	10.54 (3.09)

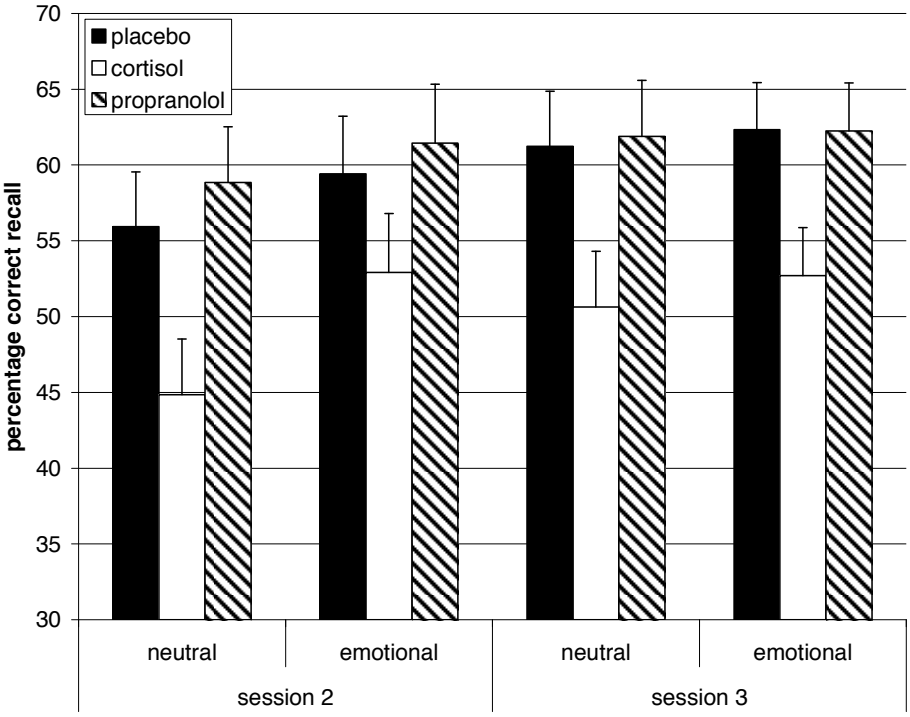
Notes: [a] *Recognition scores were calculated by subtracting the falsely recognized items from the number of correctly recognized items; neu = neutral; emo = emotional.*

In the RM-ANOVA used to test for effects of treatment on memory retrieval the last recall trial from session 1 and the free recall trials from sessions 2 and 3 were analyzed. The RM-ANOVA with session (1 vs. 2 vs. 3) and emotion (neutral vs. emotional) as within-subject variables, and group as between-subject variable was calculated for retrieval performance. A significant main effect of session was found ($F(2, 122) = 537.49$, $p < .001$), showing a decrease in performance from session 1 to 2 ($F(1, 76) = 648.80$, $p < .001$) and a slight increase in performance from session 2 to 3 ($F(1, 76) = 6.59$, $p < .02$) for all groups. This increase in recall performance from the second to third week may be due to the cued recall task that was performed after the free recall task of the second session. Furthermore, a main effect of emotion was found ($F(1, 76) = 26.83$, $p < .001$) reflecting a higher recall of emotional vs. neutral words.

No main effect of group was found. In line with our expectations, a significant group by session interaction was found ($F(3, 122) = 3.62$, $p = .013$). With planned comparison analyses for the cortisol and propranolol group separately, this interaction appeared to be significant between the placebo and cortisol group ($F(2, 77) = 6.00$, $p < .01$), but not between the placebo and propranolol group ($F(2, 89) = 0.35$, $p = .68$). To clarify in which phase the interaction effects for cortisol were apparent, separate RM-ANOVAs were conducted on session 1 vs. session 2 and session 1 vs. session 3. In the first RM-ANOVA with session 1 and session 2, and emotion as within-subject factors, and cortisol vs. placebo as between-subject factor, it was shown that memory performance in the cortisol group decreased significantly more from session 1 to session 2 than in the placebo group (time x group interaction: $F(1, 51) = 5.35$, $p = .025$), while performance on session 1 did not differ between these groups ($F(1, 51) = 1.08$, $p = .30$). This interaction was also found in the RM-ANOVA with session 1 and 3 as within-subject variable (time x group interaction: $F(1, 51) = 8.81$, $p < .01$), indicating a higher decrease in memory retrieval from session 1 to 3 in the cortisol group compared to the placebo group.

We also calculated the percentages correct recall on sessions 2 and 3 with respect to the last learning trial on session 1 (see Figure 4.3). RM-ANOVAs for the percentages correct recall on both session 2 and session 3 with emotion as within-subject factor, and cortisol vs. placebo as between-subject factor, showed that the cortisol group remembered significantly less from the last learning trial than the control group in both session 2 ($F(1, 51) = 4.17$, $p = .046$) and session 3 ($F(1, 51) = 6.60$, $p = .013$). No interaction effects with emotion were found (all ps $> .40$), indicating that the immediate and prolonged effects of cortisol on memory retrieval were similar for both neutral and emotional memory retrieval. The propranolol group did not remember less from the last learning trial than the control group in either session 2 ($F(1, 51) = 0.37$, $p = .92$) or session 3 ($F(1, 51) = .512$, $p = .48$).

To examine the change in retrieval performance from session 2 to session 3, an additional RM-ANOVA with session 2 and session 3, and emotion as within-subject factors, and group (cortisol vs. propranolol vs. placebo) as between-subjects factor was performed. No interaction between group and time was found ($F(2, 76) = 0.45$, $p = .64$), indicating that memory after treatment changed in a similar way in each group. In addition, when the percentage correct recall on session 3 was calculated with respect to session 2 and compared between groups in an ANOVA, no



Figuur 4.3. Percentage correct recall (Mean ± SEM) on session 2 and 3 with respect to the last learning trial on session 1. The cortisol group showed a lower memory performance on both session 2 and 3 compared to the placebo and propranolol groups (*p*s < .05).

effect of group was found either ($F(2, 76) = 0.41$, $p = .67$). To test whether there was a change in the reactivated items from session 2 to session 3, we also calculated the percentage correct recall on session 3 with respect to session 2 including only the words from session 3 that were also recalled on session 2. However, no further decline was found in recall of words that were reactivated during treatment ($F(2, 76) = .87$, $p = .42$.). No main or interaction effects of emotion were found either (*p*s > .65).

When conducting separate ANOVAs on the absolute scores on each of the three sessions, no differences in recall performance were found for any of the sessions between the placebo and the treatment groups (all *p*s > .10). Also, no effect of group was found on cued recall or recognition scores (all *p*s > .10).

In both sessions 2 and 3 participants were asked whether they expected a memory test. In session 2, more participants expected a memory task in the propranolol group ($F(2, 76) = 3.09$, $p = .05$). Furthermore, as mentioned in the methods section, age and depression scores differed in the control group compared to the other 2 groups. Therefore, these 3 variables were subsequently entered in the above analyses as covariates. Controlling for these possible confounding variables did not affect the main interaction between group (placebo, cortisol and propranolol) and session (sessions 1, 2, and 3) ($F(4, 144) = 3.57$, $p = .014$).

Working memory and attention

A RM-ANOVA with session (1 vs. 2) and order (forward vs. backward) as within-subject variable and group as between-subject variable was performed for the working memory scores on the digit span. Performance increased from session 1 to 2 ($F(1, 76) = 37.17$, $p < .001$) and performance on digits forward was higher than digits backward ($F(1, 76) = 19.46$, $p < .001$). No effects of group were found however (all ps $> .15$). Moreover, an ANOVA also failed to reveal significant effects of group on errors in the sustained attention task ($F(2, 76) = 1.51$, $p = .23$).

Discussion

In this study we found evidence that the retrieval impairments that have been observed as a result of cortisol administration are still observable after a wash out period of 1 week. These immediate and prolonged impairments in memory retrieval were found for the retrieval of both neutral and emotional words. These results are in line with earlier studies showing impairing effects of acute cortisol administration on memory retrieval (de Quervain et al., 2000; Het et al., 2005). They also relate to an earlier study by our group (Tollenaar et al., 2008b / Chapter 3) in which we found that stress impairs long-term memory retrieval when memories are reactivated during stress. While in the previous study stress was found to further diminish long-term memory retrieval when memory was reactivated during stress, in the present study we did not find a further decrease in memory performance after cortisol treatment. The persistence of the retrieval impairment in the cortisol group might be due to a lowered rehearsal during treatment and hence a lower re-encoding of the learned material or to the effects of cortisol on post-retrieval (reconsolidation) processes. The latter is less likely as there was no further decline in the retrieval of reactivated information, but from these data it cannot be concluded which of these two processes were involved in the memory impairments 1 week after treatment. The differences found between the long-term effects of exogenous cortisol administration and stress-induced endogenous cortisol increases may be related to the additional physiological and psychological responses that arise during stress. Furthermore, to examine whether reconsolidation specifically is affected by cortisol, future investigations should increase cortisol levels *after* memory reactivation to separate the effects on retrieval and reconsolidation, and compare the effects of cortisol not only to a placebo group, but also to a group in which cortisol is administered without reactivation, to rule out non-specific effects of cortisol on long-term memory.

In contrast to the impairing effects of cortisol, we found no immediate effect of propranolol on memory retrieval. So far, only one other study reported on the effects of propranolol on memory retrieval (de Quervain et al., 2007). In this study, propranolol did not reduce retrieval either. Furthermore, we found no indications for effects of propranolol on post-retrieval processes. That is, performance 1 week after treatment was still comparable to placebo. This is in contrast with our expectations based upon studies in which propranolol was found to affect post-retrieval processes like reconsolidation (Debiec & Ledoux, 2004; Miller et al., 2004; Przybyslawski et al., 1999). However, these studies used mostly fear conditioning paradigms, which are

not directly comparable to our declarative memory task. While fear conditioning is concerned with implicit learning, generally involves higher levels of fear, and is found to be mediated by the amygdala (Debiec & LeDoux, 2006), memory retrieval is thought to be primarily mediated by the hippocampus and prefrontal regions (Squire et al., 2004; Takashima et al., 2006), although the amygdala has also been implicated in emotional memory retrieval (Dolcos et al., 2005). A reason that might therefore explain our non-results is that propranolol may be more involved in (amygdala related) physiological and anxiety reducing mechanisms, as in fear conditioning. Although we did find a slight decrease in anxiety in the propranolol group at the end of the treatment session, this effect did not remain 1 week after treatment. Propranolol may potentially only affect declarative memory reconsolidation when related emotions and physiological responses are very strong, as in PTSD (Orr et al., 2002). That is, propranolol has been found to affect declarative memory *consolidation* in humans (Cahill et al., 1994; van Stegeren, Rohleder et al., 1998), but in those studies picture tasks were used that might have elicited more emotional arousal than our word task. Another reason that could explain the non-results, is that the administered dose of propranolol was too low. This is not a likely explanation however, since the expected physiological effects of propranolol administration were clearly observed. There was a very significant decrease in adrenergic activity measured with heart rate and blood pressure (although only on systolic blood pressure, as previously reported by Maheu et al. (2005) and van Stegeren, Rohleder et al. (2005)), but also with alpha-amylase. Alpha-amylase measured from saliva seems to be a valid and non invasive measure of adrenergic activity and is sensitive to beta-adrenergic blockade (Nater et al., 2006; van Stegeren et al., 2005). Besides the fact that propranolol induced the expected physiological effects, we have administered a dose of propranolol (80mg) that was twice as high as in the study by de Quervain et al. (2007). Taken together, even though we did not find evidence for effects of propranolol on reconsolidation, this study does not rule out that propranolol might potentially play a role in reconsolidation in humans. Reconsolidation in humans is still a relatively unstudied area and future studies using different memory paradigms will have to elucidate whether propranolol can affect post-retrieval memory processes.

Interestingly, propranolol also led to a moderate, but significant, increase in cortisol levels, which was previously reported by Maheu et al. (2004) as well. Apparently, this increase did not impair memory recall. The increase might not have been sufficiently large to cause any effects (less than 2 nmol/L cortisol), but the fact that noradrenergic activation was blocked by propranolol might have prevented cortisol increases from any impairing effects on memory as well (see also de Quervain et al., 2007).

Overall, the present findings suggest that reactivation of memories when cortisol levels are high may lead to long-term memory attenuation. This is highly relevant for the treatment of post-traumatic stress disorder (PTSD), in which a lasting diminished recall of trauma-related memories might be beneficial (de Quervain, 2007). Moreover, these findings are in accordance with clinical observations of prolonged beneficial effects of glucocorticoids in PTSD and phobias (de Quervain & Margraf, 2008). However, our findings suggest that cortisol may impact both emotional and neutral memory retrieval. The role of emotionality and valence in the

effect of cortisol on memory retrieval in humans is still unclear. That is, several other studies have found effects of cortisol on retrieval of neutral memories as well (Buss et al., 2004; de Quervain et al., 2000), while other studies found effects of cortisol primarily on emotional memory retrieval (Domes et al., 2004; Kuhlmann, Kirschbaum et al., 2005). The effects of endogenous increases of cortisol on memory retrieval seem to be dependent on the emotionality of the material or on an arousing context (Kuhlmann, Piel et al., 2005; Tollenaar et al., 2008a / Chapter 2), while high exogenous doses of cortisol might affect memory retrieval as long as the subject has a normal level of sympathetic arousal (de Quervain et al., 2007; Kuhlmann & Wolf, 2006b). With regard to the clinical setting, the impairing effects of cortisol on the retrieval of neutral information may be a potential negative side effect, and merit special attention in future clinical trials.

There are some limitations to our current investigation that merit consideration. The effects we found of cortisol on memory retrieval were all within-subject effects. They were expressed in an interaction between group and session and present in the percentage correct recall with respect to the individual last learning trial. No effects were found on absolute memory scores when the sessions were analyzed separately. Other studies have reported within-subject effects of cortisol on memory retrieval as well (Buss et al., 2004; Kuhlmann, Piel et al., 2005; Kuhlmann, Kirschbaum et al., 2005), suggesting that these effects, while subtle, are consistent. Second, the present study only included men, while disorders related to stress and memory problems like PTSD and depression are highly prevalent in women. Future studies should examine whether similar results are found in females, while taking into account hormonal fluctuations due to menstrual cycle and birth control agents. Another point is that our control group differed from the drug groups on depression and age scores despite randomization. In the control group, two participants were over 30 years leading to a higher mean. However, including both age and depression scores as covariates in the analyses did not change our results. Furthermore, the fact that the impairments in memory were prolonged up to 1 week does not necessarily mean that the memory traces are impaired indefinitely. A longer follow-up is needed to indicate whether these effects are persistent or temporary. Of interest is also whether memory losses can be restored with cues or in a different context (Bouton, 2002). Moreover, future studies should investigate the effects of multiple reactivations under treatment, as animal research has indicated this might strengthen the effects (Cai et al., 2006).

In summary, these results lend support to the hypothesis that cortisol might aid in the treatment of disorders like PTSD and phobias by diminishing (emotional) memory recall over extended time spans, but does not clarify the mechanisms through which propranolol exerts its therapeutic effects.

Chapter 5

Autobiographical memory after acute stress in healthy young men

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http://www.informaworld.com/smpp/title~content=t713683358~link=cover
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Summary

Autobiographical memories have been found to be less specific after hydrocortisone administration in healthy men, resembling memory deficits in e.g. depression. This is the first study to investigate the effects of stress-induced elevated cortisol levels on autobiographic memory specificity and experience in healthy young men. Autobiographical memories were elicited by neutral and negative cue words, with instructions to recall either recent or remote memories. No effect of psychosocial stress was found on memory specificity or experience, but cortisol increases tended to be related to less specific, recent memories elicited by neutral cue words, especially when subjects were physically aroused during memory retrieval. These results indicate that autobiographical memories are fairly resistant to an acute stressor in healthy young men, but that endogenous cortisol increases might be related to autobiographical memory retrieval. More research into the relation between endogenous cortisol increases and autobiographic memory retrieval is needed, especially in stress-related disorders.

Introduction

Dependent on the memory stage being tested, acute stress and high cortisol levels can have impairing or enhancing effects on memory. While encoding and consolidation are found to be facilitated by cortisol (e.g. Buchanan & Lovallo, 2001), retrieval and working memory are found to be impaired by acute stress (Elzinga & Roelofs, 2005; Kuhlmann, Piel et al., 2005; Tollenaar et al., 2008a / Chapter 2; Oei et al., 2006) or exogenous cortisol administration (Het et al., 2005; Lupien et al., 1999; Wolf, Convit et al., 2001). Recent studies suggest that the impairing effects of cortisol on human memory may be mediated by reduced prefrontal and medial temporal lobe (MTL) activation (de Quervain et al., 2003; Oei et al., 2007). Animal studies have shown that the impairing effects of cortisol on memory are mediated by hippocampal and prefrontal glucocorticoid receptors (Lupien & LePage, 2001), and are dependent on noradrenergic signaling of the basolateral nucleus of the amygdala (Roozendaal, Hahn et al., 2004; Roozendaal, McReynolds et al., 2004). In line with the hypothesis that noradrenergic activation is a prerequisite for cortisol effects on memory, recent studies in humans have shown that this effect is dependent on arousal elicited by the encoded stimuli and/or the environment (Elzinga & Roelofs, 2005; de Quervain et al., 2007; Tollenaar et al., 2008a / Chapter 2).

Declarative memory retrieval in humans is most often tested with word tasks, using free recall, cued recall or recognition paradigms (see also Wolf, 2008), showing fairly consistent results. However, laboratory word tasks are not necessarily an ecologically valid measure of real-life memories of one's personal past, defined as autobiographical memory (Tulving, 2002). Furthermore, disorders such as acute stress disorder, depression or Post Traumatic Stress Disorder (PTSD) that are characterized by cortisol disregulations have been related to recall of non-specific, over-general autobiographic memories (e.g. Bryant et al., 2007; Harvey et al., 1998; Kangas et al., 2005; Williams & Scott, 1988).

Based on the hierarchical model of autobiographical memory by Conway and Pleydell-Pearce (2000), one might expect that if retrieval of memory details is impaired by stress and/or cortisol, autobiographic memory retrieval might not progress to the level of event-specific knowledge and remain over-general after stress exposure or cortisol administration. Based on this same model, Williams et al. (2007) suggested three mechanisms that may underlie over-general autobiographical memories, described in the CaRFAX model. Important in the present context is that each of these processes can potentially be affected by stress or cortisol increases: First of all, specific memory retrieval requires cognitive resources and hence impaired executive functioning might lead to over-general memory retrieval (Dalgleish et al., 2007). As stress and cortisol have been found to impair working memory (i.e., an indicator of executive functioning capacity), this might thus lead to the recall of less specific memories. Secondly, decreased specificity might be a result of functional avoidance. Because stress may induce negative mood states (e.g. Kuhlmann, Piel et al., 2005), stressed individuals might try to avoid further mood disturbances by avoidance of sensory and perceptual details of negative events and hence adopt an over-general retrieval style (see Au Yeung et al., 2006 and Svaldi & Mackinger, 2003 for the negative effects of mood inductions on memory specificity). Thirdly,

rumination about self-referential attributes can also lead to lowered specificity. While ruminating, the search process might not progress to specific memories but rather move across the memory hierarchy by retrieving abstract, self-related conceptual knowledge (see also Spinhoven, Bockting et al., 2007). During a psychosocial stress test (as the Trier Social Stress Test, a test that is frequently used to induce psychosocial stress), participants are confronted with their performance in a social situation. This might lead to increased activation of self-schemas and rumination, leading to less specific memory retrieval. In sum, based on the hierarchical model by Conway and Pleydell-Pearce (2000) and the CaRFAX model by Williams et al. (2007), we might expect that stress and cortisol increases lead to over-general memory retrieval.

The only experimental study so far in which the effects of cortisol administration on the retrieval of autobiographical memories have been examined is that by Buss et al. (2004). They found that acute cortisol administration in healthy young men diminished recall of specific memories, especially in response to neutral cue words. Interestingly, the fact that it was mostly neutral autobiographical memories that were impaired by cortisol administration is not in line with previous studies using word recall in which the retrieval of emotional words was found to be most affected by cortisol (Kuhlmann, Kirschbaum et al., 2005) and stress (Kuhlmann, Piel et al., 2005; Tollenaar et al., 2008a / Chapter 2; Wolf, 2008).

The effects of acute stress and endogenously increased cortisol levels on autobiographical memory have never been studied before. Based on the predictions from the CaRFAX model, stress exposure might affect even more processes involved with the retrieval of specific memories than cortisol administration. The present study therefore investigated the effects of an acute psychosocial stressor and related cortisol increases on autobiographical memory retrieval in healthy young men. Autobiographic memory specificity and subjective emotional experience of the memories were tested, elicited by means of both neutral and negative cue words. In addition, to test whether both recent and remote memories are equally vulnerable to stress, participants were instructed to recall half of the memories from childhood while the other half from the two years before the test.

We thus expect a psychosocial stress task and its related cortisol increases to reduce autobiographical memory specificity. As a consequence, we also expect that subjective emotional experience of the memories will be rated as less intense. From the literature on the effects of cortisol on declarative memory retrieval we would expect mostly negative, emotional memories to be affected, although the study by Buss et al. (2004) found impairing effects of cortisol on neutral autobiographic memories. However, on the basis of predictions derived from the CaRFAX model, we expect effects of stress on memory specificity for both neutral and negative cue words.

Methods

Participants

Forty healthy male participants between the age of 18 and 30 (M = 21.7, SD = 3.4) were recruited at Leiden University for the present study. Females were not included in the study because of confounding effects of gender (e.g. by menstrual cycle and contraceptive pills) on cortisol responses (Kirschbaum et al., 1992). Inclusion criteria were: no reported medical or psychological problems in the past year, no reported use of medication, and no drug or alcohol abuse. There were no differences between the stress and control group on depressive and anxious symptoms as measured with the Beck Depression Inventory-II (BDI-II, van der Does, 2002; stress group: M = 8.45, SD = 1.39; control group: M = 6.45, SD = 0.97; $F(1, 38) = 1.40$, $p = 0.25$) and the Hospital Anxiety and Depression Scales (HADS, Spinhoven et al. 1997; HADS depression: stress group: M = 2.65, SD = 0.53; control group: M = 2.35, SD = 0.49; $F(1, 38) = 0.17$, $p = 0.68$; HADS anxiety: stress group: M = 4.90, SD = 0.49; control group: M = 5.10, SD = 0.63; $F(1, 38) = 0.06$, $p = 0.80$). Participants gave written informed consent before participation and were rewarded with either money or course credits afterwards. The study was approved by the Ethics Committee of the Faculty of Social and Behavioral Sciences at Leiden University.

Procedures

To avoid confounding of cortisol measurements, participants were instructed not to drink any caffeinated drinks on the morning of the study, and further not to smoke, eat and only drink water an hour before the start of the study. All testing sessions took place in afternoon, starting either at 12pm or 3pm. Participants were randomly assigned to either a stress (N = 20) or control (N = 20) condition in a between subject design. At the start of the test session, the autobiographical memory task (AMT) was practiced and the first physiological measurement was taken (t = 0 min). During the stress and control task, the experimenter was seated behind a one-way screen. After the stress task and a short break, the second physiological measurement was taken (t = 30 min). The AMT was administered by the experimenter directly after this measurement and lasted on average 30 min (M = 31.8 min, SD = 6.14 min). This was followed by the last physiological measurement (t = 60 min).

Measures

Autobiographical memory task

Autobiographic memory was measured with an adapted version from the AMT (Williams & Broadbent, 1986). Participants were given 6 negative and 6 neutral cue words (see Table 5.1) and were asked to produce a specific memory to each cue word. Specific memories were described as events that lasted less than a day and occurred at a particular time and place. As a restriction, for each valence category, participants were instructed to name 3 remote memories (from their primary school time) and 3 recent memories (from the last 2 years, except for the current day). Four pseudo random versions with valence and date instructions were constructed. Cue words were presented on a card and read aloud by the experimenter. Participants were prompted at least once to elaborate on their memory. If there was no response after 60

Table 5.1. Words used in the AMT (translated from Dutch).

Negative	Neutral
grief	grass
regret	bread
ashamed	bathe
bad	nature
hurt	library
guilt	fast

seconds 'no memory' was reported. Memories were tape-recorded for later scoring. After each memory a questionnaire on emotional experience and the age of the participant during the memory was administered (see below). Reliability of the memories was not verified. For scoring, only the first answer was used. Memories were only scored as specific when the reported event did not last longer than a day (i.e. an extended memory) and was not a repeated event (i.e. a categoric memory). Semantic associations (e.g. "I often feel sad") and memories from the wrong time period (which happened only 4 times in total) were also classified as not specific. Memory specificity was scored by a trained rater blind to condition. A random sample of 20% of all memories was double scored by an independent rater leading to an inter-rater agreement of more than 93% (Cohen's kappa = 0.82). Maximum specificity score for the total AMT was 12, with 3 points for each category (negative-remote / negative-recent / neutral-remote / neutral-recent).

To examine subjective emotional experience of the memories and to verify the valence of the memories a Dutch questionnaire was used, derived from the study by Greenberg et al. (2005) on emotional valence, intensity (arousal and physical feelings) and feelings of re-experiencing (reliving, seeing in mind, coherence, remembering-knowing).

Stress protocol

Psychosocial stress was induced using the Trier Social Stress Task, which is well known for inducing hypothalamic-pituitary-adrenal (HPA) axis and cardiovascular responses in a large part of participants (see Kirschbaum et al., 1993, for a detailed procedure). The TSST consists of three parts of each about 5 min; an anticipation/preparation period, followed by a public speech task, and a cognitive task (in the present study an arithmetic task and a working memory task) in front of an audience of 3 people with a camera and voice recorder. The control group had to write a letter for a fictitious job interview and also performed the working memory task, but with no audience present.

Physiological measures

Cortisol saliva samples were obtained at 0, 30 and approximately 60 min with reference to the stress task with Salivette collection devices (Sarstedt Germany). Saliva samples were stored at -20 °C before assay. Free cortisol saliva levels were determined with a competitive electrochemiluminescence immunoassay ECLIA, using a Modular Analytics E170 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). The functional detection limit was 2.0 nmol/l and the intra- and inter-assay variability coefficients in the measuring range were less than 10%. The analytical detection limit was 0.5 nmol/l and values below 0.5 nmol/l were not reported. Heart rate, blood pressure and subjective stress measures were taken at 0, 15 (during), 30 and 60 min with reference to the stress task using the Omron R5-I. Subjective stress experience was measured with visual analogue scales, ranging from 0 to 100 mm, on tension, mood and tiredness.

Statistical analyses

Repeated measures ANOVA's were performed to test the effect of the stress task on the physiological measures and on memory specificity and experience. Condition (stress vs. control) was set as a between subject variable and valence of the cue words (negative vs. neutral) and time period (remote vs. recent) as within subject factors. Memory experience was tested with 2 multivariate dependent variables; emotional intensity (arousal and physical feelings) and feelings of re-experiencing (reliving, seeing in mind, coherence, remembering-know). To study the effects of cortisol increases on memory specificity, areas under the curve increase (AUCi) for cortisol with respect to baseline ($t = 0$) were calculated (using the equation from Pruessner et al., 2003) and Spearman Rank correlations were performed. The overall α was set at 5%.

Results

Stress outcomes

All physiological measures showed an increase in the stress group over time compared to the control group (see Figure 5.1a-d; cortisol: $F(2, 76) = 13.59$, $p < 0.001$, heart rate: $F(3, 102) = 7{,}97$, $p < 0.001$, systolic blood pressure: $F(3, 99) = 19.42$, $p < 0.001$, diastolic blood pressure: $F(3, 99) = 13.5$, $p < 0.001$). Cortisol levels and the stress-induced increases of cortisol did not differ between groups that started at 12pm or 3pm (control group: $F(2, 36) = 0.23$, $p = .78$; stress group: $F(2, 36) = 0.62$, $p = .54$). The stress group did not report more subjective tension, sadness or tiredness after the stress task than the control group (all $ps > 0.35$).

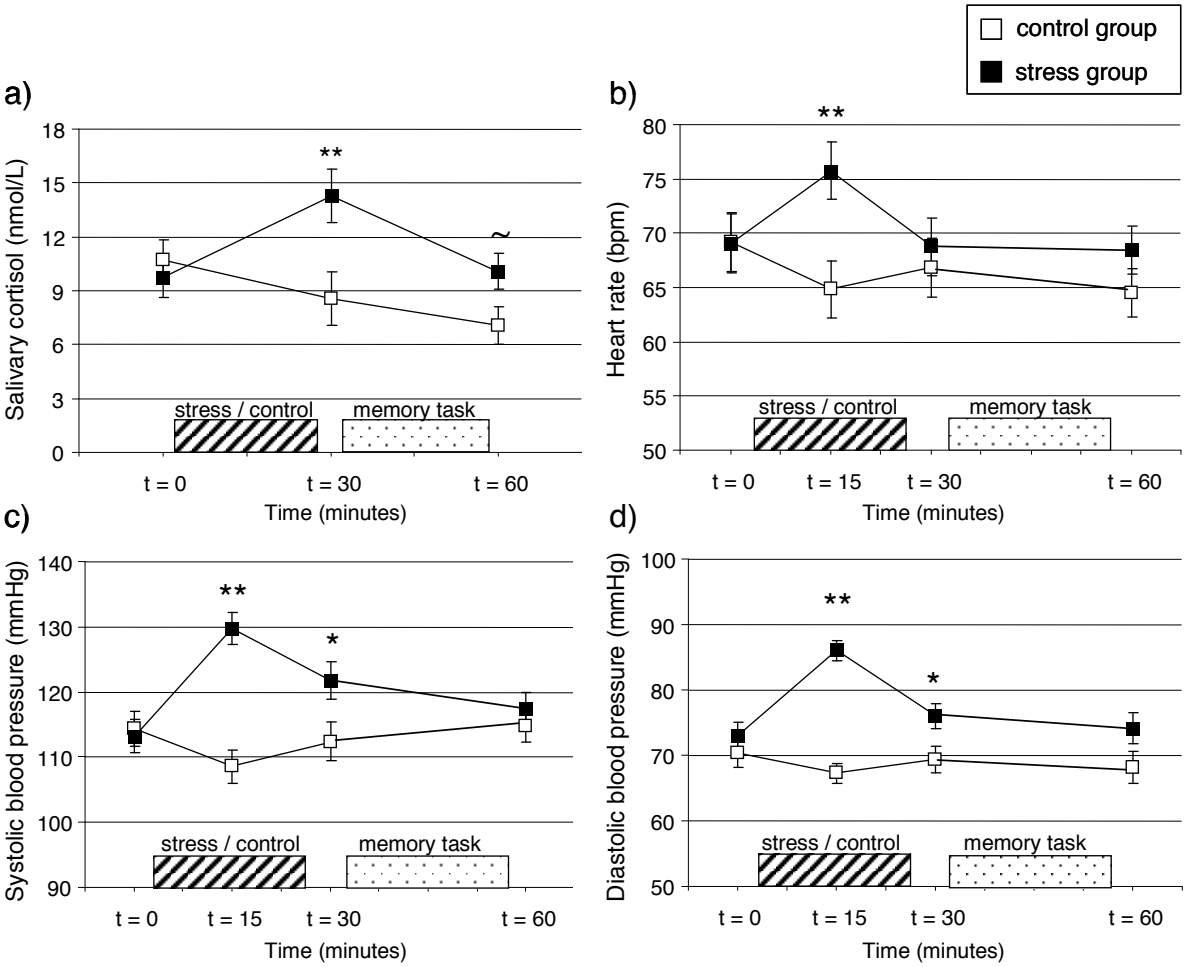

Figure 5.1. Mean (± SEM) levels of (a) free salivary cortisol (nmol/L), (b) heart rate (bpm), (c) systolic and (d) diastolic blood pressure (mmHg) levels from the start till the end of the test session.
*Notes: ** = $p < 0.01$, * = $p < 0.05$, ~ = $p < 0.10$.*

Memory outcomes

No differences were found between the stress and the control group in the number of specific memories retrieved ($F(1, 38) = 0.25$, $p = 0.62$), see Figure 5.2. No interactions between condition and valence or time period were found either ($F(1, 38) = 0.00$, $p = 1.00$; $F(1, 38) = 0.52$, $p = 0.82$). Including time of day (12pm or 3pm) as a covariate did not change these outcomes (all $ps > .56$). The repeated measures ANOVA did reveal a time period by valence interaction ($F(1, 38) = 4.31$, $p < 0.05$). Post hoc analyses showed that within the neutral valence category, remote memories were less specific than recent memories ($F(1, 38) = 8.14$, $p < 0.01$), while the specificity of remote and recent negative memories did not differ ($F(1, 38) = 0.025$, $p = 0.88$). Overall, there were no differences in the specificity of neutral and negative memories ($F(1, 38) = 0.034$, $p = 0.86$).

To control for depressive and anxious symptoms, the scores on the BDI-II and HADS were entered as covariates in the above analyses. These covariates did not affect the main and interaction effects of condition (condition: $F(1, 35) = 0.99$, $p = .33$; condition by valence: $F(1, 35) = 0.03$, $p = .88$; condition by time period: $F(1, 35) = 0.01$, $p = .92$).

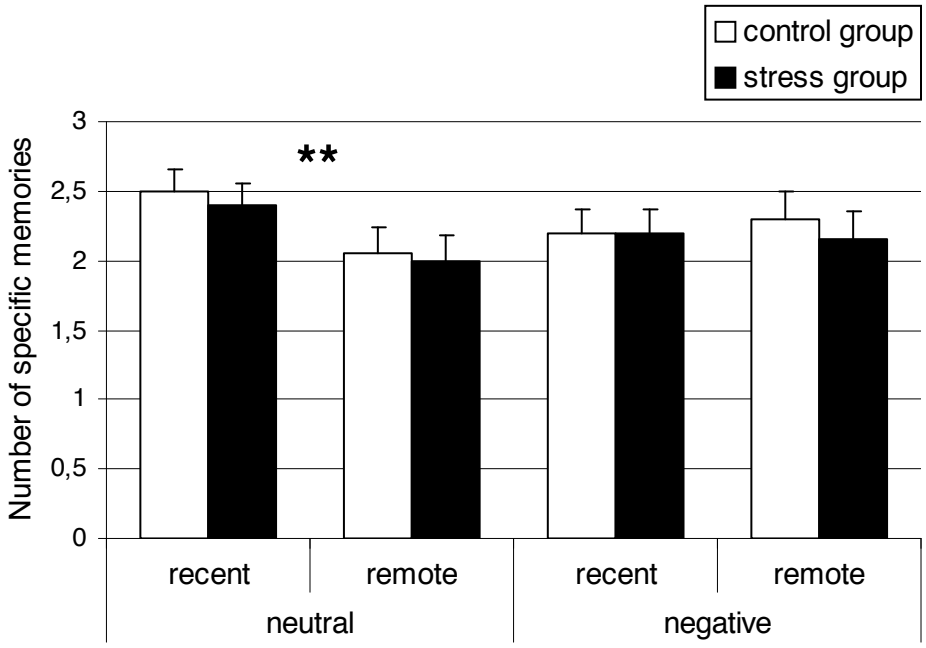

Figure 5.2. Mean number (± SEM) of specific neutral and negative autobiographic memories retrieved by the stress and control group, divided over time period (recent or remote).
*Notes: ** = significant difference between recent and remote neutral memories ($p < 0.01$).*

Memory experience was also not affected by stress. Both the repeated measures MANOVA for emotional intensity and re-experiencing did not show any main effects for condition ($F(2, 35) = 0.53$, $p = 0.59$; $F(4, 33) = 1.34$, $p = 0.28$) or any interaction effects with time period or valence (all $ps > 0.10$). Overall, negative memories were rated as more emotional intense ($F(2, 35) = 33.25$, $p < 0.001$), more negatively valenced ($F(1, 37) = 300.79$, $p < 0.001$), and with higher re-experiencing scores than neutral memories, ($F(4, 33) = 6.09$, $p = 0.001$). Recent memories tended to be rated as more emotional ($F(2, 35) = 3.13$, $p = 0.06$) and were rated with higher re-experiencing scores than remote memories ($F(4, 33) = 11.34$, < 0.001).

Cortisol and memory specificity

No significant correlations were found between memory specificity and cortisol increase (AUCi) within the stress group (see Table 5.2, left column), although there was a trend for a negative correlation between AUCi cortisol and specificity of recent, neutral memories ($rho = -0.39$, $p = 0.09$, see also Figure 5.3a). Since previous studies have shown that cortisol effects on memory are dependent on arousal elicited by the memory or by the environment (de Quervain et al., 2007; Tollenaar et al., 2008a / Chapter 2), we calculated these correlations in the group of subjects that showed heightened sympathetic arousal until the end of the memory task (at $t = 60$), indicated by a systolic blood pressure higher than at baseline (N = 14, see also Table 5.2, right column). When subjects from the stress group were physically aroused till the end of the AMT, the correlation between cortisol increase and specificity of recent, neutral memories was indeed stronger ($rho = -0.67$, $p < 0.01$, see Figure 5.3b). When this

Table 5.2. Spearman Rank correlations, Rho (*p*), between increases in cortisol due to stress and memory specificity on the AMT.

Cue		Group	
		Stress ($N = 20$)	Stress ($N = 14$) with heightened SBP at $t = 60$
Neutral	Remote	-0.06 (.82)	-0.20 (.49)
	Recent	-0.39 (.09) ~	-0.67 (.008)**
Negative	Remote	-0.27 (.25)	-0.22 (.46)
	Recent	-0.04 (.85)	-0.15 (.60)

*Note: SBP = systolic blood pressure, ** = p < 0.01, ~ = p < 0.10.*

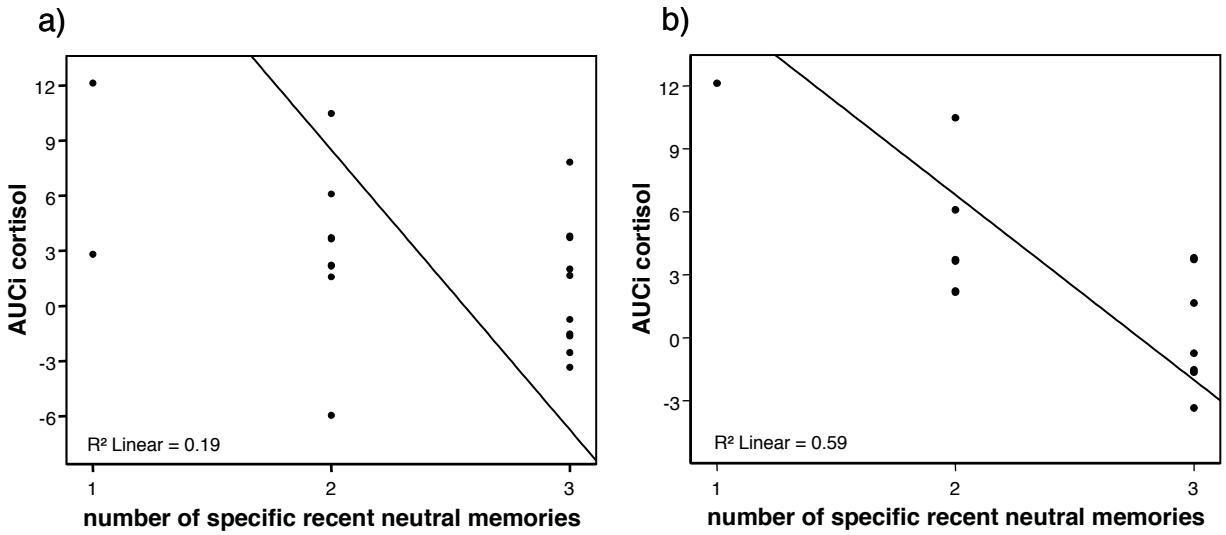

Figure 5.3. Scatter plots of the correlation between specificity of recent memories elicited by neutral cue words and increases of cortisol (AUCi) in (a) the whole stress group ($N = 20$), and (b) the stress group that still showed heightened SBP at $t = 60$ ($N = 14$).

sub-group was limited to subjects that showed a heightened heart rate and diastolic blood pressure at $t = 60$ as well, only 7 participants were left, leaving very low statistical power for correlation analyses, but showing similar results (*rho* = -0.69, $p = 0.08$). No relations between cortisol increase and any of the other types of memory were found in this these sub-groups.

In the scatter plots of Figure 5.3, it might seem that the participants that only retrieved 1 specific recent neutral memory are partly causing the correlations. When removing those participants from the data set, the correlations between cortisol and memory specificity failed to reach significance in the whole stress group but remained significant in the subgroup with heightened systolic blood pressure (whole stress group: rho = -0.29, p = 0.26; subgroup: rho = -0.58, p = 0.04).

Discussion

We found no effects of a psychosocial stressor on autobiographic memory specificity or subjective experience of the memories in healthy young men. This contradicts our expectations based on earlier findings of impaired memory retrieval after stress and cortisol administration, as well as predictions from the CaRFAX model. However, lower specificity of recent, neutral memories tended to be related to a larger increase in cortisol due to stress, especially when participants were physically aroused while retrieving their autobiographical memories. This finding should be interpreted with caution though, since it was found in a small group and we expected to find correlations of cortisol with memory specificity on negative and remote cues as well.

Several rationales can be put forward to account for our null findings regarding the group differences and the single correlation. First, the increase in endogenous cortisol was much lower than e.g. the pharmacologically induced cortisol increase in the study by Buss et al. (2004). In that study cortisol increased in average from 10.01 to 99.13 nmol/l after 10 mg of cortisol administration while in our study cortisol increased on average from 10.25 to 14.28 nmol/L after the stress task. In addition, we used a rough estimate of the cortisol increase during the memory task, as we only measured 3 time points in total that did not reflect the actual levels during the memory task. The area under the curve reflected not only the increase in cortisol but the speed of recovery during the memory task as well. A recent review by Het et al. (2005) has also shown that cortisol effects on memory may be strongest in the morning when natural cortisol levels are high due to diurnal rhythms, while the present study was carried out in the afternoon. In addition, endogenous cortisol increases might only affect autobiographical memory retrieval when physical arousal is high (see also Tollenaar et al., 2008a / Chapter 2). As shown in Figure 1, most sympathetic measures (heart rate and blood pressure) were back to baseline at the end of the autobiographical memory task. In the sub-group of participants that still showed elevated blood pressure levels at the end of the memory task compared to baseline, the association between cortisol increase and the number of specific recent neutral autobiographical memory retrieval was indeed stronger and significant. Furthermore, mood was not affected by the stressor which might be another reason why we did not find group effects, as could be expected on the basis of the functional avoidance mechanism of the CaRFAX model (see Williams et al., 2007) and earlier findings of mood inductions on autobiographical memory retrieval (Au Yeung et al., 2006; Svaldi & Mackinger, 2003). However, effects of stress on declarative memory without decreases in mood have been reported before (Tollenaar et al., 2008a / Chapter 2; Domes et al., 2004). Hence, it cannot be ruled out that the present stress task did not provide a sufficient stressor or high enough cortisol levels to affect autobiographical memory recall, even though participants were physically aroused by the stress task.

Second, this study was conducted among healthy young men and specificity scores were relatively high. The memory traces might have been too strong to be affected by stress and moderate cortisol increases. Studies performed on clinical populations that are characterized by lowered specificity (over-generality) to start with might show stronger effects of stress and cortisol increases on autobiographical

memory. Furthermore, autobiographical memory failures have been linked to the content of the cue words used to elicit autobiographical memories in previously depressed patients (Barnhofer et al., 2007). It is possible that self-referenced cue words trigger more over-general answers under stress. This would be in line with the CaRFAX model (Williams et al., 2007) predicting more abstract-conceptual thinking and rumination when self-schemas are activated. In the present study the stress task might not have elicited enough negative self-schemas, or they were no longer activated during the memory task. In addition, the model by Williams and colleagues predicts that increasing cognitive load during memory retrieval might lead to less available executive functioning capacity to retrieve specific memories. Since the memory task was performed after the stress task, executive capacities might have been sufficient again to perform the task.

A third explanation might be that our null findings are due to power problems. We based our group sizes on the large effect of cortisol on autobiographical memory in the study by Buss and colleagues (Cohen's $d > 1$, group size calculation with GPower 3.0.10), but twenty subjects per group might not have been sufficient to find effects of the stressor, with cortisol increases much lower than in the study by Buss et al. (2004). However, the fact that all F-values for the group effects were smaller than 1 indicates that bigger group sizes would probably not have led to significant effects.

While no effect of the stress task was found on autobiographical memories measured with the AMT, we did find interesting differences in subjective experience and specificity of memories, dependent on the remoteness and emotional tone of the cue words used to elicit the autobiographical memories. Both memories elicited by neutral cue words as well as memories that were instructed to be remote, were rated as less emotionally intense and were re-experienced less intensive than memories elicited by negative cue words and that were instructed to be from the last two years, in line with previous research (e.g. Sutin & Robins, 2007). Interestingly, neutral memories were recalled with less specificity when these memories were remote in comparison to recent neutral memories. In contrast, the recall of remote *negative* events was still accompanied by specific details compared to recent negative events, even when the events had taken place a long time ago. The findings that negative memories are re-experienced more intense than relatively neutral memories, and are still as specific when they are remote as when they are recent, are in line with the common finding that emotionally arousing experiences are generally well remembered. Stress hormones like adrenaline and cortisol, released by emotional arousal, appear to play an important role in enabling the significance of an experience to regulate the strength of the memory of that experience (from McGaugh, 2000).

It is interesting that in previous studies using word tasks, cortisol has been found to impair mostly the retrieval of emotional words (for an overview see: Wolf, 2008), whereas so far in studies on autobiographical memories, neutral memories are affected most (see Buss et al., 2004, and data from the present study). Possibly, emotional autobiographical memories are not as sensitive to the effects of cortisol as recently learned emotional words. This is important for clinical practice where cortisol administration is thought to have potentially beneficial effects by blocking the excessive retrieval of emotional (traumatic) memories, leading to less intrusive memories and PTSD symptoms (de Quervain & Margraf, 2008). It should be noted

though that by using the AMT, autobiographical memory retrieval is assessed by the measure of specificity. It is not possible to conclude whether the memories recalled specifically are accurate or are lacking essential information. After the stress task participants may have felt pressure to perform well on the memory task as well and possibly confabulated autobiographical memories when they could not recall a specific real-life memory. Future studies should therefore try to incorporate accuracy measures of autobiographical memories. Furthermore, in the present study an association was found between cortisol increases and recall of *recent* neutral autobiographical memories and not *remote* neutral autobiographical memories. Possibly, recalling recent specific memories is mediated by different brain processes and areas than the recall of remote specific memories and therefore differently influenced by cortisol. Future studies using functional MRI to study cortisol effects on autobiographical memories could shed more light on this issue.

Finally, stress and mild cortisol elevations might simply not affect autobiographical memory retrieval in healthy young men. The only finding in line with this option comes from a study in depressed patients that also did not find a strong association between higher basal cortisol levels and less specific autobiographical memories, but even report inverted relations, with cortisol decreases relating to less specific autobiographical memories (Barnhofer et al., 2005). Taken together, this study should be regarded as a very first step in investigating the role of stress exposure and endogenous cortisol increases on autobiographical memories. Given the importance of understanding the impaired autobiographical memory in stress-related disorders, such as depression or PTSD, this is a field that needs to be further investigated. In future studies, the effects of stress on memory could be investigated in vulnerable groups, already prone to lowered autobiographical memory specificity. Furthermore, since stress-related disorders are more prevalent in women, female participants should be included in future studies as well, to study the possible differential effects of gender on the relation between stress and autobiographical memory retrieval. Besides specificity, accuracy measures of autobiographical memory should be tried to be included as well. In addition, our study indicates that differences in remoteness and emotionality of the memories are important to take into account when studying the relation between stress, cortisol and autobiographical memory.

Chapter 6

Psychophysiological responding to emotional memories in healthy young men after cortisol and propranolol administration

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Summary

Propranolol is found to reduce physiological hyper-responsiveness in Post Traumatic Stress Disorder (PTSD), possibly by affecting reconsolidation after the reactivation of traumatic memories. Cortisol is found to attenuate declarative memory retrieval, but it is unknown whether it also reduces physiological responses to emotional memories. To examine whether the effects of propranolol on physiological responding to emotional memories can also be found in healthy controls, and to investigate the immediate and prolonged effects of cortisol on physiological responding to emotional memories, we tested these effects in 79 healthy young men. After preparing a script of a negative disturbing memory, participants were instructed to imagine this event one week later after ingestion of either 35 mg cortisol, 80 mg propranolol or a placebo. Physiological responding to the script-driven imagery was recorded. Another week later, after wash-out, the imagery was repeated again. During all 3 sessions as well as 8 months later, subjective emotional reactions to the memories were assessed. The emotionality of the memories was reduced over time, which was not affected by the treatments, however. The personal emotional script did evoke higher skin conductance responses than a neutral story, which decreased one week later, but no effects were found of either propranolol or cortisol on this responsiveness. Whereas healthy males do show psychophysiologic responding to personal emotional scripts, the effects of cortisol and propranolol on physiological responses to emotional memories might be specific to clinical groups characterized by hyper-responsiveness, like PTSD. Future studies using longer-acting doses and more elaborate reactivation procedures in both healthy men and women could shed more light on the effects of cortisol and propranolol on psychophysiological responding to emotional memories.

Introduction

Stress hormones like cortisol and (nor)adrenaline have been found to affect human memory processing (Cahill et al., 1994; Lupien & McEwen, 1997; Wolf, 2008). These effects are dependent on several variables, including the stage of memory processing and the emotionality of the memories involved. Encoding and consolidation stages seem to be enhanced by both elevated cortisol and adrenaline levels (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Cahill & Alkire, 2003; O'Carroll et al., 1999), while they are impaired after blocking adrenaline by means of selective beta-blocking agents like propranolol (Cahill et al., 1994; van Stegeren et al., 1998). On the other hand, retrieval seems to be impaired by increased cortisol levels (de Quervain et al., 2000; Het et al., 2005), while not much is known about the effects of adrenaline manipulation before retrieval (Chamberlain et al., 2006; de Quervain et al., 2007). Regardless of the memory stage, the effects of cortisol seem to be dependent on the emotionality of the memories involved. That is, effects are stronger when memories are arousing (Buchanan & Lovallo 2001; Kuhlmann, Kirschbaum et al., 2005; Kuhlmann, Piel et al., 2005) or when the testing environment elicits enough arousal (Abercrombie et al., 2005; Kuhlmann & Wolf, 2006b; Tollenaar et al., 2008a / Chapter 2). Likewise, blocking adrenergic activation impairs the encoding mostly of emotional material (Cahill et al., 1994; van Stegeren et al., 1998).

As it has been shown that encoding and retrieval can be affected by stress hormones, lately, an increasing interest in manipulating post-retrieval processes has arisen (e.g. Diergaarde et al., 2008; McCleery & Harvey, 2004). If it would be possible to affect memory traces after they have been formed and retrieved, this could improve the treatment of stress and memory related disorders, like Post Traumatic Stress Disorder (PTSD) and phobias (Debiec & Ledoux, 2006; de Quervain & Margraf, 2008). Promising in this view is animal research that has shown that stress hormones like corticosterone (a glucocorticoid that resembles cortisol, but is naturally more abundantly present in rodents), and beta-adrenergic blocking agents like propranolol can affect long-term memory when administered during or after reactivation of the existing memory traces (Abrari et al., 2008; Cai et al., 2006; Debiec & Ledoux, 2004; Maroun & Akirav, 2007; Przybyslawski et al., 1999; Tronel & Alberini, 2007; Yang et al. 2005). Processes that are thought to be influenced by these drugs are post-retrieval mechanisms like extinction and reconsolidation (Suzuki et al., 2004). While extinction may lead to new memories that are formed during habituation to emotional memories (or conditioned anxiety responses), reconsolidation is thought to be a process during which the original memory trace becomes temporarily labile after reactivation, and thus prone to change. If extinction could be enhanced or reconsolidation impaired, it should be possible to attenuate existing (traumatic and anxious) memories.

Based on the above findings, both cortisol and propranolol have been proposed to lead to lasting reductions of emotional memory traces after exposure to traumatic memories and phobias. Moreover, both substances have already been included in clinical trials. Preliminary results have indeed shown that administration of both cortisol and propranolol can diminish PTSD and anxiety symptoms. Namely, peri-operative cortisol administration reduced PTSD symptoms at 6 months after

cardiac surgery (Schelling, Kilger, et al. 2004) and repeated cortisol administration was found to reduce symptoms of re-experiencing and intensity of the traumatic memories in PTSD patients (Aerni et al. 2004) and also to reduce phobic fears (Soravia et al. 2006). Propranolol administered within hours of a traumatic experience was found to reduce subsequent physiologic responding to traumatic memories (Pitman et al., 2002) and development of PTSD symptoms (Vaiva et al., 2003). Although these studies show clinically relevant effects of cortisol and propranolol, the mechanisms through which these substances work are still unclear.

During reactivation two different memory routes could be affected that are not mutually exclusive, 1) declarative memory traces might be weakened and 2) the physiologically arousing components of emotional memories might be attenuated. While the first route is thought to be mostly mediated by the hippocampus and prefrontal cortex, the amygdala is thought to be engaged in the emotional reactions to memories, but these systems are highly interlinked (e.g. Greenberg et al., 2005). Therefore it is also of interest to know whether these routes can be affected separately. In order to test the first possibility, we previously studied the immediate and prolonged effects of both cortisol and propranolol administration on declarative memory retrieval. We found that declarative memory can be impaired long-term when memories are reactivated during high levels of stress (Tollenaar et al., 2008b / Chapter 3) or after cortisol administration (Tollenaar et al., 2009 / Chapter 4), in line with animal research (Cai et al., 2006; Maroun & Akirav, 2007). In contrast, we did not find any immediate or long-term effects of propranolol on declarative memory after reactivation (Tollenaar et al., 2009 / Chapter 4), which is consistent with findings by de Quervain et al. (2007).

In line with the idea that the physiologically arousing components of emotional memories can be attenuated, a recent study by Brunet and colleagues (2008) has shown that post-retrieval propranolol administration diminished physiological responses to script-driven imagery of traumatic memories in PTSD patients. These results might indicate that propranolol is more effective in attenuating emotional components of memories than reducing declarative memory. Het and Wolf (2007) found that cortisol administration in healthy young women led to reduced negative mood after a psychosocial stress task. They suggest that this effect might be mediated by a slight impairment in retrieving the just-experienced negative stress episode and/or from a reduced retrieval of previous negative episodes related to the stressor. This finding indicates that cortisol administration might affect the emotional experience of negative events and thus possibly also of negative memories.

To investigate whether the physiologically reducing effects of propranolol in PTSD patients can also be found in a healthy human population, and to examine whether cortisol has similar properties of attenuating the physiological components of emotional memories, we have conducted the present study. In the present study we investigated the immediate and prolonged effects of both cortisol and propranolol administration on physiological responding to script-driven imagery of negative, disturbing memories in healthy young men after reactivation of these memories, as well as on subjectively experienced emotions to these memories. We expected both propranolol and cortisol to influence post-retrieval processes leading to diminished

physiological responding to the emotional script in comparison to a neutral story one week after treatment.

Methods

Participants

Eighty-five Dutch male students were recruited through advertisements at colleges and the University of Leiden as part of a larger study on memory for which results will be presented elsewhere (Tollenaar et al., 2009 / Chapter 4; Oei et al., *submitted*). Only men were selected because of possible confounding effects of menstrual cycle and contraceptive pills on the relation of cortisol and propranolol treatment with memory (Cahill & van Stegeren, 2003; Kuhlmann & Wolf, 2005). Participants were screened before inclusion. Inclusion criteria were: no reported history of disease or psychiatric problems, no current use of prescribed medication including corticosteroid containing ointments, no chronic disease requiring medical attention including diabetes, allergies and asthma, no use of psychotropic drugs, no alcohol abuse, smoking less than 10 cigarettes per day, age between 18 and 35 years, an estimated Body Mass Index (BMI) between 19 and 26 and blood pressure levels over 100/70 mmHg. Before participation, written informed consent was obtained and after participation participants were rewarded with either course credits or a monetary compensation (40 Euros). The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre.

To minimize influences on baseline cortisol levels, participants were instructed to refrain from drinking any sweet or caffeinated drinks and eating heavy meals on the morning of the second (treatment) session. Furthermore, they were instructed not to eat or drink anything but water, and not to smoke an hour before the second session would start.

Of the 85 recruited participants, 2 men were excluded after the first session due to low blood pressure. Two participants were ill during one of the sessions and one person dropped out after the first session. We excluded one more participant due to a fire alarm on the second session during the imagery task. Hence, 79 participants completed the study. Participants were randomly assigned to one of three experimental groups in a double blind between subjects design (placebo: $N = 26$, cortisol: $N = 26$, propranolol: $N = 27$). Dependent on group, 35 mg hydrocortisone, 80 mg fast acting propranolol or a placebo was administered orally, in identical capsules. Table 6.1 shows the demographic variables of the participants per group. No differences between groups were found for BMI, anxiety (STAI-trait) and general psychopathology (Symptoms Checklist, SCL-90). Age was significantly higher in the cortisol group compared to the placebo group ($t(32) = 2.38$, $p < .05$), due to the fact that the two oldest participants (aged 30 and 32 years) were randomly assigned to the cortisol group. Depression scores on the Beck Depression Inventory (BDI-II) were marginally higher in the control group compared to both the cortisol ($t(44) = 1.74$, $p = .09$) and propranolol group ($t(50) = 1.89$, $p = .07$).

Table 6.1. Demographic variables (Mean ± SD) per treatment group.

	Placebo (N = 26)	Cortisol (N = 26)	Propranolol (N = 27)
Age	19.54 (1.39)[a]	21.35 (3.61)[a]	20.74 (2.21)
BMI	22.13 (2.38)	22.40 (1.98)	21.90 (2.31)
Depression (BDI-II)	6.54 (4.46)[b]	4.69 (3.04)[b]	4.46 (3.41)[b]
Anxiety (STAI trait)	33.81 (9.26)	33.73 (9.08)	31.56 (6.89)
Psychopathology (SCL-90)	28.50 (25.26)	28.73 (23.23)	27.81 (19.88)

Notes: BMI = Body Mass Index; BDI-II = Beck Depression Inventory II; SCL-90 = Symptom Checklist-90; [a] *Significant difference in age between the placebo and cortisol group ($p < .05$);* [b] *Marginally significant difference in depression scores between the placebo group and the cortisol and propranolol group ($p < .10$)*

Psycho-physiological measures

Saliva samples were obtained using Salivettes (Sarstedt, Germany) to measure unbound cortisol and alpha-amylase levels. Alpha-amylase has been shown to be an estimate of adrenergic activity (Nater et al., 2006; Rohleder et al., 2004) and is sensitive to beta-blockage by propranolol (van Stegeren et al., 2005). Saliva samples were stored at -20°C prior to analyses. The saliva samples were analyzed by the Kirschbaum lab, Technical University of Dresden (see Rohleder et al., 2006). One person (from the propranolol group) had a missing saliva sample and 3 people (1 from each group) had a missing alpha-amylase sample. These participants were left out of the RM-ANOVAs with cortisol or alpha-amylase as a factor.

Heart rate and blood pressure were measured with an automatic upper arm blood pressure monitor (OMRON, M6) once before (t = 0) and 3 times after pill ingestion (at t = 75, 110 and 135) to further asses adrenergic functioning. In addition to each physiological recording, participants were given a questionnaire with 7 questions on the intensity of subjective experiences of tension, anxiety, insecurity, irritation, motivation, mood and tiredness. Answers were given on Visual Analogue Scales (VAS) of 100 mm in length, leading to a score from 0 (not at all) to 100 (extremely) on each scale.

During the script-driven imagery procedures, heart rate and skin conductance level (SCL) were continuously measured at 50Hz using a stimulus presentation and physiological analyses software package developed by the University of Amsterdam (VSRRP98, http://www.test.uva.nl/ozi_psychology/index.php?Page=Software). Heart rate was measured with a finger plethysmograph on the non-dominant ring finger. SCL was measured with two 1 cm^2 electrodes attached to the middle phalanx of the index and middle finger of the same hand. SCL fluctuations (SCLfluc) were calculated in Matlab (R2007a) by peak detection on the first derivative of the SCLs after a 2nd order forward / backward 1Hz low pass filter. Because statistical analyses on the SCLfluc responses showed similar patterns to SCL responses we will not report the SCLflucs in the results section. For 9 participants, heart rate was not measured on

one or more time points due to technical failure (placebo: $N = 5$, cortisol: $N = 4$). These participants were left out of the RM-ANOVAs with heart rate responding as a factor.

Questionnaires

The BDI-II (Beck et al., 1996; van der Does, 2002) was administered to assess depressive feelings in the past 2 weeks, a Dutch version of the STAI-trait (Spielberger, 1983) to measure the level of generalized anxiety and the SCL-90 (Arrindell & Ettema, 1986) to assess psychological symptoms and general psychopathology during the last week.

A questionnaire about the personal script consisted of questions on emotional arousal, valence, re-experiencing, fear, anger, sadness, importance and how often one had thought about the event. All were measured on 7-point Likert scales, ranging from 1 (very low) to 7 (very high).

Procedure

Participants came to a lab at the Faculty of Social and Behavioral Sciences in Leiden for 3 sessions. The interval between each session was 1 week (see Figure 6.1 for an overview of the test sessions).

In the first session screening measurements of blood pressure and heart rate were taken with the OMRON after 3 rest periods of 4 min, as well as a baseline measure of heart rate and SCL during a 4 min continuous measurement period. During this first session, a personalized script was prepared in 15 min (according to methodology of: Bremner et al., 1999; Pitman et al., 1987). Participants were asked to write down a negative disturbing event that still triggered emotional feelings of anxiety, anger or fear on a script preparation form in the present tense. Participants also filled in a short questionnaire on the intensity of emotions the memory evoked. After that session, before session 2, the experimenter reviewed the writing and composed and recorded a script approximately 1 min in length for later audio playback.

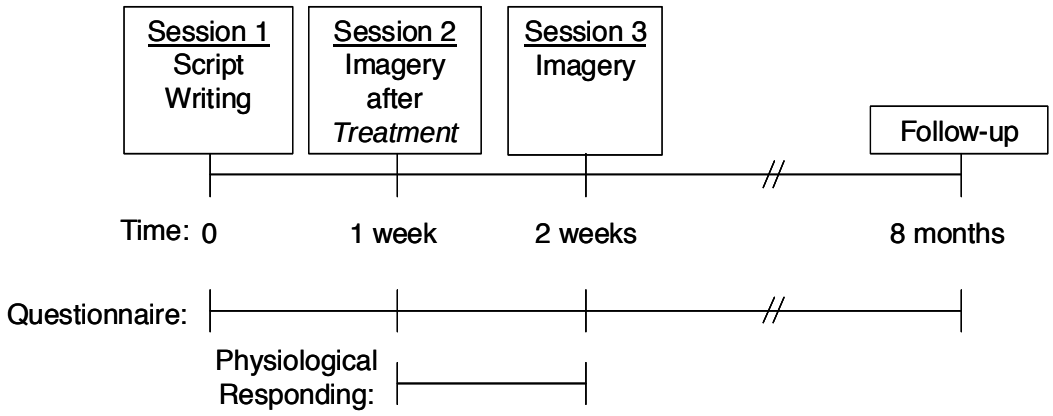

Figure 6.1. Schematic overview of the test sessions.

At the start of the second session, baseline measurements of heart rate and blood pressure were assessed and baseline saliva samples obtained. Participant then ingested a capsule containing placebo, 35 mg hydrocortisone or 80 mg propranolol. During the next 75 min, participants completed several computer based questionnaires and were instructed to remain in the lab and read (reading material was provided). At $t = 75$ mins after ingestion, participants heart rate and blood pressure were again assessed and saliva measurements obtained. At approximately $t = 90$ the script-driven imagery task took place. After a baseline period of 60 s, a neutral script, which was similar for all participants, was played with the VSRRP software program while physiological reactions were measured. Then, for 60 s participants were instructed to imagine the story they had heard. After another baseline period of 60 s, their personal script was played, followed by a 60 s period in which they had to imagine reliving the event. After the imagery task, participant filled in the emotionality questionnaire again. The neutral story was always followed by the personal script to prevent emotions elicited by the personal scripts from persisting into the neutral story. In the third session, the same procedures regarding the imagery tasks were repeated, also followed by the emotionality questionnaire. In an 8 month follow-up telephone interview, participants were asked once more to rate the intensity of the emotions related to their personal memory.

Data analysis

The effects of the treatment (placebo vs cortisol or propranolol) on physiological and subjective measures were analyzed using repeated measure (RM-) ANOVAs with time as within subject and group as between subject factors. Log transformed values were used for cortisol and alpha amylase values to account for non-normality. The change in subjective emotionality of the story over time was analyzed using an RM-MANOVA with group as between subject factor and time (session 1, session 2, session 3, and 8 months follow-up) as within subject factor. For the analyses of the physiological responding to the script-driven imagery, mean SCL and heart rate were calculated for 60 s periods by averaging measurements of 3 consecutive 20 s periods. Reactions to the neutral and personal script were calculated by subtracting the 1 min listening and 1 min imagery periods from the 1 min baseline period before the respective story. This resulted in 2 neutral change scores and 2 emotional script change scores on both sessions 2 and 3 for each group. These change scores were square-root transformed prior to analyses. We applied RM-ANOVAs with group as between subject factor and session (session 2 vs. session 3), emotion (neutral vs. personal script) and part (listening vs. imagining) as within subject factors. In a multivariate RM-analysis we included heart rate and SCL change scores as dependent variables. Then separate RM-ANOVAs were calculated for each dependent change score separately. Greenhouse-Geisser corrected p values were used when indicated by violated Sphericity. Analyses were performed with SPSS 14.0 (SPSS, Chicago, IL). The criterion for statistical significance was $p < 0.05$.

Results

Treatment effects

Cortisol administration induced the expected increase in free saliva cortisol levels, as indicated by a significant time by group interaction between the cortisol and placebo groups ($F(2, 112) = 345.96$, $p < .001$, see Table 6.2). Cortisol did not affect alpha-amylase levels, heart rate and systolic (SBP) or diastolic (DBP) blood pressure (respectively: $F(2, 93) = 2.34$, $p = .10$; $F(2, 87) = 1.86$; $p = .71$; $F(2, 113) = 0.56$; $p = .59$; $F(2, 114) = 1.71$, $p = .18$), see also Figure 6.2a-d.

Propranolol lowered adrenergic activation as expected, indicated by significant time by group interactions between the propranolol and placebo group for alpha-amylase, heart rate and systolic blood pressure (respectively: $F(2, 111) = 3.88$, $p < .02$; $F(2, 101) = 16.25$; $p < .001$; $F(2, 101) = 10.76$; $p < .001$). All measures declined stronger over time in the propranolol group compared to the placebo group (see Figures 6.2a-d). The general decline in adrenergic activation in all groups from $t = 0$ to $t = 75$ might be due to the 75 min restful waiting period. Although diastolic blood pressure also showed a time by group interaction ($F(2, 125) = 4.15$, $p < .02$), post hoc t-test revealed no significantly lower diastolic blood pressure at any of the time points (all *ps* > .09). Propranolol also slightly increased free saliva cortisol over time compared to the control group ($F(2, 94) = 12.74$; $p < .001$, see Tollenaar et al., 2009 / Chapter 4).

No effects of treatment over time were found on subjective feelings of tension, anxiety, insecurity, irritation, motivation, mood and tiredness (all *ps* >.39). We did find an interaction effect of group with time on anxiety, although not significant ($F(5, 186) = 2.21$, $p = .06$), suggesting that the propranolol group reported lower anxious feelings over time compared to the control group. However, post hoc t-tests revealed no significantly lower anxious feelings at any of the time points in the propranolol group versus the control group (all *ps* > .11). Furthermore, all treatments were well tolerated and participants were not aware which treatment they received (Pearson χ^2 (6) = 5.71, $p = .46$).

Table 6.2. Free salivary cortisol in nmol/L (± SEM) in each treatment group.

Group	Time			
	$t = 0$	$t = 75$	$t = 110$	$t = 135$
Placebo	9.11 (0.96)	5.00 (0.45)	4.34 (0.39)	4.58 (0.44)
Cortisol	7.47 (0.71)	206.61 (17.53) [a]	134.79 (9.01) [a]	99.37 (5.55) [a]
Propranolol	8.01 (0.47)	6.38 (0.78)	8.29 (1.05) [b]	9.67 (1.27) [b]

Notes: [a] *Significant increase in cortisol levels in the cortisol group versus the placebo group (p < .001);* [b] *Significant increase in cortisol levels in the propranolol group versus the placebo group (p < .001).*

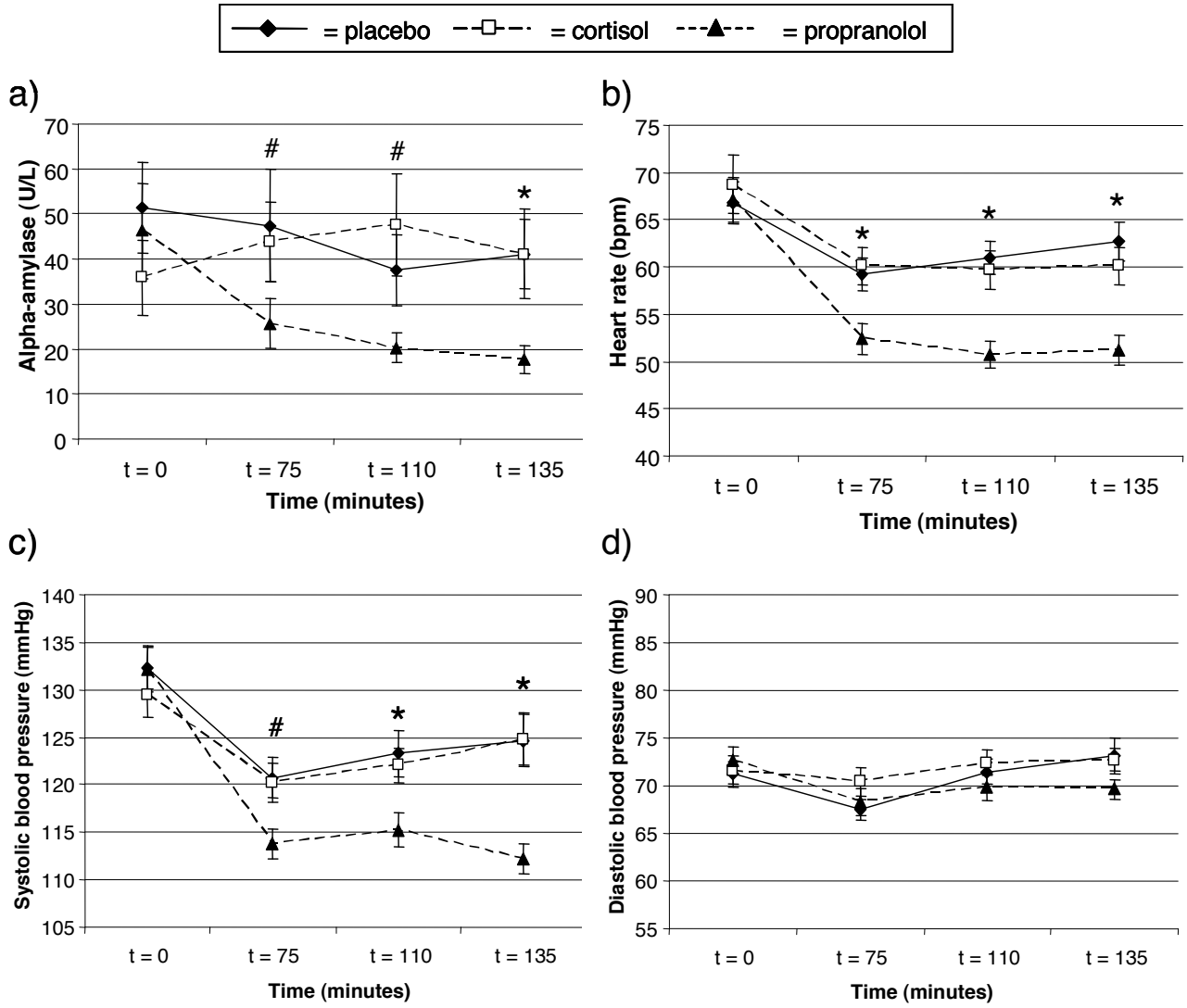

Figure 6.2. Physiological measures (Mean ± SEM) of adrenergic activation on session 2, before treatment (*t* = 0) and after treatment (*t* = 75, 110 and 135 min). a) Alpha-amylase. b) Heart rate. c) Systolic blood pressure. d) Diastolic blood pressure.

*Notes: U/L = Units per Liter; bpm = beats per minute; mmHg = millimeter of Mercury; * = significant difference in the propranolol versus the placebo group (p < .01); # = significant difference in the propranolol versus the placebo group (p < .05).*

Baseline values

In the first session a baseline period was measured for heart rate and SCL. No differences were found between the groups (MANOVA: $F(6, 148) = 0.67$, $p = .61$; separate *ps* > .34, see Table 6.3). The groups also did not differ on any of the emotionality ratings that were given to their personal script on the first session (MANOVA: $F(16, 140) = 0.47$, $p = .96$; all separate *ps* > .16, see Table 6.3).

Emotional ratings over time

To study the change over time in the subjective emotionality ratings related to the personal script, we performed a RM-MANOVA with time (session 1, session 2, session 3, and follow-up) as within subject factor and group as between subject factor. Follow-up was completed by 74 participants. The overall MANOVA revealed no significant group by time interactions ($F(42, 1272) = 0.72$, $p = .91$), but significant declines over time ($F(21, 627) = 5.43$, $p < .001$), reflected in significant Univariate ANOVA tests for all measures (all *ps* < .001, except for Fear $p < .02$). The decline in subjective emotional appraisals was already significant at session three ($F(14, 302) = 3.90$, $p < .001$).

Table 6.3. Mean (± SD) physiological and subjective baseline values on session 1 for the three treatment groups.

Baseline value (Session 1)	Group[a]		
	Placebo	Cortisol	Propranolol
Heart rate (bpm)	65.85 (8.27)	64.33 (8.61)	65.17 (9.54)
SCL (µS)	16.91 (5.54)	18.42 (6.16)	16.18 (6.13)
Arousal	4.04 (1.54)	4.54 (1.61)	4.33 (1.82)
Negative valence	4.81 (1.47)	4.96 (1.56)	5.15 (1.32)
Re-experiencing	4.19 (1.47)	4.31 (1.35)	4.26 (1.38)
Fear	2.54 (1.27)	2.81 (1.58)	2.85 (1.46)
Anger	2.77 (1.68)	3.42 (1.65)	3.37 (1.84)
Sadness	3.77 (1.77)	4.08 (1.85)	4.41 (1.62)
Importance	3.65 (1.92)	4.27 (1.89)	4.63 (1.74)
Thought about	4.19 (1.55)	4.54 (1.68)	4.56 (1.34)

Notes: SCL = Skin Conductance Level; bpm = beats per minute; µS = microSiemens; on the subjective emotionality ratings, minimum scores were 1 (very low) and maximum scores were 7 (very high); [a] *No effects of group on any of the baseline measures were found (all ps > .16).*

Physiological responses to the scripts

Figure 6.3 shows the raw heart rate and SCL responses to both the neutral and personal emotional script in the three groups. For every session responses were divided into the listening and imagery responses. The multivariate RM-MANOVA on heart rate and SCL showed a main effect of emotion ($F(2, 66) = 24.38$, $p < .001$), a main effect of day ($F(2, 66) = 4.84$, $p < .02$), a main effect of part ($F(2, 66) = 36.36$, $p < .001$) and a day by emotion interaction ($F(2, 66) = 5.08$, $p < .01$), as well as a part by day ($F(2, 66) = 3.52$, $p < .05$) and a part by emotion interaction ($F(2, 66) = 5.64$, $p < .01$). No main or interaction effects of group were found (all ps $> .10$), thus not revealing the expected emotion by group or day by emotion by group interactions.

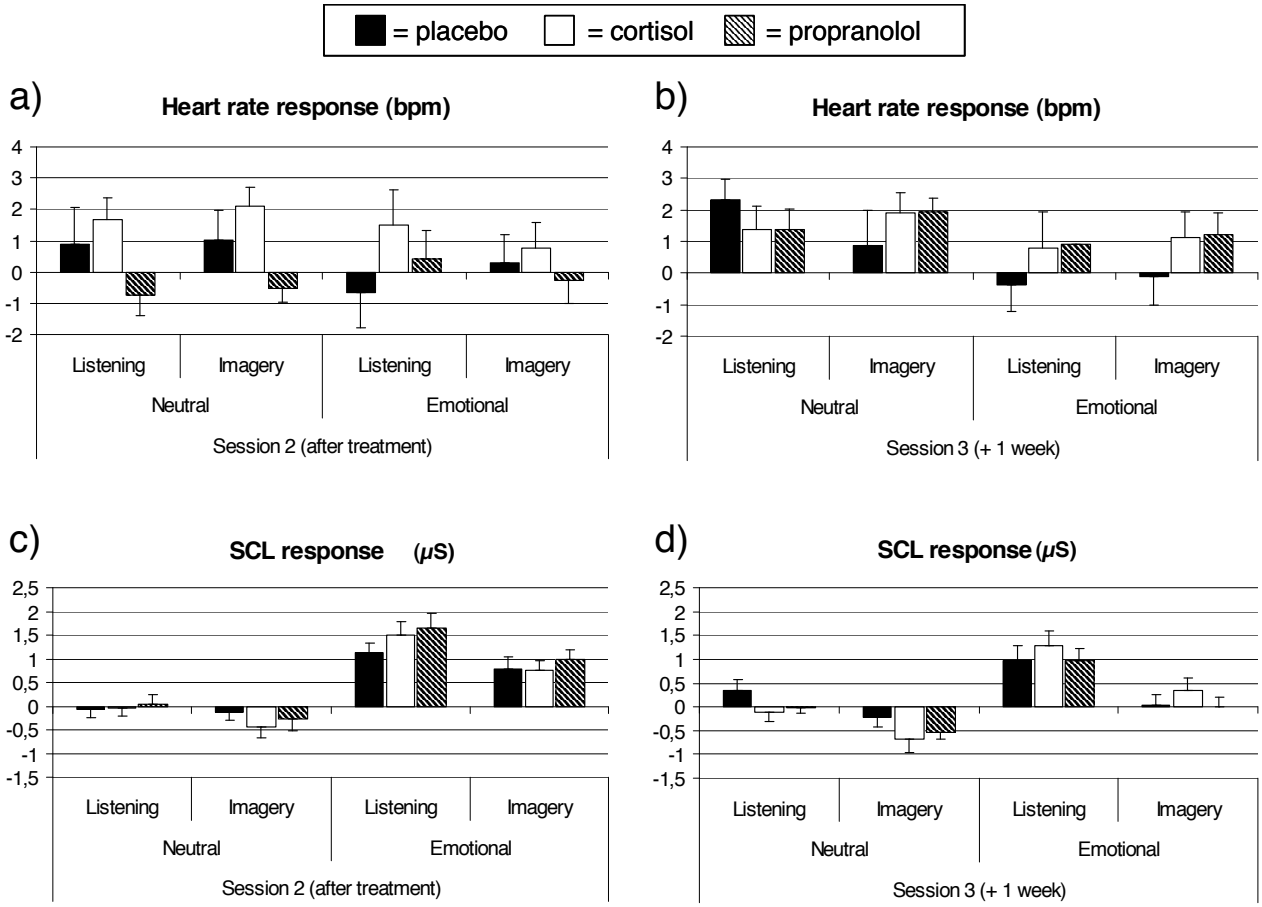

Figure 6.3. Heart rate (a / b) and SCL (c / d) responses to both the neutral story and personal emotional script in the three groups on both session 2 (after treatment - left) and session 3 (1 week later - right). Responses were divided in listening and imagery responses.

Abbreviations: bpm = beats per minute; μS = microSiemens.

The separate RM-ANOVA for SCL responses revealed the same main and interaction effects as in the RM-MANOVA (days: $F(1, 76) = 8.99$, $p < .01$; emotion: $F(1, 76) = 53.01$, $p < .001$; part: $F(1, 76) = 92.95$, $p < .001$; day by emotion: $F(1, 76) = 7.56$, $p < .01$), part by day ($F(1, 76) = 9.43$, $p < .01$) and part by emotion interaction $F(1, 76) = 18.46$, $p < .001$). Overall, the emotional script seemed to elicit higher SCL responses than the neutral story, and on the third session responses were smaller than on the second session. To examine the day by emotion interaction, we split the analyses by emotion. It was revealed that there was no day effect for the neutral story ($F(1, 76) = 0.033$, $p = .86$), but a significant day effect for the emotional script ($F(1, 76) = 13.98$, $p < .001$), with lower SCLs to the emotional script on the third session compared to the second session (see also Figure 6.3c and d). Furthermore, overall responses to the listening part were higher than to the imagery part. To examine the part by day and the part by emotion interactions, we split the analyses by part. During both the listening and imagining part, the main emotion effect was present, although slightly stronger during listening than imagery (respectively $F(1, 76) = 75.04$, $p < .001$ and $F(1, 76) = 31.90$, $p < .001$). The day by emotion interaction was also present for both parts, but the main effect for day was only present for the imagery part ($F(1, 76) = 23.30$, $p < .001$; listening part: $F(1, 76) = 1.22$, $p = .27$), reflecting a greater decrease in physiological response over time for imagery.

For heart rate responses we found no effects of day, emotion or part (all *ps* > .24) as in the RM-MANOVA, but we did find a significant part by emotion by group interaction ($F(2, 67) = 3.16$, $p < .05$) and a trend for a part by day by group interaction ($F(2, 67) = 2.54$, $p = .09$). When breaking the analyses up in the listening and imagery part, we found a marginally significant emotion effect only during imagery ($F(1, 75) = 3.64$, $p = .06$), with higher heart rate change scores for the neutral compared to the emotional story (see Figure 6.3a and b). Furthermore, during imagery we found a trend for a day by group interaction ($F(2, 75) = 2.84$, $p = .07$), revealing that the propranolol group showed higher heart rate responses on session 3 compared to session 2 ($F(1, 26) = 6.98$, $p < .02$), while this affect was not present in the control and cortisol groups (*ps* > .80).

Because differences between groups in age and BDI scores were (borderline) significant, we entered them as covariates in the above analyses. Still none of the expected group effects revealed significance (all *ps* > .29), and the day by group interaction during the imagery part remained borderline significant for heart rate ($F(2, 72) = 3.01$, $p = .06$).

Discussion

The present study examined the immediate and prolonged effects of both cortisol and propranolol administration on physiological responses to script-driven imagery of negative, disturbing memories in healthy young men after reactivation of these memories. No diminishing effect of either propranolol or cortisol on psycho-physiological responding to the script driven imagery of emotional memories was found. The subjective emotional experience of the memories was not affected by cortisol or propranolol either. Even though propranolol was found to attenuate

physiological responding (heart rate and SCL) to traumatic scripts in PTSD patients (Brunet et al., 2008), we did not find such effects to negative emotional scripts in healthy young men. These results also contrast the finding by Het and Wolf (2007) that cortisol can affect the emotional experience of negative events and the finding that cortisol can reduce the intensity of traumatic memories (Aerni et al., 2004). However, methodological differences between these studies and our study might have caused these conflicting findings, as we will discuss below.

While our hypotheses regarding cortisol and propranolol were not confirmed, we were able to evoke physiological responses to personal emotional scripts in a healthy male population. That is, the emotional scripts led to significantly higher SCL responses than a neutral story, although not to any significant increases in heart rate. Heart rate even seemed to be lower during imagery of the emotional script than during the neutral script. This is in line with previous research showing heart rate decelerations during attention to emotional (auditory) stimuli in healthy humans (Bradley & Lang, 2000; Palomba et al., 1997; van Stegeren et al., 2002). Within the propranolol group heart rate responses to the neutral and emotional script were higher one week after treatment than during treatment. This is probably due to the fact that propranolol administration on the second session lowered heart rate and adrenergic functioning overall, and might thus have led to lower heart rate responses in the second session. Therefore, these sessions cannot be compared validly. Overall, the SCL responses were higher when participants listened to their script than when imagining the script, and responses to imaging decreased more over time. This might be important for future studies using script-driven imagery tasks to take into account, since the two processes could be differently affected by drug treatment.

Furthermore, the subjective emotional and arousal responses to the memories decreased steadily over time, from session 1 to session 3 and 8 months later, and likewise the SCL responses to the emotional scripts decreased from the second to the third session. However, while physiological reactions to the emotional memories might have diminished over time, participants might also have been less surprised the second time they heard their story (on the third session). That is, even though subjects knew their memory would be part of the study, on the second session they were not aware they would hear an audio version of it.

There might be several reasons why cortisol and propranolol did not attenuate physiological responses to script-driven imagery of negative, disturbing memories in healthy young men after reactivation of these memories. First of all, heart rate was not heightened in response to the emotional script in the placebo group on either of the two sessions, and SCLs were not heightened in response to emotional imagery in the placebo group on the last session. Therefore, cortisol and propranolol could only act on SCL responses during the second (treatment) session and on SCL responses to the listening part during the third session, on which we expected the reconsolidation effects. The negative disturbing events that the young, healthy males in our studies had described were probably much less intense than traumatic memories in PTSD populations and hence attenuation of physiological responding might not have been possible due to floor effects. However, we did elicit consistent SCL responses during listening to the emotional scripts and these negative memories were described as overall important and were thought about more often than other memories. This might

lead to consideration of a second possibility, namely that the dose was too low to affect the physiological responding. In the studies by Brunet et al. (2008) and Pitman et al. (2002) multiple doses or longer-acting propranolol were used. These studies involved patient groups that are characterized by high blood pressure and heart rate levels overall, and therefore larger doses of propranolol might be needed to reduce physiological responding. However, reconsolidation of emotional memories could possibly take longer than a few hours, and longer-acting doses might therefore be needed to affect these reconsolidation processes. Cortisol on the other hand, was given in lower doses in the clinical studies by Aerni et al. (2004) and Schelling, Kilger, et al. (2004), but the doses were given daily instead of once, leading to active cortisol during potentially multiple memory reactivations. While in the present study the dose may have been too low or short-acting to obtain reductions in physiological arousal in response to personalized scripts, in the same population as the present study we did find long-term impairing effects of the 35mg cortisol dose on declarative memory retrieval (Tollenaar et al., 2009 / Chapter 4). Taken together, this might lead to the possibility that cortisol only affects declarative memory retrieval and not the physiologically arousing components of the memory. Perhaps longer-acting doses or more frequent administration of cortisol and propranolol might lead to attenuating physiological responding in a healthy population as well.

Our study differed in several aspects to the study in PTSD patients by Brunet et al. (2008). As mentioned, different and longer-acting doses of propranolol were used than in our study. Furthermore, in the study by Brunet et al., propranolol was given after the script preparation procedure that was used to reactivate the event. In our study, the script was prepared a week before drug administration and only the listening to, and imagining of, the memory, which lasted 2 minutes, was used to reactivate the memory under the influence of the drugs. This might have been a suboptimal reactivation procedure to affect reconsolidation. In addition, reactivation of the memory a week before treatment might have promoted extinction of, or habituation to, the memory. Hence, it cannot be ruled out that the reactivation procedure was not optimal to find immediate or prolonged effects of cortisol or propranolol on physiological responding.

Another issue that merits consideration is the timing of the drugs. In the present study we administered cortisol and propranolol before reactivation. This way both retrieval and post-retrieval processes are within the active time window of the drugs. In animal research these substances are usually given after reactivation to only affect post-retrieval processes. In the study by Brunet et al. (2008) a similar post-reactivation approach was taken, although in the clinical trials mentioned in the introduction (Aerni et al., 2004; Pitman et al., 2002; Soravia et al., 2006; Vaiva et al., 2003), cortisol and propranolol were administered during a longer time span or before retrieval as well. The fact that in this study drugs were active during both retrieval and post-retrieval processes could potentially explain our non-results with regard to the prolonged effects of cortisol and propranolol. That is, if memory retrieval is reduced by cortisol or propranolol, the emotional memories might not be sufficiently reactivated and hence reconsolidation processes could possibly not be blocked. However, psychophysiological responding under the influence of cortisol did not seem to be affected, indicating the emotional memories were adequately reactivated at

the time of the treatment. Propranolol, on the other hand, did lower heart rate responses during treatment, which may have reduced the possibility to affect reconsolidation processes.

A last factor to take into account is that we studied only males. Females might be more reactive to imagery of emotional memories or more sensitive to the effects of cortisol and propranolol, although in the clinical studies no gender effects are reported. Het and Wolf (2007) did find attenuating effects of cortisol on the experience of a negative emotional event in healthy women while we did not find such effects on negative emotional memories in men, suggesting gender may indeed play an important role. Our negative findings on cortisol and propranolol could also be due to power problems. However, our group sizes compare well to the clinical studies, and as reported above before we did find an impairing effect of cortisol on declarative memory retrieval in the same population.

We were not able to find an attenuating effect of cortisol on physiological responding to memories in healthy men, but whether cortisol can attenuate physiological responding in PTSD remains unknown. Potentially the attenuating effects of cortisol are only present in individuals that are hyper aroused. Future studies using cortisol or propranolol in healthy populations could use different or longer-acting doses, more frequent administration or different timing protocols with regard to reactivation of the memories. Future studies could also investigate more elaborate reactivation paradigms or consider vulnerable populations to elicit higher emotional responses to the memories. In addition, both males and females should be included in future investigations. Conditioning paradigms would also be a good way to measure and replicate animal studies on post-retrieval processes. At this point there are only preliminary data available, showing reducing effects of propranolol on a conditioned fear response in healthy subjects when administered during reactivation of the fear memory (Miller et al., 2004).

To conclude, the present study was able to measure physiological responding to script-driven imagery of emotional memories on two consecutive occasions in healthy young men, reflected in heightened SCL responses and lowered heart rate responses. Furthermore, we measured the subjective emotional responses to these memories over a long time span of 8 months. Reductions in emotional appraisal of the memories were shown within 3 weeks and even further up to 8 months. We did not find any immediate or prolonged effects of either cortisol or propranolol on these physiological and subjective measures. We might conclude that the effect of propranolol on physiological responses to emotional memories is specific to clinical groups characterized by hyper responsiveness, like PTSD, although differences in study designs might partly explain these divergent findings. Furthermore, the effects of cortisol on physiological responses to emotional memories in clinical groups should still be explored, in addition to its effect on declarative memory retrieval. More knowledge on the mechanisms behind propranolol and cortisol in treating disorders like PTSD and phobias might lead to more efficient and safe use of these drugs (for discussions see Glannon, 2006; van Stegeren, 2005).

Chapter 7

General Discussion

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 my 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.

What have we learned about emotional memory retrieval?

In the introduction we hypothesized that cortisol might impair the retrieval of emotional memories, and its effects are thought to be mediated by the hippocampus. This could also lead to a less intense emotional experience of the memories. While we indeed found cortisol to impair declarative memory retrieval (chapter 2 and 4), we did not find cortisol to affect the emotional experience of personal, autobiographical memories (chapter 3 and 6), including the physiological responding to these emotional memories. Potentially, the memories can still be judged and experienced as emotional, even though they lack in (contextual) content. This might be due to retrieval mechanisms modulated by areas other than the hippocampus such as the amygdala and the prefrontal cortex (Kensinger & Corkin, 2004; LeDoux, 2000).

Propranolol has been found to affect the learning of emotional material (Cahill et al., 1994; van Stegeren et al., 1998) and might also reduce the strength of an emotional memory trace after its reactivation (as was found in animal studies: Debiec & LeDoux, 2004; Przybyslawski et al., 1999). However, we did not find immediate or delayed effects of propranolol on declarative memory retrieval (chapter 5). Neither did we find any effects of propranolol on subjective and physiologic responding to emotional memories (see chapter 6). While animal and patient studies have both shown a reduction in physical responses to emotional memories when propranolol was administered after reactivation, we could not find such effects in healthy human subjects. Even though differences in design might account for our non results, it may also be that the memory task we used did not elicit enough emotional arousal to begin with. Furthermore, propranolol might be more active in diminishing arousal responses to fear memories. Animal studies are usually based on fear conditioning paradigms and in PTSD, fear conditioning to reminder cues of the trauma might be in place. The memories that were recalled in our healthy group probably did not elicit high fear responses. So the possibility remains that propranolol might be more active in amygdala-dependent tasks compared to the (more hippocampus-dependent) memory tasks used in our studies. Fear conditioning paradigms in healthy subjects might shed more light on this issue, especially combined with declarative knowledge on such cue related fears.

As for the discussion on the differences between retrieval of recent and remote memories, the present studies have provided minimal information. Previously we discussed the difference in specificity between recent and remote neutral and emotional autobiographical memories (chapter 5). Furthermore, even though in chapter 2 a distinction was made between recent and remote memories, the effects of stress were difficult to compare as the retrieval of recent memories reached a ceiling effect. In chapter 3 we did find a contrasting long-term effect of stress on the retrieval of both recent and remote memories, indicating that at different time intervals stress can either have a beneficial or detrimental long-term effect. This could be due to either consolidation or reconsolidation mechanisms that are differentially effective during the retrieval of recent and remote memories, and also to different brain areas supporting the retrieval of these memories. Again, future studies employing neuro-imaging techniques may provide more insight into these underling mechanisms.

We also sought to investigate whether autobiographical memories are as vulnerable to stress as episodic memories that were created in the lab. Autobiographical memories might be less vulnerable as they are usually based upon strong, often repeated, and well remembered events. On the other hand, they often have an emotional component to them which theoretically makes them more vulnerable to cortisol increases (according to Roozendaal's model). The one study we did on the effects of stress on the retrieval of autobiographical memories did not confirm this hypothesis. The only memories that were slightly less specific due to cortisol increases were recent *neutral* memories. Potentially these memories are the least consolidated (compared to remote memories and emotional memories) and therefore most vulnerable to cortisol increases. Whether these effects are mediated by the hippocampus or more prefrontal areas remains to be studied. It does show that it is not that easy to generalize findings on laboratory word tasks to real life memories. As recently many neuro-imaging studies are performed on the retrieval of autobiographical memories, it should be relatively straightforward to incorporate measures of stress hormones levels or to study the effects of cortisol administration in combination with such techniques.

Could and *should* we use cortisol or propranolol in clinical practice?

Findings so far

In the introduction and in chapters 5 and 6 we have mentioned several clinical studies that investigated the effects of cortisol and propranolol on the development and treatment of post-traumatic stress disorder (PTSD), as well as mood and phobic disorders, based on the effects of these drugs on memory. So far, these studies have indicated that propranolol might lead to a reduction in the development of PTSD or to a reduction in the symptoms of PTSD (Brunet et al., 2008; Pitman et al., 2002; Vaiva et al., 2001). Cortisol is found to reduce the development of PTSD (Aerni et al., 2004; Schelling, Kilger, et al., 2004; Weis et al., 2006) and to lead to a reduction in phobic fears (Soravia et al., 2006) and mood (Het & Wolf, 2007). It should be noted though that many of these studies can be considered as pilot studies. For example, some studies were not randomized (Vaiva et al., 2001), showed very small effect sizes (Pitman et al., 2002), or were performed in very small groups (Aerni et al., 2004). Furthermore, only Aerni et al. and Brunet et al. studied the effects of these drugs after PTSD had already developed. Studies on the effectiveness of cortisol and propranolol in the treatment of PTSD are thus still in a very early stage. Despite the preliminary conclusions that can be drawn from these studies, the results are promising. However, the mechanisms by which cortisol and propranolol are beneficial in treating these disorders (i.e. by way of reducing memory retrieval and reconsolidation) are still only speculative (de Quervain, 2007; de Quervain & Margraf, 2008; Diergaarde et al., 2008; McCleery & Harvey, 2004; Schelling et al., 2004) and very much in need of empirical proof. That is, it remains to be seen whether these drugs actually treat or augment treatments of these disorders by means of affecting memory. Our data suggest that in healthy men, cortisol might lead to reduced memory retrieval of both neutral and emotional memories created in the laboratory and to reduced specificity of neutral autobiographical memories. Whether cortisol can also reduce the retrieval of

highly emotional autobiographical memories in patient populations remains unknown. Moreover, if neutral memories would be affected during treatment as well, this might be an unwanted side-effect. Furthermore, we did not find propranolol to affect declarative memory retrieval, and neither to reduce the emotional experience of autobiographical memories in healthy men. While traumatic memories might be differentially affected by propranolol, none of the clinical studies has actually measured declarative recall of the traumatic memories. As there is increasing interest in this area of research, fundamental memory studies and clinical trials might advance well by joining their efforts.

Memory effects vs. other effects

As mentioned above, cortisol and propranolol might potentially augment treatment of PSTD by blocking excessive retrieval of emotional memories and/or by reducing the reconsolidation of these memories. In some respects, propranolol seems a more natural way to reduce emotional memory recall. Propranolol is thought to attenuate the emotional experience of emotional (traumatic) memories, possibly without directly affecting declarative memory, while cortisol is found to directly impair the recall of emotional memories, which might be less beneficial. However, both drugs seem to contradict the idea of exposure, a method that has long been used in the treatment of PTSD and phobias (McCleery & Harvey, 2004). While full exposure (in combination with cognitive therapy) can lead to better processing of distressing memories, blocking full exposure to emotional memories seems unwanted. That is, suppressing emotional memories may reduce distress in the short-term, but can lead to maintenance of PTSD symptoms in the long-term (Holmes et al., 2007). However, the first clinical studies with propranolol and cortisol have indicated no such effects. Potentially, cortisol and propranolol exert positive clinical effects via other mechanisms than attenuating memory.

It might well be that cortisol administration can help to restore cortisol imbalances found in psychiatric disorders. Namely, it has been found that PTSD patients show lower endogenous cortisol levels (Bremner et al., 2003; Mason et al., 1986; Yehuda, 2001; Yehuda et al., 1998), although not all studies show this effect (Pitman & Orr, 1990; Shalev et al., 2008; Young & Breslau, 2004), and also that reduced cortisol reactions to traumatic experiences predict the development of PTSD (Delahanty et al., 2000; Yehuda et al., 1998). Abnormalities in HPA axis functioning might contribute to the development of this disorder (de Kloet et al., 2005; Olff et al., 2006; Yehuda, 2001) and cortisol administration could potentially normalize the system (e.g. Yehuda et al., 2007). On the other hand, depression is mainly characterized by heightened cortisol levels (Young, 2006; Young & Breslau, 2004) and is highly comorbid with PTSD. These disorders might thus each need a different approach to treat and in the case of comorbidity, more should be known with regard to the underlying HPA axis abnormalities before treatment with cortisol is considered (Yehuda et al., 1996). Dysregulation of noradrenergic functioning has also been implicated in PTSD (Elzinga & Bremner, 2002; O'Donnell et al., 2004; Yehuda et al., 1992), and propranolol might reduce the hyperactivity of the noradrenergic neural networks, including the amygdala.

Another possible mode of action for cortisol and propranolol might be the prefrontal cortex. PTSD patients might have a lowered activity of the prefrontal cortex, which could lead to hyperactivity of the amygdala and to more intrusions by emotional memories (Elzinga & Bremner, 2002; Shin et al., 2004). Cortisol administration has been found to reduce emotional distraction during working memory performance in healthy men (Oei et al., *submitted*), indicating it might affect the interaction between prefrontal and amygdalar functions. Imaging studies should shed more light on this finding. Propranolol might reduce the impairing effects of high levels of noradrenaline on prefrontal functioning (Arnsten, 1998). Furthermore, besides affecting memory retrieval and reconsolidation, cortisol might also lead to a better consolidation of traumatic memories, especially when administered soon after the traumatic event (as was done in most clinical studies described above). Traumatic events that are stronger consolidated might lead to less fractioned memories that are better processed.

Lastly, cortisol has been found to enhance extinction of fear memories in rodents (Cai et al., 2006; Yang et al., 2005). That is, when given during or after memory reactivation, fear associations become less strong. As problems in the extinction of conditioned fears are thought to be part of PTSD development (Blechert et al., 2007; Garakani et al., 2006), cortisol might also have beneficial effects by working on this mechanism. Likewise, propranolol might enhance extinction instead of suppressing reconsolidation, although animal studies explain the fear reducing effects mostly in the light of reconsolidation.

To summarize, both cortisol and propranolol might be beneficial for the treatment of PTSD through mechanisms other than their influence on memory retrieval and reconsolidation. This makes these drugs even more interesting for clinical practice, but also for fundamental research studying the relation between neuro-modulators and cognitive and emotional functioning.

Advantages vs. disadvantages

While so far it remains unclear whether the assumed beneficial effects of cortisol and propranolol in the treatment of PTSD are due to their impact on memory retrieval and reconsolidation, it is important to realize the potential ethical concerns surrounding this issue. As mentioned in the introduction, it is important to know what happens to emotional memories after treatment with these drugs. The studies described in the present thesis suggest that declarative memory retrieval is blocked by cortisol and can have lasting effects, as was found for the retrieval of both neutral and emotional memories. It might not be desirable that patients forget parts of the traumatic experience(s). Either because it may lead to less integration of the event with personal experiences and schemas of the world, or because it raises the ethical question whether we may ‘erase’ memories. However, patients themselves might wish nothing more than to forget the event they experienced. It should be mentioned here that so far the impairing effects of cortisol have only been shown on laboratory memory tasks, and the extent to which it can impair autobiographical and traumatic memories remains to be investigated. Likewise, the extent to which propranolol actually affects the recall of the declarative aspects of emotional memories and their subjective evaluations in the aftermaths of trauma is still to be elucidated.

When considering whether or not we should be able to change memories, it has to be noted that within cognitive therapy, memories are also being restructured and even imagery rescripting is used to change automatic thoughts and feelings associated with the traumatic memories. Cognitive behavioral therapy has even been shown to modify the neural circuitry associated with anxiety disorders (Paquette et al., 2003). Cortisol and propranolol could aid or accelerate these processes. Therefore, combinations of therapy and pharmacological treatments with cortisol or propranolol (or other drugs implicated in reconsolidation: see Diergaarde et al., 2008) should be studied as well.

So as to conclude whether we could and should use cortisol and propranolol in clinical practice, we advise that more research should be done on the working mechanisms of these drugs and the possible (long-term) benefits and risks before they become routinely used in clinical practice. By incorporating well designed memory tasks in clinical trials besides testing clinical symptoms, a first step could be made. However, in cases where short-term gains may outweigh long-term side-effects, these drugs (that both have been safely used before in treating physical diseases) could prove to be valuable in the near future.

Suggestions for future research

Research often provides new questions and as seen in the previous chapters there are still many issues to be resolved. We here suggest several areas that are of specific interest in the study of stress hormones and memory retrieval.

The impact of stress and stress hormones on memory retrieval has mostly been studied in homogenous male populations, and the present thesis did so as well. As mentioned in the introduction, we acknowledge this as a shortcoming, since most stress related disorders (like PTSD and depression) are more prevalent in women than men. However, studying these effects in a homogenous group is a first step in further understanding the relation between cortisol and memory. As the results of these studies might be of interest to clinical practice, where a large part of patients is female, these studies need replication and comparison in females.

With respect to stress hormones, the present thesis has focused on cortisol and the interaction with sympathetic arousal. In addition, the effects of blocking (nor)adrenergic activity by means of propranolol was studied. However, the role of noradrenalin increases on the retrieval and reconsolidation of (emotional) memories in humans still remains to be investigated. This could provide more insight into the pathophysiology of PTSD in which an overactive noradrenergic system might be related to emotional memory intrusions (Elzinga & Bremner, 2002; Pitman et al., 2000; Pitman & Delahanty, 2005). On the other hand, the effects of suppressing cortisol levels on memory retrieval may also be of interest, as hyper-reactivity or high basal cortisol levels have been found in psychiatric disorders like depression. In this regard it may also be fruitful to precisely examine which cortisol levels or doses are impairing and which are beneficial for emotional memory retrieval, as cortisol effects on memory have been found to be inverted U-curve dependent (Lupien & McEwen, 1997). Furthermore, other hormones that are involved in the stress response

(mediating the cortisol outcomes), like corticotrophin releasing hormone (CRH) and adrenocorticotropin hormone (ACTH) might be directly involved in memory processing (Croiset et al., 2000; Pitman et al., 2000; Pitman & Delahanty, 2005) and could be related to memory functioning as well.

With respect to methodological issues regarding memory measurement, more precise measures of autobiographical memories should be used that measure memory on different and additional levels than specificity. Above, we referred to current paradigms used in neuro-imaging studies, which include memory tasks using real life pictures and temporal studies of memory retrieval. Even more precise studying of autobiographical events could aid in studying more subtle memory effects (e.g. using the Autobiographical Memory Interview: Kopelman et al., 1990). Future studies on (autobiographical) memory retrieval should try as much as possible to combine measures of accuracy, subjective emotional experience and physiological responding to the memories, instead of using different tasks (as was done in the present thesis).

Another promising area will be the study of conditioned fear memories in combination with declarative fear memories. Studying which brain areas are mostly involved in these memory processes, as well as the sensitivity of these processes to both cortisol and propranolol could shed more light on the mechanisms of these two drugs in clinical practice.

An important aspect that has not been discussed so far is the role of context in the effects of cortisol and propranolol on (conditioned and declarative) memory retrieval. Extinction has been found to be sensitive to the context in which the memories/associations were learned and reactivated (Bouton, 2004, Bouton et al., 2006; Effting & Kindt, 2007). Long-term effects of cortisol on memory retrieval might also only be found in the same context as the one in which the memories where reactivated. In the study from chapter 5, all testing was done in the same lab, with the same experimenter for each participant. Even though we found long-term effects of cortisol and stress on memory retrieval, it might very well be that in a different context / setting, memory retrieval would be renewed. However, the follow-up in the study from chapter 3 was done via a telephone interview. Every participant was thus in a new context when long-term memory effects were assessed and found. Future studies investigating the long-term effect of stress hormones on memory should try to assess the effects of context change as well.

Findings on memory reconsolidation and the involvement of stress hormones in this process in animals, mostly rodents, have given a great advantage to research in humans. Animal models can be tested and memory paradigms can be imitated. Important in this respect is to mention that animals studies on reconsolidation use slightly different timing protocols for the administration of drugs around the retrieval of memories when studying reconsolidation than we have done in the present studies. Reconsolidation effects of propranolol in rodents have only been found when given after memories were first fully activated, and likewise extinction effects of cortisol were found to be strongest after full reactivation of the memories. When given before retrieval, less clear results were found (Cai et al., 2006). In our studies we gave propranolol and cortisol before retrieval, thereby not differentiating between effects on retrieval and post-retrieval processes. Retrieval was furthermore affected by cortisol, leading to incomplete reactivation and potentially to a less optimal situation

for cortisol to affect post-retrieval processes like reconsolidation. However, in clinical practice it is easier to administer drugs on a continuous basis or before treatment, than to exactly time the drugs to be active just after exposure treatment or every time a patient has remembered or relived the traumatic event. Our studies have shown that at least for cortisol, the administration before retrieval can have long-term attenuating effects, but future studies could investigate whether administration of cortisol or propranolol after reactivation can lead to stronger memory effects.

Lastly, it is important to remember that individual differences can play a role in memory processing, stress reactions and possibly in the interplay between the two. Innate differences in the development of brain areas related to memory abilities, as well as in the psychological and physiological reactivity to stress may make some individuals more vulnerable to stress and memory problems than others. For example, personality traits like neuroticism and extraversion have been associated with brain activity in response to emotional stimuli (Canli et al., 2001) and self-esteem was found to relate to cortisol responses to stress (Pruessner et al., 1999). Genetic variations (e.g. the BDNF val66met polymorphism) have been related to differences in memory performance (Goldberg et al., 2008) and brain activity (Hariri & Weinberger, 2003), and may explain relations between hippocampal activity and memory performance (Egan et al., 2003; Hariri et al., 2003). Genetic differences might also cause exaggerated or blunted cortisol and adrenergic responses in reaction to life stressors (de Rijk et al., 2006; Wust, van Rossum, et al., 2004), potentially making certain people more vulnerable to the development of psychiatric disorders and cognitive problems (McCleery & Harvey, 2004; Wust, Federenko, et al., 2004). In this respect it is also important to note that the relation between stress and memory is not strictly causal. While stress may cause memory problems, memory capacity can also influence responses to stress. For example, working memory capacity is related to the inhibition of emotional intrusions (Brewin & Beaton, 2002; Wessel et al., 2008) and memory deficits are found to be predictive of the development of PTSD (Halligan et al., 2002; Kleim & Ehlers, 2008). Furthermore, earlier traumatic experiences have also been found to affect future reactions to stress (Resnick et al., 1995), which can lead to higher chances of developing anxiety disorders (Yehuda et al., 1998). Individual differences in brain development, personality, stress responsiveness and memory abilities, as well as earlier experiences are thus important to account for in research on stress and memory, and in the future this might lead to customized psychological and medical treatments based on personal characteristics.

To end

In short, the present thesis has tried to shed more light on the working mechanisms of stress, cortisol and propranolol on human memory retrieval. Most importantly, we found acute stress and a single cortisol administration to have long-term impairing effects on memory for neutral and emotional information that was learned and reactivated in a controlled laboratory situation. Future studies should shed more light on the generalizability of these findings to real life settings and clinical practice. And as neuro-imaging techniques are getting more and more advanced, in the near future, the underlying brain mechanisms of emotional memory retrieval and the neurobiological impact of stress could be unraveled.

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Samenvatting

De impact van stresshormonen op het ophalen van emotionele herinneringen

In dit proefschrift hebben we de directe en langetermijneffecten van stress en stresshormonen op het ophalen van emotionele herinneringen onderzocht. Met emotionele herinneringen bedoelen we de herinnering aan gebeurtenissen die emoties opriepen. Tijdens het ophalen van zulke herinneringen kunnen opnieuw emoties worden beleefd, maar soms is het alleen nog feitelijke informatie zonder gevoelens die herinnerd wordt. Gedurende stressvolle periodes of onder grote druk kan het lastig zijn om belangrijke informatie te herinneren, bijvoorbeeld tijdens een examen of een sollicitatie. Eerder onderzoek heeft aangetoond dat het ophalen van herinneringen inderdaad negatief wordt beïnvloed door stress. Met name het ophalen van emotionele informatie lijkt lastig te zijn. Deze effecten worden toegeschreven aan het stresshormoon cortisol in combinatie met het stresshormoon noradrenaline. Als deze hormonen worden vrijgemaakt in het lichaam tijdens stress, werken ze vervolgens in op hersengebieden die van belang zijn voor het geheugen, zoals de hippocampus, de amygdala en de prefrontale cortex (zie Figuur 1.1).

De effecten van het stresshormoon cortisol op het ophalen van (emotionele) herinneringen zijn niet altijd even duidelijk. Dit uit zich bijvoorbeeld wanneer soms zowel neutrale als emotionele herinneringen worden beïnvloed en soms alleen emotionele herinneringen. Daarnaast weten we niet of dit negatieve effect van cortisol ook te vinden is op oude herinneringen. Oudere herinneringen zijn misschien op andere plekken in het brein vastgelegd dan recente herinneringen en zijn daardoor wellicht minder kwetsbaar voor stresshormonen. Eerder onderzoek heeft zich voornamelijk gericht op herinneringen aan informatie die kort daarvoor was aangeleerd. Bijna al het onderzoek naar stress, cortisol en het ophalen van herinneringen is gedaan in laboratoria, waar herinneringen worden gecreëerd door de onderzoeksgroep woorden of verhaaltjes te laten inprenten die ze vervolgens moeten ophalen. Of herinneringen uit het echte leven (het zogenaamde autobiografische geheugen) ook negatief worden beïnvloed door cortisol is nog onduidelijk. We vroegen ons ook af of er langetermijneffecten zijn van stress en cortisol op het geheugen. Wellicht is het negatieve effect slechts tijdelijk. Onderzoek bij dieren heeft echter aangetoond, dat cortisol ook effect heeft op processen die zich tijdens en direct na het ophalen van herinneringen afspelen. Eén van die processen wordt herconsolidatie genoemd. Wanneer een herinnering wordt opgehaald, zou het mogelijk kunnen zijn dat stress of cortisol de her-opslag van deze herinnering beïnvloedt en dus op lange termijn de herinnering verandert.

In dit proefschrift hebben we geprobeerd bovenstaande onduidelijkheden te onderzoeken. We hebben niet alleen gekeken naar het stresshormoon cortisol, maar ook naar een medicijn dat de activiteit van noradrenaline in de hersenen blokkeert, genaamd propranolol. In dieronderzoek is aangetoond, dat dit medicijn de

herconsolidatie van emotionele (angst) herinneringen kan afzwakken. Als we dit medicijn toedienen tijdens het ophalen van herinneringen, zouden er dus ook langetermijnveranderingen kunnen plaatsvinden. Deze veranderingen zouden zich kunnen uiten in de herinnering aan wat er feitelijk gebeurde, maar ook in de emotionele beleving die daarmee gepaard ging. Zowel propranolol als cortisol zouden dus mogelijk op de lange termijn tot een vermindering van emotionele herinneringen kunnen leiden. Dit idee wordt momenteel toegepast in experimenteel klinisch onderzoek bij patiënten met een posttraumatische stress stoornis (PTSS). Patiënten met PTSS hebben last van overmatig sterkte emotionele herinneringen aan hun trauma. Dit leidt onder andere tot flashbacks en intrusies. Als deze herinneringen afgezwakt zouden kunnen worden door cortisol of propranolol, zou dit kunnen leiden tot een betere behandeling van deze stoornis. Het is van belang precies te weten wat er met het geheugen gebeurt tijdens het gebruik van deze medicijnen, voordat ze veilig gebruikt kunnen worden in de klinische praktijk. In dit proefschrift hebben we getracht hier meer duidelijkheid in te brengen.

We zullen nu kort de bevindingen uit hoofdstuk 2 tot en met 6 bespreken. In hoofdstuk 2 hebben we onderzocht of stress het ophalen van zowel neutrale als emotionele herinneringen en of stress zowel recente als oude herinneringen beïnvloedt in gezonde jongemannen. Ook keken we naar de invloed van cortisoltoenames door stress op het geheugen, rekening houdend met de fysieke spanning van de mannen (door verhoogde noradrenaline). Deze spanning hebben we gemanipuleerd door de geheugentaak af te nemen tijdens en na de stresstaak. Op allebei de momenten was cortisol verhoogd, maar de fysieke spanning, gemeten via hartslag en bloeddruk, nam snel af na de stresstaak. We vonden dat stress leidt tot het verminderd ophalen van emotionele woorden die 5 weken eerder waren aangeleerd. Dit stemt overeen met eerder onderzoek waarbij vooral emotionele herinneringen worden beïnvloed door stress en het voegt aan deze eerdere onderzoeken toe dat ook oudere herinneringen worden beïnvloed door stress. De toename in het stresshormoon cortisol hing samen met een slechtere prestatie op de geheugentaak, maar alleen tijdens de stresstaak, als de fysieke spanning nog hoog was. Dit duidt op een interactie tussen de twee stress hormonen, cortisol en noradrenaline. De woorden die 1 dag eerder werden aangeleerd werden nog zo goed onthouden, dat we geen duidelijke conclusies konden trekken over de effecten van stress of cortisol hierop.

In hoofdstuk 3 beschrijven we een vervolgstudie op de studie uit hoofdstuk 2 om te onderzoeken of er 6 maanden na de start van het onderzoek nog effecten waren van de stresstaak. We vonden inderdaad dat in de groep die woorden 5 weken na het aanleren hadden opgehaald tijdens stress, na 6 maanden nog steeds een verminderde prestatie vertoonden en zelfs een verdere verslechtering lieten zien ten opzichte van de controle groep. Dit duidt erop dat stress langetermijneffecten kan hebben en daarnaast eventueel de herconsolidatie van herinneringen beïnvloedt. Of cortisol een rol speelde in deze langetermijneffecten bleef onduidelijk.

In hoofdstuk 4 hebben we gekeken of cortisol inderdaad langetermijneffecten heeft op het ophalen van herinneringen. We keken in deze studie naar de geheugenprestatie een week nadat de herinneringen opgehaald waren onder invloed van cortisol. We vonden dat cortisol direct het ophalen van herinneringen

verminderde vergeleken met een placebo groep en verder dat dit een week later nog steeds te meten was. We keken ook naar het effect van propranolol op het ophalen van herinneringen. We vonden geen directe of langetermijneffecten van propranolol op het geheugen. Dit zou kunnen betekenen dat propranolol niet de herconsolidatie van herinneringen bij mensen beïnvloedt, maar het zou ook kunnen dat het alleen de emotionele beleving van herinneringen beïnvloedt. Dit werd onderzocht in hoofdstuk 6.

In hoofdsstuk 5 onderzochten we of stress ook het ophalen van autobiografische herinneringen beïnvloedt. In onderzoek naar autobiografische herinneringen wordt niet gekeken naar het accuraat ophalen van herinneringen, omdat dit moeilijk te achterhalen is, maar naar de specificiteit van de herinneringen. Dit houdt in dat wordt gekeken of de herinneringen specifiek verwijzen naar een enkele gebeurtenis op één bepaalde dag. We vonden echter geen effect van stress op de specificiteit van de herinneringen of op de subjectieve emotionele beleving van deze herinneringen. Dit geeft aan dat bevindingen van geheugentaken uit het lab niet altijd te generaliseren zijn naar het autobiografische geheugen. Cortisoltoenames waren matig gerelateerd aan minder specifieke neutrale herinneringen. Wellicht zijn deze herinneringen het minst sterk en dus het meest kwetsbaar voor stress.

In hoofdstuk 6 keken we ook naar autobiografische herinneringen, maar dan in hoeverre de fysieke emotionele reacties op deze herinneringen worden beïnvloed door cortisol en propranolol. Dit werd gemeten door middel van veranderingen in hartslag en huidgeleiding tijdens het luisteren naar en inbeelden van een negatieve, vervelende gebeurtenis uit het eigen leven van de onderzoeksgroep. Hoewel deze taak wel fysieke reacties teweegbracht, vonden we geen directe of langetermijneffecten van cortisol en propranolol op deze reacties in vergelijking met een placebo groep. Dit is in tegenstelling tot onderzoek bij PTSS patiënten waarin propranolol leidde tot verminderde fysieke reacties op het inbeelden van hun traumatische ervaring. De invloed van cortisol op deze fysieke reacties bij PTSS patiënten is nog nooit onderzocht en blijft dus onduidelijk.

Met de onderzoeken beschreven in dit proefschrift hebben we enkele van de onduidelijkheden kunnen ophelderen met betrekking tot de effecten van stress op het geheugen, maar een aantal zaken blijven nog open voor toekomstig onderzoek. Een eerste punt is dat onze onderzoeken allemaal gedaan zijn bij mannen. Wat de effecten van stress, cortisol en propranolol op het geheugen bij vrouwen zijn kunnen we hieruit niet concluderen, omdat vrouwen anders kunnen reageren op stress en stress hormonen vanwege hun menstruatiecyclus en vanwege het mogelijke gebruik van hormonale anticonceptie. Eerdere onderzoeken hebben ook aangetoond dat het geheugen van vrouwen anders beïnvloed kan worden door stresshormonen. Gezien de interesse vanuit de klinische praktijk voor het gebruik van cortisol en propranolol bij PTSS, een klinische populatie die grotendeels uit vrouwen bestaat, is het van belang de relatie tussen deze stoffen en het geheugen ook in vrouwen uit te zoeken.

Een tweede punt is dat we vooral hebben gekeken wat de rol was van een toename in cortisol en een afname in noradrenaline bij het ophalen van herinneringen. Het experimenteel verlagen van cortisol kan ook interessant zijn, om te zien of de negatieve effecten van stress op het geheugen te blokkeren zijn. Daarnaast is er

weinig bekend over de toename van noradrenaline op het ophalen van herinneringen, wat meer inzicht zou kunnen geven in de acute effecten van stress. Tijdens stress worden verder nog een reeks andere hormonen vrijgemaakt, die ieder ook hun eigen effecten zouden kunnen hebben. Ook neuro-imaging onderzoek (met bijvoorbeeld EEG of fMRI) kan meer inzicht geven in welke hersengebieden precies beïnvloed worden door deze hormonen.

Onze belangrijkste bevinding is dat acute stress en een enkele dosis cortisol, maar niet propranolol, langetermijneffecten kan hebben op het neutrale en emotionele geheugen voor woorden. In toekomstig onderzoek naar de effecten van stresshormonen en propranolol op het geheugen zou het autobiografische geheugen beter onderzocht moeten worden, zowel op de inhoudelijke kant als op de fysieke en emotionele reacties bij herinneringen. Dit zal meer inzicht kunnen geven in de toepassing van cortisol en propranolol in de klinische praktijk. Het is onder andere van belang om te weten wat er verandert aan herinneringen en of dit wenselijk is in de behandeling van de patiënt. Tenslotte is het van belang om rekening te houden met individuele verschillen tussen mensen (zowel genetisch te verklaren of door eerdere ervaringen) die de relatie tussen stress en het geheugen kunnen beïnvloeden.

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Curriculum Vitae

Marieke Suzanne Tollenaar was born on December 29, 1979, in Huizen, the Netherlands. She graduated from high school (Atheneum level) at the Mendelcollege in Haarlem in 1997. In September 1998 she started the study of Psychology at the Free University (VU) in Amsterdam and finished her doctoraal (Master's equivalent) in Biological Psychology in January 2003 (Cum Laude) within the group of Prof. Dorret Boomsma. After working for almost a year as a researcher in the Department of Medical Psychology at the Academic Medical Center (AMC) of Amsterdam in 2003, she returned to her studies after obtaining a scholarship for a semester abroad (University of California Berkeley, USA). In Berkeley she studied the evolution of cognitive functions and did an internship in brain dissection at the lab of Prof. Terrence Deacon.

Upon her return to the Netherlands, she started her PhD in the fall of 2004 in the department of Clinical, Health and Neuropsychology at Leiden University. Her PhD studies focused on the effects of stress hormones on the retrieval of emotional memories and were supervised by Prof. Philip Spinhoven, Dr. Bernet Elzinga (both Leiden University) and Prof. Walter Everaerd (University of Amsterdam). As of January 2009 she is working as a post-doctoral researcher at the Department of Developmental Psychology of the Radboud University in Nijmegen with Dr. Carolina de Weerth and Prof. Marianne Riksen-Walraven.