Modulating emotional memories: Influence of stress and interference learning on brain potentials during encoding and retrieval

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Content

Abstract

There is multiple evidence that emotionally arousing events are preferentially processed, and better remembered than neutral events. In the present dissertation I investigated whether those strong emotional memories are affected by acute and chronic stress. Moreover, I was interested in whether already established emotional memories can be changed by behavioral intervention.

According to the modulation hypothesis, emotionally arousing events promote attention and memory processes via noradrenergic and glucocorticoid actions. Recent models suggest that stress hormones differentially impact mnemonic processing, namely encoding, (re-) consolidation and memory retrieval, depending on timing and duration of the stressor relative to the learning experience. Acute stress around the time of encoding has been found to enhance memory, whereas chronic stress has been associated with memory impairments. Furthermore, consolidated memories are not resistant to modifications. Following reactivation, memories can turn into an unstable state and undergo a process called reconsolidated in order to persist. During this vulnerable state, memories are prone to modification, for instance by pharmacological blockade or interference learning.

Here, the modulation of newly formed emotional and neutral memories as well as existing emotional and neutral memories was investigated in a well-established picture viewing and recognition memory paradigm using behavioral and neurophysiological measures (event-related potential, ERPs). More elaborative processing of emotional, relative to neutral stimuli has been related to the late positive potential (LPP). During encoding of emotional and neutral pictures, enhanced LPPs (starting at about 400 ms after stimulus onset) are usually observed for emotionally arousing relative to neutral pictures, indicating preferential attention allocation and processing. During recognition, correctly

recognized old items evoke larger ERP amplitudes than correctly identified new items. This difference, the ERP old/new effect, was used to measure mnemonic processing during retrieval. The ERP old/new effect over centro-parietal sensor sites (400-800 ms) has been associated with recollection processes, and is enhanced for emotional, compared to neutral materials. Three studies are presented, that investigated 1) the influence of acute stress prior to encoding on long-term memory and its neural correlates, 2) the impact of chronic stress on encoding and memory, and 3) the influence of interference on already established memories (reconsolidation), always contrasting emotionally arousing and neutral scenes. Study 1 investigated subsequent recognition memory after encoding following acute stress using a socially evaluated cold pressure test, while study 2 tested the influence of chronic stress investigating breast cancer survivors about two years after cancer treatment. In study 3, one day after encoding, reconsolidation of the reactivated picture memory was targeted with an interfering learning task. In all three studies, recognition memory was tested one week later. High-density electroencephalograms (EEGs; 257 electrodes) were recorded to measure brain potentials.

The results showed, in line with previous research, that emotionally arousing scenes were preferentially processed, as indicated by larger LPPs, and were better remembered than neutral scenes, as indicated by enhanced memory performance and larger ERP old/new differences. Experiencing acute stress prior to encoding enhanced the centro-parietal ERP old/new effect for emotionally arousing pictures at recognition, corroborating that acute stress facilitates memory for emotional scenes (Study 1). In contrast, attenuated LPPs for unpleasant pictures and impaired memory performance for arousing pictures were observed in breast cancer survivors (Study 2), indicating altered attention to emotion and subsequent emotional memory storage in chronically stressed individuals. When memory reactivation was followed by an interfering learning task, recognition memory and ERP old/new differences were attenuated for emotionally arousing scenes, selectively,

showing the possibility that emotional memories might be modulated by behavioral interventions (Study 3).

The results of all three studies are discussed and integrated into a model of memory modulation by stress and interference. The results highlight the importance of understanding the role of emotional arousal in the processes of memory formation, retrieval and reconsolidation. Moreover, shedding light on the differential effects of acute and chronic stress, interference and their possible interactions might help to prevent and even modify impairing memories that are one of the major concerns in stress- and fear-related mental disorders.

Zusammenfassung

Eine Vielzahl von Studien zeigt, dass emotional erregende Ereignisse mehr Aufmerksamkeit auf sich ziehen, dadurch bevorzugt verarbeitet und folglich auch besser erinnert werden, als neutrale Ereignisse. Die sogenannte Modulationshypothese postuliert, dass die Effekte von emotional erregenden Ereignissen auf Aufmerksamkeits- und Gedächtnisprozesse über eine Aktivierung von Noradrenalin und Glukokortikoiden vermittelt werden. Stresshormone beeinflussen Gedächtnisprozesse, also die Aufnahme, die (Re-)Konsolidierung und den Abruf, in unterschiedlicher Weise. Dieser Einfluss ist dabei abhängig vom zeitlichen Zusammenhang zwischen Stress und Lernerfahrung, sowie der Dauer des Stressors. Akuter Stress fördert die Informationsaufnahme und Gedächtniskonsolidierung, während chronischer Stress mit Gedächtniseinschränkungen in Verbindung gebracht wird. Werden einmal konsolidierte Inhalte reaktiviert, so erreichen sie erneut einen instabilen Zustand und müssen rekonsolidiert werden, um dauerhaft im Gedächtnis zu bleiben. In diesem instabilen Zustand sind die Inhalte beispielsweise durch eine pharmakologische Blockade oder eine neue, konkurrierende Lernaufgabe (Interferenzlernen) modifizierbar.

In der vorliegenden Arbeit wurde die Modulation von emotionalen und neutralen Gedächtnisinhalten durch akuten und chronischen Stress sowie durch Blockade der Rekonsolidierung anhand eines gut etablierten Paradigmas (Bildbetrachtung und Wiedererkennenstest) untersucht. Neben Verhaltensmaßen für die Gedächtnisleistung wurden hochauflösende Ereigniskorrelierte Potentiale (EKPs) als ein neuronaler Indikator während des Gedächtnistests erhoben. Während der Einspeicherung emotionaler und neutraler Bilder werden üblicherweise erhöhte späte Positivierungen (Late Positive Potentials, LPPs; beginnend bei etwa 400 ms nach Stimulusbeginn) beim Betrachten emotionaler im Vergleich zu neutralen Bildern beobachtet, was für eine verstärkte Aufmerksamkeitszuwendung und Verarbeitung dieser Reize spricht. Während

des Wiedererkennens evozieren korrekt wiedererkannte alte Items größere EKP-Amplituden als richtig eingestufte neue Items (Old/New Effekt). Der Old/New Effekt über zentroparietalen Sensoren (400-800 ms) wurde mit Aktivierungen im Hippocampus und exaktem Wiedererkennen in Verbindung gebracht. Er tritt verstärkt bei emotionalen Inhalten auf.

Drei Studien werden vorgestellt, die 1) den Einfluss von akutem Stress während der Enkodierung auf das Langzeitgedächtnis und dessen neuronale Korrelate, 2) den Einfluss von chronischem Stress auf Enkodierung und Gedächtnis und 3) den Einfluss von Interferenz auf die Rekonsoliderung bereits gespeicherter Gedächtnisinhalte untersuchen. Dabei wurden jeweils emotionale und neutrale Reize gegenübergestellt. Studie 1 untersuchte das Wiedererkennen von Reizen, die in Folge des sozial evaluierten Kaltwassertests unter Stress enkodiert wurden, während Studie 2 den Einfluss von chronischem Stress in einer Gruppe Brustkrebsüberlebender ca. zwei Jahre nach der Krebsbehandlung untersuchte. In Studie 2 wurden die 24 Stunden zuvor bereits gesehenen Bilder erneut präsentiert, um die Gedächtnisspur zu reaktivieren. Interferenzlernen zielte hier auf eine Blockade der Rekonsolidierung ab. In allen drei Studien erfolgte nach einer Woche ein unerwarteter Wiedererkennenstest. Ein hochauflösendes Elektroenzephalogramm (EEG; 257 Sensoren) wurde in den Untersuchungen abgeleitet.

Im Einklang mit früheren Untersuchungen wurden emotional erregende Bilder bevorzugt verarbeitet. Dies spiegelte sich in größeren LPPs wider. Emotional erregende Bilder wurden besser wiedererkannt als neutrale Bilder, was mit einem stärkeren Old/New Effekt verbunden war. Akuter Stress vor Enkodierung ging mit einem größeren zentroparietalen Old/New Effekt für emotional erregende Bilder einher, was dafür spricht, dass akuter Stress die Gedächtnisspeicherung für emotionale Erlebnisse fördert

(Studie 1). Im Gegensatz dazu wurden geringere LPPs für unangenehme Bilder und eine eingeschränkte Gedächtnisleistung für emotional erregende Bilder bei Brustkrebsüberlebenden beobachtet (Studie 2), was auf eine veränderte Verarbeitung in chronisch gestressten Personengruppen hinweist. Interessanterweise (Studie 3) führte Interferenzlernen nach Reaktivierung zu selektiven Einschränkungen in der Gedächtnisleistung und dem Old/New Effekt für emotionale Bilder. Dies weist auf die Möglichkeit hin, dass etablierte emotionale Erinnerungen durch Verhaltensinterventionen beeinflusst werden können.

In dieser Arbeit werden die drei Studien vor dem Hintergrund der aktuellen Forschung diskutiert und die Befunde in ein Modell zur Gedächtnismodulation durch Stress integriert. Die Ergebnisse unterstreichen die Bedeutung von emotionaler Erregung auf die Gedächtniskonsolidierung und Rekonsolidierung. Darüber hinaus tragen sie zum Verständnis der unterschiedlichen Einflüsse von Stress auf Gedächtnisprozesse bei, die bei der Entstehung und Aufrechterhaltung von stress- und angstbezogenen psychischen Störungen eine Rolle spielen.

1. Emotion, attention and memory

Emotional events do not only automatically capture attention, they are also better remembered than neutral episodes. From an evolutionary perspective, the fast and automatic detection of a poisonous snake in the grass or the tastiest berries in the undergrowth is important for survival. This automatic, cue-driven selective attention for emotionally arousing events has been described as *motivated attention* (Lang, 1995; Lang, Bradley, & Cuthbert, 1997). Moreover, it would be wise to recall the first life-threatening snake encounter with as much detail as possible, or the location of the sweetest berries for future energy supply. Thus, it is reasonable that emotionally arousing events are preferentially processed and consolidated, resulting in better long-term memory performance (Bradley, Greenwald, Petry, & Lang, 1992; Brown & Kulik, 1977; James, 1890; LaBar & Cabeza, 2006). This emotional memory advantage has been reported for varying retention time intervals (Dolcos, LaBar, & Cabeza, 2005; Weymar, Löw, & Hamm, 2011; Weymar, Löw, Melzig, & Hamm, 2009), increasing from immediate to delayed recognition (Bradley et al., 1992; Sharot & Phelps, 2004; Sharot & Yonelinas, 2008).

McGaugh and colleagues hypothesized that the amygdala plays a key role in modulating the effects of emotional arousal on memory consolidation (McGaugh, 2004; McGaugh, Cahill, & Roozendaal, 1996). According to this *modulation hypothesis*, emotionally arousing experiences lead to (nor-) adrenaline and glucocorticoid release (see Figure 1). These neuromodulators further mediate interactions between (basolateral) amygdala and other brain regions, especially the medial temporal lobe (MTL, including the hippocampus), which are fundamental for enhanced memory consolidation (McGaugh, 2000; McGaugh, 2004; McIntyre, McGaugh, & Williams, 2012). There is now multiple evidence from lesion (Adolphs, 2000; Cahill, Babinsky, Markowitsch, & McGaugh, 1995; Phelps, LaBar, & Spencer, 1997) and pharmacological studies using beta-

adrenergic antagonists (Hurlemann et al., 2010; Weymar et al., 2010) supporting the modulation hypothesis.

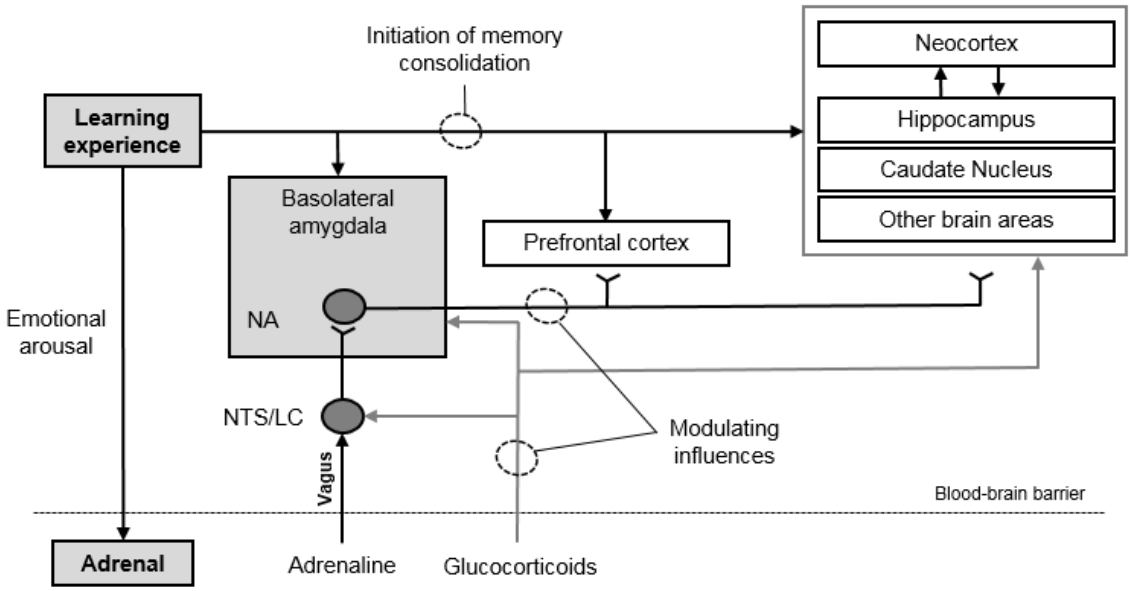

Figure 1. Memory modulation hypothesis. Learning experiences initiate memory consolidation processes in different brain regions and stress hormones from the adrenal gland (adrenaline, glucocorticoids) are released. Via noradrenaline (NA), the amygdala is stimulated, further modulating memory consolidation (NTS= Nucleus tractus solitarii; LC= Locus coeruleus; adapted from Schwabe et al., 2012 and McGaugh, 2000).

For example, participants with left amygdala lesions showed selective declarative memory impairments for emotionally arousing information when tested 24 hours later, compared to healthy controls (Adolphs, 2000). Moreover, beta-adrenergic blockade using propranolol (which acts centrally, crossing the blood-brain barrier) during encoding interferes with long-term memory for an emotionally arousing, but not an affective neutral story, one week later (Cahill, Prins, Weber, & McGaugh, 1994; Van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998). Further evidence for the modulation hypothesis can be drawn from neuroimaging studies relating amygdala and MTL activation to superior

memory for emotional stimuli (Dolcos, LaBar, & Cabeza, 2004; Hamann, Ely, Grafton, & Kilts, 1999; Ritchey, Dolcos, & Cabeza, 2008).

1.1 Event-related potentials of picture processing and memory

Event-related potentials (ERPs) provide measures of neural activity with high temporal resolution, and are thus well suited to investigate attention and memory processes as well as their modulation by emotional contents (Voss & Paller, 2008). According to dimensional models of emotion (Lang, Bradley, & Cuthbert, 1990; Russell, 1980), emotional experiences can be described on at least two dimensions of valence (or pleasure) and arousal. Whereas valence spans from very unpleasant (triggering avoidance) via neutral to very pleasant (motivating approach), arousal reflects the intensity of the experience from absolute calm to intense excitement. The International Affective Picture System (IAPS) has been widely used to investigate the distribution of emotional responses in affective space (Lang, Greenwald, Bradley, & Hamm, 1993). The IAPS provides a set of photographs with standard ratings on the two dimensions of valence and arousal, and induces reliable behavioral and physiological changes that can be associated with the basic emotional dimensions of valence and arousal (Lang, Bradley, & Cuthbert, 1998; Lang et al., 1993). These pictures, however, do not only prompt emotional expression and physiological response mobilization, they also can be used to study effects of emotional perception during stimulus encoding and memory, measuring brain potentials during picture viewing (encoding) and subsequent recognition (memory).

1.1.1 Brain activity during encoding of emotional scenes

During viewing of emotional pictures the late positive potential (LPP), a positive going waveform over centro-parietal sensors, starting around 400 ms post-stimulus, is consistently elevated compared to the LPP during viewing of neutral stimuli (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Schupp et al., 2000; Schupp, Flaisch,

Stockburger, & Junghöfer, 2006) (see Figure 2). This LPP modulation is supposed to reflect motivated attention with higher attention allocation to, and preferential processing of emotionally arousing, compared to neutral stimuli (Schupp et al., 2006; Schupp, Junghöfer, Weike, & Hamm, 2004).

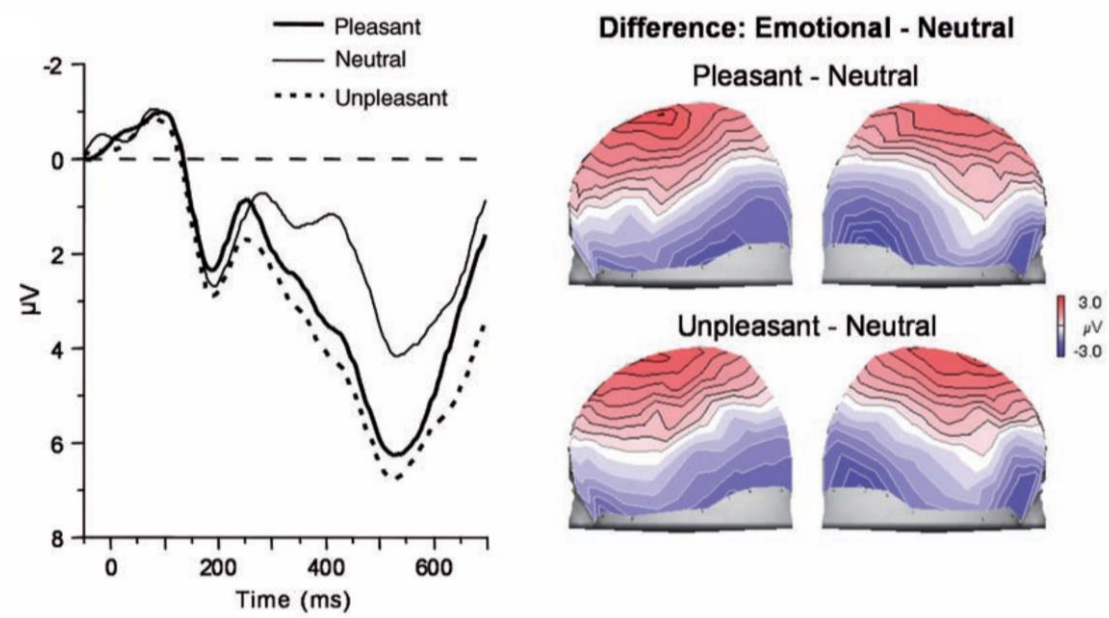

Figure 2. The late positive potential (LPP). Left: Mean ERPs averaged across a centro-parietal sensor for unpleasant, neutral, and pleasant pictures. Right: Difference scalp topographies (emotional-neutral) in the time window from 400-600 ms (adapted from Schupp et al., 2006).

Functional magnetic resonance imaging and ERP studies found a widely distributed network, especially a functional connectivity with reentrant organization between amygdala and visual cortex, to be active during emotional picture viewing (Keil et al., 2009; Sabatinelli, Lang, Bradley, Costa, & Keil, 2009). Using simultaneous fMRI and ERP recordings, emotional pictures evoked enhanced LPPs and larger activity in the amygdala and prefrontal cortex, compared to neutral pictures (Liu, Haiqing, McGinnis, Keil, & Ding, 2012). Also, LPP deflections during emotional picture viewing were

associated with fMRI activation in parietal visual, lateral occipital, and infero-temporal areas, relating the elevated LPP during emotional picture processing to activity in the visual cortex, thus corroborating the assumption that larger LPPs reflect enhanced perceptual sensitivity for motivational relevant stimuli (Sabatinelli, Lang, Keil, & Bradley, 2007).

The LPP magnitude has also been related to subsequent memory performance (Dolcos & Cabeza, 2002; Weymar, Schwabe, Löw, & Hamm, 2012). During encoding, subsequently remembered items elicit more positive going late ERPs than subsequently forgotten items, which has been labeled the *subsequent memory effect* (Paller, Kutas, & Mayes, 1987). Like the LPP magnitude per se, the subsequent memory effect is also modulated by emotion. For example, Dolcos & Cabeza (2002) reported not only more positive going ERPs during emotional picture viewing but also a larger subsequent memory effect for emotionally arousing compared to neutral pictures, relating memory performance to initial processing. Measuring ERPs during recognition of pictures that have been presented before would allow tracing the neural bases of this facilitated memory performance during retrieval.

1.1.2 Recognition: The ERP old/new-effect

Recognition memory paradigms where previously encountered items (*old*, in contrast to new items) are presented again and have to be correctly identified (Eichenbaum, Yonelinas, & Ranganath, 2007; Yonelinas, 2002), have successfully been used to investigate item memory performance. During recognition memory testing, ERPs evoked by correctly recognized old stimuli show more positive going waveforms compared to correctly identified new materials. This ERP difference is known as the *old/new effect* (Warren, 1980). Two temporally and spatially distinct ERP components have been linked to the so-called dual-process model of recognition memory: An early old/new effect over

frontal scalp sites (300-500 ms), possibly reflecting familiarity (the feeling of knowing an item) and a later centro-parietal old/new effect starting at about 400 ms following stimulus onset that has been associated with recollection experience (remembering an item) and hippocampus-dependent recognition (Curran, 2000; Eichenbaum et al., 2007; Rugg et al., 1998; Weymar & Hamm, 2013; Yonelinas, 2002). Emotionally arousing picture stimuli reliably elicit larger late ERP old/new differences over centro-parietal scalp sites compared to neutral pictures even after longer retention intervals up to one year after encoding, suggesting that the emotion memory advantage is more related to recollection rather than familiarity (Weymar & Hamm, 2013; Weymar et al., 2011, 2009; see Figure 3).

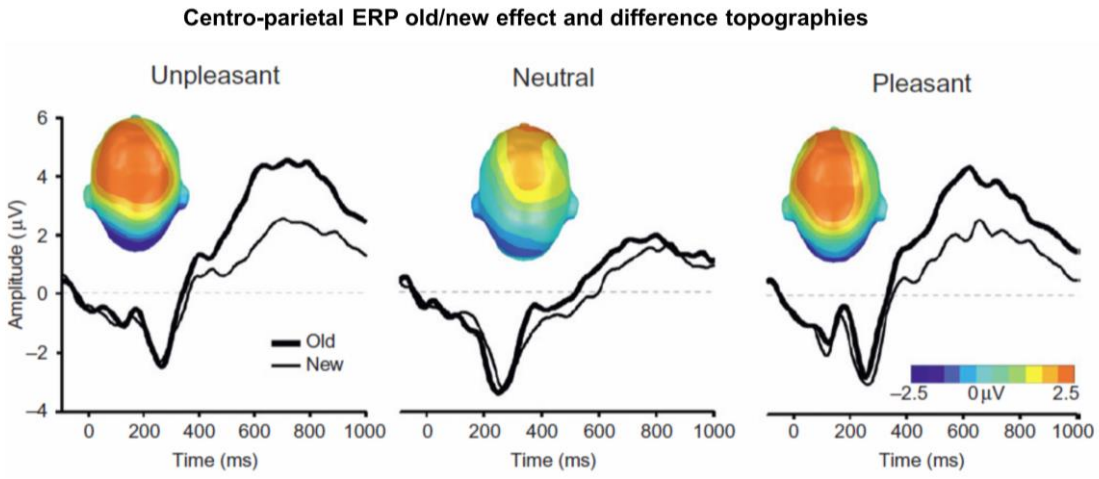

Figure 3. Centro-parietal ERP old/new effect and difference topographies (old minus new) for unpleasant, neutral and pleasant pictures (adapted from Weymar & Hamm, 2013).

Moreover, the increased late centro-parietal old/new effect for emotional items was associated with better recognition memory performance, increased recognition confidence and remember judgments (LaBar & Cabeza, 2006; Weymar et al., 2011,

2009). Supporting the modulation hypothesis, beta-adrenergic blockade during picture viewing resulted in a reduced centro-parietal old/new effect (500-800 ms) for emotionally arousing pictures when recognition was tested one week later (Weymar et al., 2010). There is multiple evidence from fMRI and lesion studies that memory retrieval might involve brain activation patterns similar to encoding, and is linked to parietal cortex and hippocampal activity (Cabeza, Ciaramelli, Olson, & Moscovitch, 2008; Dolcos et al., 2005; Düzel, Vargha-Khadem, Heinze, & Mishkin, 2001; Ritchey, Wing, Labar, & Cabeza, 2012; Sterpenich et al., 2009). In line, source analyses also revealed larger activation in parietal cortical areas for correctly recognized old, relative to new pictures, and larger old/new differences for emotional compared to neutral pictures (Weymar et al., 2010).

2. Influences of Stress on Attention and Memory

Besides the initiation of multiple adaptation processes in the body, stress differentially affects attention, learning and memory, depending on the duration and timing of the stressful event (Joëls, Fernandez, & Roozendaal, 2011; Roozendaal, McEwen, & Chattarji, 2009; Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012). An integrative model of the differential effects of stress on memory was proposed by Schwabe et al. (2012) and is depicted in Figure 4.

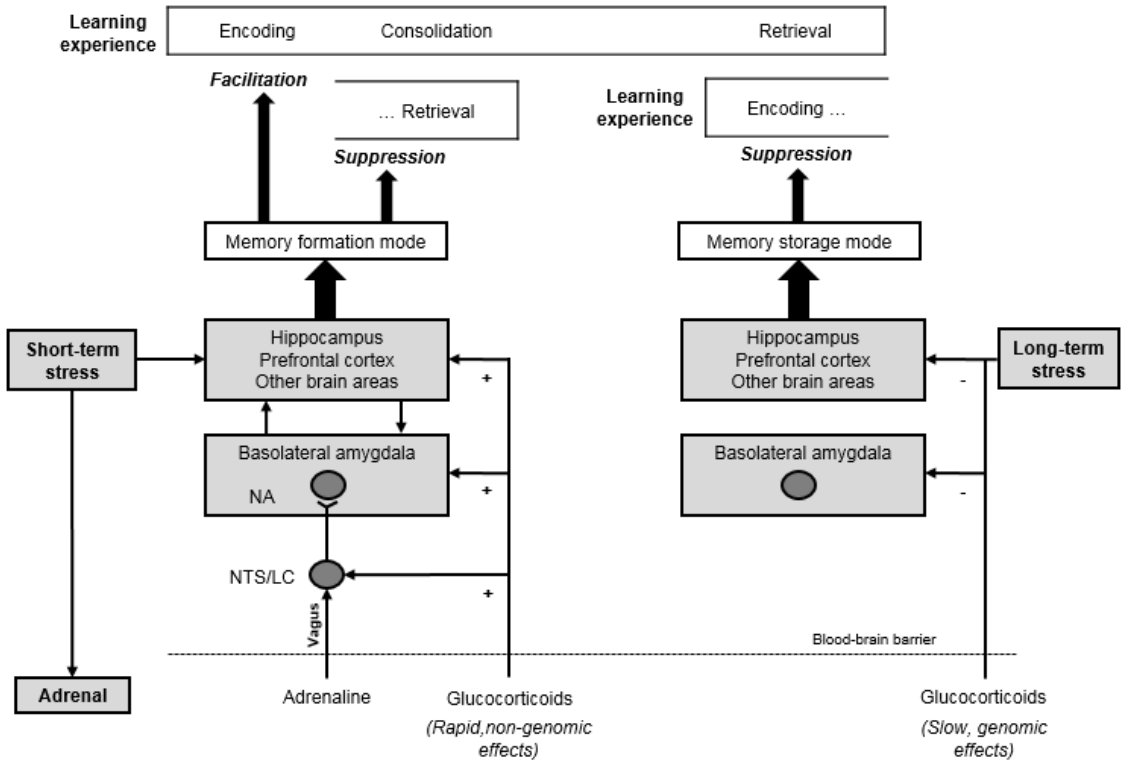

Figure 4. Integrative model of short- and long-term effects of stress on memory. NA= noradrenaline; NTS= nucleus tractus solitarii; LC= locus coeruleus (adapted from Schwabe et al., 2012).

2.1 Acute stress

Acute stress activates the autonomic nervous system (ANS) and the hypothalamo-pituary-adrenal (HPA) axis, resulting in a fast release of (nor-)adrenaline, corticotropin releasing hormone and glucocorticoids (cortisol in humans) (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Schwabe, Joëls, et al., 2012). Short-term stress administered around the time of encoding has been found to enhance memory consolidation processes, especially for emotionally arousing events. In contrast, acute stress impairs retrieval of previously consolidated memories (for reviews, see Roozendaal, 2002; Schwabe, Joëls, et al., 2012). When an acute stressor is experienced, the brain is supposed to enter a *memory formation mode* in which encoding is facilitated whilst retrieval of already established memories is suppressed (Schwabe, Joëls, et al., 2012) (see Figure 4). It has been suggested that

noradrenergic activation, evoked by emotionally arousing stimuli, is essential for the memory enhancing effect of acute pre-encoding stress (Abercrombie, Speck, & Monticelli, 2006; Joëls et al., 2006). For example, cortisol administration or stress induction with a social evaluative, uncontrollable stressor shortly prior to picture viewing enhanced subsequent memory performance for emotionally arousing, but not for neutral episodes when memory was tested unexpectedly one week later (Buchanan & Lovallo, 2001; Payne et al., 2007). And, using a 24-hour retention interval, unpleasant pictures were better recalled then neutral pictures at least at trend level, when the Socially Evaluated Cold Pressure test was administered 20 minutes prior to encoding, suggesting that stress facilitates mnemonic processing particularly of emotionally arousing items (Weymar et al., 2012). The Socially Evaluated Cold Pressure Test combines physical (3 min hand immersion in 0-2°C cold water) and social (videotaping and evaluation by an unsociable experimenter) stressors, resulting in reliable stress responses as indicated by self-report, changes in the autonomic nervous and the endocrine system (Schwabe, Haddad, & Schachinger, 2008). Weymar et al. (2012) reported that SECPT exposure also resulted in enhanced LPPs during encoding for emotionally arousing, compared to neutral pictures, which were related to better subsequent memory, supporting the assumption that acute stress promotes motivated attention and consolidation processes (Weymar et al., 2012). As acute stress prior to encoding enhanced neural correlates of emotional picture processing and promoted emotional memory at the behavioral level (Weymar et al., 2012), this should also be reflected in the neural signature during later recognition memory testing. Therefore, Wirkner et al. (2013, see Manuscript 1, Appendix A) examined the influence of acute pre-encoding stress on ERP correlates of long-term memory (Wirkner, Weymar, Löw, & Hamm, 2013). Again, participants were exposed to the SECPT or a non-stressful control procedure (3 min hand immersion in lukewarm water, friendly experimenter, no videotaping) prior to presentation of emotional and

neutral scenes. Ninety pictures (30 unpleasant, 30 neutral, and 30 pleasant) were presented for 3000 ms each, and no mention of a later memory test was made (incidental encoding). One week later, the old pictures of the encoding session were presented intermixed with 90 new pictures (30 unpleasant, 30 neutral, and 30 pleasant) and participants were instructed to indicate whether they had seen the picture before, or not. In parallel, ERPs were recorded. During recognition, the ERP old/new-effect over centro-parietal sensor sites (400-800 ms), but not over frontal areas (300-500 ms) was enhanced in the stress group, compared to controls. The magnitude of the centro-parietal ERP old/new difference was positively related to experienced stress during encoding, and was especially enhanced for emotionally arousing pictures in high-stressed individuals, suggesting that intense stress experience during encoding particularly facilitates consolidation and recollection of emotional memories (Wirkner et al., 2013, see Manuscript 1; Appendix A). How these findings can be integrated in the model proposed by Schwabe et al. (2012; Figure 4) will be discussed in the final summary section.

2.2 Chronic stress

In the animal model, impairing effects of chronic stress or long-term glucocorticoid application, on learning and memory have been demonstrated (Park, Campbell, & Diamond, 2001; Woolley, Gould, & McEwen, 1990; for review, see Roozendaal et al., 2009). For instance, chronic stress as induced by five week predator exposure and random housing led to worse performance in the radial water maze task in rats (for procedure, see Park, Campbell, & Diamond, 2001), suggesting that long-term stress impairs spatial learning. And, detrimental effects of long-term glucocorticoid application on the hippocampus, including dendritic atrophy and reduced neurogenesis, have been observed in rodents (Woolley et al., 1990; for reviews, see Lupien & Lepage, 2001; McEwen & Magariños, 1997). Similarly, in healthy humans, longer glucocorticoid exposure resulted in memory impairments (Newcomer, Craft, Hershey, Askins, & Bardgett, 1994;

Wolkowitz et al., 1990). When subjects were given prednisone (a synthetic glucocorticoid) over five days in a placebo-controlled study, verbal memory was affected, suggesting that long-term elevated glucocorticoid levels may lead to cognitive impairments (Wolkowitz et al., 1990). But, for ethical reasons, transfer of experimental manipulations of chronic stress from animal to human research is difficult. Therefore, human research has mainly focused on clinical groups, showing that elevated levels of glucocorticoid were associated with cognitive impairments and reduced hippocampal volumes (for review, see Wolkowitz, Reus, Canick, Levin, & Lupien, 1997). Starkman et al. (1992) reported that hippocampus volumes, measured via MRI, were negatively related to plasma cortisol in patients suffering from Cushing's syndrome (prolonged hypercortisolemia), and that smaller hippocampus volumes were associated with impaired verbal memory performance (Starkman, Gebarski, Berent, & Schteingart, 1992).

Also, in mental disorders, alterations in HPA-axis functioning have been observed (for review see (Wingenfeld & Wolf, 2010). For example, depressed patients showed altered HPA-axis functioning with chronically elevated cortisol levels (for review, see Holsboer, 2001). Interestingly, chronically elevated cortisol levels have been associated with hippocampal atrophy in depressed patients, depending on the duration of the depression (Sheline, Wang, Gado, Csernansky, & Vannier, 1996). Moreover, there is evidence for elevated hair cortisol in depressed individuals (Dettenborn et al., 2012; Herane Vives et al., 2015; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Hair cortisol is a biological marker of long-term systemic cortisol levels, and the number of studies investigating hair cortisol levels in clinical groups increases (Stalder et al., 2012; Steudte et al., 2013). Besides the aforementioned clinical conditions, populations experiencing major critical life events provide another opportunity to investigate the effects of long-term stress on brain processes. Breast cancer diagnosis and treatment is very stressful and

an emotionally challenging life event. Breast cancer survivors often report cognitive impairments, as well as symptoms of depression, anxiety, and fatigue after recovery of their primary cancer treatment. In our study (Wirkner et al., under review, see Manuscript 2, Appendix A) we investigated breast cancer survivors two years following primary cancer treatment, Likewise, reports of increased symptoms of depression, anxiety, and fatigue were found, but also elevated hair cortisol levels compared to healthy controls (matched for age and education). Although there is increasing research on chronic stress on cognitive functioning in general, little is known about the influence of chronic stress on emotion processing and memory and the underlying neural mechanisms so far. Therefore, Wirkner et al. also investigated encoding and recognition memory of emotional and neutral pictures in breast cancer survivors. In neuropsychological testing, breast cancer survivors showed impaired verbal memory corresponding to impaired memory accuracy for emotionally arousing pictures in the recognition memory test described above, relative to controls. Replicating previous findings, emotionally arousing pictures elicited larger LPPs compared to neutral ones in breast cancer survivors and controls. However, decreased LPPs were observed during viewing of unpleasant pictures in breast cancer survivors relative to healthy controls. Similarly reduced LPPs have been found in response to unpleasant emotional stimuli in healthy subjects, when they are instructed to deploy their attention while processing these pictures (Hajcak, Dunning, & Foti, 2009; Hajcak, MacNamara, & Olvet, 2010). Also, attenuated LPPs were found in response to emotional words and threatening faces in depressed individuals, indicating emotional disengagement (Blackburn, Roxborough, Muir, Glabus, & Blackwood, 1990; Foti, Olvet, Klein, & Hajcak, 2010; Hajcak, Bress, Foti, Kujawa, & Klein, 2015). Thus, breast cancer survivors show a comparable pattern of brain activation during stimulus encoding, which might point to attentional deployment from unpleasant stimuli. Perhaps, this pattern might be related to the high hair cortisol levels, reflecting chronic stress.

Naturally, cortisol levels peak in the morning and rapidly decrease over the day, forming a characteristic diurnal cycle. Interestingly, breast cancer patients showed a slower decrease in salivary cortisol levels from waking until bedtime, compared to controls, suggesting a dysregulation in the circadian rhythmicity (Abercrombie et al., 2004; Spiegel, Giese-Davis, Taylor, & Kraemer, 2006). Moreover, cortisol response to an acute stressor was surprisingly low in breast cancer patients (Giese-Davis et al., 2006; Spiegel et al., 2006). Based on these findings, Andreotti et al. (2015) proposed a multifactorial integrative model of stress biology and neuropsychological functioning in cancer patients: When confronted with acute stressors, the adaptation to environmental or situational changes is blunted under conditions of chronically elevated glucocorticoid levels and in combination with the flattened diurnal rhythm (Abercrombie et al., 2004; Andreotti, Root, Ahles, Mcewen, & Compas, 2015; Spiegel et al., 2006). The blunted adaptive response to acute stressors has been related to higher fatigue and anxiety in breast cancer (e.g., Bower et al., 2005), for review, see (Andreotti et al., 2015). In our study (see Manuscript 2, Appendix A), breast cancer survivors also showed elevated long-term cortisol levels, attentional deployment during encoding of unpleasant stimuli and impairments in emotional recognition memory, although, unfortunately, no correlational patterns could be tested due to the small sample size. But, there is evidence that avoidance of aversive experience is indeed related to higher chronic emotional distress and anxiety in breast cancer patients (Iwamitsu et al., 2005). And, in a four month longitudinal study, individuals with higher acceptance levels were less likely to develop depressive symptoms when experiencing stressful life events (Shallcross, Troy, Boland, & Mauss, 2010). Thus, chronically stressed individuals, who show blunted responses to acute stress and emotional challenge, and who cope with experiential avoidance (resulting in temporary relief), might even exhibit higher distress and negative affect in the long run (Cohen, 2013; Iwamitsu et al., 2005; Shallcross et al., 2010).

3. Reconsolidation

One of the major challenges of clinical interventions is the modification of unpleasant, intrusive trauma related or fear memories in trauma- and stressor-related disorders (Parsons & Ressler, 2013). But how can these well-established memories be targeted? Following reactivation, consolidated memories return to an unstable state during retrieval and have to be stabilized again into a persisting memory (Misanin, Miller, & Lewis, 1968; for reviews, see Nader & Einarsson, 2010; Nader & Hardt, 2009). Thus, there is a certain time window (lasting up to six hours following reactivation) in which established emotional long-term and fear memories are vulnerable to modifications and can be targeted by reconsolidation blockade using pharmacological and behavioral interventions (Agren, 2014; Agren et al., 2012; Chan & Lapaglia, 2013; Kindt, Soeter, & Vervliet, 2009; Schiller et al., 2010). Interestingly, memory impairments for emotional pictures following pharmacological reconsolidation blockade using the beta-adrenergic antagonist propranolol, were associated with altered amygdala and hippocampus activation (Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012; for review, see Schwabe, Nader, & Pruessner, 2013). Besides pharmacological interventions, new learning following reactivation has been found to impair (Wichert, Wolf, & Schwabe, 2011) or update consolidated memories (Hupbach, Gomez, Hardt, & Nadel, 2007; Hupbach, Hardt, Gomez, & Nadel, 2008; Wichert, Wolf, & Schwabe, 2013; see Figure 5).

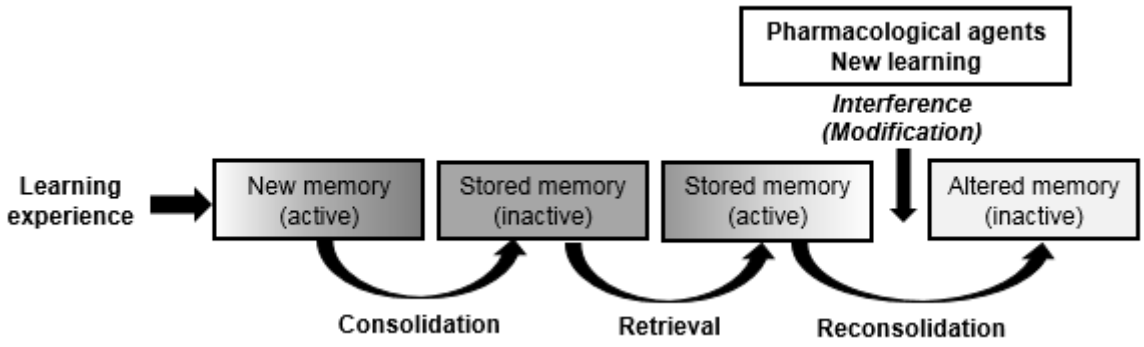

Figure 5. Memory consolidation and reconsolidation. Following retrieval, stored memories re-enter an active state and become vulnerable to modification during reconsolidation (adapted from Schwabe et al., 2014).

For example, Schwabe & Wolf (2009) reported that a behavioral intervention (new learning) interfered with reconsolidation of episodic memories. Participants who were instructed to memorize an unfamiliar story immediately after recalling (and thus reactivating) emotional and neutral autobiographical memories from their past (from 24 h up to 2 weeks), showed worse memory for the neutral autobiographical information one week later, relative to participants without interference learning or participants without initial memory reactivation (Schwabe & Wolf, 2009).

In order to test whether new learning can also interfere with emotional memory reconsolidation, Wirkner et al. (2015, see Manuscript 3, Appendix A) targeted memory for incidentally encoded emotional and neutral pictures after a brief reactivation. On day 1 of the experiment, all participants viewed ninety emotional and neutral pictures (each picture was presented for 3 seconds). Participants were randomly assigned to one of the four experimental groups: Reactivation, Reactivation + Interference, Non-Reactivation + Interference, and Control. 24 hours later (day 2), in the experimental groups Reactivation and Reactivation + Interference, the old memory was shortly reactivated using a new reactivation method: rapid serial visual presentation (RSVP). In the RSVP, pictures were

presented in a continuous stream of 3 Hz, resulting in an overall reactivation duration of 30 seconds. RSVP provides a short and elegant method for memory reactivation without further increasing learning and memory storage (Potter, 2012). Moreover, during RSVP, the perceptual system is confronted with rapidly changing information, and because fast temporal resolution is required, emotionally salient stimuli pop out in the stream (Junghöfer, Bradley, Elbert, & Lang, 2001; Schupp et al., 2004). So, this reactivation procedure was used to prevent increased learning and to selectively target emotional memories. Participants in the Reactivation + Interference group performed a new learning task (90 unfamiliar emotional and neutral pictures, presented for 3 seconds each) immediately after RSVP, whereas participants in the Non-Reactivation + Interference group only performed the new learning task without previous RSVP. Participants in the Control group omitted day 2. One week following picture encoding (day 3), recognition memory was tested in all participants and ERPs were recorded in order to investigate the underlying neural correlates. Picture recollection was selectively impaired for emotional relative to neutral contents when reactivation was followed by interference learning (Reactivation + Interference group), compared to reactivation alone (Reactivation group) (Wirkner, Löw, Hamm, & Weymar, 2015, see appendix A). In line, interference learning after RSVP resulted in smaller centro-parietal ERP old/new differences for emotional, but not for neutral pictures. As the late ERP old/new effect has been related to recognition-based hippocampus-dependent recollection (Weymar & Hamm, 2013), these findings suggest that new learning of emotional and neutral scenes after reactivation using RSVP especially interfered with reconsolidation of emotionally arousing pictures. To some extent, returning to the lab context 24 hours later alone also seemed to have reactivated the initial memory trace, as new learning without RSVP reactivation (Non-Reactivation + Interference group) also impaired recognition memory performance for emotionally arousing pictures at the behavioral level (Wirkner et al., 2015).

Regarding stress effects, it has been discussed that the impact of stress on reconsolidation might be similar to stress influences on consolidation processes. Indeed, there is some evidence that emotional memory consolidation and reconsolidation share the same neural substrates, both requiring noradrenaline-dependent activation of amygdala and hippocampus (Agren et al., 2012; Kroes, Strange, & Dolan, 2010; Strange & Dolan, 2004; Strange, Hurlemann, & Dolan, 2003). But, findings from studies investigating stress and glucocorticoid effects on reconsolidation in rodents and human subjects are inconclusive (Akirav & Maroun, 2013; Maroun & Akirav, 2008; Meir Drexler et al., 2014; Meir Drexler & Wolf, 2016). In some human studies, acute psychosocial or cold pressure stress enhanced reconsolidation of neutral verbal materials (Bos, Schuijer, Lodestijn, Beckers, & Kindt, 2014; Coccoz, Maldonado, & Delorenzi, 2011). Interestingly, impaired reconsolidation of neutral (but not emotionally relevant) autobiographical information was found following the SECPT (Schwabe & Wolf, 2010), suggesting opposing effects of stress on reconsolidation of emotionally arousing and neutral materials. Thus, the emotional memory advantage is possibly promoted by acute stress during both, consolidation and reconsolidation processes.

4. Integrative summary and future directions

The present thesis focused on the differential influence of acute (Study 1) and long-term stress (Study 2) on memory for emotionally arousing and neutral pictures, and on emotional memory modulation following reactivation (Study 3), using event-related potentials and behavioral measures. In this summary, the main results are put in the context of the "integrative model of stress effects on memory" (Schwabe, Joëls, et al., 2012) (Figure 6).

First, according to the model, short-term stress facilitates memory encoding and consolidation, resulting in better subsequent memory. And, noradrenergic activation

(emotional arousal) is essential for this memory enhancing effect. Rapid noradrenaline and glucocorticoid release are supposed to shift the brain into a *memory formation mode*, in which new encoding is facilitated (Roozendaal et al., 2009; Schwabe, Joëls, et al., 2012). Corroborating this assumption, in study 1, participants exposed to an acute stressor prior to encoding showed enhanced late centro-parietal ERP old/new differences during recognition of emotionally arousing pictures, relative to controls, indicating enhanced hippocampus-dependent recollection (Weymar & Hamm, 2013). No differences were observed for neutral pictures, indicating that acute stress selectively promotes encoding and consolidation for emotionally arousing stimuli (Wirkner et al., 2013).

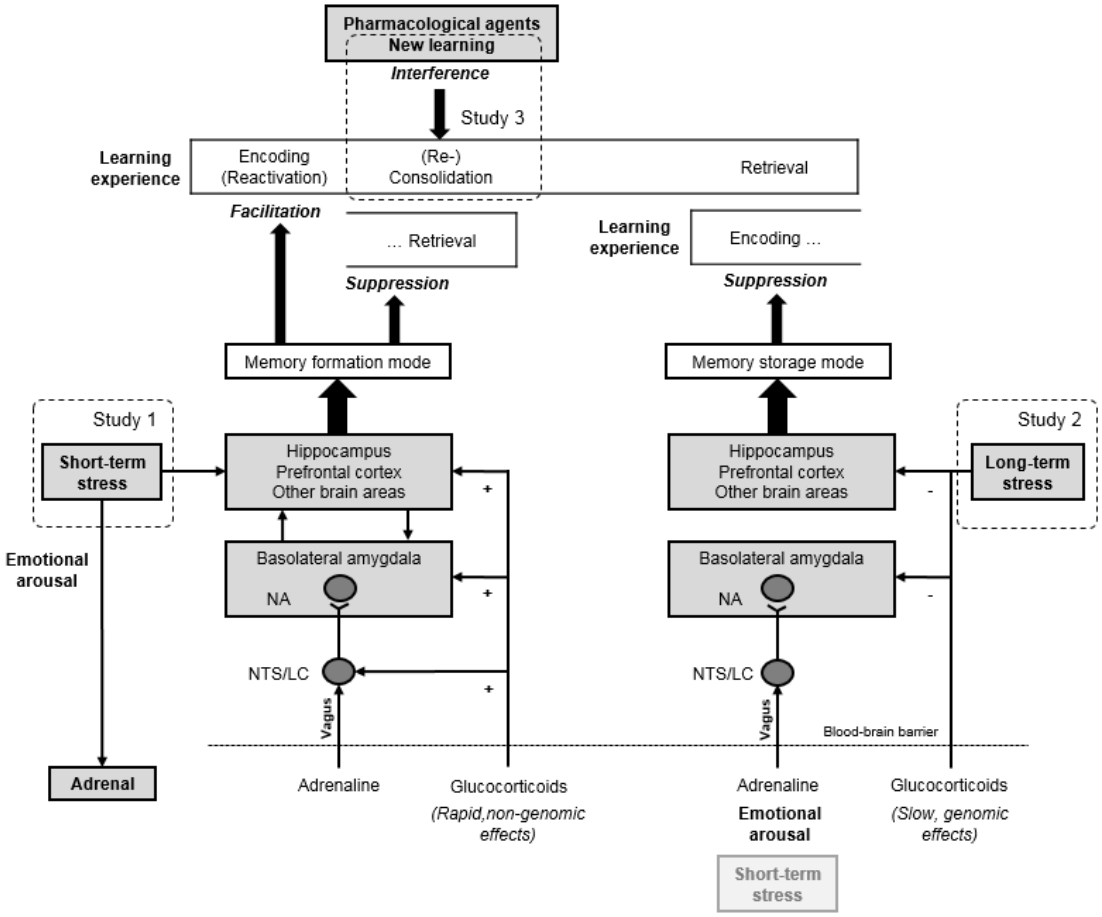

Figure 6. A link to the integrative model of stress on memory: Effects of short (Study 1) and long-term stress (Study 2), and interference learning (Study 3) on memory (re)consolidation. NA= noradrenaline; NTS= nucleus tractus solitarii; LC= locus coeruleus (adapted from Schwabe et al., 2012).

Second, long-term stress has been found to suppress both memory encoding and retrieval via detrimental effects on brain structures involved in memory (especially the hippocampus) (Wolkowitz et al., 1997). Therefore, in study 2, neuropsychological functioning as well as encoding and recognition memory of emotional stimuli were investigated in breast cancer survivors. This group was selected because diagnosis and treatment are physically and emotionally challenging over a long period of time. Indeed, elevated hair cortisol levels as a marker of long-term stress were observed in breast cancer survivors, compared to healthy controls. Breast cancer survivors were more anxious and depressed than controls and showed impairments in verbal memory and in recognition memory for emotionally arousing pictures, suggesting that long-term stress may impair memory (Figure 6). In addition, brain potentials during encoding indicated attentional deployment from unpleasant pictures in breast cancer survivors. There is evidence that the adaptation to emotionally challenging events is blunted in chronically stressed individuals (Andreotti et al., 2015). The results from study 2 suggest that encoding and retrieval may be especially impaired for emotionally arousing stimuli in chronically stressed individuals. But, to date, the interactions between chronic and acute stress are not well understood and the conclusions from study 2 remain speculative, as, unfortunately, the small sample size did not allow further testing for specific associations.

Third, it is known that reactivation renders established memories prone to modification (Nader, 2015). Study 3 investigated, whether new learning selectively interferes with reconsolidation of emotional. Indeed, new learning following brief reactivation especially affected later memory for emotionally arousing (but not neutral) pictures (Wirkner et al., 2015). To date, there is some evidence, that acute stress improves memory reconsolidation and can even enhance reconsolidation of emotional relative to neutral memories (Coccoz et al., 2011; Schwabe & Wolf, 2010). Thus, reconsolidation might be linked to the integrative model as shown in figure 6. But, more research on the effects of

stress and stress hormones on reconsolidation processes is required (Akirav & Maroun, 2013; Meir Drexler & Wolf, 2016) and this link should therefore be treated with caution.

Finally, all three studies provide multiple evidence for higher attention allocation to and more elaborated processing of emotionally arousing stimuli, and the emotional memory advantage on the behavioral and neural level, further supporting the modulation hypothesis (McGaugh, 2004; Roozendaal & McGaugh, 2011).

To sum up, the results of the present thesis suggest that acute stress around the time of encoding enhances emotional memory, whereas chronic stress might impair encoding and recollection of emotionally arousing memories. Moreover, established emotional memories can successfully be targeted by interfering learning following reactivation. Understanding the role of acute and long-term stress on (emotional) memory formation and the modification of (unwanted) emotional memories following reactivation, are crucial to shed light on the formation of stress-related mental disorders, therefore providing new prevention and treatment approaches. Definitely, more data are needed to test the model of short- and long-term stress on encoding, consolidation, and reconsolidation of emotionally arousing and neutral memories. Besides disentangling the differential effects of acute and chronic stress and their interactions, prevention and modification of impairing memories should be one of the main research focuses in order to target stress-related mental disorders. To further examine emotional memory formation and modification, future research may apply varying time points of short-term stress induction in the processes of picture memory acquisition, retrieval and reconsolidation. These paradigms should also be conducted in individuals with and without chronic stress or stress-hormone exposure, in order to investigate possible interaction processes. Then, stress influences and memory modulation by means of reactivation blockade may also be investigated in mental disorders.

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Appendix A: Publications

(Peer-reviewed journal articles)

Manuscript 1

Wirkner, J., Weymar, M., Löw, A., & Hamm, A. O. (2013). Effects of Pre-Encoding Stress on Brain Correlates Associated with the Long-Term Memory for Emotional Scenes. *PLoS ONE*, *8*, e68212.

Manuscript 2

Wirkner, J., Weymar, M., Löw, A., Hamm, C., Struck, A.-M., Kischbaum, C., & Hamm, A. O. (under review). Cognitive functioning and emotion processing in breast cancer survivors and controls: An ERP pilot study.

Manuscript 3

Wirkner, J., Löw, A., Hamm, A. O., & Weymar, M. (2015). New learning following reactivation in the human brain: Targeting emotional memories through rapid serial visual presentation. *Neurobiology of Learning and Memory, 119*, 63–68.

Manuscript 1

Effects of Pre-Encoding Stress on Brain Correlates Associated with the Long-Term Memory for Emotional Scenes

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Effects of Pre-Encoding Stress on Brain Correlates Associated with the Long-Term Memory for Emotional Scenes

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Abstract

Recent animal and human research indicates that stress around the time of encoding enhances long-term memory for emotionally arousing events but neural evidence remains unclear. In the present study we used the ERP old/new effect to investigate brain dynamics underlying the long-term effects of acute pre-encoding stress on memory for emotional and neutral scenes. Participants were exposed either to the Socially Evaluated Cold Pressure Test (SECPT) or a warm water control procedure before viewing 30 unpleasant, 30 neutral and 30 pleasant pictures. Two weeks after encoding, recognition memory was tested using 90 old and 90 new pictures. Emotional pictures were better recognized than neutral pictures in both groups and related to an enhanced centro-parietal ERP old/new difference (400–800 ms) during recognition, which suggests better recollection. Most interestingly, pre-encoding stress exposure specifically increased the ERP old/new-effect for emotional (unpleasant) pictures, but not for neutral pictures. These enhanced ERP/old new differences for emotional (unpleasant) scenes were particularly pronounced for those participants who reported high levels of stress during the SECPT. The results suggest that acute pre-encoding stress specifically strengthens brain signals of emotional memories, substantiating a facilitating role of stress on memory for emotional scenes.

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Introduction

Acute stress initiates various bodily adaptation processes to establish physiological homoeostasis and affects cognitive processes such as attention, learning and memory. The link between stress and cognitive functioning has been the focus of intensive basic and clinical research. Experiencing episodes of extreme stress can lead to mental disorders such as post-traumatic stress disorder (PTSD), which is characterized by severe impairment in cognitive functioning and memory (e.g., intrusive recollections and hyper-arousal) [1].

However, studies that investigated the effects of stress and elevated stress hormone levels on learning and memory in healthy populations have reported mixed findings [2–4]. Acute stress prior to memory retrieval can impair memory performance [5,6]. Kuhlmann et al. found that free recall of previously learned negative and positive words, but not of neutral words, was impaired after experiencing a psychosocial stressor shortly prior to memory testing [6].

In contrast, acute stress and stress level glucocorticoid doses around the time of encoding have been shown to improve later memory performance for emotionally arousing events in humans and animals [4,7,8]. For instance, exposure to cold pressure stress immediately after encoding high arousing emotional and neutral IAPS pictures led to enhanced memory recall for emotional pictures, but not for neutral pictures one week later [9]. Similarly, Payne et al. found that pre-encoding psychosocial stress facilitated memory for an emotional story while recognition for the neutral episode was impaired. These discrepant findings for emotional and neutral materials have been discussed to result from differential effects of stress and stress hormones on brain regions involved in either attention or memory control [8]. Likewise, cortisol administration, shortly before picture viewing, enhanced later incidental memory performance for pleasant and unpleasant compared to neutral pictures [10]. Memory enhancement after pre-encoding stress was also shown for emotional words; conversely, memory for neutral words were impaired [11]. Additionally, studies with neutral stimulus materials found mixed results or even failed to show such memory enhancing effects [12,13]; therefore suggesting that noradrenergic activation due to emotional arousal seems to be essential for the memory enhancing effects of pre-encoding stress [14–16].

A key advantage of ERPs compared to other brain imaging techniques is that they provide measures of neural activity with extraordinary time resolution in real time and thus makes them ideally suited to examine neural events responsible for human memory [17]. ERPs of the retrieval of previously encoded items have been traditionally studied in recognition memory tasks, where old and new items are presented. It has been repeatedly shown that ERPs elicited by recognized old items evoke more positive going waveforms than those elicited by correctly classified new items [18–20]. Early research suggests a dual-process model

where recognition memory is assumed to be based on familiarity (i.e. the feeling of knowing an item), and recollection, characterized by detailed item recognition and supposed to require hippocampal involvement [21]. Linking the ERP data to the dual-process model of recognition memory the ERP old/new effect was separated into two topographically and temporally distinct components: an early effect over frontal electrode sites, peaking between 300 and 500 ms and a late centroparietal old/new effect starting at about 400 ms after stimulus onset. There is multiple evidence associating the late centroparietal old/new effect with recollective experience and hippocampus-dependent recognition [20,22–24], whereas the assumption of the early frontal component reflecting familiarity processes is still under debate [17,25].

Recent picture memory studies using longer retention intervals from 24 hours up to one year [26–30] suggest that the centroparietal old/new effect is modulated by emotional arousal, showing higher old/new differences for emotional pictures compared to neutral pictures, in accordance with better memory performance for these stimuli [31]. Moreover, the centroparietal effect for emotional contents is related to increased confidence [28,29] and remember judgments, supporting the functional association of the parietal old/new difference with recollective experience and the role of recollection in emotional memory [32].

In the present study, we examined the influence of acute pre-encoding stress on brain dynamics associated with the long-term memory (two-week interval) for emotionally arousing and neutral scenes. In accordance with our previous ERP long-term recognition memory studies [26,28], we predicted better memory performance for emotionally arousing compared to neutral pictures. For ERPs, we expected to find larger centro-parietal positivity for old, compared to new pictures during recognition, with larger ERP old/new differences for emotional relative to neutral pictures. If stress during encoding facilitates memory consolidation for emotionally arousing events [15] we further expected enhanced recognition and larger ERP old/new effects following acute pre-encoding stress selectively for emotionally arousing pictures. Recognition and brain potentials of neutral pictures, on the other hand, were expected to be unaffected or even impaired [11] by the stress manipulation.

Materials and Methods

Ethics Statement

Participants provided informed written consent for the protocol approved by the Review Board of the University of Greifswald and received financial compensation for participation. The study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki) printed in the British Medical Journal (18 July 1964).

Participants

Fifty-two healthy students (23 females) from the University of Greifswald (mean age: 23.0 years, range: 18–30, 4 left-handed, mean body-mass-index (BMI): 22.2, range 19–27 kg/m^2) participated in the study. Exclusion criteria were checked in a standardized telephone interview and included smoking, current or lifetime diagnosis of mental disorders, medical conditions and medication intake within the prior three weeks, and during study participation. Participants were instructed to refrain from physical exercise, meals and caffeine intake within 3 h prior to the experimental sessions. Female participants reported a regular cycle with six subjects in follicular and 17 in luteal phase. All participants had normal or corrected-to-normal vision.

Table 1. Sample characteristics.

	Control	Stress
n	26	26
Mean (SEM) age [years]	23.6 (.61)	22.5 (.64)
Sex (male/female)	15/11	14/12
Female menstrual cycle		
follicular/luteal phase	2/9	4/8
Handedness		
left/right	24/2	24/2
Mean (SEM) BMI [kg/m^2]	22.3 (.39)	22.0 (.34)

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Stress protocol and control condition

Participants were randomly assigned to either the stress or control condition. Groups did not differ for age ($F_{(1,51)} = 1.1$, $p = .19$), sex, handedness, BMI and menstrual cycle for females (all $F_{(1,51)} < 1$; see Table 1).

In the stress condition, participants (N = 26) were exposed to the Socially Evaluated Cold Pressure Test (SECPT) as described by Schwabe et al. [33]. Participants were monitored by a rather cold and unsociable experimenter and were asked to immerse their right hand, including the wrist, into ice water (temperature: 0–2°C) for 3 min (or until they could no longer tolerate it). During hand immersion, participants were videotaped, asked to look straight into the camera and told that video recordings would later be analyzed for facial expressions. Several studies have shown that the SECPT is an effective stress induction method that leads to significant elevations in autonomic arousal, salivary cortisol and subjective stress ratings [34–36]. Participants in the control condition (N = 26) immersed their right hand including the wrist for 3 min in warm water (35–37°C). They were neither videotaped nor monitored by an unfamiliar experimenter. To validate the efficacy of the SECPT, cardiovascular measures (heart rate and blood pressure) were recorded manually (using Riva-Rocci technique) immediately before (pre), during and after (post) SECPT or warm water test. Participants then rated on a scale from 0 ("not at all") to 100 ("very much") how stressful, painful and unpleasant the previous procedure was and how difficult it was to keep the hand immersed in the water.

Stimulus materials

Stimuli consisted of 180 pictures (60 unpleasant, 60 neutral and 60 pleasant pictures) taken from the International Affective Picture Series (IAPS) [37] and the Emotional Picture Set (EmoPicS) [38] (IAPS and EmoPicS Numbers. Set 1: Unpleasant: 1019, 1220, 1300, 1932, 2352,2, 3019, 3064, 3102, 3110, 3150, 3180, 3190, 3191, 3195, 3530, 6210, 6212, 6313, 6560, 6571, 8180, 9042, 9230, 9301, 9490, 9561, 9599, 9902, 9910, 9921; Neutral: EP278, 2026, 2038, 2357, 2390, 2512, 2513, 2850, 2890, 5130, 5390, 5535, 5593, 5726, 5800, 7037, 7041, 7150, 7205, 7207, 7234, 7491, 7495, 7546, 7550, 7595, 7900, 7920, 9210, 9360; Pleasant: 1463, 1540, 1710, 1811, 2040, 2158, 2160, 2208, 2300, 4604, 4611, 4640, 4647, 4652, 4658, 4659, 4681, 5470, 5621, 5626, 8030, 8160, 8170, 8180, 8260, EP075, 8470, 8490; Set2: Unpleasant: 1052, 1201, 1304, 1726, 1931, 3015, 3051, 3062, 3100, 3101, 3140, 3160, 3225, 3250, 3261, 6260, 6370, 6410, 6563, 6821, 9008, 9440, 9520, 9560, 9570, 9600, 9622, 9630, 9635,1, 9908; Neutral: EP308, 2190, 2206, 2273, 2383, 2595, 2749, 2840, 2870, 2980, 5120, 5510, 5635, 5711, EP345, 5875,

6000, 7038, 7130, 7160, 7179, 7233, 7490, 7493, 7500, 7510, 7547, 7590, 7710, 9401; Pleasant: 1440, 1590, 1720, 1722, 2058, 2075, 2080, 2340, 2345, 4598, 4599, 4645, 4651, 4656, 4687, 4693, 4694, 4800, 5629, 8021, 8041, 8080, 8161, 8185, 8186, 8190, 8300, 8370, 8380). Two stimulus sets were carefully matched according to their normative hedonic valence and arousal ratings (see IAPS and EmoPicS norms for both sexes; set 1: mean hedonic valence = 2.6, 5.1 and 7.1; mean arousal = 6.1, 3.2 and 5.9 for unpleasant, neutral and pleasant pictures, set 2: mean hedonic valence = 2.6, 5.2 and 7.1; mean arousal = 6.0, 3.2 and 5.9). Additionally, both sets were matched for semantic categories (e.g., pictures of attack, mutilations, neutral people, objects, adventure, and erotic couples). The picture sets were counterbalanced during encoding so half of the sample viewed each of the two picture sets.

Each picture set consisted of 90 pictures (30 unpleasant, 30 neutral and 30 pleasant pictures, respectively). 21 pictures were added before (7 unpleasant, 7 neutral, 7 pleasant) and after (7 unpleasant, 7 neutral, 7 pleasant) picture presentation to avoid serial position effects on subsequent memory performance. These pictures were not included in the analyses.

In addition, individual hedonic valence and arousal ratings for all pictures in our sample were obtained to check for correspondence with normative ratings of the IAPS and EmoPicS. As expected, unpleasant pictures were rated as more unpleasant (Mean valence: 2.8) than neutral (Mean valence: 4.9; $F_{(1,51)} = 475.08$, $p < .001$) and pleasant (Mean valence: 6.8, $F_{(1,51)} = 883.74$, $p < .001$) pictures. Additionally, emotional pictures (pleasant, Mean arousal: 4.5; unpleasant, Mean arousal: 5.8) were rated as more arousing than neutral pictures (Mean arousal: 2.4; $F_{(1,51)} = 349$, $p < .001$). As in our previous study [36], there were no group differences between SECPT and control condition regarding hedonic valence ($F_{(1,25)} = 2.6$, $p = .118$) and arousal ($F_{(1,25)} < 1$) ratings. No differences were observed between both picture sets.

Procedure

All experimental sessions took place in the afternoon between 1 and 5 pm. After participant's arrival at the laboratory, heart rate and blood pressure measurements were taken. Then, participants were exposed to either the SEPCT or control condition. Heart rate and blood pressure were measured during hand immersion. Participants then rated how stressful, painful and unpleasant the previous situation was and how difficult it was to keep the hand immersed in the water. In addition, heart rate and blood pressure were recorded again. During the following encoding session, 90 pictures were presented on a 20-inch computer screen for 3000 ms with a random inter-trial interval (ITI) of 2000, 2500 or 3000 ms. A 500 ms fixation cross preceded the onset of each picture to ensure that participants fixated the center of the screen. The pictures were presented in pseudorandom order for each participant with the restriction that no picture from the same valence category was presented in two consecutive trials. Participants were instructed to attentively watch the pictures and to avoid eye blinks and body movements during ERP measurement. No mention of a memory test was made (incidental encoding).

Two weeks after the encoding session participants returned to the lab for memory testing. After attaching the EEG electrodes, a recognition test was conducted during which 90 previously seen pictures (30 unpleasant, 30 neutral, and 30 pleasant pictures) were presented randomly intermixed with 90 new pictures that were matched for content, valence and arousal. Each picture was displayed for 3000 ms and preceded by a 500 ms fixation cross. Participants were instructed to attentively watch the pictures and to avoid eye blinks and body movements during ERP measurement. Following each picture, participants had to indicate whether they had seen the picture before or not, by pressing either a "yes" or "no" button. The assignment of left and right button presses to yes/no responses was counterbalanced across participants. After recognition, participants were asked to rate all previously seen pictures for their subjective hedonic valence and arousal using the SAM rating procedure [39].

Electrophysiological recording

EEG signals were recorded continuously from 257 electrodes using an Electrical Geodesics (EGI) HydroCel high-density EEG system with NetStation software on a Macintosh computer. The EEG recording was digitized at a rate of 250 Hz, using the vertex sensor Cz as recording reference. Scalp impedance for each sensor was kept below 30 kΩ. All channels were bandpass filtered online from 0.1 to 100 Hz. Offline reduction was performed using EMEGS [40] and included lowpass filtering at 40 Hz, artifact detection, sensor interpolation, baseline correction, and conversion to the average reference [41]. Stimulus-synchronized epochs were extracted from 100 ms before to 1200 ms after picture onset and baseline corrected (100 ms prior to stimulus onset).

ERPs were computed for the three emotional picture categories (unpleasant, neutral and pleasant) in each experimental group (stress vs. control). Only trials with correct responses (correctly recognized old pictures and correctly classified new pictures, respectively) were included in ERP averages.

Data analysis

To identify sensor clusters representative for the old/new effect, visual inspection and single-sensor waveform analyses were used in concert. On the basis of this inspection, ANOVAs, including the factors Emotion (unpleasant vs. neutral vs. pleasant), Memory (old vs. new) and Group (stress vs. control), were calculated for each time point and each individual sensor [28]. Based on these results and guided by previous studies [22,26,28], two time windows and electrode clusters (Figure 1) were selected for further statistical analyses. An electrode cluster over frontal sites (including EGI sensors 5, 6, 7, 8, 13, 14, 15, 16, 17, 21, 22, 23, 24, 28, 29, 198 and 207) was selected for the early time window (300–500 ms) and a centro-parietal electrode cluster (including EGI sensors 45, 53, 79, 80, 81, 88, 89, 90, 100, 101, 129, 130, 131, 132, 142, 143, 144 and 257) was selected for the late time window between (400–800 ms).

Mean ERP amplitudes of both scalp clusters in the corresponding time windows were analyzed separately using an ANOVA involving the within-subject factors Emotion (unpleasant vs. neutral vs. pleasant) and Memory (old vs. new) as well as the between-group factor Group (stress vs. control).

In addition, group differences were observed for old and new neutral and unpleasant pictures in the time window between 250 and 300 ms over the frontal electrodes (see Figure 1). Mean N200 amplitudes were analyzed using an ANOVA involving the within-subject factors Emotion (unpleasant vs. neutral vs. pleasant) and Memory (old vs. new) and the between-group factor Group (stress vs. control).

For behavioral performance, hit rates, false alarm rates and the discrimination index Pr for recognition were analyzed using an ANOVA involving the factors Emotion (unpleasant vs. neutral vs. pleasant) and Group (stress vs. control). All analyses were conducted with SPSS 19.0 (IBM, Armonk, NY, USA).

For effects involving repeated measures, the Greenhouse-Geisser procedure was used to correct violations of sphericity.

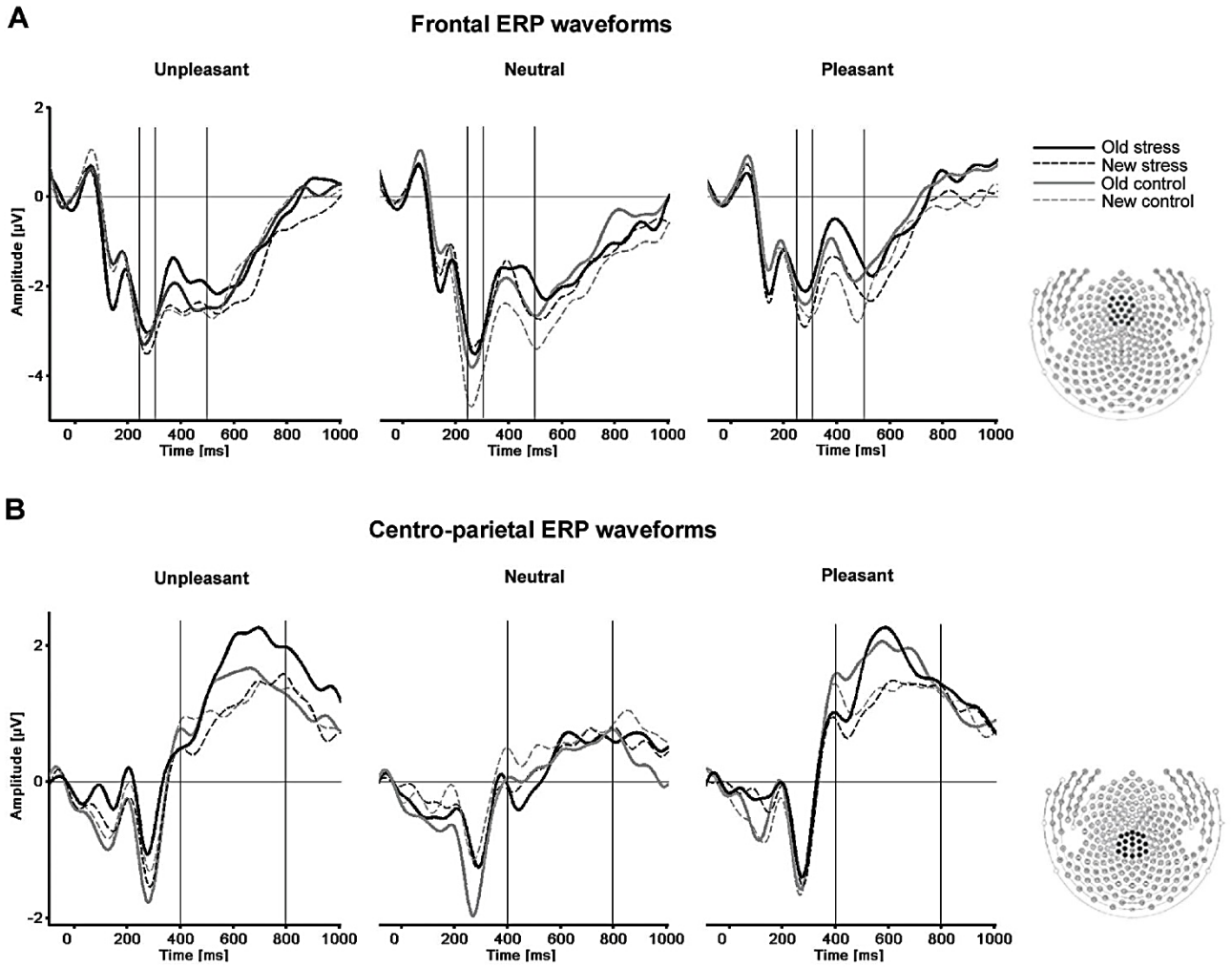

Figure 1. Grand average ERPs waveforms at frontal (A) and centro-parietal (B) sensor clusters for old (thick line) and new (dotted line) unpleasant, neutral and pleasant pictures in stressed (black lines) and control (grey lines) participants.
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Results

Stress ratings and cardiovascular responses to the SECPT/warm water control

Autonomic and subjective measurements indicated a successful stress induction by the SECPT (see Table 2).

Stress ratings. As expected, participants rated the hand immersion in the SECPT condition as significantly more stressful ($F_{(1,50)} = 46.29$, $p < .001$), painful ($F_{(1,50)} = 104.94$, $p < .001$) and unpleasant ($F_{(1,50)} = 67.63$, $p < .001$) than the participants in the warm water control condition. In addition, hand immersion was harder to tolerate in the stress group ($F_{(1,50)} = 59.25$, $p < .001$) compared to the warm water control group.

Heart rate and blood pressure. Exposure to the SECPT also resulted in significantly stronger increases in heart rate (Time×Group, $F_{(2,50)} = 4.14$, $p < .05$), systolic (Time×Group, $F_{(2,50)} = 69.85$, $p < .001$) and diastolic (Time×Group, $F_{(2,50)} = 8.69$, $p < .01$) blood pressure compared to the control group. Moreover, during hand immersion, participants in the SECPT group showed significantly elevated autonomic reactions (heart rate: $F_{(1,50)} = 6.80$, $p < .05$; systolic blood pressure: $F_{(1,50)} = 15.48$, $p < .001$; diastolic blood pressure: $F_{(1,50)} = 50.63$, $p < .001$) compared to the control group (see Table 2). No significant group differences were observed immediately before and after hand immersion, supporting the view that the observed group differences were specifically induced by the stress test.

Recognition: Behavioral data

Table 3 lists memory performance for old and new pictures as a function of picture content and experimental group.

Recognition memory performance. As expected for hit rate (hr), a main effect of Emotion ($F_{(2,100)} = 42.44$, $p < .001$) indicated that pictures with emotional contents were better remembered than neutral pictures. Unpleasant pictures (Mean (hr) = .81) were better recognized than pleasant (Mean (hr) = .74) pictures ($F_{(1,50)} = 24.36$, $p < .001$). Consistent with hit rate, correct discrimination (Pr) between old and new pictures was better for emotional compared to neutral pictures ($F_{(2,100)} = 47.66$, $p < .001$). False alarm rates differed between emotional contents ($F_{(2,100)} = 4.21$, $p < .05$) and were slightly higher for pleasant compared to unpleasant pictures. No group effects were observed for false alarms ($F_{(1,50)} < 1$, $p = .434$) or hit rates ($F_{(1,50)} = 3.21$, $p = .079$). In contrast, a main effect for group ($F_{(1,50)} = 6.94$, $p < .05$) indicated that picture discrimination (Pr) was overall better

Table 2. Subjective stress ratings and autonomic measures during and after the SECPT/warm water control condition.

	Control	Stress
Stress ratings		
Stressful	6.9 (2.3)	**45.8 (5.2)****
Painful	3.8 (1.8)	**59.2 (5.1)****
Unpleasant	8.5 (3.4)	**61.5 (5.4)****
Hard to tolerate	9.6 (3.7)	**57.7 (5.0)****
Heart rate (bpm)		
During hand immersion	63.7 (1.5)	**71.6 (2.5)***
After hand immersion	63.7 (1.8)	64.9 (1.8)
Systolic blood pressure (mmHg)		
During hand immersion	121.5 (1.3)	**129.7 (1.6)****
After hand immersion	121.1 (0.9)	118.6 (1.5)
Diastolic blood pressure (mmHg)		
During hand immersion	79.6 (0.6)	**89.5 (1.2)****
After hand immersion	79.3 (0.7)	77.8 (1.0)

Subjective assessments were measured using a scale from 0 ("not at all") to 100 ("very much"). Data represent means (SEM). Bold indicates significantly higher values in stress compared to control group (*p<.05, **p<.001).
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Table 3. Memory: Behavioral Data.

	Control	Stress
Hit rate		
Unpleasant	.84 (.02)	.78 (.03)
Neutral	.66 (.04)	.58 (.04)
Pleasant	.78 (.03)	.70 (.03)
FA rate		
Unpleasant	.15 (.02)	.17 (.02)
Neutral	.16 (.02)	.20 (.02)
Pleasant	.19 (.02)	.20 (.02)
Pr		
Unpleasant	.69 (.02)	.61 (.03)
Neutral	**.51 (.03)***	.39 (.03)
Pleasant	.58 (.02)	.50 (.03)

Numbers represent means for hit and false alarm rates and discrimination Pr for each picture type (SEM). Bold indicates significantly higher values in control compared to stress group (*p<.05).
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in the warm water control compared to the stress group. Post hoc tests showed that discrimination was better for neutral pictures in the controls compared to stress group ($F_{(1,50)} = 7.01$, $p<.05$); however these group differences did not occur for emotionally arousing pictures (F1,50) = 2.83, p = .10).

Recognition: ERP data

Figure 1 illustrates the grand average ERPs for correctly recognized old and new pictures of two representative sensor clusters as a function of picture content (unpleasant, neutral and pleasant pictures) and group (stress vs. control).

N200. In the time window from 250 to 300 ms, new pictures evoked a larger ERP negativity than old pictures (Memory: $F_{(1,50)} = 9.18$, $p<.01$) over frontal sensor sites. A significant interaction (Emotion×Memory×Group: $F_{(2,100)} = 3.228$, $p<.05$) indicated that novel neutral pictures prompted a larger N200 than correctly recognized old neutral pictures in the control group ($F_{(1,25)} = 4.50$, $p<.05$). This effect did not occur in the SECPT group ($F_{(1,25)}<1$, $p = .731$). In contrast, new unpleasant pictures prompted a larger negativity than correctly recognized old unpleasant pictures in the SEPCT group but not in the controls ($F_{(1,25)} = 4.32$, $p<.05$; Control group: $F_{(1,25)}<1$, $p = .718$). For pleasant scenes, the N200 in response to novel pictures, compared to old, did not differ between stress and control group (Memory×Group: $F_{(1,50)} = 1.20$, $p = .278$).

Early old/new effect. In the early time window from 300 to 500 ms, correctly recognized old pictures prompted more positivity, relative to correctly classified new pictures over frontal (Memory: $F_{(1,50)} = 39.30$, $p<.001$), but not over centro-parietal sensor sites (Memory: $F_{(1,50)}<1$, $p = .770$). No main effects of group or any interactions were significant over frontal ($F_{(1,50)}<1$, $p = .542$) and centro-parietal sensors ($F(1,50)<1$, $p = .450$) in the early time window.

Late old/new effect. In the late time window between 400 and 800 ms, there was a prominent old/new effect (Memory: $F_{(1,50)} = 19.45$, $p<.001$) over frontal electrodes but no interaction with emotion ($F_{(2,100)}<1$, $p = .50$). No group differences were observed between SECPT and control group ($F_{(1,50)}<1$, $p = .943$).

For centro-parietal sensors, correctly recognized old pictures showed greater ERP positivity than new pictures (Memory: $F(1,50) = 13.45$, $p<.01$). Notably, this old/new difference was modulated by emotion (Emotion×Memory: $F_{(2,100)} = 5.46$, $p<.01$) with emotional pictures showing larger old/new differences than neutral pictures ($F_{(1,50)} = 9.06$, $p<.01$) (see Figure 2). The old/new difference for unpleasant pictures was significantly larger in the stress group compared to the non-stressed control group ($F_{(1,25)} = 4.45$, $p<.05$). These group differences were not observed during correct recognition of neutral or pleasant pictures. The mean amplitude changes during correct recognition of old and new pictures as a function of emotion and group are listed in Table 4.

Experienced stress and the centro-parietal ERP old/new effect. To further examine the relationship between acute pre-encoding stress and memory, correlational and median split analyses were conducted. As a measure of overall experienced stress, the mean average of the four subjective stress ratings (stressful, painful, unpleasant and tolerance difficulty) was calculated. Significant correlations were observed between the reported stress level and the centro-parietal old/new effect (difference score: old minus new) for emotionally arousing pictures (Pearson correlation: Early time window: $r = .51$, one-tailed, $p<.001$; Late time window: $r = .40$, one-tailed, $p<.05$, see Figure 3A), but not for neutral ones in the SECPT group (Pearson correlation for unpleasant pictures: Early time window: $r = .38$, one-tailed, $p<.05$; Late time window: $r = .32$, one-tailed, $p = .056$ and Pearson correlation for pleasant pictures: Early time window: $r = .36$, one-tailed, $p<.05$; Late time window: $r = .266$, one-tailed, $p = .094$). As expected, no significant correlations between these variables were observed in the control group. Also no significant correlations were observed between reported stress levels and the frontal ERP old/new difference in both time windows.

A median split was performed for the overall reported stress level in the SECPT group (Median = 57.50), in which participants were divided into a high stress (Mean: 75.0) and low stress group (Mean: 37.11). Figure 3 (B and C) illustrates the influence of

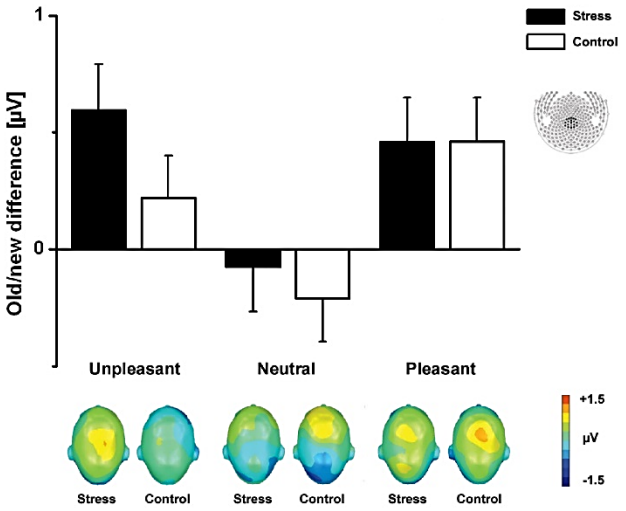

Figure 2. ERP old/new differences. The upper section shows the ERP old/new effect (old minus new) of the mean amplitudes recorded over the centro-parietal cluster in the 400–800 ms time window for stress and control group. Error bars represent standard error of the means (SEM). The lower section displays the corresponding scalp topographies of the ERP difference separately for the three picture categories (top view) and group.
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experienced stress (high vs. low) on the late centro-parietal old/new differences for emotionally arousing and neutral pictures.

Discussion

In the present study, we examined the influence of acute pre-encoding stress on brain potentials of long-term memory for emotional and neutral scenes. Correct recognition of previously seen pictures evoked enhanced positivity over centro-parietal sensor sites in the late time window (400–800 ms) compared to viewing of new pictures. This late old/new difference was more pronounced for emotional picture contents. Interestingly, although memory performance was not generally facilitated by stress exposure, we found that acute pre-encoding stress specifically enhanced the late centro-parietal old/new effect for emotional (unpleasant) pictures. Furthermore, participants experiencing high levels of stress showed higher ERP old/new differences for emotionally arousing (unpleasant and pleasant) pictures, but not for neutral pictures. Taken together, pre-encoding stress prompts an enhanced old/new effect during recognition of emotional pictures that varies with individual stress levels.

Correct recognition of previously presented compared to correctly rejected new pictures was associated with enhanced ERP positivity over centro-parietal sensor sites (400–800 ms). This late old/new difference over posterior sensor sites has been reliably described as an electrophysiological correlate reflecting retrieval processes with specific episodic recollection [20] because it is modulated by depth of processing, correct source memory, "remember" and high confidence judgments [27,28,42]. As in earlier studies using longer retention intervals, memory performance was better for emotional compared to neutral contents [28,29,31,43]. Moreover, enhanced memory for emotional pictures was reflected in greater ERP old/new differences during recognition of emotional compared to neutral pictures over centro-parietal sensor sites in the late time window, suggesting that emotional stimuli are better recollected than neutral stimuli [19,27,28,30,44].

Previous work has indicated that stress influences learning and memory processes depending on the exact timing of the stressor [45]. When experienced in the context and around the timing of the learning episode, acute stress has been suggested to promote selective attention processes and to further enhance memory consolidation, particularly for emotional contents [7,9,10,15,36,46]. Overall, memory performance was not better in the stress, compared to control, group. In contrast, picture discrimination was significantly better for participants receiving the warm water relative to participants receiving the social and cold pressor stress, an effect that was most reliable for neutral contents. These data replicate previous findings which demonstrate that administration of stress and stress hormone doses around the time of encoding impair memory performance for neutral stimuli [11,12]. Similar results have been described by Payne et al., where recognition memory performance for a neutral slide show was selectively impaired in stressed subjects [8]. Payne et al. suggested that pre-encoding stress exposure preserves or even enhances emotional aspects of an episode whereas memory for neutral information is disrupted; therefore pointing to a qualitatively different memory formation under stress experience [7]. Recent data suggest that reduced memory for neutral events after acute stress might be related to changes in adrenergic and noradrenergic signaling in hippocampus and midbrain circuits during memory formation [47,48].

Even though the ERP old/new effect for neutral pictures did not differ between stressed and control participants, we found that new neutral pictures prompted an enhanced negativity (250 to 300 ms) compared to old neutral pictures in control participants.

Table 4. ERPs for correctly classified old and new pictures averaged over centro-parietal sensors (400–800 ms).

	Control		Stress		High stress		Low stress	
	Old	New	Old	New	Old	New	Old	New
Unpleasant	1.39	1.17	1.67	1.07	1.87	.93	1.46	1.21
	(.19)	(.23)	(.22)	(.18)	(.35)	(.28)	(.27)	(.23)
Neutral	.38	.59	.32	.39	.20	.34	.43	.44
	(.19)	(.20)	(.21)	(.15)	(.36)	(.19)	(.25)	(.25)
Pleasant	1.77	1.31	1.65	1.19	1.86	1.05	1.44	1.33
	(.20)	(.20)	(.20)	(.22)	(.27)	(.34)	(.29)	(.30)

Data represent means in µV (SEM).
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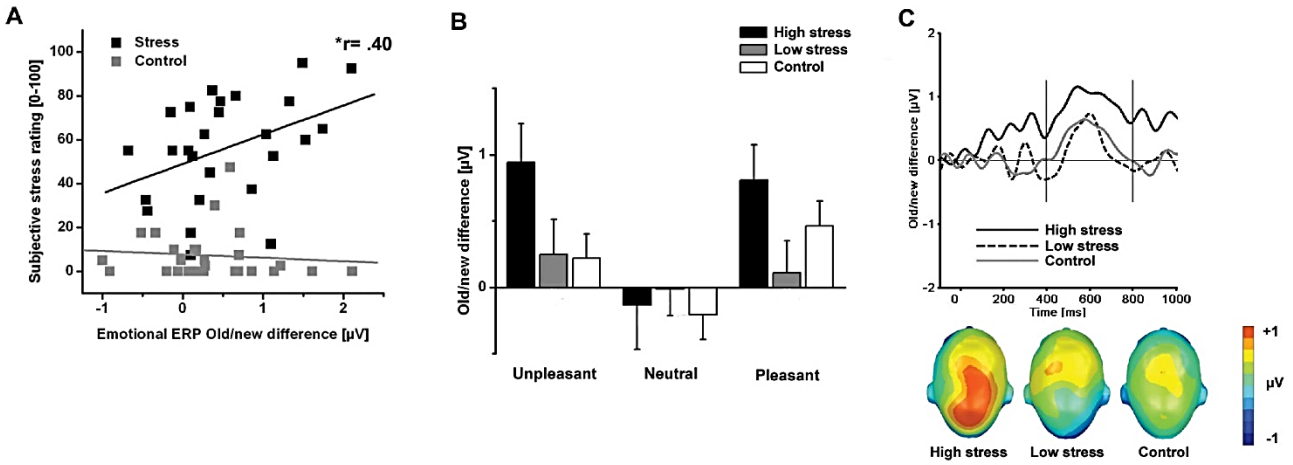

Figure 3. Subjective stress and centro-parietal ERP old/new effect. A. Experienced stress predicts enhanced centro-parietal ERP old/new difference for emotional pictures (400–800 ms) in the stress group. Correlations between (averaged) subjective stress ratings and centro-parietal ERP old/new effect (400–800 ms) for emotional pictures in both experimental groups (stress vs. controls). B. ERP old/new differences averaged over centroparietal sensors (400–800 ms) for unpleasant, neutral and pleasant pictures in high and low stressed participants and control group. Error bars indicate SEM. C. ERP difference waveforms (old-new) averaged over centroparietal sensors for emotional pictures in high stressed (black line), low stressed (dotted line) and control (grey line) participants. The lower section displays the corresponding scalp topographies of the ERP difference separately for the three groups.
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A fronto-central N200 has been discussed as an ERP component reflecting perceptual novelty during information processing, because it is elicited when a perceptual mismatch between the repetitive standard and an infrequent target is detected or when visual stimuli with novel features are presented [49–51]. No differences between neutral old and novel pictures were observed in stressed participants indicating that acute stress during encoding might affect later visual novelty detection (because of less specific memory representation) for neutral contents [47].

Although memory performance for emotional picture contents was not enhanced in the stress group on the behavioral level, we observed enhanced late centro-parietal ERP old/new differences for unpleasant pictures following pre-encoding stress exposure, indicating that the neural signature of the memory trace is enhanced for unpleasant stimuli encoded in the context of stressful experiences [15,46]. Collapsing recollection and familiarity based answers during our recognition task could have prevented better behavioral memory performance for emotionally arousing pictures after pre-encoding stress. We suggest that stress might enhance recollection based recognition for emotionally arousing pictures as reflected in the ERP data. Different behavioral measures to tap recollection and familiarity (remember/know judgments, confidence ratings) could be helpful for disentangling the differential effects of acute stress on later behavioral memory performance.

Another line of research has described enhanced memory based on mood-congruency [52], in which affective stimuli that are encoded in congruent with a current mood state are better remembered than incongruent stimuli [53]. Consistent with this model, experiencing an unpleasant painful cold pressor stress might selectively enhance memory for unpleasant cues only, as indicated by the larger ERP old/new difference. On the other hand, additional correlation and median split analyses revealed that the enhanced ERP old/new difference was not selectively related to unpleasant scenes, since it was also found for pleasant materials. Participants reporting more stress during SECPT exposure showed enhanced ERP old/new difference amplitudes for emotionally arousing contents, but not for neutral ones. This finding suggests that, if the stress experience is intense enough, pre-encoding stress specifically facilitates episodic retrieval for emotionally arousing materials. Recent ERP and fMRI studies suggest that stress increases perceptual vigilance [36,54–59] that might foster memory consolidation, particularly for emotional stimuli [60]. Replicating previous data [36], men showed larger late positive potentials (LPPs) during viewing of unpleasant stimuli following the cold pressor stress; therefore supporting the interpretation of more elaborative processing after stress. Women did not show this enhancement in the LPP. An elaborate discussion of these sex differences would, however, go beyond the scope of the current manuscript.

The amygdala is assumed to be the key brain structure for mediating stress effects on attentional networks and memory formation, interacting with several brain structures [such as the primary visual cortex, the prefrontal cortex, and the hippocampus] triggered by locus coeruleus-originating noradrenaline innervation [60,61]. Corroborating these assumptions, van Marle et al. found enhanced connectivity between amygdala and locus coeruleus activation during resting state after administering a short psychological stressor, suggesting a prolonged state of hypervigilance after stress that may facilitate salience and memory formation [56]. The present study did not test whether the findings for the ERP old/new effect during retrieval was mediated by stress- induced changes in noradrenaline or glucocorticoid activity during memory formation; however, the SECPT has been used in previous studies as an efficient stress induction method that leads to significant elevations in both autonomic arousal and salivary cortisol [33,35]. Furthermore, the ERP old/new effect of unpleasant pictures has been related to sympathetic activation during encoding [26], making it feasible that the combined action of noradrenaline and glucocorticoids on brain systems of attention and memory formation led to later changes in the ERP old/new effect in the present study.

From an evolutionary perspective, remembering the emotionally arousing aspects of a stressful experience is important for survival. But, experiencing highly arousing, life-threatening

episodes under conditions of extreme stress can result in exceptionally strong, over-consolidated traumatic (fear) memories (re-experienced in flashbacks or nightmares). These memories often lack the integration of specific neutral context information, and thus may lead to the development of Posttraumatic Stress Disorder (PTSD) and an overgeneralization of fear [1,48]. Interestingly, the individually perceived intensity of the traumatic event has been deliberated in the current DSM-5 debate to play an important role in the development of PTSD [62,63].

To summarize, acute exposure to stress significantly increased the late centro-parietal old/new effect during retrieval of emotionally arousing pictures. Moreover, this effect was most pronounced in participants reporting high subjective stress experience. These findings suggest that recollection of emotional memories seems to be particularly facilitated when the stressful event around the time of encoding is also evaluated as intense, stressful and unpleasant.

Author Contributions

Conceived and designed the experiments: JW MW AL AOH. Performed the experiments: JW. Analyzed the data: JW MW AL. Wrote the paper: JW MW AL AOH.

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Cognitive functioning and emotion processing in breast cancer survivors and controls: An ERP pilot study

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COGNITIVE FUNCTIONING AND EMOTION PROCESSING IN BREAST CANCER SURVIVORS AND CONTROLS: AN ERP PILOT STUDY

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Running head: Emotion processing in breast cancer survivors

Abstract

Diagnosis and treatment of breast cancer is a very emotionally aversive and stressful life event, which can lead to impaired cognitive functioning and mental health. Breast cancer survivors responding with repressive emotion regulation strategies often show less adaptive coping and adverse outcomes.

We investigated cognitive functioning and neural correlates of emotion processing using event-related potentials (ERPs). Self-report measures of depression, anxiety, and fatigue, as well as hair cortisol as an index of chronic stress were assessed. Twenty breast cancer survivors (BCS) and 31 carefully matched healthy controls participated in the study. After neuropsychological testing and subjective assessments, participants viewed 30 neutral, 30 unpleasant and 30 pleasant pictures and ERPs were recorded. Recognition memory was tested one week later.

BCS reported stronger complaints about cognitive impairments, and more symptoms of depression, anxiety, and fatigue. Moreover, they showed elevated hair cortisol levels. Except for verbal memory, cognitive functioning was predominantly in the normative range. Recognition memory performance was decreased in cancer survivors especially for emotional contents. In ERPs, survivors showed smaller LPP amplitudes for unpleasant pictures relative to controls in a later time window, which may indicate less elaborative processing of this material.

Taken together, we found cognitive impairments in BCS in verbal memory, impaired emotional picture memory accuracy and reduced neural activity when breast cancer survivors were confronted with unpleasant materials.

Further studies and larger sample sizes, however, are needed to evaluate the relationship between altered emotion processing and reduced memory in BCS in order to develop new treatment strategies.

Introduction

Over the last two decades cancer- and treatment-related changes in cognitive functions, such as attention and memory, and psychological well-being have gained increasing interest in breast cancer research. Breast cancer patients often report cognitive impairments with varying prevalence across studies, depending on different definitions and study designs (Shilling, Jenkins, & Trapala, 2006). Cognitive impairments were observed in about 15 to 25 % of breast cancer patients following chemotherapy (Ahles, Root, & Ryan, 2012). Mainly, the domains of attention, learning and memory were affected (Ahles et al., 2012; Pullens, De Vries, & Roukema, 2009). Verbal learning and memory were especially impaired in BCS tested shortly after treatment (Quesnel, Savard, & Ivers, 2009; Shilling, Jenkins, Morris, Deutsch, & Bloomfield, 2005; Vearncombe et al., 2009), but also after longer time intervals up to twenty years following chemotherapy (Bender et al., 2006; Koppelmans, Breteler, et al., 2012; Von Ah et al., 2009; Wefel, Saleeba, Buzdar, & Meyers, 2010).

Imaging studies investigating the neurobiological correlates of cognitive functioning in BCS have produced mixed findings (Kaiser, Bledowski, & Dietrich, 2014). Using magnetic resonance imaging (MRI), structural changes have been observed in gray and white matter up to 21 years after primary treatment (Abraham et al., 2008; de Ruiter et al., 2011; Koppelmans, de Ruiter, et al., 2012). Impaired recognition memory and hyporesponsiveness of the lateral posterior parietal cortex, related to deficient attentional capacities, was also found ten years following chemotherapy (de Ruiter et al., 2011). However, some studies reported recovery from these impairments over time (Inagaki et al., 2007; McDonald, Conroy, Ahles, West, & Saykin, 2010). In addition to brain imaging findings there is also evidence for altered event-related potentials (ERPs) during information processing in chemotherapy-treated breast cancer patients. Studies reported longer latencies and amplitude differences in the P3 waveform for patients, compared to

controls, suggesting changes in the allocation of processing resources and attentional deficits, but results were inconsistent (Kam et al., 2015; Kreukels et al., 2006, 2008).

Besides cognitive changes, depression, anxiety and fatigue are the most common impairing conditions following breast cancer diagnosis and treatment (Ho, Rohan, Parent, Tager, & McKinley, 2015; Maass, Roorda, Berendsen, Verhaak, & De Bock, 2015). Higher distress, anxiety and depression in breast cancer patients have been related to the use of repressive emotion regulation strategies, like emotion suppression or avoidance (Iwamitsu et al., 2005; Li et al., 2015; Wang et al., 2014).

Stress itself is also affecting cognitive functioning and emotion processing (McGaugh, 2015). Chronic stress can have detrimental effects on memory acquisition and consolidation, resulting in cognitive deficits and even hippocampal atrophy (Osborne, Pearson-Leary, & McNay, 2015; Roozendaal & McGaugh, 2011). Critically, chronic stress has been associated with impaired cognitive performance in breast cancer survivors (Fagundes, LeRoy, & Karuga, 2015), who failed to show memory enhancing effects of acute post-training stress for an emotional narrative, compared to controls (Andreano, Waisman, Donley, & Cahill, 2012).

In the present study we investigated cognitive performance in BCS and healthy controls using neuropsychological testing. As hair cortisol provides an elegant measure of recent chronic stress, we collected hair samples (Steudte et al., 2013). Moreover, self-perceived deficits in attention and memory performance, measures of fatigue, depression and anxiety, and brain dynamics of emotion processing were assessed. To test affective processing in BCS, we used a well-established picture-viewing paradigm (Cuthbert et al., 2000; Schupp et al., 2000), in which participants viewed pictures of emotional and neutral scenes. Free viewing of emotional pictures reliably prompt enhanced late positive potentials (LPPs) starting around 400 ms and lasting for several seconds. This slow LPP

is assumed to reflect increased attention allocation and elaborative processing (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Schupp et al., 2000), which can be also modulated by emotion regulation, when attention is drawn away from emotionally arousing aspect of a scene (Hajcak, Bress, Foti, Kujawa, & Klein, 2015). Because repressive emotion regulation strategies are observed in breast cancer survivors (Iwamitsu et al., 2005; Li et al., 2015; Wang et al., 2014), reduced LPPs might be visible in the ERPs for this group, relative to controls. One week after picture encoding, we also tested recognition memory (Weymar & Hamm, 2013). Previous studies found that emotional pictures are better recollected than neutral ones, in part due to deeper elaboration during encoding (Dolcos & Cabeza, 2002; Weymar et al., 2012; Wirkner, Löw, Hamm, & Weymar, 2015; Wirkner, Weymar, Löw, & Hamm, 2013). If reduced LPPs are observed during encoding of emotional pictures, this might also affect subsequent memory performance.

Taken together, we expected that BCS experience more long-term stress, depression, anxiety and fatigue compared to controls. Impairments in the cognitive domains of attention and memory were predicted, which may be specific for emotionally arousing materials.

Methods

Participants

Fifty-three women participated in the study. Two control participants had to be excluded from analysis because of missing recognition memory data. The final sample consisted of twenty female breast cancer survivors from the Psychooncological Outpatient Unit of the University Medicine Greifswald. Breast cancer survivors had undergone medical treatment including surgery, chemotherapy and endocrine therapy no longer than seven years ago (Median duration post diagnosis: 3.2 years; Median duration

post chemotherapy: 2.3 years). Thirty-one healthy controls were recruited via bulletin boards and were matched for sex, age, education and handedness (Table 1). All participants had normal or corrected-to-normal vision. Participants provided informed written consent for the study protocol approved by the Review Board of the Medical Faculty of the University of Greifswald in accordance with the provisions of the World Medical Association Declaration of Helsinki. All participants received financial compensation for participation.

Procedure

On the first day, participants underwent neuropsychological testing and filled in the questionnaires. To assess neuropsychological functioning in the attention domain, the following subtests of the validated German computer-based test series measuring non-verbal attention (Test Battery for Assessment of Attention, TAP) (Zimmermann & Fimm, 1992) were used: Alertness, Divided attention, and Go/Nogo. The Working memory task of the TAP and the subtests Digit span forwards and backwards (Wechsler Memory Scale revised; WMS-R) (Härting et al., 2000) were used to assess working memory and short term memory. Verbal memory was tested using the Logical memory I & II of the WMS-R and the German verbal learning and memory test (Verbaler Lern- und Merkfähigkeitstest, VLMT) (Helmstaedter, Lendt, & Lux, 2001).

Participants were asked to complete the trait version of the State-Trait Anxiety Inventory (STAI) (Laux, Glanzmann, Schaffner, & Spielberger, 1981; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), the revised Beck-Depression-Inventory (BDI-II) (Hautzinger, Keller, & Kühner, 2009), the German questionnaire for self-perceived deficits in attention (Fragebogen erlebter Defizite der Aufmerksamkeit, FEDA) (Zimmermann, Merser, Poser, & Sedelmeier, 1991) and the Multidimensional Fatigue Inventory (MFI-20) (Smets, Garssen, Bonke, & De Haes, 1995). Participants also rated their overall ability to concentrate and their general retentiveness/memory performance

on an analogue scale from 0 (“very bad”) to 100 (“very good”). Participants also provided sociodemographic and treatment (breast cancer survivors only) data.

On the second day, the free picture viewing paradigm was conducted. To determine retrospective cortisol levels of all participants, hair samples were collected before picture viewing (for procedure, see Steudte et al., 2013).

Participants were seated in a dimly lit sound-attenuated cabin, viewing a 20” computer monitor (1024 x 768, 60 Hz), located 1.5 m in front of the viewer. All participants then viewed ninety pictures (30 unpleasant, 30 pleasant, 30 neutral). During encoding, all 90 pictures as well as 42 buffer pictures were presented for 3000 ms with a random inter-trial interval (ITI) of 2000, 2500 or 3000 ms. A 500 ms fixation cross preceded every picture onset to ensure that participants fixated the center of the screen. The pictures were presented in pseudorandom order for each participant with the restriction that no picture from the same valence category was presented on two consecutive trials. Participants were instructed to attentively watch the pictures. No mention of a memory test was made (incidental encoding). For ERP recording, participants were instructed to avoid eye blinks and body movements.

One week after the encoding session, all participants underwent a recognition memory test in which ninety old pictures (from the encoding session) were presented randomly intermixed with ninety new pictures (30 unpleasant, 30 pleasant, 30 neutral pictures). Each picture was displayed for 3000 ms and preceded by a 500 ms fixation cross. Participants were instructed to decide whether each picture had been previously presented or not and to press an “old” button if they remembered the pictures, or else a “new” button. The assignment of left and right button presses to old/new responses was counterbalanced across participants.

After the recognition procedure, participants were asked to rate all pictures for their experienced pleasure (valence) and arousal using a computerized SAM rating

procedure (Bradley & Lang, 1994). All subjects reported that no memory test was expected. Finally, participants filled in the cognitive self-rating questionnaire for perceived concentration and memory function (analogue scale).

Stimulus materials

Experimental stimuli for picture viewing and recognition memory testing consisted of 270 pictures (90 unpleasant, 90 neutral and 90 pleasant pictures) taken from the International Affective Picture Series (IAPS; Lang, Bradley, & Cuthbert, 2008) and the Emotional Picture Set (EmoPicS; Wessa, Kanske, Heissler, & Schönfelder, 2010). Two stimulus sets, each consisting of 90 pictures (30 unpleasant, 30 neutral and 30 pleasant pictures) were matched according to their normative valence and arousal ratings (see IAPS and EmoPicS norms for females; Set 1: mean valence = 2.7, 5.1 and 7.1, mean arousal = 6.1, 3.2 and 5.9; Set 2: mean valence = 2.7, 5.2 and 7.1, mean arousal = 6.1, 3.2 and 5.9; for unpleasant, neutral and pleasant pictures, respectively) and for semantic categories (e.g. attack, mutilation, neutral people, objects, adventure, and erotic couples). Forty-two additional pictures were added prior to (7 unpleasant, 7 neutral, 7 pleasant) and after (7 unpleasant, 7 neutral, 7 pleasant) the 90 encoding pictures to avoid serial position effects on subsequent memory performance. These buffer pictures were not included in the analyses. The two picture sets were used to serve either as encoding picture set or as a new picture set during recognition memory testing. Valence (Likert-Scale ranging from "1 – very unpleasant" to "9 – very pleasant") and arousal ratings (Likert-Scale ranging from "1 - very calm" to "9 – very aroused") for each picture were assessed after the recognition memory task to ensure that the ratings of our sample corresponded with the standard ratings of the pictures.

EEG recording

EEG signals were recorded continuously from 257 sensors using an Electrical Geodesic system (EGI, Eugene, OR, USA) and digitized at a rate of 250 Hz, using the

vertex sensor (Cz) as recording reference. Scalp impedance for each sensor was kept below 30 kΩ. All channels were bandpass filtered online from 0.1 to 100 Hz. Offline reduction was performed using Electro Magneto Encephalography Software (EMEGS) (Peyk, De Cesarei, & Junghöfer, 2011) and included lowpass filtering at 40 Hz, artifact detection, sensor interpolation, baseline correction, and conversion to the average reference (Junghöfer, Elbert, Tucker, & Rockstroh, 2000). Based on artifact detection, on average, approximately 78%, 84.8% and 81.8% trials in the BCS group and 81.4% 79.9% and 77.4% trials in the control group were included in each picture category condition (unpleasant, neutral and pleasant, respectively).

Data analysis

Neuropsychological testing and questionnaires

The WMS-R, VLMT and all self-report questionnaires were analyzed according to the given instructions and norms. Subscales for FEDA and MFI-20 were calculated as recommended in the manuals. Data were analyzed using univariate ANOVAs including the between-subjects factor Group (BCS vs. Control).

Hair cortisol

Hair cortisol measures were available from 17 BCS and 27 control participants who provided written informed consent and sufficient hair probes for analysis. Hair cortisol was analyzed at the Technische Universität Dresden (LAB service; Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009). Data of six months hair cortisol storage (one cm hair length corresponds to approximately one month; Steudte et al., 2013) was available. Hair cortisol data was analyzed in an ANOVA involving the within-subjects factor Time (Segment A: 1-3 months before sampling vs. Segment B: 4-6 months before sampling; Steudte et al., 2013) and group (BCS vs. Control).

Event-related potentials

Stimulus-synchronized epochs were extracted from 100 ms before to 1200 ms after picture onset and baseline corrected (100 ms prior to stimulus onset). For picture encoding, ERP signals were computed for each sensor and participant for picture categories (unpleasant, neutral and pleasant) in each experimental group (BCS and Control). Based on the results of the waveform analyses and guided by previous research (Schupp et al., 2000; Schupp et al., 2007; Weymar et al., 2012), time windows and electrode clusters were selected for further statistical analyses accordingly. LPPs (early time window: 400-800 ms, late time window: 800-1200 ms, chosen after visual waveform inspection; centro-parietal EGI-sensors: 8, 9, 17, 44, 45, 53, 80, 81, 90, 131, 132, 144, 185, 186, 198, 257) were analyzed using ANOVAs including the within-subject factor emotion (Unpleasant vs. Neutral vs. Pleasant) and the between-subject factor Group (BCS vs. Control).

Emotional memory assessment

For assessing behavioral memory performance, hit rates (the probability that an old item is correctly classified as old) and false alarm rates (the probability that a new item is incorrectly classified as old) were calculated for the behavioral recognition data for each participant and valence category (Snodgrass & Corwin, 1988). Additionally, as a measure of memory accuracy, we calculated the discrimination index Pr subtracting the false alarm rates from the hit rates. High Pr values [p(hit) – p(false alarm)] indicate better ability to discriminate between old and new items and represent memory accuracy (Snodgrass & Corwin, 1988). For hit rates and the discrimination index Pr, ANOVAs were conducted involving the within-subject factor Emotion (Unpleasant vs. Neutral vs. Pleasant) and the between-subjects factor Group (BCS vs. Control).

Affective judgements

Valence and arousal ratings were analyzed separately involving the within-subject factor Emotion (Unpleasant vs. Neutral vs. Pleasant) and the between-subjects factor Group (BCS vs. Control).

All analyses were performed with SPSS 22.0 (IBM, Armonk, NY, USA). For effects involving repeated measures, the Greenhouse-Geisser corrections were applied where relevant. For post-hoc single comparisons Bonferroni-corrected p-values are reported.

Results

Neuropsychological testing and questionnaires

Results of the self-report measures are presented in Table 2. Breast cancer survivors reported more symptoms of depression (BDI-II) and trait anxiety (STAI) compared to controls. Moreover, BCS reported elevated levels of fatigue in all subscales of the fatigue questionnaires (FEDA, MFI-20). BCS evaluated their memory performance and their ability to concentrate (ratings were obtained after the memory task) to be lower in comparison to controls (Concentration: $F(1,49)= 20.82$, $p< .001$; Memory: $F(1,49)= 6.14$, $p= .017$). Except for the omission errors in the Go/Nogo task, in which breast cancer survivors missed more targets than controls (see Table 3 for neuropsychological test results), BCS and matched controls did not differ in attention, alertness, and working memory performance.

BCS showed poorer performance in verbal memory tasks (VLMT and the WMS-R logical memory) compared to the control group. Poorer performance was also observed in the digit span (forwards) subscale but no differences were obvious in the digit span (backwards) suggesting that impairments in short term memory were less pronounced than in verbal memory.

Hair cortisol analysis

Cortisol analysis (Figure 1) showed that breast cancer survivors had increased hair cortisol levels over the last six months before testing compared to controls (Group: ($F(1,39)= 7.40$, $p= .010$). Comparing hair segments from the past 1 to 3 month (Segment A) with the past 4 to 6 month (Segment B) before sampling (see procedure by Steudte et al., 2013), hair cortisol levels were lower in the control group ($p< .001$), whereas cortisol levels in BCS did not change over time ($p= .236$) and remained stable at a high level (Time x Group: $F(1,39)= 8.24$, $p= .007$). Follow-up analyses confirmed that group differences were only statistically significant in the 4-6 month period before sampling (Segment B: $p= .004$), but not for the time window from 1-3 month (Segment A: $p= .119$).

Event-related potentials (LPP)

Waveforms and scalp distributions of the LPP are depicted in Figure 2 and Figure 3. Replicating many previous ERP studies, emotional, relative to neutral pictures prompted larger late positive potentials in the time window between 400 and 800 ms relative to neutral pictures (Emotion: $F(2,98)= 28.13$, $p< .001$; Unpleasant BCS: mean= 1.07 µV; Unpleasant control: mean= 1.18 µV; Neutral BCS: mean= .52; Neutral control: mean= .60; Pleasant BCS: mean= 1.23 µV; Pleasant control: mean= 1.39 µV; Unpleasant vs. Pleasant: $p= .16$; Unpleasant vs. Neutral: $p< .001$; Pleasant vs. Neutral: $p< .001$). This emotion effect did not differ between breast cancer patients and controls in the overall analysis (Group: $F(1,49)< 1$, $p= .652$); Emotion x Group ($F(2,98)< 1$, $p= .911$). In the later time window from 800 tp 1200 ms, the enhanced LPP for emotional scenes remained stable (Emotion: $F(2,98)= 13.26$, $p< .001$). Emotional pictures (Unpleasant BCS: mean= .79 µV; Unpleasant control: mean= 1.38 µV; Pleasant BCS: mean= 1.13 µV; Pleasant control: mean= 1.46 µV) showed more positive going ERP waveforms compared to neutral pictures (BCS: mean= .63 µV; Control: mean= .81 µV; Unpleasant vs. Neutral: $p< .001$; Pleasant vs. Neutral: $p< .001$; Unpleasant vs. Pleasant: $p= .099$). Interestingly –

in contrast to pleasant (p= .29) and neutral scenes (p= .48) – LPPs were smaller for unpleasant pictures in BCS compared to controls (p< .05). The overall Emotion x Group interaction, however, was not significant (F(2,98)= 1.72, p= .188). Also, no main effect of group was observed (F(1,49)= 2.35, p= .132).

Emotional memory assessment

Results of the recognition memory task one week later showed a memory advantage for emotional pictures replicating prior studies. Unpleasant pictures (BCS: mean= .74; Control: mean= .80) and pleasant pictures (BCS: mean= .67; Control: mean= .77) were better remembered than neutral pictures (BCS: mean= .61; Control: mean= .67). Hit rates for unpleasant pictures were also higher than for pleasant pictures (Emotion: F(2,98)= 20.39, p< .001; all post-hoc comparisons: p< .05). Hit rates did not differ between groups (Group: F(1,49)= 2.81, p= .10; Emotion x Group: F(2,98)<1, p= .661).

Discrimination index Pr (hits minus false alarms) was higher for unpleasant (BCS: mean= .45; Control: mean= .55) compared to neutral (BCS: mean= .39; Control: mean= .43) and pleasant pictures (BCS: mean= .31; Control: mean= .48; all p< .05). Pr did not differ between pleasant and neutral pictures (Emotion: F(2,98)= 12.39, p< .001). Overall, breast cancer survivors showed poorer memory accuracy compared to controls (Group: F(1,49)= 6.95, p< .05). A significant interaction between Emotion and Group (F(2,98)= 3.63, p= . 031) indicated that this impairment was driven by emotional contents (Pleasant: p= .001; Unpleasant: at trend level, p= .066), but not neutral contents (p= .30).

Affective judgements

As expected, valence ratings differed as a function of picture category. Pleasant pictures were rated as more pleasant than neutral and unpleasant pictures (BCS: valence ratings = 2.07; 6.85; 7.30; Control: valence ratings = 2.29; 6.85; 7.20; for unpleasant, neutral, and pleasant pictures respectively; Emotion: F(2,98)= 617.9, p< .001; all post-hoc comparisons: p< .001). Valence ratings did not differ between both groups (Group:

F(1,49)< 1, p= .497; Emotion x Group: F(2,98)= 2.54, p= .105). Unpleasant (BCS: mean= 7.17; Control: mean= 6.61) and pleasant pictures (BCS: mean= 3.29; Control: mean= 3.73) were rated as more arousing than neutral pictures (BCS: mean= 2.58; Control: mean= 2.47; Arousal: F(2,98)= 373.0, p< .001). Replicating previous findings with women in the age range of the current sample (Cuthbert et al., 1994; Gong &Wang, 2016), unpleasant pictures were rated as significantly more arousing than pleasant pictures (F(1,50)= 303.50, p< .001). These differences in the arousal ratings between unpleasant and pleasant pictures were significantly larger in the BCS group (Emotion x Group: F(2,98)= 4.37, p= .019). Since breast cancer survivors rated the unpleasant pictures as more arousing and the pleasant pictures as less arousing than the age matched control group the main effect of group was not significant (F(1,49)< 1, p= .819).

Correlational analyses

Subjective ratings of concentration and memory performance were correlated with trait anxiety ratings (Concentration: r= -.588, p= .006; Memory: r= -.523, p= .018) in the BCS group, indicating that higher anxiety was associated with a more self-perceived decline in cognitive performance. Self-reported fatigue in the BCS group was correlated with depression (correlation coefficients ranging from r= .445 to r= .751, all p< .05; except for the MFI subscale general fatigue: r= .383, p= .10) and trait anxiety (ranging from r= .556 to r= .828, all p< .05; except for FEDA reduced energy subscale: r= .186, p= .43). BDI-II and STAI were highly correlated (r= .677, p= .001) in BCS. Depression and trait anxiety were not related to neuropsychological test performance in BCS. Notably, self-rated memory performance in BCS was not correlated with verbal memory performance in the logical memory and the verbal learning and memory tests. Moreover, no further significant relationships were observed for hair cortisol levels, subjective measures, neuropsychological and behavioral performance and ERPs in BCS.

Discussion

In the present study we investigated neuropsychological performance and brain dynamics associated with emotion processing in breast cancer survivors and healthy controls.

Viewing unpleasant and pleasant pictures elicited larger late positive potentials compared to neutral pictures, replicating many previous ERP studies (Cuthbert et al., 2000; Schupp et al., 2000; Schupp, Flaisch, Stockburger, & Junghöfer, 2006). In a later time window, however, BCS showed smaller LPP amplitudes for unpleasant pictures compared to controls. Similarly reduced ERP responses to emotional words (Blackburn, Roxborough, Muir, Glabus, & Blackwood, 1990) and threatening faces (Foti, Olvet, Klein, & Hajcak, 2010) have been observed in depressed individuals, and interpreted as emotional disengagement (Hajcak et al., 2015). Such an interpretation is supported by studies investigating the effect of emotion regulation strategies on brain potentials. LPPs evoked by unpleasant stimuli were consistently reduced when participants were instructed to draw their attention away from the arousing aspect of these pictures (Hajcak, Dunning, & Foti, 2009; Hajcak, MacNamara, & Olvet, 2010). Utilizing reappraisal strategies also reduced LPPs to emotionally arousing pictures compared to a free viewing condition (Hajcak & Nieuwenhuis, 2006). Suppression of unpleasant emotions is widely observed in breast cancer patients and has indeed been related to heightened anxiety and depression, resulting in more frequent reports of psychological distress (Cohen, 2013; Iwamitsu et al., 2005). In the present study BCS also reported more symptoms of depression trait anxiety and had also higher hair cortisol compared to controls, indicating elevated levels of distress. Although major depressive disorder has been associated with increased cortisol secretion, only few studies focused on long-term cortisol measures. They have produced mixed results with some evidence for increased hair cortisol levels

in depressed individuals (Dettenborn et al., 2012; Herane Vives et al., 2015; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). As self-ratings and hair cortisol levels were not associated with ERPs and not linked to anxiety and depression, the reduction in the LPP to unpleasant stimuli cannot be fully explained by influences of chronic stress or depression alone. Instead, the present findings might point to an emotion regulation strategy in BCS characterized by reduced elaborative and extended processing of unpleasant contents (remember, there were no group differences in the early LPP and overall group differences). The interpretation of an active withdrawal of attention is supported by the rating data (affective judgments). Replicating previous findings, unpleasant pictures were rated as more arousing than pleasant pictures by women in the age range of the current sample (see Cuthbert et al., 1994; Gong & Wang, 2016). This effect was amplified in breast cancer survivors who rated unpleasant pictures as more (and pleasant pictures as less) arousing than age matched controls, a finding that has also been observed in patients with post-traumatic stress disorder (PTSD; Adenauer et al., 2010). In the same study, Adenauer and colleagues (2010) also found reduced brain activity in visual processing areas in response to aversive, which parallels the reaction pattern of cancer survivors in the current study.

Memory performance was better for emotionally arousing compared to neutral pictures (c.f., LaBar & Cabeza, 2006; Weymar, Löw, & Hamm, 2011; Weymar, Löw, Melzig, & Hamm, 2009). However, BCS, in comparison to controls, showed poorer discrimination ability, particularly for pleasant and unpleasant (at trend level) pictures. As mentioned above, decreased LPPs were found in BCS for unpleasant pictures during encoding. LPP magnitudes were previously associated with deeper encoding processing and better later recognition memory performance (Palomba, Angrilli, & Mini, 1997; Dolcos & Cabeza, 2002; Weymar et al., 2012). Possibly, impaired emotional memory tested one week after encoding could be a result of emotional avoidance and thus less

elaborative processing, at least for unpleasant scenes. However, impaired memory discrimination was especially found for pleasant stimuli and not related to ERPs during encoding. Since emotional avoidance alone does not account for the present recognition memory findings differences in memory consolidation (see stress explanation below) might be another explanation in this effect.

Recognition memory was not only impaired for emotional materials, but also for neutral contents (words and stories) in verbal memory testing in BCS, compared to controls. This is in line with previous studies when verbal learning and memory was tested immediately (Shilling et al., 2005) and one year after primary cancer treatment (Von Ah et al., 2009). In a longitudinal examination, acute cognitive declines shortly after chemotherapy, but also in a one-year follow-up mainly affected learning and memory performance (Wefel et al., 2010). Even twenty years after chemotherapy, performance is impaired in BCS compared to non-cancer controls in immediate and delayed verbal memory testing (Koppelmans, Breteler, et al., 2012). Although, in the present study, verbal memory was significantly impaired in BCS compared to controls, performance was still in the normative range. In the domains of attention and working memory, BCS showed almost no impairments compared to controls. In addition, ERPs during picture processing for neutral contents did not reveal group differences suggesting similar attention allocation and processing for neutral but not for emotionally arousing stimuli.

Recently, Andreotti et al. proposed an integrative model of stress biology and neuropsychology in cancer patients, highlighting the multifactorial genesis of cancer-related cognitive changes (Andreotti, Root, Ahles, Mcewen, & Compas, 2015). The authors suggest that elevated chronic levels of cortisol (which were also observed in the present study), combined with a flattened diurnal rhythm (Abercrombie et al., 2004; Spiegel, Giese-Davis, Taylor, & Kraemer, 2006), account for difficulties adapting to environmental or situational changes when individuals are confronted with acute

stressors. In line, a recent study found no emotional memory enhancement following post-training stress in BCS (who also showed a blunted glucocorticoid response to the stressor) compared to healthy controls (Andreano et al., 2012). Thus, the present data may indicate that chronic stress hinders memory consolidation for emotionally arousing unpleasant and pleasant scenes. Moreover, this lack of adaptive processing might promote the emotional avoidance effect in BCS. When stressed, individuals frequently tend to avoid unpleasant feelings, a behavioral strategy which seems to be helpful at first glance (Shallcross, Troy, Boland, & Mauss, 2010). But, as a long-term consequence, emotional avoidance has been found to increase distress, anxiety and negative affect whereas mindfulness and acceptance of unpleasant facts showed beneficial effects (Iwamitsu et al., 2005; Shallcross et al., 2010). In line, cancer patients and breast cancer survivors frequently report high levels of emotion suppression and higher distress (Cohen, 2013; Iwamitsu et al., 2005; Tamagawa et al., 2013), which might contribute to cognitive impairments. Therefore, cognitive-behavioral and acceptance-based approaches might be especially beneficial to promote adaptive emotion regulation strategies in BCS, enhancing psychological well-being and targeting perceived cognitive impairments.

There are some limitations to the study. In the present study, most correlations were observed between the different self-report measures, but self-report was not related to most of the neuropsychological performance measures, or ERP waveforms. In line, Jenkins et al. (2006), did not observe significant associations between self-reported psychological distress and subjective cognitive impairments and objective testing. Also, no significant associations with hair cortisol measures were observed. Unfortunately, the small sample size did not allow testing for complex interactions, and, conclusions are further limited by the cross-sectional study design. To support the emotional avoidance hypothesis, it would have also been useful to assess self-report of (habitual) emotion

regulation strategies and eye movements during picture viewing as a marker for attention deployment from emotionally arousing picture locations (e.g. Ferri et al., 2013).

To further test the model proposed by Andreano et al. (2012), which suggests a blunted response to acute stress (e.g. emotional challenging events) in BCS, it would be useful to have additional assessment of physiological markers like salivary alpha-amylase, cortisol, or autonomic measures in response to emotional picture viewing. In addition, physiological responses to an experimentally induced stressor (e.g. cold pressure test, Andreano et al., 2006) could be included in future study designs. Here, with hair cortisol measures, we provide only one physiological marker that is limited to assumptions about associations with long-term cortisol exposure.

Finally, recruitment of BCS via bulletin boards (university medicine, support group) may have resulted in a sampling bias including only women who experience cognitive or psychological impairments, and who were therefore especially interested in participating in the study. As Shilling et al. (2006) noted, reports of objective cognitive impairments vary across study designs. Thus, conclusions about emotion processing and memory from this pilot study might be strongly limited to a subgroup of BCS women who report (neuro-) psychological complaints. To rule out selection biases, a larger sample should be recruited preferably at diagnosis and examined in a longitudinal design. Moreover, to test whether the observed effects would be specific for breast cancer survivors it would be desirable to compare BCS patients with other chronically ill individuals (like depressed patients).

Taken together, breast cancer survivors showed higher depression, trait anxiety and fatigue measures and reported more subjective cognitive complaints than healthy controls. Significant group differences were observed for long-term cortisol and verbal memory, but not in the neuropsychological testing of attention and working memory. In both groups emotional pictures elicited larger LPPs than neutral pictures, indicating

preferential processing of this material. In the late time window, however, LPPs were reduced when BCS were confronted with unpleasant pictures, which may indicate emotional avoidance for these pictures that were also rated as more arousing by the patients. While arousal ratings were increased, recognition memory performance was impaired in BCS for emotional contents, particularly for pleasant contents that were also rated as less arousing compared to the control group.

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Tables

Table 1. Sample characteristics

	BCS	**Control**	**P**
Mean age [years]	52.75 (N= 20)	51.74 (N= 31)	.498
Range	44-59	41-62	
Education [years] N (%)			.403
8	2 (10.0)	1 (3.2)	
10	12 (60.0)	16 (51.6)	
12	6 (30.0)	14 (45.2)	
Handedness N (%)			
Left	3 (15.0)	1 (3.2)	.127
Right	17 (85.0)	30 (96.8)	
Menopause N (%)	20 (100)	25 (80.6)	.061
Breast cancer treatment			
Mean (SD) time since diagnosis	3.43 (1.9)	-	
Mean (SD) time since treatment	2.83 (1.9)	-	
Chemotherapy N (%)	20 (100)	-	
Hormonal therapy N (%)	15 (75)	-	
Radiotherapy N (%)	17 (85)	-	

Sociodemographic data for breast cancer survivors (BCS) and control participants.

Table 2. Self-report measures.

	BCS	Control	P
STAI (trait)	48.40 (2.09)	31.67 (1.70)	**.000**
BDI-II	16.20 (1.64)	4.57 (1.34)	**.000**
FEDA			
Distractibility	42.20 (2.73)	57.55 (2.19)	**.000**
Reduced energy	21.45 (1.06)	26.23 (1.06)	**.006**
Fatigue and deceleration in practical activities	26.35 (1.09)	37.13 (.87)	**.000**
MFI-20	62.67 (2.54)	33.26 (2.54)	**.000**
General fatigue	14.10 (.67)	7.55 (.64)	**.000**
Physical fatigue	13.60 (.68)	6.40 (.68)	**.000**
Reduced activity	11.65 (.70)	5.53 (.72)	**.000**
Reduced motivation	10.75 (.66)	5.50 (.66)	**.000**
Mental fatigue	13.25 (.88)	6.75 (.88)	**.000**
Subjective ratings of			
Concentration	54.50 (3.42)	74.52 (2.75)	**.000**
Memory performance	50.50 (4.41)	64.52 (3.54)	**.017**

Mean (SEM) values for the State-Trait-Anxiety Inventory (STAI; trait), the Beck-Depression-Inventory (BDI-II), fatigue scales (Fragebogen erlebter Defizite der Aufmerksamkeit, FEDA; Multidimensional Fatigue Inventory, MFI-20) and subjective ratings for breast cancer survivors (BCS) and controls. Bold highlights significant group differences.

Table 3. Neuropsychological testing

	BCS	Control	P
Alertness			
Median RT without alert [ms]	332.65 (28.77)	300.96 (12.09)	.266
SD RT without alert [ms]	61.00 (10.38)	43.46 (3.13)	.071
Median RT with alert [ms]	339.85 (.24)	306.57 (12.74)	.194
N anticipations with alert	.60 (.23)	.29 (.09)	.164
Working Memory			
N missings [T-value]	49.65 (2.61)	50.78 (2.57)	.764
N errors [T-value]	46.95 (2.31)	40.00 (1.67)	.465
Divided attention			
Missings visual [T-value]	50.75 (1.47)	52.36 (.49)	.246
Missings auditive [T-value]	38.35 (1.27)	38.96 (.88)	.628
Missings visual-auditive [T-value]	42.75 (1.18)	44.75 (.62)	.113
Go/Nogo			
N comission error [T-value]	49.00 (1.20)	51.39 (.83)	.097
N omission error [T-value]	37.58 (.70)	38.96 (.06)	**.021**
Median RT [T-value]	44.05 (1.76)	47.21 (1.74)	.223
VLMT			
Learning [T-value]	54.53 (1.33)	63.39 (1.09)	**.000**
Consolidation [T-value]	47.24 (1.82)	52.23 (1.50)	**.040**
Recognition [T-value]	53.95 (1.57)	58.61 (1.30)	**.027**
WMS-R			
Digit span (forwards) [PR]	33.90 (6.22)	53.32 (5.26)	**.021**
Digit span (backwards) [PR]	60.84 (6.69)	61.61 (5.51)	.930
Logical memory I [PR]	67.12 (6.39)	84.66 (5.40)	**.042**
Logical memory II [PR]	61.40 (6.26)	89.02 (5.29)	**.002**

Mean (SEM) specific values for the Testbatterie zur Aufmerksamkeitsprüfung (TAP: Alertness, Working memory, Divided attention, Go/Nogo), verbal memory (Verbaler Lern- und Merkfähigkeitstest, VLMT), Wechsler memory scale (WMS-R), and statistical analysis comparing breast cancer survivors (BCS) and controls. RT= reaction time, PR= percentage rank.

Figure captions

Figure 1

Mean hair cortisol concentration in pg/mg 1 to 3 (Segment A) and 4 to 6 (Segment B) month prior to testing for breast cancer survivors (BCS; black bars) and controls (white bars); *p< .05 (between-group contrast). Error bars represent standard error of mean.

Figure 2

ERP waveforms averaged over representative sensors placed over centro-parietal sites (see sensor map) when unpleasant (red lines), neutral (black lines) and pleasant (blue lines) pictures were presented to breast cancer survivors (BCS, left) and controls (right).

Figure 3

Upper graph: ERP waveforms for representative centro-parietal electrode cluster (see sensor map) and scalp topographies for breast cancer survivors (BCS; red lines) and controls (black lines) during viewing unpleasant, neutral and pleasant pictures. Significant group differences between BCS and controls in the late LPP for unpleasant pictures are highlighted by the grey area.

Lower graph: Bar charts depict mean ERP amplitudes for unpleasant, neutral and pleasant pictures averaged over central sensor sites in the time windows 400-800 ms (left) and 800-1200 ms (right) for breast cancer survivors (BCS; black bars) and controls (white bars). Error bars represent standard error of mean.

Figure 4

Recognition memory performance: Hit rate (left) and discriminability (Pr: hit-false alarm) are displayed for unpleasant, neutral and pleasant pictures. Black bars represent breast cancer survivors (BCS; white bars = controls). Error bars represent standard error of mean.

Figure 1.

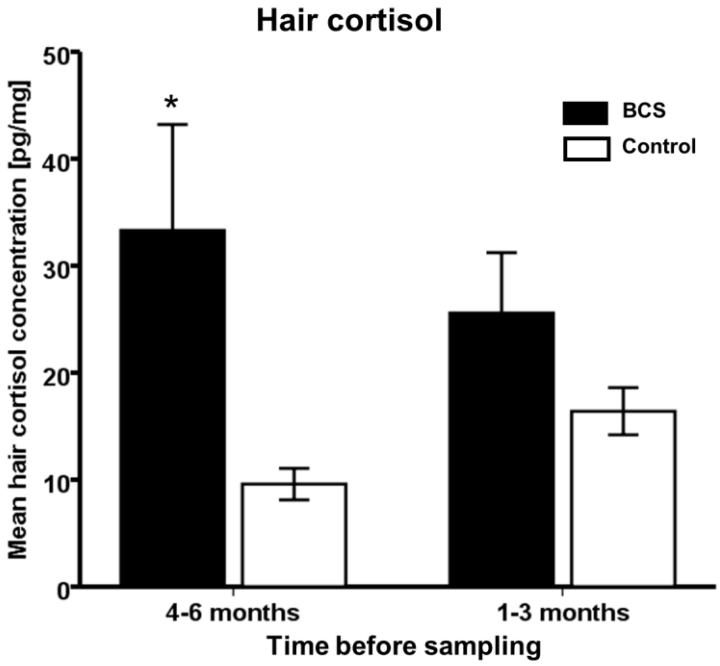
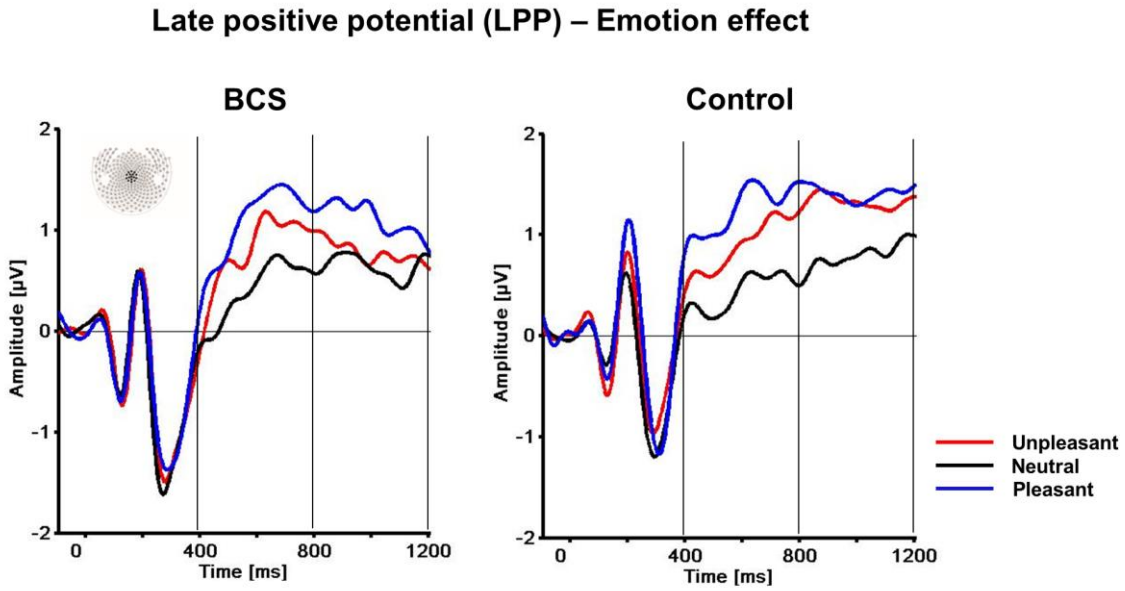


Figure 2.



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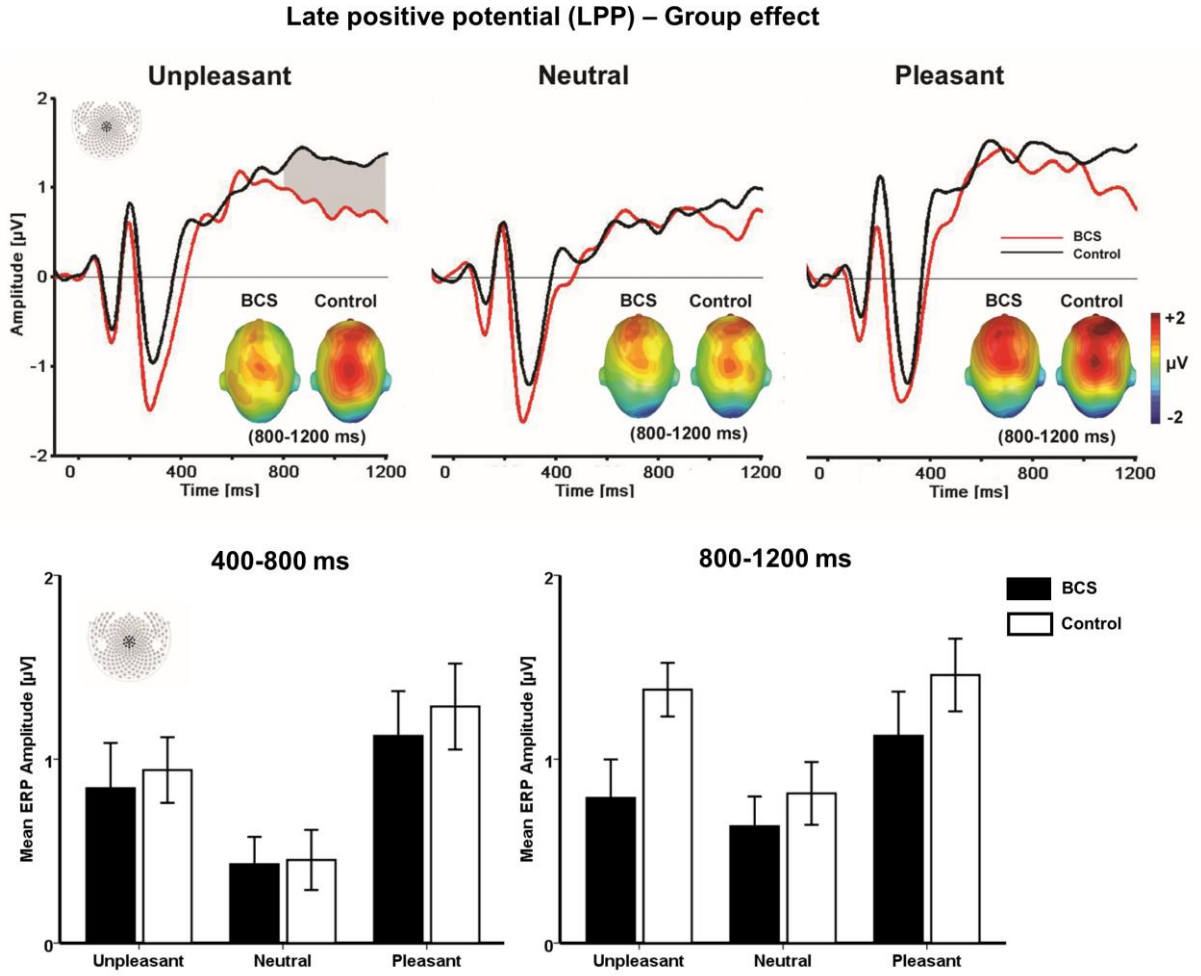
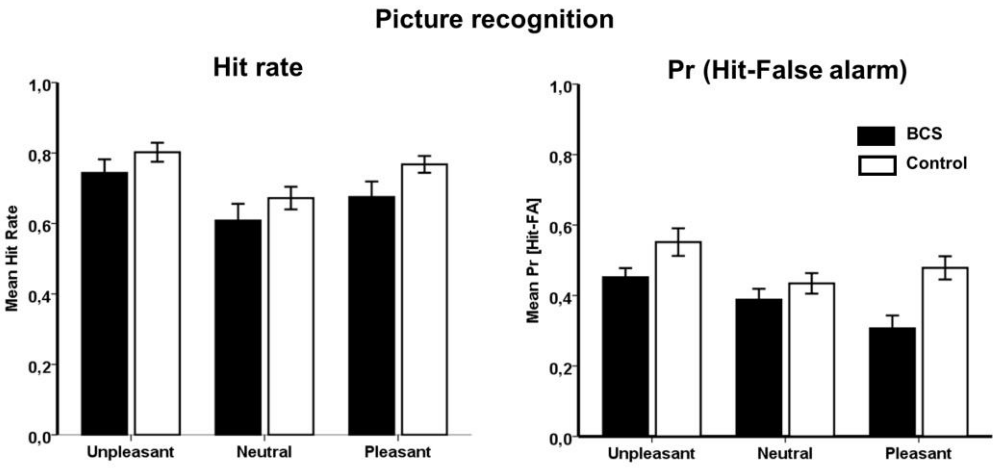


Figure 4.



Manuscript 3

New learning following reactivation in the human brain: Targeting emotional memories through rapid serial visual presentation.

Janine Wirkner, Andreas Löw, Alfons O. Hamm, & Mathias Weymar

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New learning following reactivation in the human brain: Targeting emotional memories through rapid serial visual presentation

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ABSTRACT

Once reactivated, previously consolidated memories destabilize and have to be reconsolidated to persist, a process that might be altered non-invasively by interfering learning immediately after reactivation. Here, we investigated the influence of interference on brain correlates of reactivated episodic memories for emotional and neutral scenes using event-related potentials (ERPs). To selectively target emotional memories we applied a new reactivation method: rapid serial visual presentation (RSVP). RSVP leads to enhanced implicit processing (pop out) of the most salient memories making them vulnerable to disruption. In line, interference after reactivation of previously encoded pictures disrupted recollection particularly for emotional events. Furthermore, memory impairments were reflected in a reduced centro-parietal ERP old/new difference during retrieval of emotional pictures. These results provide 0neural evidence that emotional episodic memories in humans can be selectively altered through 0behavioral interference after reactivation, a finding with further clinical implications for the treatment of anxiety disorders.

1. Introduction

Changing unpleasant or even traumatic memories is one of the major challenges for clinical interventions (Parsons & Ressler, 2013). It is a well-established finding that the formation of emotional long-term memories is mediated by the adrenergic system and depends on the interaction between amygdala and hippocampus (McGaugh, 2000). Following retrieval consolidated memories return to an unstable state and have to be stabilized again into a persisting memory, a process that is known as memory reconsolidation (Nader, Schafe, & LeDoux, 2000). Recent animal and human research has successfully targeted the modification of conditioned fear memories through blockade of reconsolidation after reactivation of these memories by pharmacological agents, such as beta-adrenergic receptor blockers (Nader & Hardt, 2009; Nader et al., 2000). To date, psychophysiological and emerging behavioral evidence exists for successful blockade of reconsolidation in humans using pharmacological or behavioral interventions (Chan & LaPaglia, 2013; Schiller et al., 2010; for review see Agren, 2014). For instance, it has been shown that new learning after reactivation of previously learned material may impair (Wichert, Wolf, & Schwabe, 2011) or update (Hupbach, Gomez, Hardt, & Nadel, 2007) memory. Despite promising behavioral evidence only few brain imaging studies (Agren et al., 2012; for review see Schwabe, Nader, & Pruessner, 2014) investigated human brain function underlying altered memory representations following reconsolidation blockade in humans. Agren et al. (2012) found that behavioral disruption of fear reconsolidation significantly decreased memory trace activity in the amygdala. Using pharmacological reconsolidation blockade, Schwabe, Nader, Wolf, Beaudry, and Pruessner (2012) found that impairments in memory for emotional materials were associated with altered amygdala and hippocampus activation.

In the present study we used event-related potentials (ERPs) to investigate the brain dynamics underlying episodic emotional memories. ERPs provide non-invasive measures of neural activity with high time resolution (ms range) and are thus well suited to examine the neural networks underlying human memory (Voss & Paller, 2008). In recognition memory tasks it is a key finding that ERPs during the retrieval of previously encoded "old" items evoke more positive going waveforms than correctly classified "new" items (Rugg et al., 1998). This so-called ERP old/new effect is most prominent over centro-parietal brain sites, starting at about 500 ms post-stimulus, and has been associated with hippocampus-dependent explicit recollection (Düzel, Vargha-Khadem, Heinze, & Mishkin, 2001). Numerous studies have found that the late ERP old/new effect is specifically enhanced for emotionally arousing compared to neutral stimuli (Weymar & Hamm, 2013), an effect that can be abolished by pre-encoding beta-adrenergic blockade (Weymar et al., 2010). The beta-adrenergic system also

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mediates the reconsolidation of conditioned fear memory in humans (Kindt, Soeter, & Vervliet, 2009).

Here, we tested whether encoding of new information after reactivation of previously encoded memories interferes with the reconsolidation of reactivated emotional and neutral episodic memories and whether this interference can be traced in the neural signature of episodic memory. We applied a new method for memory reactivation – rapid serial visual presentation (RSVP). During this task all 90 previously encoded pictures were presented in a rapid stream so that the entire reactivation lasted only for 30 s (Versace, Bradley, & Lang, 2010). Based on previous research four experimental groups were included in the design (see Fig. 1). In two groups old memories were reactivated using the 30 s RSVP after one day and reconsolidation of these old memories were interrupted after 10 min by an interfering task (encoding of new emotional and neutral scenes) in one group but not in the other. Two groups without the reactivation manipulation were added as control groups. One of these groups just received the interference task on day two to assess the unique influence of the interference task.

We expected that new learning, compared to no learning, would impact reconsolidation of previously reactivated pictures. Because pictures were reactivated through RSVP – favoring the processing of salient stimuli – we predicted that particularly emotional memories would be affected by the interference task resulting in impaired recognition memory performance and smaller centro-parietal old/new difference in the ERPs.

2. Material and methods

2.1. Participants

Eighty-eight individuals participated in the study. Exclusion criteria were checked in a standardized telephone interview and included current or lifetime diagnosis of mental disorders, current medical conditions and medication intake during study participation. Participants who missed the second (after 1 day) and/or third session (after 7 days) were excluded and were not tested further ($n = 8$). The final sample included 80 healthy male participants (mean age: 24.1 years, range: 19–31, 4 left handed). All participants had normal or corrected-to-normal vision. Participants provided informed written consent for the protocol approved by the Review Board of the University of Greifswald in accordance with the provisions of the World Medical Association Declaration of Helsinki and received financial compensation for participation.

2.2. Stimulus materials and procedure

Stimuli consisted of 270 pictures (90 unpleasant, 90 neutral and 90 pleasant pictures) taken from the International Affective Picture Series (IAPS) (Lang, Bradley, & Cuthbert, 2008) and the Emotional Picture Set (EmoPicS) (Wessa et al., 2010). Three stimulus sets, each consisting of 90 pictures (30 unpleasant, 30 neutral and 30 pleasant pictures) were carefully matched according to their normative valence and arousal ratings (see IAPS and EmoPicS norms for males; Set 1: mean valence = 3.1, 5.1 and 7.1, mean arousal = 5.7, 3.3 and 6.0; Set 2: mean valence = 3.0, 5.1 and 7.1, mean arousal = 5.8, 3.2 and 5.8; Set 3: mean valence = 2.9, 5.2 and 7.0, mean arousal = 5.8, 3.2 and 5.9 for unpleasant, neutral and pleasant pictures respectively). The three sets were also matched for semantic categories (e.g. attack, mutilation, neutral people, objects, adventure, and erotic couples).

Forty-two additional pictures were added before (7 unpleasant, 7 neutral, 7 pleasant) and after (7 unpleasant, 7 neutral, 7 pleasant) the encoding picture presentation (day one) to avoid serial position effects on subsequent memory performance. These pictures were not included in the analyses. The three picture sets were counterbalanced across participants and the four experimental groups to serve either as encoding picture set, interference learning set or as a new picture set during recognition memory testing.

Individual hedonic valence and arousal ratings for all pictures were obtained to control for group differences in our sample (Bradley & Lang, 1994). As expected, unpleasant pictures were rated as more unpleasant (mean valence: 2.7) than neutral (mean valence: 5.5; $F_{(1,78)} = 1010.33$, $p < .001$) and pleasant (mean valence: 6.8, $F_{(1,78)} = 1366.67$, $p < .001$) pictures. Pleasant pictures were more pleasant than neutral pictures ($F_{(1,78)} = 363.69$, $p < .001$). Additionally, emotional pictures (unpleasant (mean arousal: 5.6) and pleasant (mean arousal: 4.5)) were rated as more arousing than neutral pictures (mean arousal: 2.1; $F_{(1,78)} = 746.47$, $p < .001$). Hedonic valence ($F_{(6,150)} < 1$, $p = .81$) and arousal ratings ($F_{(6,150)} < 1$, $p = .91$) of the pictures in the three sets did not differ between the four experimental groups. During the encoding session, 90 pictures as well as the 42 buffer pictures were presented on a 20-inch computer screen for 3000 ms with a random inter-trial interval (ITI) of 2000, 2500 or 3000 ms. A 500 ms fixation cross preceded every picture onset to ensure that participants fixated the center of the screen. The pictures were presented in pseudorandom order for each participant with the restriction that no picture from the same valence category was presented on two consecutive trials.

On the first day of the experiment, all participants encoded ninety pictures (30 unpleasant, 30 pleasant, 30 neutral) and were randomly assigned to one of four experimental groups (Fig. 1). Participants were instructed to attentively watch the pictures. No mention of a memory test was made (incidental encoding).

Twenty-four hours later, participants in the *Reactivation group* ($n = 20$) returned to the lab for reactivation of the previously seen pictures using RSVP (3 Hz). The rationale for this approach was as follows: Because memory for emotional scenes is exceptional even

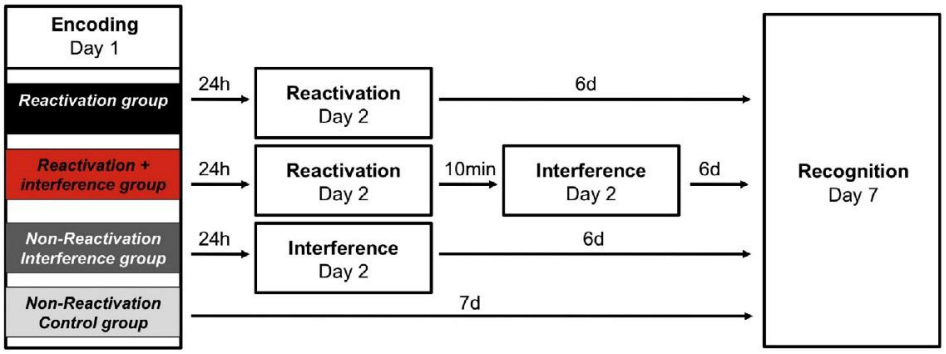

Fig. 1. Experimental design. The four experimental groups (Reactivation, Reactivation + interference, Non-Reactivation Interference, Non-Reactivation Control) and time intervals between the experimental sessions (Encoding, Reactivation, Interference learning task and Recognition).

after prolonged delays (Weymar, Löw, & Hamm, 2011) and repetition (Ferrari, Bradley, Codispoti, Karlsson, & Lang, 2013) as well as longer presentation times result in enhanced memory performance (for review see Potter, 2012), we assumed that longer picture presentation in the reactivation task would result in unwanted ceiling effects in memory. Thus, using RSVP, the current reactivation manipulation is a very low level process that might not promote additional deep encoding. We also assumed that salient (emotional) memories would pop out during this reactivation procedure (Schupp, Junghöfer, Weike, & Hamm, 2004), making them especially vulnerable to reactivation blockade. Before the RSVP procedure, participants were instructed to attentively watch the pictures they had seen the day before with the exception that all pictures are presented in a rapid serial stream (no mention of a memory test was made). Participants in the *Non-Reactivation Interference group* ($n = 20$) were instructed to memorize a new picture set (30 unpleasant, 30 pleasant, 30 neutral pictures; interference task) without reactivating the pictures they had seen the day before. Participants performing the interference learning task were instructed to memorize the set of 90 new pictures in order to recall them after the presentation. Picture presentation was identical to the previous encoding session. Immediately after the interference learning, participants were instructed to write down all pictures they could remember within 15 min and with as much detail as they could remember so that an outsider could identify the picture (free recall).

Participants in the *Reactivation + interference group* ($n = 20$) performed the interference task immediately after reactivation in order to target the reconsolidation window (Schiller et al., 2010). Participants in the *Non-Reactivation Control group* ($n = 20$) did not come to the lab the second day (Fig. 1).

One week after the encoding session, all participants underwent a recognition memory test in which ninety old pictures that were encoded on day one were presented randomly intermixed with ninety completely new pictures (30 unpleasant, 30 pleasant, 30 neutral pictures). Each picture was displayed for 3000 ms and preceded by a 500 ms fixation cross. Participants were instructed to indicate by a button press, whether they had already seen the picture during the first experimental session ("old") or had never seen it before ("new") and to rate their recognition confidence on a Likert scale ranging from 0 ("not confident") to 10 ("absolutely confident"). Participants who had performed the interference learning task were told that no picture from the interference learning set was included in the recognition memory test. The assignment of left and right button presses to "old"/"new" responses was counterbalanced across participants. During recognition memory testing, ERPs were recorded. After application of the EEG sensor net, participants were instructed to attentively watch the pictures and to avoid eye blinks and body movements during ERP measurement. After recognition, participants were asked to rate all pictures for their subjective hedonic valence and arousal using a computerized SAM rating procedure (Bradley & Lang, 1994). All subjects reported that no memory test was expected.

2.3. Apparatus and data analysis

EEG signals were recorded continuously from 257 sensors using an Electrical Geodesic system (EGI, Eugene, OR, USA) and digitized at a rate of 250 Hz, using the vertex sensor (Cz) as recording reference. Scalp impedance for each sensor was kept below 30 kΩ. All channels were bandpass filtered online from 0.1 to 100 Hz. Offline reduction was performed using Electro Magneto Encephalography Software (EMEGS) (Peyk, DeCesarei, & Junghöfer, 2011) and included lowpass filtering at 40 Hz, artifact detection, sensor interpolation, baseline correction, and conversion to the average reference (Junghöfer, Elbert, Tucker, & Rockstroh, 2000). Stimulus-synchronized epochs were extracted from 100 ms before to 1200 ms after picture onset and baseline corrected (100 ms prior to stimulus onset). ERP signals were computed for each sensor and participant for picture categories (emotional and neutral) in each experimental group (Reactivation, Reactivation + interference, Non-Reactivation Interference, Non-Reactivation Control). Because a similar pattern of results was observed for unpleasant and pleasant pictures in behavior and ERPs, both picture contents were merged into one condition (emotion). Only trials with correct responses (correctly recognized old pictures and correctly classified new pictures, respectively) were included in ERP averages.

Based on the results of the waveform analyses and guided by previous research (Weymar, Löw, Melzig, & Hamm, 2009; Weymar et al., 2011), a time window from 500 to 800 ms within a central electrode cluster (including EGI sensors 8, 9, 17, 45, 81, 132, 186, 198, and 257) where the differences between old and new conditions were maximal was selected for further statistical analyses. Mean ERP amplitudes of the selected scalp clusters were then analyzed using an ANOVA involving the within-subject factors Emotion (Emotional vs. Neutral), Memory (Old vs. New) and the between-subjects factor Group (Reactivation vs. Reactivation + interference vs. Non-Reactivation Interference vs. Non-Reactivation Control).

For behavioral performance, hit rates (the probability that an old item is correctly classified as old) and false alarm rates (the probability that a new item is incorrectly classified as old) were calculated for the behavioral recognition data for each participant and emotion (Snodgrass & Corwin, 1988). Additionally, as a measure of memory accuracy, we calculated the discrimination index Pr subtracting the false alarm rates from the hit rates. High Pr values [p(hit) – p(false alarm)] indicate better ability to discriminate between old and new items and represent memory accuracy according to Snodgrass and Corwin (1988). Because previous research suggested that memory for emotional pictures is based on recollection rather than familiarity processes (e.g., Weymar & Hamm, 2013), we expected that interference after reactivation would specifically disrupt this hippocampus-dependent system. The parietal ERP old/new difference has been related to recollection-based recognition processes. Recognition memory performance (item memory), however, is often based on both recollection and familiarity. In accordance with previous work we therefore analyzed hit rates and discrimination index (Pr) taking into account only the highest confidence ratings (e.g., rated "10") to obtain a purer behavioral index of recollection-based recognition (Weymar et al., 2009, 2011; Yonelinas, 2002). High confidence hits and Pr were analyzed by employing separate ANOVAs including the within-subject factor Emotion (Emotional vs. Neutral) and the between-subject factor Group (Reactivation vs. Reactivation + interference vs. Non-Reactivation Interference vs. Non-Reactivation Control). In addition, recognition memory performance (hits and Pr) was analyzed irrespective of subjective recognition confidence.

All analyses were performed with SPSS 22.0 (IBM, Armonk, NY, USA). For effects involving repeated measures, the Greenhouse-Geisser procedure was used to correct violations of sphericity. For post hoc single comparisons Bonferroni-corrected p-values are reported.

3. Results

3.1. Behavioral data

Table 1 presents hit rates and discrimination indices for high confidence ratings as an index for recollection based remembering. As expected, emotional pictures were better recognized than neutral pictures (Hit rates: $F_{(1,76)} = 13.00$, $p < .01$; Pr: $F_{(1,76)} = 10.53$,

Table 1
Recognition memory performance.

High confidence	Reactivation	Reactivation + interference	Non-Reactivation Interference	Non-Reactivation Control
Hit rate				
Emotional	.51 (.05)	.33 (.05)	.35 (.05)	.42 (.04)
Neutral	.37 (.06)	.31 (.06)	.35 (.05)	.29 (.05)
FA rate				
Emotional	.02 (.00)	.02 (.01)	.02 (.01)	.01 (.00)
Neutral	.01 (.01)	.01 (.00)	.01 (.00)	.01 (.01)
Pr (Hit-FA)				
Emotional	.49 (.04)	.31 (.04)	.33 (.04)	.41 (.04)
Neutral	.36 (.06)	.31 (.06)	.34 (.05)	.28 (.05)

Numbers represent means for high confidence hit and false alarm (FA) rates and discrimination index Pr (Hit rates minus false alarm rates) for emotional and neutral pictures (SEM).

$p < .01$). Importantly, these differences in the behavioral indices in recollection based memory performance for emotional and neutral pictures varied across groups as indicated by a significant interaction between emotion and group ($F_{(3,76)} = 2.91$, $p < .05$; $F_{(3,76)} = 3.35$, $p < .05$ for hit rates and Pr, respectively). For high confidence ratings there were also significant differences between groups in their hit rates for emotional ($F_{(3,76)} = 3.16$, $p < .05$), but not for neutral scenes ($F_{(3,76)} < 1$, $p = .765$). The same pattern of results was obtained for the discrimination index Pr with significant group differences for emotional ($F_{(3,76)} = 3.46$, $p < .05$) but not for neutral pictures ($F_{(3,76)} < 1$; $p = .739$). Critically, high confidence hit rates and memory discrimination decreased when reactivation was followed by the interference task compared to reactivation alone (all $p < .05$; see Fig. 2A for high confidence Pr). The remaining post hoc single comparisons were not significant.

When analyzing recognition memory responses irrespective of subjective confidence, pictures depicting emotional contents were also better remembered than neutral pictures after one week (Hit rates: $F_{(1,76)} = 51.61$, $p < .001$). Hit rates did not differ between groups ($F_{(3,76)} = 1.50$, $p = .223$) in this analysis but a significant interaction was observed between emotion and group ($F_{(3,76)} = 2.82$, $p < .05$). Emotional pictures were recognized better than neutral pictures in all groups except the group with interference manipulation and no reactivation (Emotional vs. Neutral; Reactivation: $F_{(1,19)} = 34.20$, $p < .01$; Reactivation + interference: $F_{(1,19)} = 6.25$, $p < .05$; Non-Reactivation Interference: $F_{(1,19)} = 2.78$, $p = .112$; Non-Reactivation Control: $F_{(1,19)} = 27.15$ $p < .001$; Means (SEM) (Emotional/Neutral) for Reactivation: .86 (.02)/.72 (.04); Reactivation + interference: .80 (.02)/ .73 (.04); Non-Reactivation Interference: .81 (.02)/.76 (.03); Non-Reactivation Control: .79 (.02)/.63 (.03)). Post-hoc single comparisons revealed no differences between the four experimental groups for neither emotional nor neutral pictures. Picture discrimination (Pr) was also better for emotional compared to neutral pictures (Emotion: $F_{(1,76)} = 23.92$, $p < .001$). A significant interaction between emotion and group ($F_{(3,76)} = 3.06$, $p < .05$) indicated that the emotion effect in memory discrimination was only prominent in groups without interference (Emotional vs. Neutral; Reactivation: $F_{(1,19)} = 21.53$, $p < .001$; Reactivation + interference: $F_{(1,19)} = 1.12$, $p = .302$; Non-Reactivation Interference: $F_{(1,19)} < 1$, p=.459; Non-Reactivation Control: $F_{(1,19)} = 14.72$, $p < .01$; Means (SEM) (Emotional/Neutral) for Reactivation: .66 (.03)/.53 (.04); Reactivation + interference: .64 (.02)/.61 (.04); Non-Reactivation Interference: .61 (.03)/.58 (.03); Non-Reactivation Control: .64 (.03)/.50 (.05)). Again, all other post hoc single comparisons did not reach significance.

3.2. Evoked brain potentials

Fig. 2 (panels B and C) illustrates grand average ERPs for correctly recognized old and new pictures for the selected sensors as a function of picture content (Emotional pictures involve pleasant and unpleasant pictures) and experimental group as well as the averaged ERP old minus new differences. In the time window from 500 to 800 ms correctly recognized old pictures prompted a larger ERP positivity than new pictures (Memory: $F_{(1,76)} = 83.81$, $p < .001$). This old/new difference was modulated by emotional content with emotional pictures showing larger old/new differences than neutral pictures ($F_{(1,76)} = 4.13$, $p < .05$). Corroborating our behavioral findings, interference after reactivation (relative to reactivation without interference) significantly reduced the ERP old/new effect for emotional pictures (Reactivation vs. Reactivation + interference: $F_{(1,38)} = 4.18$, $p < .05$), but not for neutral contents (Reactivation vs. Reactivation + interference: $F_{(1,38)} < 1$, $p = .41$; Fig. 2B), a pattern also present when comparing the Reactivation + interference group to Non-Reactivation Controls (Emotional pictures: $F_{(1,38)} = 10.11$, $p < .01$; Neutral pictures: $F_{(1,38)} = 1.77$, $p = .23$). Interference without reactivation did not produce such an effect when compared to reactivation or control groups, neither for emotional nor for neutral pictures (all $F_{(1,38)} < 1$).

4. Discussion

In sum, the present study provides electrophysiological evidence that emotional episodic memories in humans can be selectively altered through behavioral interference after reactivation. We found that very brief reactivation (30 s) of incidentally encoded emotional scenes renders the old memory trace susceptible to behavioral interventions, such as interfering new encoding (Hupbach et al., 2007). It is important to note that during RSVP of neutral and emotional pictures, the perceptual system is confronted with rapidly changing sensory information demanding rapid resolution and only emotionally salient information pop out (Junghöfer, Bradley, Elbert, & Lang, 2001; Schupp et al., 2004). Accordingly, incidentally acquired emotional memories, but not neutral memories, might have received prioritized attention that promoted the implicit processing (and susceptibility) of these scenes. Intriguingly, the impairing interference effect was selective for emotional pictures remembered with high confidence, suggesting that RSVP, compared to other reactivation methods (Wichert, Wolf, & Schwabe, 2013a,b) particularly facilitates reactivation of emotional rather than neutral recollected memories. Based on findings relating the parietal ERP old/new effect to remember judgments and correct source memory (for review, see Friedman & Johnson, 2000), only high confidence responses might reflect hippocampus-dependent explicit recollection processes (Düzel et al., 2001). Indeed, significant group differences in behavioral memory performance only occurred for those emotional items that were recognized with high confidence.

Unexpectedly, although not significant, performing the interference task without previous reactivation also seemed to result in

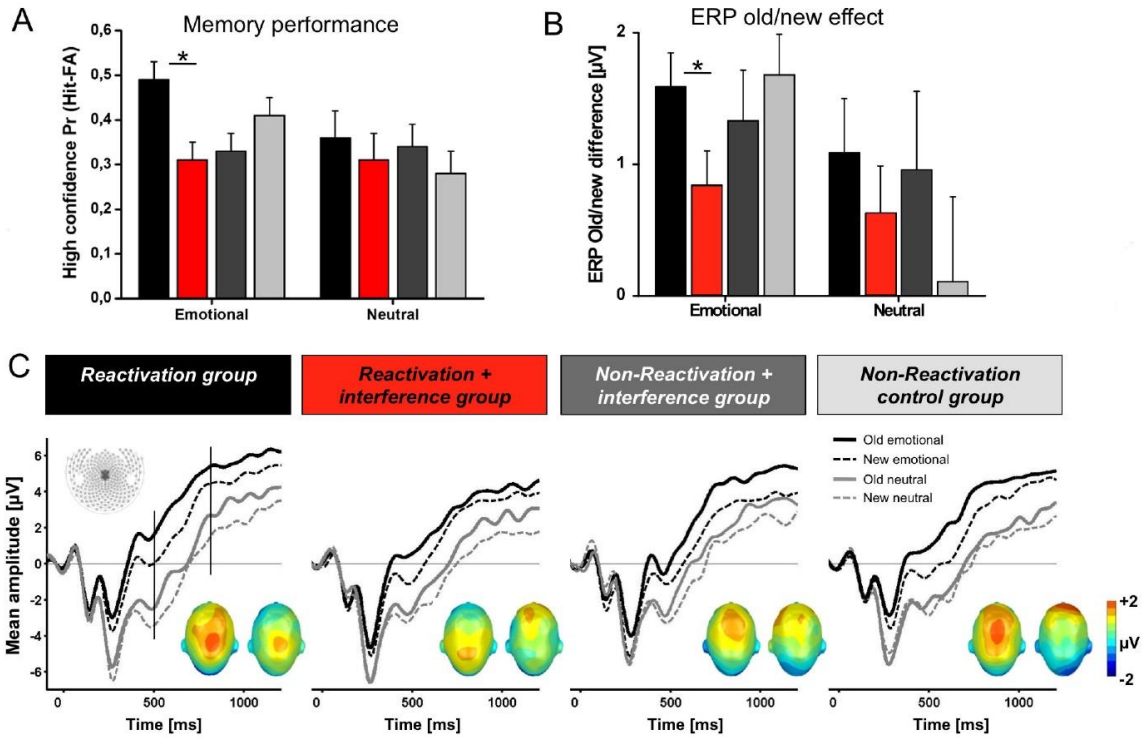

Fig. 2. Interfering learning after reactivation selectively affects recognition memory for emotional pictures. (A) Memory performance (High confidence Pr) in the four experimental groups for emotional and neutral pictures (*$p < .05$). (B) ERP old minus new differences averaged over centro-parietal sensor sites in the time window from 500 to 800 ms for emotional and neutral pictures in the four experimental groups (*$p < .05$). (C) Mean ERP amplitudes over selected central sensors in the four experimental groups for old (solid lines) and new (dotted lines) emotional (black) and neutral (grey) pictures, and old minus new difference topographies for emotional (left) and neutral (right) pictures.

lower memory performance for emotional pictures compared to reactivation alone. One possible explanation for this finding could be the influence of the spatial context as a reminder (during day 2) as suggested by Hupbach, Hardt, Gomez, and Nadel (2008) which might have been sufficient to reactivate the old memory making it vulnerable to interference. This could have been especially true for the emotionally arousing scenes (emotional memory effect; Weymar & Hamm, 2013). Testing always took place in the same environment (EEG lab) because of additional EEG measurements during encoding, reactivation and interference learning to control for possible group differences (No significant group differences were observed in the ERPs; data are not reported here). In addition, no memory differences were observed between both interference groups (the Reactivation + interference and Non-reactivation + interference). It is possible that the reminder presentation, albeit briefly presented, has nevertheless strengthened the original memory (c.f., Forcato, Fernandez, & Pedreira, 2013) hereby counteracting the effective interference.

In the ERPs, however, the reconsolidation effect was more straightforward. ERP old/new differences were smaller for emotional pictures over centro-parietal electrodes when interference followed reactivation. Behavioral measures usually reflect the output of many different individual cognitive processes and thus might not be as sensitive as ERPs (e.g., Luck, 2005; Newsome, Dulas, & Duarte, 2012; Weymar et al., 2010). There is vast literature relating the late ERP old/new effect to recognition based recollection (episodic retrieval of contextual details), as it is modulated by correct source, high confidence and remember judgments (reviewed by Voss & Paller, 2008). Therefore the present data indicate that interference following reactivation selectively impaired the re-stabilization of emotional episodic memories resulting in impaired explicit recollection at testing one week later as reflected in impaired memory performance (high-confidence responses) and smaller ERP old/new differences.

The ERP old/new effect has been linked to parietal cortex and a cortico-hippocampal network that is activated during episodic memory retrieval (Vilberg & Rugg, 2009; for review see Cabeza, Ciaramelli, Olson, & Moscovitch, 2008). Because the amygdala is a key structure mediating emotional memory consolidation and reconsolidation (Dębiec, Bush, & Ledoux, 2011; Nader & Hardt, 2009; Parsons & Ressler, 2013), it is likely that reconsolidation of emotionally arousing memories is mediated by noradrenaline-dependent amygdala and hippocampus activation (Agren et al., 2012; Kroes, Strange, & Dolan, 2010; Schwabe et al., 2012; Strange & Dolan, 2004).

Based on these assumptions, the present results suggest that reconsolidation blockade by encoding new information also depends on the interplay of the aforementioned brain structures and that emotional arousal might be required. Moreover, the present study has clinical implications for the treatment of anxiety disorders aiming at the modification of highly unpleasant or even traumatic memories. Reactivating emotionally arousing episodes during therapy (even at a very low level of processing and by considering additional contextual factors of the initial learning episode as a reminder) might be critical for subsequent successful memory modification by behavioral interventions, avoiding side-effects of alternative pharmacological blockade of reconsolidation.

Finally, there are two limitations. First, we only used male participants in order to exclude potential influences of sex differences in neuroscience research (for review see Cahill, 2006). Second, we provide no measure of the initial level of learning due to the incidental learning task. Thus, different levels of initial learning could account for the observed memory effects. To control for group differences during encoding, free recall procedures (e.g., Wichert

et al., 2013a) and explicit learning tasks with performance control after learning (e.g., Hupbach et al., 2007) were used in previous studies. In order to rule out possible group differences during encoding in the present study we analyzed the late positive potential (LPP) as measure of attention processing that has also been associated with memory performance (Weymar, Schwabe, Löw, & Hamm, 2012). However, we found larger LPPs in response to emotional pictures compared to neutral ones replicating many previous studies (e.g., Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Schupp et al., 2004), but no group differences.[1] This result indicates that encoding of pictures did not differ between groups at least at the level of elaborated processing.

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[1] Replicating previous findings, emotional pictures prompted larger LPPs compared to neutral pictures ($p < .001$) suggesting facilitated attention to emotional stimuli (Schupp et al., 2004). Importantly, there were no group differences or any interactions between emotion and group in the LPPs (all $F < 1$) during the encoding suggesting that group differences in memory performance cannot be explained by differences during encoding.

List of Publications

(Peer reviewed journal articles)

Wirkner, J., Weymar, M., Löw, A., & Hamm, A. O. (2013). Effects of pre-encoding stress on brain correlates associated with the long-term memory for emotional scenes. *PLOS ONE, 8*, e68212.

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