Modulation of emotional episodic memory in humans. Evidence from event-related potential studies.

I n a u g u r a l d i s s e r t a t i o n

zur

Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Ernst-Moritz-Arndt-Universität Greifswald

vorgelegt von

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geboren am 10.05.1983

in Szczecin (Polen)

Greifswald, 07.05.2015

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Tag der Promotion: 30.10.2015

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1 Introduction

Emotions are inevitable in our lives. The existence of every human is organized, in the first place, by his biological needs and, in the second place, by his social-cognitive motives and goals (Bower, 1992). Human actions are guided by instrumental plans that aim at fulfilling the needs and at achieving the goals. Emotions, as inherent nervous mechanisms influenced by evolution, play a control role in pursuing these instrumental plans. Emotions provide to an organism the information whether its internal and/or external environment is beneficial or harmful to its plans (Bower, 1992). In this sense, emotions are *"... evolution's way of giving meaning to our lives*" (*Ibid*., pg. 4) and as evolutionary mechanisms steer the organism's interaction with the environment influencing its biological status, motivation, social behavior, and cognitive processes.

According to Bower (1992) an organism, in order to operate successfully in its environment, must be able to prioritize its needs and motives according to their importance and urgency. One of the mechanisms that support the successful prioritization is memory for past events. Memory is governed by attention – and attention in part by, after Bower (1992), "interestingness". "Interesting" means on one hand anomalous and distinctive or, on the other hand, affectively arousing or meaningful. When memory becomes enhanced by an "interesting", meaningful event or environmental feature, the organism has better chances to react appropriately in a known environment, and to conclude about acting rules in an unknown and uncertain, also possibly hostile, environment in the future.

The focus of this dissertation is human episodic memory, as one of the cognitive processes that undergoes strong emotional influences. This fact had been long known, even before it has been scientifically proven.

"*In medieval times, before writing was used to keep historical records, other means had to be found to maintain records of important events (...). To accomplish this, a young child about seven years old was selected, instructed to observe the proceedings carefully, and then thrown into a river. In this way, it was said, the memory of the event would be impressed on the child and the record of the event maintained for the child's lifetime*" (McGaugh, 2003, pg. ix).

Although not justifiable with nowadays ethical standards, this historical report stresses the strengthening role of strong emotions in the formation of long-lasting memories.

However, the goal of the interaction between the powerful emotional system and other cognitive systems is not only the instrumental strengthening of memory by emotions, animalistic survival in a hostile environment, or making sure that our genes will survive. Emotions not only serve as direction signs in the memory for executing instrumental plans. With the following story, I would like to demonstrate that the interaction between memory and emotion, at least for human beings, also serves a more sophisticated purpose.

Recently, I was telling my friend, Marzena, about my plans of visiting my brother who happens to live in Canada. During my story, she began to be very excited. Her eyes became brighter, movements faster; I got the impression that something in what I was saying was very important to her. I asked, and in a response she told me her story. Back in her childhood, when her family lived in communistic Poland, Marzena's father went to work in Canada every now and then. She can lively remember the time, when her father was abroad, how she missed him. Most of all, she can remember, how much excited she was every time the father was about to come back and how happy she was when he was at home. Since being a little girl, Marzena has been dreaming about visiting the country, which she associates these ambivalent feelings with. Her whole life, she has not had the opportunity to go to Canada. Until now: The international company, which Marzena works at, has recently started a new project in North America. Of course, Marzena volunteered and the chances that she will be leading the Canadian part of the enterprise are relatively high. Once again, the prospect of visiting, and probably living in Canada, makes her extremely excited.

As Radvansky (2006) elaborates, *"…memories help to define who we are. Our opinions, attitudes, likes, and dislikes are a result of our previous experience. Memory is the repository of these experiences and the sharpener of our actions in the future*" (pg. 133). In case of my friend, strong emotional reactions in the late past and thus strong emotional memory have shaped Marzena's likes and attitudes. They have influenced her particular desire to go to Canada one day, which has become her personal goal, and now they are making her conduct the career path towards achieving this goal.

These two stories illustrate the impact of emotions on cognition. The medieval story describes the impact of strong emotional arousal on memory. Marzena's story not only stresses the long-term impact of emotional experiences on memory, which in her case is vivid after over thirty years, but also gives us some insight into the extent of the impact potential of emotional experience through shaping our lives.

The topic of this dissertation is modulation of episodic memory in humans. Especially, I examine the impact of emotional contents on electrophysiological markers of long-term episodic memory processes in three consecutive event-related potential studies, using emotional and neutral pictorial stimulus material.

Studying emotional influences on long-term memory not only gives us insights in the basal processes that steer the emergence of long-lasting memories. It also provides clues for further research aiming at explaining how these basal processes influence complex behavioral patterns: on the one hand, "positive" behavior like pursuing goals motivated by prior emotional experiences (like the story of my friend), on the other hand, "negatively" motivated behavioral patterns like mental disorders associated with highly unpleasant and arousing events in the past, best exemplified by post-traumatic stress disorder.

In the next sections, I summarize the theoretical background of memory research, concentrating on episodic memory, and present the important aspects of memory for emotional events, further called for readability reasons emotional memory. Then, I shortly describe the event-related potentials (ERPs) technique used in the studies reported here and will review the main findings about how these electrophysiological phenomena reflect memory processes as well as emotional influences on them. Consequently, I report and discuss data from studies, which I conducted as first investigator together with my colleagues at the Department of Biological and Clinical Psychology at the University of Greifswald and at the Department of Clinical Pharmacology at the University Medicine Greifswald. Eventually, I discuss the summarized results and open issues as well as stress the importance of emotional memory research for clinical implications.

The first study addresses spontaneous remembering of emotionally relevant material. This study shows that, while explicit remembering of emotional material after a

longer retention interval is based on recollection processes, spontaneous remembering, measured in an indirect recognition memory test, is probably mediated by familiarity processes, as indexed by relevant ERP signatures. The second study raises some methodological aspects of recognition memory in a crossover or within-subject design, which is rather unusual for psychological research. The study shows, that it is possible to reliably explicitly test episodic memory processes, when implementing two consecutive incidental encoding sessions and an unexpected recognition memory test one week later. This enabled us employing the tested paradigm in a pharmacological beta-blocker study in a crossover design. In the literature, there is strong evidence that especially episodic memory based on recollection profits from emotional influences and that this emotional enhancement of memory can be reduced by a pharmacological beta-blockade. These findings come from studies in which direct memory tests were conducted, i.e. explicit memory was assessed. In the first study reported here we observed emotional modulation of episodic memory based on familiarity and not on recollection in the indirect test. Therefore, the third, pharmacological study, as thought to test modulation of memory based on recollection, was conducted using explicit memory testing. This third study provides insights into the role of neurotransmitter in enhanced memory for emotional events. For reasons of subjects' availability, which I explain in detail further in the text, we were only able to assess eight participants. This, of course, did not enable me to validate and interpret the collected data. Thus, the reported data are analyzed in an explorative manner. The preliminary results suggest, in line with previous findings, that the emotional memory enhancement indexed by greater old minus new differences in ERPs for emotional than neutral stimuli can be reduced by a pharmacological blockade, stressing the important role of the noradrenergic system and the amygdala in mediating the emotional influences on episodic memory formation.

Part I – Background information

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2 Memory – theoretical frame

In his book "Memory and Emotion", James L. McGaugh describes memory as a "*lasting consequence of an experience*" (McGaugh, 2003; pg. 3). As he further explains, in a more specific sense, "*memory is the consequence of learning from an experience – that is, the consequence of acquiring new information*" (*Ibid*., pg. 3). The apparently simple definition of memory implicitly uncovers the great complexity of the memory system. Memory is not a unitary cognitive function; it is a system that consists of components with subcomponents which have evolved "*as a result of environmental selection pressures, to handle* [different jobs]" (Radvansky, 2006; pg. 14-15, changes: AJ). Firstly, there are different types of information, one can remember, and different ways in which information acquisition takes place. Secondly, the information may be saved in different forms and the remembered information expressed variously. Lastly, the memory trace can be short- or long-living. Over years, the various aspects of memory have led memory theoreticians and researchers to numerous models of memory, which can be classified in taxonomic systems, depending on their phenomenological characteristics, the information stored as well as the encoding and retrieval processes associated with a particular memory form.

A major contribution to understanding of different memory functions was made by Brenda Milner who, together with William Scoville (Scoville & Milner, 1957) studied and reported on probably the most famous neurological patient, Henry Gustav Molaison (†2008, previously known as H.M.). Henry Molaison had had large parts of the medial temporal lobe (MTL) bilaterally removed as a treatment of severe epilepsy, he had been suffering for a great part of his life. This surgical intervention caused, though alleviating the seizures, irreversible memory impairment. This impairment, however, was restricted to one form of memory, the declarative memory. The finding led to the conclusions that, first, memory is a cognitive function distinct from other cognitive abilities (Squire, 2009), and second, because the brain damage caused impairment in only some functions of the memory, there are distinctive and in-parallel functioning memory systems within the memory as a whole.

The modal model of memory was proposed in the late 60' by Richard Atkinson and Richard Shiffrin (Atkinson & Shiffrin, 1968). The nowadays most common classification of memory interprets the modal model by incorporating its temporal characteristic and dividing the memory system into sensory, short-term (STM) and long term-memory (LTM). It is a rather general theory of memory, a heuristic accepted by most researchers (Radvansky, 2006), not an accurate theory of memory.

The shortest memory stores, which last for only fractions of seconds, are visual and auditory stores, labeled in 1967 by Neisser iconic and echoic memory (Baddeley, 1990). These sensory systems are based on a particular sensory modality and constitute an integral part of perceptual processes. Their function is to store information for further analyses (*Ibid*.).

The next step of memory storage is the short term-memory. The STM is a temporary storage of small amounts of material over a short, limited period of time. Related to the STM is the working memory concept. The working memory is a system for temporary maintenance and manipulation of information (Baddeley, Eysenck, & Anderson, 2009) which is useful in performing various complex cognitive tasks, like learning, reasoning and comprehending (Baddley, 1990). Working memory, as a mental workspace, provides basis for thought and is able to interact with and use the resources of the STM and long-term memory (Baddeley et al., 2009)

When the information is processed within the STM or working memory, a temporary memory trace emerges which can either just fade or be "relocated" into long-term memory in a process called consolidation. The memory consolidation is a conversion of an active memory trace into a relatively inactive memory trace (cf., section 2.1.2).

In the Triarchic Theory of Memory, Tulving (1985) distinguishes three different, interconnected but independently operating types of long-term memory. The main focus of procedural memory is the way, "*how things are done*" (*Ibid.*, pg. 2). It's the learning, retaining and utilizing of perceptual, cognitive, and motor skills. "*Symbolically representable knowledge*" (*Ibid*., pg. 2) about the world is saved within semantic memory. Episodic memory comprises remembering of personally experienced events

(*Ibid*.). For Tulving, these types of memory differ not only in the contents and tasks but also in the level of consciousness, namely anoetic, noetic, and autoneoetic[1], respectively.

Procedural memory is anoetic (not-knowing) in the sense, that organisms are capable of registering, internally representing and responding to the aspects of the present environment. Anoetic consciousness is spatially and temporally bound to actual events. There is no reference to external stimuli or intrinsic states that are absent at the time. Noetic consciousness, which characterizes semantic memory, enables an organism to cognitively operate on objects and events even if they are not present. An organism is aware of the process itself, as well as the acquisition and retrieval of information. Autonoetic consciousness, in contrast, accompanies episodic memory and enables a person to go on a "mental time travel". A person is aware of his own past and of what can happen in the future, irrespective of time and space. Possession of autoneoetic consciousness is further necessary for remembering: "*organisms can behave and learn without (autoneoetic) awareness, but they cannot remember without awareness*" (*Ibid*., pg. 5).

The long-term memory is a complex cognitive system, consisting of separable subsystems with the capacity to store information over longer time periods. A very useful and perhaps most popular classification of LTM is the one proposed by Squire (1992). The author makes a distinction between declarative and non-declarative forms of memory. The declarative (explicit) memory is based on intentional learning and retrieval processes; it's the memory for events and facts. In contrast, non-declarative (implicit) memory is based on incidental or procedural learning processes, expresses in performance and is not dependent on conscious retrieval.

The taxonomy of long-term memory, which in a broad sense corresponds with Tulving's level-of-consciousness dependent division, is schematically displayed in Figure 1. Memory as a system can be fractioned into functional subcomponents with "*different operating principles*" (Squire, 2009; pg. 12711) and different tasks.

Non-declarative or implicit memory is difficult to articulate but with great impact on organism's functioning, although it does not involve or depend on conscious remembering (Eichenbaum, 2008). It encompasses procedural memory (i.e., skills and

[1] *Noema* derives from the Greek word *νόημα* meaning thought or what is thought about.

habits), priming processes (i.e., perceptual and semantic priming), associative learning (i.e., classical and instrumental conditioning), and non-associative learning (i.e., reflexes). Implicit memory is a system that enables proper and fast reactions to external and internal stimuli as well as execution of relevant sequence of actions when performing a task without the necessity of conscious awareness. Although declarative and non-declarative memory may refer to the same situation, they express differently. The non-declarative memory expresses in performance, the declarative one in recollection (Squire, 2009).

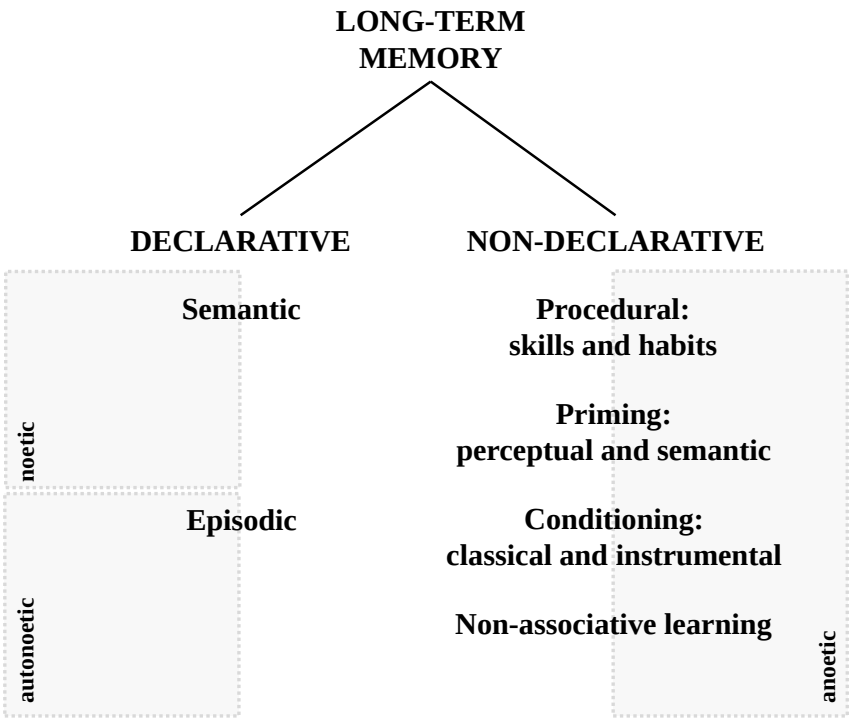

Figure 1. Long term memory systems.

Declarative (explicit) memory, in turn, is responsible for recollection of facts and past events (Gabrieli, 1998), being always accompanied by an awareness of the act of remembering. The contents of both semantic and episodic memory can be articulated. Moreover, cognitive memory (as termed by Eichenbaum to stress the opposition to non-declarative memory), as a flexible and inferential instance, enables generalization across personal memories and previous knowledge and by bringing this information to mind helps solving new problems (Eichenbaum, 2008) Semantic memory is a generalized, stable knowledge about the world, facts and principles. It is highly interrelated, shared among people and long-lasting. It is independent of time or space of acquisition and

stored without this contextual information; however it requires conscious awareness – in Tulving's terminology it depends on noetic consciousness. Episodic memory comprises specific events in our lives; it is compartmentalized and personalized as well as prone to forgetting. It is tied to the time and place in which the information was learned and requires additional knowledge of the self, as the entity to which past events can be referenced (i.e., it is accompanied by Tulving's autoneoetic consciousness).

2.1 Episodic memory

The term "episodic memory" was introduced by Endel Tulving in the 1970s as an ability to recollect events and episodes of individuals' life and to differentiate it from semantic memory as generalized knowledge of the world (Baddeley, Conway, & Aggelton, 2002). Both types of memory, semantic and episodic, allow fast encoding of great amounts of complex, diverse kinds of new information. The stored information is abstract, can be accessed flexibly, and, as stated before, used inferentially. The two kinds of memory are also functionally interconnected. Over time, memories in episodic memory can accumulate to form semantic memory (Baddeley et al., 2009). In addition, memory for an event can fade from episodic memory, but the knowledge acquired during this event stays preserved (Conway, Cohen, & Stanhope, 1992).

The episodic memory system represents that what most people understand as memory. It extracts and stores the contents from one's experiences, i.e., episodes and their context, and allows one to answer the questions related to oneself: What? Who? When? Where? It supports the ability to consciously recollect and report on facts and events that one has experienced. Thus, the most prominent characteristic of episodic memory is its ability of "mental time travel" (Tulving, 1985), i.e., it enables to replay the flow of experienced events, to consciously explore them and vividly relive the previous experience (Tulving, 2002).

There are three main properties that distinguish episodic memory from other memory types. All were newly summarized by Rudy (2014). First, episodic memory supports storage and conscious recollection of contextual information for later retrieval. The memory search can be initiated intentionally or incidentally, without prior intention.

Conscious recollection means that the act of retrieval from memory is accompanied by an awareness of remembering, i.e., a sense that the memory trace has been activated. Additionally, the sense of recollection emerges, when the retrieved memory contains contextual information, like time or place of encoding, or additional features of the event (e.g., emotional state, thought, etc.). Furthermore, the contextual information enables a replay of the experience and allows one to state that one recollects the event. Episodic memory plays the crucial role in storing and retrieving the contextual information. Second, the episodic memory system has the ability to automatically capture information about the events, simply while exploring and experiencing. The process of encoding information into episodic memory does not require any intention; it works on the basis of attention and experience. While learning, episodic memory captures information about the learning event. For instance, while we memorize a phone number, episodic memory incidentally captures the situational information. Third, episodic memories are incidental and unique in nature. The episodic memory system acquires information about a single episode, while protecting from interference with other representations. The episodes are stored separately, even if there are many overlapping details, e.g., many different instances of a situation.

Memories, as a result of experience, are created, because memory traces in the brain emerge. This is possible thanks to synaptic plasticity, a property of synapses to undergo modification as a result of experience. Although a single synapse is not the fundamental unit of memory, changes in synaptic strength among groups of neurons can represent experience (Rudy, 2014). There are two major mechanisms that aim at explaining the synaptic plasticity: reflex modification and long-term potentiation (LTP). While the reflex modification and its associated molecular and behavioral mechanisms, as researched and described by Eric Kandel[2] (Kandel, 1976, 2001), are applicable in case of reflexive responses, the LTP, originally discovered in the hippocampus, is the candidate mechanism for explaining the synaptic plasticity in formation of long-term episodic memories. LTP is "*an increase in synaptic efficacy that persists for hours to more than*

[2] Kandel explored the reflex modification mechanisms on a sea slug Aplysia californica, an invertebrate, which has a relatively simple brain with very large neurons. In investigating the gill withdrawal reflex he observed several phenomena, among others the habituation of the reflex. After indentifying the exact connections that participate in the gill withdrawal reflex, Kandel and his colleagues were able to identify the locus in the circuit that had been changed when the reflex changes. This locus was a particular synapse, whose strength of connection has weakened as a result of the reflex habituation.

day after the delivery of a brief induction stimulus" (Dudai, 2004; pg. 148). The efficacy is expressed as a magnitude increase of synaptic potentials after short stimulation (Hasselmo, 2012). Short term changes involve modification of existing proteins and long-term changes depend on gene expression, i.e., formation of new proteins. The LTP is considered a mechanism influencing learning, short- and intermediate-term memory at the cellular level, observed in many types of synapses (Dudai, 2004). Although the exact role of LTP is still controversial and lively discussed in the literature (Davachi & Danker, 2014; Dudai, 2004; Rudy, 2014), the fact that long-term synaptic changes can be induced by a single brief stimulation appears consistent with the potential role of the LTP in the formation of episodic memory (cf., Rudy, 2014).

Intact episodic memory depends on functioning subcortical structures, mostly in the area of the medial temporal lobes (MTL). Amnesia can be highly selective for episodic memory and leave all other functions spared. Patient K.C., described by Tulving and Markowitsch in 1998 (cited after: Eichenbaum, 2008) suffered a closed head injury with widespread cortical and subcortical damages, including the MTL. Almost all of his cognitive abilities remained intact, with the exception of episodic memory. He had no ability to recollect old experiences and form new episodic memories.

The already mentioned patient Henry Molaison (cf., section 2) underwent a surgery, in which his temporal lobes were removed. After the surgery, although most of his cognitive functions remained intact, the MTL lesion caused severe anterograde amnesia. The patient was not able to acquire new memories in the declarative domain. Initially, he registered new experiences, but they were only maintained over a short period of time. The memory vanished when the patient became distracted or memory span was exceeded. Molaison could remember some of his childhood memories, but retrograde amnesia was also present. This had disconnected him from most of his personal past. He was able to acquire new motor skills, but could not remember learning it. From a surgery report made by William Scoville and a magnetic resonance imaging (MRI) study conducted over several years (Corkin, Amaral, Gonza, Johnson, & Hyman, 1997) we know that mainly the hippocampus and the surrounding regions of the neocortex were removed bilaterally. These MTL structures, most prominently the hippocampus, the entorhinal, perirhinal, and parahippocampal cortex (often referred to as hippocampal formation due to their high anatomical connectivity and associated function)

are involved in formation of long-term episodic memories. They receive distinct patterns of inputs from other neocortical and subcortical regions and all participate in the formation of episodic memories (Davachi & Danker, 2014) .

2.1.1 The Hippocampus and the Memory As Reinstatement model (MAR)

Within the hippocampal formation, the hippocampus plays a crucial role in episodic memory. It is embedded in a neural system that interacts with other brain regions. Its intrinsic organization and its synaptic connections are unique (Lavenex & Amaral, 2000). The neural system supporting episodic memory is hierarchically organized with the highest level of information integration in the hippocampus. Information flow proceeds from sensory modal cortex through the perirhinal, parahippocmpal and entorhinal cortex to the hippocampus. The hippocampus is at the top of the hierarchy and recieves a wide range of information from different cortical regions. The information received is highly processed and amodal. The earlier processing stations are necessary for the hippocampus to receive the information and can therefore be considered a part of the episodic memory system. It is also hypothesized that the lower-level processing stations are involved in familiarity-based recognition (Lavenex & Amaral, 2000).

The Memory As Reinstatement model (MAR; Davachi & Danker, 2014) combines current theories and knowledge about episodic memory processes and aims at explaining how episodic memories are formed and later accessed.

During the experience of an event, neural cortical and subcortical patterns are activated, which represent sensory-perceptual experience of the event and accompanying actions, thoughts and emotions. The neural activation from the cortex and subcortical structures converges into the MTL and the hippocampus. On this way, a "microrepresentation" of the current episode emerges in the hippocampus. A successfully encoded experience (information about the event and its context) results in a neural representation of the particular experience in the hippocampus (hippocampal neural pattern, HNP) and a corresponding neural representation in the cortex (cortical neural pattern, CNP). The neural patterns (i.e., particular neural connections) in the hippocampus and the cortex become strengthened by means of LTP. The HNPs contain the critical connections which allow to later access the CNPs and to attribute the experience to a

particular time, place (Tulving's "when" and "where") and additional contextual information. This forms the base prerequisites for episodic memory, i.e., without the HNP it is difficult to recover the CNP for a specific experience.

Episodic memory retrieval proceeds in three steps: 1. Cue processing, 2. Reinstatement of the HNP, and 3. Reinstatement of the CNP. Primarily, retrieval processes are cued by external or internal cues, like emotion, a detail of the memorized event, thought, or state. Cue representations are supported by cortical regions. These representations in the cortex serve as a key unlocking the neural patterns for episodes containing the cue. Next, the retrieval cue triggers the hippocampal pattern completion. In this way, the HNP that has been established during encoding and subsequent strengthening becomes reinstated. Eventually, the reinstatement of the HNP reinstates the CNP. This final reactivation of a neural pattern for a particular event results in a subjective feeling of remembering.

2.1.2 Memory formation

There are multiple stages that build the temporal development of memory formation. Once an event or information has been perceived and encoded it may become a memory trace. First, the newly formed memory is labile and sensitive to modulation, i.e., interferences or strengthening. For memory to become stable, a time dependent consolidation process (i.e., post-acquisition stabilization of new memories) is needed. On molecular and cellular levels, synaptic consolidation requires neurons to produce new RNA and proteins (i.e., gene expression) and induces changes of synaptic efficacy (Nader & Hardt, 2009). As a result of the cellular consolidation, the consolidation on cognitive level is referred to as a transition from a labile short-term memory to a stable long-term memory.

The memory, considered as a continuous memory trace formation process, can be tested and influenced on all processing stages (see McGaugh & Roozendaal, 2008 for a review). Thus, with experimental modulations one can aim at the encoding stage, the consolidation stage, and the retrieval processes (as well as reactivation and reconsolidation[3]).

[3] Memory reactivation and reconsolidation processes, as intrinsic stages of memory formation, are referred to once again in the final discussion.

In the laboratory environment, the interference of memory formation may be caused by electroconvulsive shock (Duncan, 1949), inhibition of gene expression (reviewed in Tronson & Taylor, 2007), inactivation (or lesions) of brain regions (LaBar & Phelps, 1998; Roozendaal & McGaugh, 1997), new learning (Schwabe & Wolf, 2009), and/or blockade of neurotransmitter systems (Cahill, Prins, Weber, & McGaugh, 1994; Liang, Juler, & McGaugh, 1986; Weymar at al., 2010a). In the opposite, post-training stress (Cahill, Gorski, & Le, 2003), pre-encoding stress (Wirkner, Weymar, Löw, & Hamm, 2013) emotional valence and arousal of stimuli (Bradley, Greenwald, Petry, & Lang, 1992; Schaefer, Pottage, & Rickart, 2011; Schupp, Junghöfer, Weike, & Hamm, 2003), activation of particular neurotransmitter systems (Andreano & Cahill, 2006; Buchanan & Lovallo, 2001; Nielson & Jensen, 1994) positively influence the formation of memory.

2.1.3 Recognition memory

Recognition memory, as a particular form of episodic memory, is the ability to correctly decide whether a stimulus has been encountered before. In particular it is "*…a matching process in which the contents of the environment are compared with the contents of memory*" (Radvansky, 2006; pg. 55).

Usually, the tests of recognition memory include both old and new items (i.e., items learned in the encoding session and items not encountered in the encoding session) and force study participants to decide, if the presented item is an old or a new one. The tests are designed for the subjects to show their skills in making correct discriminations. New items serve as distractors and provide valuable information about how much a person's recognition judgment can be trusted. False recognition of distractors reflects guessing and introduces a certain bias in a measurement. Therefore, in recognition performance testing, a common method of correction for guessing is a subtraction of false positive responses (incorrect guesses) from correctly recognized items.

Recognition memory testing emerges from signal detection theory (SDT; Green & Swets, 1966), which evolved from auditory perception research. The SDT proposes that memory traces have strength values that reflect their activation in memory and which dictate how familiar they seem. The familiarity levels of the items vary and also new

items have some level on familiarity (e.g., because they are known from outside of the experiment, or are similar to old items). On average, old items are more familiar than new items, but, because the distributions of familiarity of old and new items overlap, it is necessary to choose a response criterion (familiarity or ”oldness” criterion), when making a recognition judgment. A subject’s tendency to answer “old” to new items more often reflects the liberal criterion. The conservative bias is expressed by less new items recognized than old ones.

The SDT assesses the ability to detect the signal (accurate memories) and discriminate it from noise (inaccurate memories). Thus, items correctly recognized as old are called “hits”. New items correctly recognized as “new” are called “correct rejections”. Items incorrectly recognized as old are called “false alarms”, and not recognized old items – “misses”.

As opposed to single process models of recognition memory, which the SDT is a good example for (Yonelinas, 2001), dual-process theories of recognition comprise two independent processes – familiarity and recollection – which both contribute to recognition memory (Mandler, 1980; Yonelinas & Jacoby, 2012; Yonelinas, 2001).

The difference between the two processes can be best exemplified by the common experience (Yonelinas, 2002) of meeting a person on the street. The person may seem familiar and we may be sure that we know her, but we can remember neither her name nor any other fact about her. Alternatively, in addition to the feeling of knowing we may also recall her name, the situation the last time we saw her, as well as lots of other fact about her. The first situation described refers to familiarity, the second to recollection processes.

Whereas in single process models the recognition performance is measured in the “yes-no” approach, dual process models have utilized different, more sophisticated ways of measuring the contribution of single processes to recognition performance. One of this methods is the “remember/know” procedure (Tulving, 1985), which conceptualizes these two processes. During recognition memory assessment, subjects are asked to make memory judgments on basis of their subjective convictions about the memory source and report whether they “remember” the particular item or simply “know” it. “Remembering” means recollecting episodic information about the study event, vividly remembering the

item and specific details accompanying it. “Knowing”, in turn, means recognizing the item without recollective experience, i.e., being aware that the item has been encountered before, but without any additional information about it (Schaefer et al., 2011; Yonelinas, 2002).

There are several dual-process models dealing with recollection and familiarity as independent processes supporting retrieval (cf., Yonelinas, 2002). Although each treats the processes slightly different, there are some common characteristics of the two processes arising from the models (reviewed and summarized in Yonelinas, 2002).

Familiarity and recollection are independent processes. Although both are initiated in parallel at the time of retrieval, familiarity is faster than recollection, which requires a longer and more exhaustive search in memory. Familiarity is described as depending on memory strength (i.e., how familiar the item or the event seems) and the process runs automatically. In opposition to recollection, is not that prone to distraction during retrieval. Recollection reflects retrieval of specific information about the remembered event – it is a more elaborated, controlled process. There is also a relatively broad agreement that recognition performance based on familiarity tends to diminish fast. Recognition memory based on recollection is, in turn, relatively long-lasting.

Some authors (reviewed by Yonelinas, 2002) argue that familiarity and recollection depend on perceptual and conceptual processes, respectively. However, some models discuss familiarity as depending on both perceptual and conceptual processes (*Ibid.*). Tulving (1985, 2002) proposes in turn, that recollection arises from episodic memory and familiarity relies on semantic memory, i.e., the abstract knowledge that an event had happened. Thus, the two types of retrieval are associated with different types of consciousness: recollection is accompanied by autonoetic and familiarity by noetic consciousness (Gardiner & Richardson-Klavehn, 2000).

Nevertheless, familiarity is strongly bound to a perceptual match between the test item and a stored representation. Therefore, changes in stimulus properties (e.g., changes in modality) or similarities between two different items affect recognition performance based on familiarity (Eichenbaum, 2008). Recollection incorporates the meaning of the stimulus, context and other associations, resulting in vivid reminiscence. Thus, the processes depend differently on the levels of processing (Craik & Lockhart, 1972) during

encoding: recollection profits from deeper encoding, whereas familiarity is relatively independent of the processing levels. Recollection as a controlled process, in comparison to familiarity, is more prone to distractions during retrieval. Familiarity, as a threshold-based process, is sensitive to response bias (Eichenbaum, 2008; Radvansky, 2006).

Over years, a lot of research has been made concerning the neural substrates of recollection and familiarity, and finally process dissociations procedures combined with lesion studies and functional brain imaging methods have come to relatively clear results (Yonelinas & Jacoby, 2012). There is a strong agreement that the medial temporal lobes contribute to episodic recognition memory (Brown & Aggelton, 2001; Scoville & Milner, 1957; Thompson & Kim, 1996). More specifically, the hippocampus plays a crucial role in recollection, and the surrounding cortex is essential in familiarity processes. Animal studies indicate that the hippocampus mediates recollection-like processes in rats (Fortin, Wright, & Eichenbaum, 2004). Lesion studies with patients suffering brain damages selective to hippocampus only have shown great recollection impairments, while familiarity was intact (reviewed in Yonelinas & Jacoby, 2012). In contrary, familiarity disruptions, with intact recollection, have been observed in a patient with a selective damage of the perirhinal cortex and a spared hippocampus (Bowles, Crupi, Mirsattari, Pigott, Parrent, Pruessner, Yonelinas, & Ko, 2007). Functional MRI (fMRI) studies in healthy adults support these results, showing that the hippocampus and the parahippocampal cortex are mainly associated with recollection and the perirhinal cortex with familiarity (reviewed in Eichenbaum, Yonelinas, & Ranganath, 2007; Skinner & Fernandes, 2007; Voss & Paller, 2010; Wais, 2008). Moreover, there is strong evidence for the assumption that the frontal lobes, especially the dorsolateral prefrontal cortex, and the parietal cortex play an important role in recognition processes. Their exact specializations and function dissociations, however, remain under debate (Skinner & Fernandes, 2007; Vilberg & Rugg, 2009).

3 Emotion and memory

Emotions have a strong impact on our lives, influencing not only our biological reactions but also our perception, learning, and memory. According to Panksepp (1998), emotions, which rest upon evolutionary early mechanisms, enable animals' adaptive reactions. The emotional impact on cognition manifests in many ways. For example, mood-congruent information is better attended and information is better retrieved when emotional states during encoding and retrieval overlap (Bower, 1992). In behavior, the strength of emotional arousal can influence the performance in a task, which is well known in psychology as Yerkes-Dodson law. The performance rises with the rise of emotional arousal up to a certain level of arousal, before it disintegrates rapidly. The optimal arousal level for easy tasks is higher than for hard tasks (Yerkes & Dodson, 1908). Moreover, people have the tendency to remember highly arousing incidents. Emotional stimuli are salient and attracting attention, which results in better memory for emotional events (LaBar & Cabeza, 2006).

The phenomenon of emotion-related advantage in memory is the focus of the following sections. For reasons of better readability the memory for emotional events will be called shortly emotional memory.

3.1 Emotional memory

Events, situations and stimuli that trigger emotional reactions or are associated with emotional reactions hold a privileged status in memory and therefore constitute the core of one's experience and personal history (Atkins & Reuter-Lorenz, 2008; LaBar & Cabeza, 2006). Emotion theorists claim that emotions can be described in two[4] orthogonal, independent dimensions (Bradley, 2009; Lang, Greenwald, Bradley, & Hamm, 1993) – valence (ranging from pleasant through neutral to unpleasant) and arousal (varying between calm and excited). These two factors are considered to cover the entire spectrum of emotion in a two-dimensional space (Weymar & Hamm, 2013).

[4] The third dimension with one can characterize the emotionality of stimuli is dominance (Bradley & Lang, 1994).

Therefore, this operationalization of emotion is often used in emotional memory research. The impact of these aspects on memory has been thoroughly studied in the last decades (see LaBar & Cabeza, 2006 for a review). Data suggest that emotional arousal is the primary factor influencing enhanced encoding of stimuli (Bradley et al., 1992; Cahill & Anderson, 2009; Hamann, 2001; LaBar & Cabeza, 2006), but some studies also report valence effects (Zald, 2003). It is therefore hypothesized that enhancing effects of arousal and valence on memory rely on two distinct neural mechanisms (Kensinger & Corkin, 2004; Liu, Huang, McGinnis, Keil, & Ding, 2012). Vast research in two memory domains, non-declarative (mainly fear conditioning in animals and humans) and declarative (mainly episodic memory in humans), delivers most prominent results. Here, human behavioral, lesion, neuroimaging and pharmacological studies on emotional memory are selectively reviewed. Additionally, two important concepts, the motivational model of emotion (Bradley, 2009) and the neuromodulation hypothesis (McGaugh, 2004) are summarized and the central role of the amygdala in emotional memory processes is stressed. Electrophysiological findings about the prioritized processing of and enhanced memory for emotional events, as directly relevant to the objectives of own research, are reported in a separate section (cf., section 4.2).

Subjectively, people have the impression that they better remember emotional than neutral events in their lives (Holland & Kensinger, 2010). In fact, research on autobiographical memory (i.e., explicit memory of episodes from the personal past) has shown a superiority of emotional over neutral personal episodes in memory (Berntsen & Rubin, 2002). Brown and Kulik (Brown & Kulik, 1977) studied the vividness of memory of the assassination of J.F. Kennedy in 1963. They showed that, over a decade later, most people remembered the situation in which they were when they learned the news. These results were confirmed even years later (Winograd & Killinger, 1983). However, a similar study conducted by Talarico and Rubin (2003) on the memories related to the attacks on the World Trade Center on September, 9, 2001 revealed that emotional memories related to the attacks faded to the same grade as neutral memories from the same time. What differentiated the emotional memories from the neutral ones was not the accuracy but subjective feeling of recollection, rated vividness and confidence. This is in line with the later findings showing that remembering emotional events is associated with enhanced recollective

experience and not necessarily always with higher memory accuracy (Phelps & Sharot, 2008; Weymar et al., 2010a). Enhanced memory for personal events is not only limited to public events. Also individual experiences that are accompanied by emotion, are better remembered. In a study, Brewer (1988) asked students to record events at random times during the day, over a 13 days period. When tested for memory of these events after longer retention intervals (23 and 46 day later), the students better remembered these events that were associated with emotional arousal, independently of valence. In addition, personal occurrences associated with extremely high emotional arousal, like medical emergencies, traumatic experiences, or life-time events, retain vividly and long-lasting in memory (e.g. Peterson & Whalen, 2001; Peterson & Bell, 1996; Pillemer, Goldsmith, Panter, & White, 1988; Blackburne-Stover, Belenky, & GiUigan, 1982).

Not only personally relevant emotional events are better remembered. Experimental laboratory research on memory also indicates prioritized processing (Hamann, 2001; Vuilleumier & Driver, 2007) and an enhancement of memory processes in the presence of emotional contents (LaBar & Cabeza, 2006). Prioritized processing of emotional stimuli can be well illustrated by attentional blink (Cahill & Anderson, 2009). In perceiving, the primary stimulus identification is followed by a short gap in perceptual awareness. This phenomenon can cause impairment in the perception of the following stimulus, if it occurs within the attentional gap. This "attentional blink" is diminished for emotionally arousing stimuli of both negative and positive valence (Anderson, 2005). Thus, emotional stimuli tend to capture attention, which in turn impacts their further elaborated processing and eventually results in better memory. Moreover, during encoding, the engagement of attentional resources for processing of emotional stimuli tends to inhibit the processing of the surrounding (Ellis, Detterman, Runcie, McCarver, & Craig, 1971), especially preceding neutral stimuli (Strange, Hurlemann, & Dolan, 2003). Prioritized processing of emotional stimuli has also been shown in electrocortical studies, where emotional stimuli in early perceptual processing stages are associated with enhanced event related potentials as compared with neutral stimuli (Olofsson, Nordin, Sequeira, & Polich, 2008). This is generally interpreted in means of capturing attention by emotionally relevant stimuli.

In laboratory testing of memory performance, a variety of emotional and neutral stimuli, including words (Strange & Dolan, 2004; Windmann & Kutas, 2001), natural scenes (Koenig & Mecklinger, 2008; Tapia, Carretié, Sierra, & Mercado, 2008; Weymar, Löw, Melzig, & Hamm, 2009), faces (Anderson, Yamaguchi, Grabski, & Lacka, 2006a; Liu, Chen, & Ward, 2014), narrated slide shows (Cahill et al., 1994), slide stories (Christianson & Loftus, 1991), and videos (Kamboj, Oldfield, Loewenberger, Das, Bisby, & Brewin, 2014) has been used.

Across numerous studies, it has been shown that free recall of emotionally arousing stimuli is enhanced as compared to neutral stimuli (Bradley et al., 1992; Cahill & McGaugh, 1995). Bradley and colleagues (1992) demonstrated that highly arousing emotional pictures were better recalled immediately after learning, as well as a year later, as compared to low-arousing stimuli. In recognition studies, the memory performance pattern is similar as in recall tasks. Usually, hit rates and discrimination performance are higher for emotionally arousing than for neutral stimuli (e.g., Weymar et al., 2009). Moreover, originally neutral material that is accompanied by high subjective arousal ratings is remembered better than material accompanied by low arousal ratings (Anderson, Wais, & Gabrieli, 2006b) Additionally, correct recognition of highly arousing stimuli is rather based on recollection than familiarity (Ochsner, 2000; Schaefer et al., 2011; Sterpenich Albouy, Darsaud, Schmidt, Vandewalle, Dang Vu, Desseilles, Phillips, Degueldre, Balteau, Collette, Luxen, & Maquet, 2009; Weymar et al., 2010a), and emotional stimuli are remembered with greater confidence (Weymar et al., 2009), although there are also findings which suggest that enhanced recollection for highly emotional arousal is driven by more liberal response bias (Dougal & Rotello, 2007) or high recollective experience (Phelps & Sharot, 2008).

Various studies have shown that the emotional enhancement of memory is present when memory testing takes place immediately (Schaefer, Fletcher, Pottage, Alexander, & Brown, 2009; Versace, Bradley, & Lang, 2010; Weymar, Bradley, El-Hinnawi, & Lang, 2013), but these effects seem to hold longer, ranging from hours and weeks to months (Anderson et al., 2006a; Ochsner, 2000; Schaefer et al., 2011; Weymar et al., 2009; Wirkner et al., 2013). Several studies demonstrated even longer lasting memory effects (Bradley et al., 1992; Dolcos, LaBar, & Cabeza, 2005; Weymar, Löw, & Hamm, 2011). The long-lasting enhancement of memory for highly

arousing pictures seems to rely on recollection processes, in which the memory of the stimulus is accompanied by additional contextual information and high confidence ratings (Weymar et al., 2011). Moreover, a number of studies have indicated that longer retention intervals, and thus consolidation processes are particularly beneficial for the storage of emotional material (see LaBar & Cabeza, 2006).

The memory advantage for emotional contents is dependent on prioritized processing of emotionally salient stimuli (Lang & Bradley, 2010; Schupp, Flaisch, Stockburger, & Junghöfer, 2006; Weymar, Bradley, Hamm, & Lang, 2014; Weymar & Hamm, 2013). From an evolutionary perspective, it seems reasonable that emotionally significant events or objects, such as danger, diseases, mating partners or food, are highly effective cues to capture ones attention in order to further facilitate adequate reaction. Similarly, a good remembering of an emotionally arousing (stressful, threatening or pleasurable) situation may result in a desired response in similar situations in the future.

Bradley (2009) proposes that emotion, as an organism's disposition to respond effectively to a changing environment by giving priority to one kind of action while interrupting ongoing behavior (Frijda, 1987), is organized around two motivational systems: defensive and appetitive. These systems have evolved to mediate interaction with the environment, which can be, respectively, threatening or promoting survival of the individual and the species. The motivational systems, which are shared by all mammals, rely on phylogentically old limbic structures (LeDoux, 1992a; 2012). In threatening situations, the activation of the defensive system results in escape, withdrawal, or attack. In turn, behaviors like ingestion, copulation, or care-giving indicate an activated appetitive system in circumstances promoting survival. Conceptual, biphasic organization of the motivational systems along two dimensions of emotion, valence and arousal, enables indexing the type of the system and the intensity of this activation, respectively (Bradley, Codispoti, Cuthbert, & Lang, 2001). While viewing emotional pictures, people rate each stimulus along those two dimensions. Stimuli rated as pleasant are associated with the activation of the appetitive system, whereas stimuli rated as unpleasant are associated with the defensive system. When activation of either of the system is low, rated arousal is low and stimuli are rated as "unemotional". The tendency to act and the mobilization of the organism is low as well. With increasing arousal, the

mobilization rises, preparing the organism to act (Lang et al., 1993). This is further associated with rising subjective arousal ratings. Moreover, with rising emotional intensity, the significance of the stimulus increases, resulting in stronger orienting reaction towards the particular stimulus (see Hamm, Schupp, & Weike, 2009 for a review) which is indexed by greater skin conductance changes, heart rate deceleration, potentiated startle reflex and enhanced event-related potentials associated with emotionally significant pictures (e.g., Löw, Lang, Smith, & Bradley, 2008; Schupp et al., 2003).

Various studies have shown the involvement of the amygdala, a structure in the medial temporal lobe, in emotional processing and memory (LaBar & Cabeza, 2006; Murray, 2007; Zald, 2003). Already at the front end of processing (i.e., perceiving) of emotional stimuli, the amygdala shows an enhanced activation (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Zald, 2003). Animal lesion and pharmacological studies (Ferry & McGaugh, 1999; LeDoux, 2000; Málková, Gaffan, & Murray, 1997; Roozendaal & McGaugh, 1997) have proved the role of the amygdala in encoding and retrieval of emotional memory. Moreover, functional neuroimaging (Strange & Dolan, 2004; Strange, Hurlemann, & Dolan, 2003; van Stegeren, Goekoop, Everaerd, Scheltens, Barkhof, Kuijer, & Rombouts, 2005), lesion and pathology (LaBar & Phelps, 1998; Markowitsch, Calabrese, Würker, Durwen, Kessler, Babinsky…, & Gehlen, 1994), and pharmacological studies (cf., van Stegeren, 2008) have supported this assumption in humans.

Key insights in the connections between anatomical structures and functions of emotional memory, especially the role of the amygdala come from relatively sparse studies on patients with selective amygdala damage (as a consequence of bilateral amygdala pathology due to Urbach-Wiethe syndrome), post-surgical studies in temporal lobectomy patients with unilateral damages to MTL[5] (LaBar & Phelps, 1998) and studies on Klüver-Bucy syndrome. The Klüver-Bucy syndrome develops from large lesions in temporal lobes and results in dramatic changes in emotional behavior. First observed in monkeys by Brown and Schafer in 1888 (cited after: LeDoux, 1992b), it turns normal wild and fierce monkeys into tame, indifferent hypoemotional creatures. Additionally, it

[5] However, damages to MTL may also cause a general amnesia (cf., section 2.1) which obviously makes studying of episodic emotional memory functions in many cases impossible (LaBar & Cabeza, 2006).

causes changes in dietary and sexual behavior. The first observations suggested that the amygdala plays a role in assigning motivational and emotional significance to sensory stimuli. The Urbach-Wiethe syndrome is a rare hereditary, congenital condition characterized by deposits of hyaline material mostly in the skin and oral mucosa, and in the MTL. In some cases it targets the amygdala selectively. The rare patients with the Urban-Wiethe syndrome show impairments in long-term emotional memory manifesting in recall and recognition tasks (Markowitsch et al., 1994).

The amygdala modulates the encoding and the storage of hippocampal-dependent episodic memories (Phelps, 2004). FMRI studies continuously demonstrate an enhanced amygdala response to emotional stimuli as compared to neutral ones (see Hamann, 2001 for a review). This response is very fast (LeDoux, 2002), and can occur without awareness (Whalen et al., 1998) and irrespective of attentional focus (Anderson, Christoff, Panitz, De Rosa, & Gabrieli, 2003). The amygdala receives the information about the emotional salience in the early stages of stimulus processing (LeDoux, 1992a) and, due to connections to the visual cortex (Amaral, Behniea, & Kelly, 2003), influences later perception, enhancing perceptual encoding of emotional relevant material (Phelps, O'Connor, Gatenby, Grillon, Gore, & Davis, 2001). As a consequence, several studies reported a correlation between an increased activity of the amygdala at encoding and later memory enhancement for emotional material (Cahill et al., 1996; Canli, Zhao, Brewer, Gabriel, & Cahill, 2000; Hamann, Ely, Grafton, & Kilts, 1999).

Next to encoding, amygdala also has an impact on the eventual memory formation process: consolidation. Animal models suggest that the enhanced amygdala activation for emotional stimuli is followed by an increased activity of hormonal stress systems (Cahill, Haier, Fallon, Alkire, Tang, Keator, Wu, & McGaugh, 1996; McGaugh, McIntyre, & Power, 2002). Stress hormones activate adrenergic receptors in the basolateral amygdala influencing the hippocampal consolidation (McGaugh, 2004). In humans, it has been demonstrated that administration of either agonists or antagonists of (nor-)adrenergic receptors, increases or attenuates the emotional memory modulation (see van Stegeren, 2008 for a review).

It has been suggested that by both effects – the enhancement of encoding and the modulation of consolidation – the amygdala plays a crucial role in prioritized hippocampus-dependent episodic memory storage of emotional events. In this way, it

influences the prioritized retrieval of emotional memories (Phelps, 2004). In fact, Strange and Dolan (2004) demonstrated enhanced amygdala and hippocampus activation during encoding of emotional words as compared to neutral words in an fMRI study. During retrieval, only the hippocampus showed increased activation while retrieving emotionally arousing words.

The enhanced amygdala response to emotionally arousing stimuli involves ß-adrenergic receptors. These receptors are sensitive to noradrenaline (norepinephrine, NA), which plays an important regulatory role in emotional arousal (Aston-Jones, Rajkowski, Kubiak, Valentino, & Shipley, 1996). Most NA-containing cells in the human brain are located in the brainstem's locus coeruleus, LC (Berridge & Waterhouse, 2003). The LC, through its extensive connections to the higher brain structures, has, amongst others, a great impact on the amygdala's functional activity. The LC shows a phasic response to both positive and negative salient stimuli. In the presence of these stimuli, the LC neurons rapidly increase their firing rates for a short period and more NA is released along the noradrenergic pathways. As a result, also β-adrenergic receptors in the amygdala are activated, which in turn temporarily modulates neural activity of the interconnected structures, like for example the hippocampus (de Rover, Brown, Boot, Hajcak, van Noorden, van der Wee, & Nieuwenhuis, 2012).

The arousal effects on the processing of emotional stimuli, as modulated by centrally acting NA, are functionally connected with peripheral arousal and epinephrine, EPI (McIntyre, McGaugh, & Williams, 2011). Although even in highly stressful situations the EPI access into the brain is limited (McIntyre et al., 2011), there are neural mechanisms enabling the peripheral hormone to regulate NA in the central nervous system. EPI's actions stimulate peripheral vagus nerve fibers that innervate adrenal glands and project to the brain (Coupland, Parker, Kesse, & Mohamed, 1989). Ascending vagal fibres in the brain reach the nucleus of the solitary tract in the brain stem (Sumal, Blessing, Joh, Reis, & Pickel, 1983). The following activation influences noradrenergic activity through direct connections between the nucleus of the solitary tract and the LC within the brain stem (Van Bockstaele, Peoples, & Telegan, 1999).

Over years of research, McGaugh and colleagues (McGaugh, McIntyre, & Power, 2002; McGaugh, 2004; McIntyre et al., 2011; Roozendaal & McGaugh, 1997;

McGaugh, Cahill, & Roozendaal, 1996) established the neuromodulation hypothesis (cf., Figure 2).

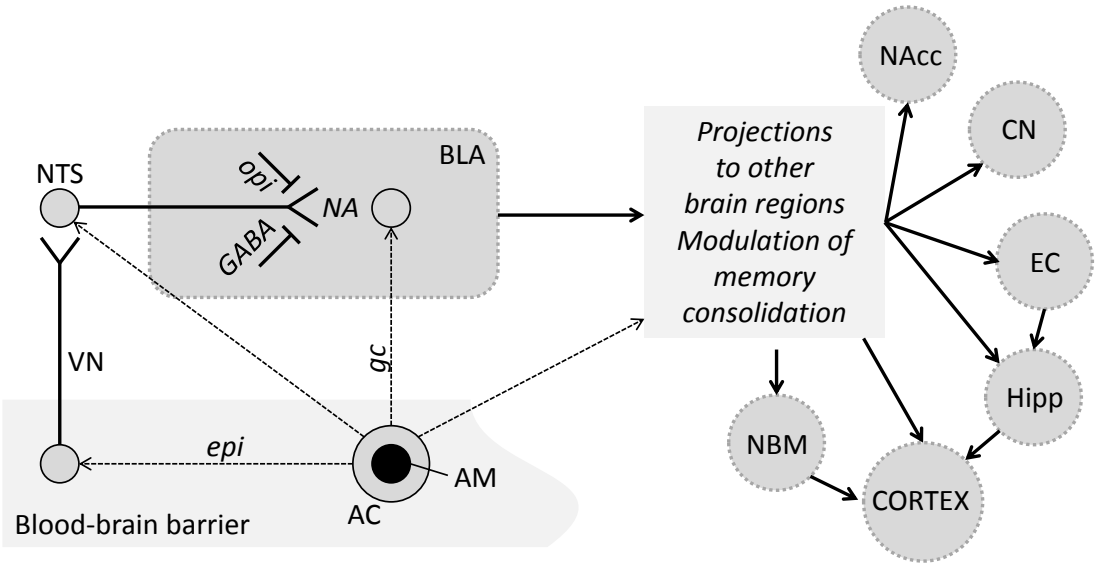

Figure 2. Schematic summary of the core role of the amygdala and the neuromodulatory influences on the memory storage. Emotional arousal releases peripheral epinephrine (from AM) and cortisol (from AC). Epinephrine activates receptors of the VN which through NTS connections activates NA release in the BLA. Cortisol passes easily through the blood-brain barrier, modulating memory formation. Within the BLA, neuromodulatory transmitters (e.g., GABA and opioids) inhibit the release of NA (blocking these neurotransmitters increases the activation of the NA, strengthening its influences on consolidation). The BLA has a variety of connections to other brain regions, influencing on direct and indirect way the memory consolidation. Abbreviations: AC – adrenal cortex, AM – adrenal medulla, BLA – basolateral amygdala, CN – caudate nucleus, CORTEX – other cortical regions, EC – entorhinal cortex, epi – epinephrine, GABA – gamma aminobutyric acid, gc – glucocorticoids, Hipp – hippocampus, NA – noradrenaline, NAcc – Nucleus accumbens, NBM – Nucleus basalis of Meynert, NTS – nucleus of the solitary tract, opi – opioids, VN – vagus nerve. On the basis of McGaugh et al., 1996, 2003, 2004.

It focuses on the core role of the amygdala and stress hormones in emotional processing and emotional memory formation. This view is supported by early research findings, showing that the retention of recently acquired information is altered by post-training treatments. A learning situation activates many hormonal, transmitter and neuromodulatory systems, which in turn can be affected, among others, by drugs, hormones or electrical stimulation administered after training, which in turn may enhance or impair the retention of information (reviewed by McGaugh, Introini-Collison, Cahill, Kim, & Liang, 1992). Up to date, there is a great body of evidence, which originates from animal research, that has been supported by numerous human pharmacological and imaging studies (McGaugh & Roozendaal, 2008; McGaugh, 2004; McIntyre et al., 2011). In short, the reports indicate that the basolateral nucleus of the amygdala (BLA) mediates the modulating effects of adrenal stress hormones (EPI and glucocorticoids) and their

interactions on emotional processing. Moreover, the BLA mediates the consolidation of different forms of emotional memory modulated by influences of various classes of neurotransmitters (noradrenergic, cholinergic, glucocorticoid, GABAergic, opioid peptiergic). The consolidation of long-term emotional memory, among others explicit memory of emotionally arousing experiences, results from influences of the BLA on other brain regions (e.g., the hippocampus) involved in memory formation.

In line with McGaugh's model, it has been shown in humans that mainly the activation of β-adrenoreceptors in the amygdala as a result of emotional arousal, is involved in memory storage of emotionally vivid stimuli (Cahill et al., 1996; Cahill et al., 1994; Debiec & LeDoux, 2006; Maheu, Joober, Beaulieu, & Lupien, 2004; Przybyslawski, Roullet, & Sara, 1999; Strange & Dolan, 2004; Strange et al., 2003; van Stegeren et al., 2005; van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998; Weymar, Löw, Modess, Engel, Gründling, Petersmann, Siegmund, & Hamm, 2010b). The stimulation of the noradrenergic system results in an enhancement of memory for emotional material, whereas a blockade of the adrenergic system causes a reduction thereof (see van Stegeren, 2008 for a review).

In one of the first pharmacological studies in humans, Cahill and colleagues (1994) used slides with either a highly arousing, emotional or a neutral narration. Before watching one version of the narrated slides, participants were administered with either a β-receptor blocker (beta-blocker) or a placebo. The authors found that a relatively small dose of the beta-blocker (40 mg) resulted in a decrement in memory performance for the normally elevated performance for emotional version of the slides. With this study, Cahill et al. demonstrated that the enhancing effect of arousing, emotional material depends on the noradrenergic system. Later, van Stegeren and colleagues (1998) demonstrated that enhanced emotional memory involves the activation of central β-adrenoreceptors, while the peripheral activation of the adrenergic system is not sufficient herein. Moreover, an fMRI study by van Stegeren et al. (2005) supported the hypothesis, that noradrenalin mediates amygdala activity while processing emotional pictures and that this activity can be disrupted by noradrenergic antagonists. In two recent pharmacological studies, Schwabe and colleagues (Schwabe, Nader, & Pruessner, 2013; Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012) proved that that both the feeling of remembering (2013) and memory performance (2012) of emotional material may be attenuated by beta-blocker

application before reactivation of already formed memories, i.e., in later stages of memory formation.

4 ERPs and emotional memory

4.1 EEG and ERP technique[6]

While neural imaging methods such as fMRI or positron emission tomography allow quite exact localization of brain functions and their connectivity, they are not useful tools in examining the time course of cognitive processes. In contrast, event related potentials (ERPs) permit a precise quantification, in the millisecond range resolution, of characteristics of neural activity. They are an accurate measure for describing the temporal dimension of information processing. The ERP components may be identified as a function of experimental manipulations based on their temporal and spatial (scalp) distribution. The ERPs can be computed by means of signal averaging techniques after the EEG had been non-invasively recorded from the scalp.

The ERPs are small voltage changes (1-30 microvolt) embedded in the electroencephalographic brain activity, which are induced in response to a variety to sensory, cognitive and motor processes. They reflect summated postsynaptic potentials from large cell assemblies. The potentials that are measured on the scalp surface most likely originate from cortical pyramidal cells in the cortex, which are aligned perpendicular to the scalp. When the neurons all have a similar orientation, fire synchronously, and all receive the same type of input (excitatory or inhibitory), their dipoles (i.e., a region of positive charge separated from a region of negative charge; extracellular voltage near dendrites, caused by excitation of postsynaptic neurons, is more negative than elsewhere along the nerve cell) will be spatially aligned and will summate, resulting in a measurable potential on the scalp surface. An electric dipole that is present in a conductive medium, as the brain is, generates electric current that is conducted until it reaches the surface (the so called volume conduction), where the voltage can be measured. The voltage, which is always present on the scalp, depends on the position and orientation of the generator dipole as well as on the resistance of elements in the head (skin, skull, eye holes, etc.)[7]. Because electromagnetic waves, per definition, travel at the

[6] The paragraph is based on (Friedman & Johnson, 2000; Jackson & Bolger, 2014; Luck, 2005).

[7] High resistances that are caused by the skin are one of the main sources of noise in EEG recordings. Therefore, it is extremely important to ensure that the impedances between the electrode and the scalp are

speed of light, the measured voltages on the scalp reflect the underlying processes online, i.e. with a negligible delay after the moment they happen. This phenomenon enables depicting the brain processes with high temporal resolution. On the other hand, the problem of poor spatial resolution and the impossibility of precise localization of the origin of a particular ERP[8] is clarified by the forward and inverse problems. The forward problem refers to the possibility of computing the voltage distribution on the scalp, when the location and spatial orientation of dipole generator in the brain is known. In contrary, it is not possible to tell the orientation and localization of a dipole, when only the information about the scalp distribution is provided – a fact known as the inverse problem. This is because there is not just one dipole (or set of dipoles) that can explain the observed voltage distribution.

4.2 Event related potentials as signatures for prioritized processing of emotional contents

Behavioral studies of encoding have shown that the depth of processing correlates with successful retrieval (Craik & Lockhart, 1972). Moreover, deep semantic processing results in better recollection-based recognition as compared to recognition based on familiarity (Tulving, 1985). Similar to deep processing, emotional significance of stimuli facilitates encoding as well, leading to enhanced memory performance when compared with neutral stimuli (Bradley et al., 1992).

Numerous studies demonstrated consistently and reliably that ERP components reflect this differential processing of emotional compared to neutral pictures. Schupp and colleagues (Schupp et al., 2003) have shown that already early encoding stages, as marked by the early posterior negativity (EPN, starting about 150 ms after stimulus onset over occipital sensor sites), can be influenced by emotion, reflecting the selective processing of emotional material as well as perceptual categorization up to the level of semantic meaning. In this study, the EPN was most pronounced for highly emotional stimuli with evolutionary significance (erotic scenes, mutilations, threat). Moreover, the

kept at a minimum. For mechanisms explaining the high-impedance dependent noise, please refer to Luck (2005, pg.116 ff.).

[8] Of course, there are some methods which enable estimating the source of ERPs, like model fitting techniques or probabilistic approaches (cf. Luck, 2005). For a review see Grech et al. (Grech et al., 2008).

prioritized encoding of emotional material was preserved, even if the attention was directed towards neutral material.

On later encoding stages, positive-going potentials emerge over centro-parietal scalp regions starting about 300-400 ms, peaking about 500-700 ms after stimulus onset and lasting over several seconds, even if the stimulus is no longer present (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000). These late positive potentials (LPPs) are associated with processing of the higher order perceptual information and encoding processes (Kok, 1997). The LPPs are modulated by stimulus significance (Bradley, 2009; Cacioppo, Crites, Gardner, & Berntson, 1994) and, consequently, by emotional arousal. The modulatory effect of emotion on the LPP manifests in enhanced waveform positivities for emotional stimuli (pleasant and unpleasant) as compared to neutral stimuli (de Rover et al., 2012; Keil et al., 2002; Weymar et al., 2010b). The highest emotional minus neutral differences are observable for emotionally high arousing, motivationally significant, like mutilation or erotic stimuli (Schupp, Stockburger, Codispoti, Junghöfer, Weike, & Hamm et al., 2007; Schupp, Junghöfer, Weike, & Hamm, 2004). Moreover, the amplitude of the LPP usually increases monotonically with participants' ratings of subjective emotional arousal, regardless of whether the stimuli were experienced as pleasant or unpleasant (Cuthbert et al., 2000; Schupp et al., 2000). The emotional modulation of the LPP is quite reliable (cf. Bradley, 2009), as demonstrated by Codispoti and colleagues (Codispoti, Ferrari, & Bradley, 2007). In this study, although the amplitude of the LPPs diminished somewhat with repetition of the stimuli, enhanced LPPs for emotional, significant (as compared to neutral) pictures persisted even after 90 presentations of the same stimuli in one session, leaving the difference between emotional and neutral LPPs unchanged. In addition, even brief (25 ms) presentations of visual stimuli result in LPP differences between emotional and neutral contents (Codispoti, Mazzetti, & Bradley, 2009), suggesting enhanced emotional engagement in stimulus processing. On the basis of available findings, it has been proposed that enhanced LPPs for emotional stimuli reflect increased allocation of attentional resources to motivationally relevant stimuli (Leite et al., 2012) as well as sustained processing enabling thorough encoding of emotional material (Foti, Hajcak, & Dien, 2009).

Widespread brain activations, including occipital, parietal, inferotemporal cortices and the amygdala, are observed while viewing emotional pictures (cf., Liu et al., 2012). It is also well established that amygdala plays an important role in encoding emotional stimuli (Hamman, 1999). When comparing ERPs and fMRI results in separate sessions, several studies showed correlations between LPPs and BOLD (blood oxygen-level-dependent) activity in the lateral occipital, parietal, and inferotemporal cortices (Sabatinelli, Lang, Keil, & Bradley, 2007). In a recent study, Liu et al. (2012) demonstrated in direct comparisons, when fMRI and EEG were simultaneously recorded, that the LPPs are generated and modulated by broad cortical and subcortical networks associated with visual and emotional processing, including the visual cortices, the temporal orbitofrontal and insular cortices, and the amygdala. The heightened amygdala activity may be an important factor in mediating the emotional enhancement of the LPPs, probably by modulating the cortex activity (de Rover at al., 2012).

During retrieval, the ERP old/new effects have gained great significance in emotional memory research. Basically, they consist of two potentials and are the difference between the ERP curve for correctly recognized old stimuli (i.e., hits) and correctly categorized new stimuli (i.e. correct rejections) in recognition memory tasks. The ERP old/new effects are associated with successful recognition processes and reflect the memory advantage of remembered stimuli compared to the new, unknown ones. The logic behind this comparison, and not simply comparing ERP curves for old (all studied) and all new stimuli, is that, for new items, any interaction of a retrieval cue with the memory trace can be excluded (Mecklinger, 2006). The effects have been observed for various types of stimuli including words, objects, people, faces, abstract visual patterns etc. (Curran & Cleary, 2003; Groh-Bordin, Zimmer, & Mecklinger, 2005; Herzmann & Curran, 2011, 2013; Nessler, Mecklinger, & Penney, 2001; Rugg, Mark,Walla, Schloerscheidt, Birch, & Allan, 1998; Ventura-Bort, Löw, Moltó, Poy, Hamm, & Weymar, in preparation; Windmann & Kutas, 2001).

The old-new differences are present when the recognition test is conducted directly after encoding (Langeslag & Van Strien, 2008; MacKenzie & Donaldson, 2007; Wilding & Rugg, 1996) as well as after longer retention intervals of 1 to 24 hours or 1 week (Curran, DeBuse, Woroch, & Hirshman, 2006; Curran & Friedman, 2004; Weymar et al., 2009). The old/new effects after longer retention intervals are regarded as

a neural correlate for retrieval of information from a long-term memory (Rugg & Curran, 2007)

When considering familiarity and recollection processes in long-term recognition memory, two functionally and spatiotemporally distinguishable ERP old/new effects arise during memory testing (Curran, 2000). The early old/new effect emerges at about 300 ms after stimulus onset, lasts over the later temporal development of the potentials, and is most prominent over central frontal electrode sites. The mid-frontal memory component varies with the familiarity strength (Woodruff, Hayama, & Rugg, 2006), is a marker of stimuli encoded shallowly (Rugg et al., 1998) and is associated with "know" answers (i.e., recognition without contextual retrieval; Düzel, Yonelinas, Mangun, Heinze, & Tulving, 1997), low confidence responses (Weymar et al., 2009) and false alarms (Wolk et al., 2006) in memory recognition tests. Therefore, it is thought to reflect (implicit) memory recognition processes based on familiarity [9] arising from global similarity between study and test items (Mecklinger, 2000; Nessler et al., 2001). Single-neuron recordings in monkeys and fMRI findings in humans suggest familiarity-sensitive neuron assemblies in perirhinal regions of the MTL (Henson, Cansino, Herron, Robb, & Rugg, 2003; Xiang & Brown, 1998), but these processes start at earlier (~90 ms) processing stages than ERP familiarity signatures. At greater latencies (~250 ms), neuronal responses that better fit the time-course of the frontal old/new effect have also been found in monkeys in various prefrontal areas (Xiang & Brown, 2004). In addition, Yonelinas and colleagues (Yonelinas, Otten, Shaw, & Rugg, 2005) have demonstrated in an fMRI study that lateral prefrontal activity was modulated by the strength of familiarity in humans. Taken together, the neural origins of the mid-frontal effects seem to be in one or more regions of the prefrontal cortex (Rugg & Curran, 2007).

The later old/new effect which emerges centro-parietally, starts about 400-500 ms after stimulus onset and reaches highest amplitudes about 500-700 ms. It is considered to be a neural correlate of explicit recollective processes, as it is associated with high confidence ratings (Weymar et al., 2009), "remember" answers (Weymar, et al., 2010a), and correct source judgments (Wilding & Rugg, 1996). In addition, it occurs only for stimuli subjected to deep study (Rugg et al., 1998). The spatial distribution of the parietal

[9] For an alternative interpretation see Voss & Paller (2008).

old/new effect suggests lateral parietal lobes as its neural origin. In fact, fMRI studies have demonstrated recollection-sensitive activations in these regions. Additionally, direct functional parallels between fMRI and ERP results, as well as source analyses provide further support for these findings (Herron, Henson, & Rugg, 2004; Vilberg & Rugg, 2009; Wagner, Shannon, Kahn, & Buckner, 2005; Woodruff et al., 2006; Weymar et al., 2010b; Yonelinas et al., 2005).

When considering the early old/new effect, it has been shown that ERPs reflecting familiarity processes are relatively independent of the emotional significance of stimuli (Maratos, Allan, & Rugg, 2000; Weymar et al., 2009). In contrast, the centro-parietal ERP old/new effect is modulated by emotional content. Old emotional stimuli, both pleasant and unpleasant, are associated with a more positive-going waveform than the waveforms associated with old neutral stimuli, resulting with greater old-new differences for emotional than neutral stimuli (Schaefer et al., 2011; Weymar et al., 2009). The old/new effect is usually enhanced as a function of emotional arousal, with greater effects for highly arousing stimuli (Weymar et al., 2011), but there is also evidence that the greatest old-new differences are present for emotional stimuli rated as moderately arousing (Schaefer et al., 2009). In addition, the emotional modulation effect in memory related ERPs does not only rely on the emotional content of the stimuli, but also on the emotional status of the subjects and contextual information in which the stimuli are presented. Wirkner et al. (2013) have shown that pre-encoding stress enhances the already elevated old/new effect for unpleasant material. The emotional modulation is best observable when memory testing takes place after longer retention intervals, probably reflecting the emotional memory enhancing effect of consolidation (cf., section 3.1). The emotional enhancement of the ERP old/new effect is known to be long-lasting, and such effects were even observed after 6 months delay (Weymar et al., 2011).

As mentioned in section 3.1, the emotional memory enhancement, as also apparent in ERPs, depends at least in part on the heightened amygdala activation as well as stress hormones and neurotransmitter (noradrenergic) activity. Therefore, it is possible to disrupt the normal enhancement of memory (and associated amygdala activation) by a blockade of the noradrenergic system (for a review see Chamberlain, Müller, Blackwell, Robbins, & Sahakian, 2006). In ERPs, it has also been shown, that a pharmacological

blockade of the noradrenergic system by propranolol diminishes the enhanced ERP old-new difference for highly emotional stimuli (Weymar et al., 2010b).

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Part II – Research reports

In the following, own research on the modulation of emotional episodic long-term memory is presented. The first study investigates spontaneous remembering of emotional events. In the second experiment, a crossover design for memory recognition studies is tested. The third study, with its preliminary results, gives insights into the noradrenergic influences on emotional memory.

In reporting the results of the own research I use the personal pronouns in the first person plural to stress the fact that all studies (design, conducting of the experiments, results and interpretation of them) have been discussed in our working group. However, in every case I was the main investigator and in case of study 1 the main author of the manuscript as well.

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5 Study 1: Spontaneous remembering of emotionally relevant stimuli

This study has already been published in Cognitive Neuroscience (Jaworek, Weymar, Löw, & Hamm, 2014). For the purpose of this thesis, some parts have been changed.

5.1 Background information and research objectives

Two recent ERP studies investigated explicit and implicit retrieval of emotional and neutral scenes using immediate memory tests (Ferrari, Bradley, Codispoti, Karlsson, & Lang, 2012; Weymar et al., 2013). In both studies, enhanced ERP old-new differences were found for emotional and neutral pictures over centro-parietal electrodes during explicit retrieval. However, when the post-encoding task involved an implicit retrieval condition, such as free viewing (Ferrari et al., 2012; Weymar et al., 2013) or an active decision task (one vs. more people-categorization; Weymar et al., 2013), only previously presented emotional pictures were associated with greater ERP positivity than new pictures, again over central and parietal electrodes. Hence, both studies suggest that for immediate testing, emotional pictures seem to spontaneously trigger recollection even when the task does not explicitly probe episodic memory. The question arises, however, whether spontaneous remembering also persists after longer retention intervals or only occurs during immediate testing. Spontaneous remembering of past events may also play a maladaptive role in trauma and stressor related disorders, in which environmental cues prompt intrusive distressing memories, dreams and flashbacks (Ehlers & Clark, 2000). Knowing the neural correlates of involuntary retrieval in healthy populations might also help in understanding these intrusion symptoms.

In the present study, we tested spontaneous retrieval of emotional and neutral pictures following a longer retention interval (one week). Following incidental encoding of emotional and neutral scenes, one group performed a recognition task, in which participants decided whether a picture has been previously seen (old) or not (new); a second group performed a categorization task (cf., Weymar et al., 2013) in which

participants decided whether a picture contained one or more people. In accordance with earlier studies using longer retention intervals (Weymar et al., 2011, 2009; Wirkner et al., 2013) we expected to find greater centro-parietal ERP old-new differences for emotional pictures, compared to neutral pictures, during explicit recognition. For implicit recognition (categorization task), one hypothesis was that, following a longer retention interval, the active categorization decision might interfere with implicit retrieval, thus reducing the old-new ERP differences. However, because emotional pictures are generally better remembered than neutral pictures in delayed tests (Bradley et al., 1992; Dolcos et al., 2005; Weymar et al., 2011), previously presented emotional pictures may spontaneously trigger episodic memory retrieval even in a delayed test, reflected in enhanced ERP old/new effects for emotional scenes.

5.2 Method

5.2.1 Participants

Participants were forty healthy (20 female and 20 male) students from University of Greifswald. They had normal or corrected-to-normal vision. Two subjects were rejected from further analyses due to memory performance under chance-level or poor EEG data quality. The remaining sample consisted of thirty-eight subjects (18 male, mean age: 24.2, one left-handed). The participants provided written informed consent for the study approved by the Review Board of the University of Greifswald and received financial compensation or course credits for the participation in the study.

5.2.2 Stimulus material

Overall, 168 photographs[10] were selected from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008) and the Emotional Picture Set (EmoPicS; Wessa, M., Kanske, P., Neumeister, P., Bode, K., Heissler, J., Schönfelder, 2010), consisting of 56 unpleasant (mutilation, death, diseases, attack scenes), 56 pleasant (erotic couples, erotic and attractive males and females, romance, adventure, sports), and

[10] For numbers of pictures from the picture sets see Appendix I.

56 neutral pictures (neutral people). All pictures contained people (half of the pictures depicted one person, the other half more than one person). Two sets of 84 pictures were carefully matched based on hedonic valence and arousal, and also according to their semantic content and number of people in the picture (single vs. more).

Individual hedonic valence and arousal ratings of all pictures in our sample were obtained to check for correspondence with the normative ratings of the IAPS and EmoPicS. Mean rating values are summarized in Table 1. As expected, unpleasant pictures were rated as more unpleasant, than neutral, $F(1,37) = 423.51$, $p < .001$, and pleasant, $F(1,37) = 619.18$, $p < .001$, pictures, and pleasant as more pleasant than neutral pictures, $F(1,37) = 34.84$, $p < .001$. Emotional pictures were rated as more arousing than neutral pictures, unpleasant vs. neutral: $F(1,37) = 300.17$, $p < .001$; pleasant vs. neutral: $F(1,37) = 181.66$, $p < .001$; unpleasant vs. pleasant: $F(1,37) = 65.30$, $p < .001$. There were no differences in SAM-ratings between both experimental groups.

Table 1. Individual valence and arousal ratings.

Emotional content	Valence	Arousal
Unpleasant	2.19 (.60)	6.34 (1.52)
Pleasnt	6.47 (.67)	4.85 (1.53)
Neutral	5.78 (.76)	2.29 (.86)

Note: Standard deviations are given in parentheses.

5.2.3 Procedure

The experimental procedure is schematically depicted in Figure 3.

The experiment was divided in two parts: An encoding task in the first session was followed by either a direct recognition or a categorization task (indirect recognition) in the second session one week later (parallel groups). The assignment of the participants to the tasks in the second session was counterbalanced. The participants were seated in a reclining chair in a sound-attenuated, dimly lit room. During both sessions, while the EEG was recorded, participants were to avoid eye blinks and body movements during ERP measurement.

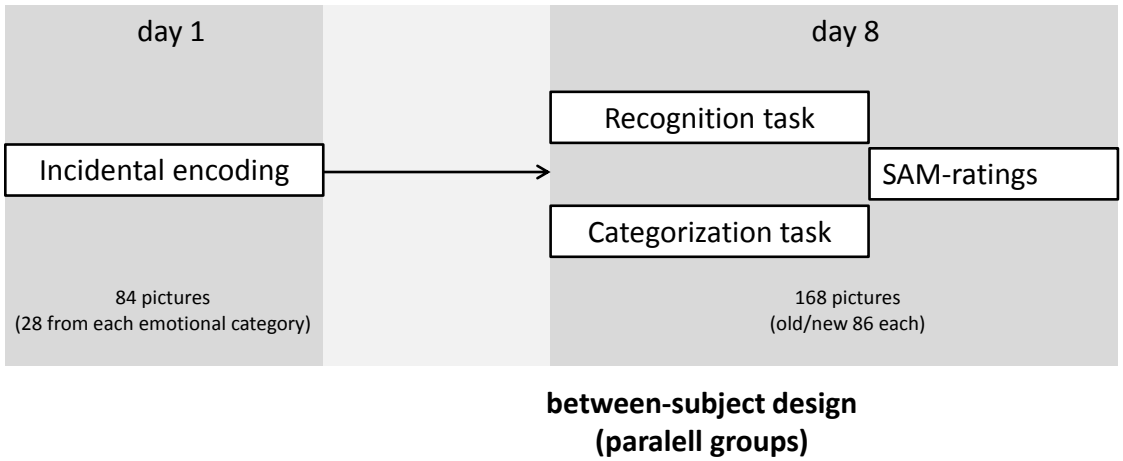

Figure 3. Scheme of the experimental procedure introduced in the study. Description in text. The recognition and categorization tasks were conducted in parallel groups. Abbreviations: SAM – Self-Assessment Manikin.

In the first session all participants viewed 84 emotionally arousing and neutral pictures presented for 3000 ms, with an intertrial interval (ITI) of 2000, 2500, or 3000 ms on a 21 in computer screen. A fixation cross was presented for 500 ms before every picture with no ITI 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. The participants were asked to watch the pictures attentively, no mention of memory test was made (incidental encoding).

In the second session the same sets of stimuli were used for both tasks, containing 84 old pictures intermixed with an equal number of new photographs. The stimuli were displayed for 3000 ms each with a preceding 500 ms fixation cross. The order of stimuli was pseudorandomized as well, with the restrictions that a picture from one valence category occurs at last twice in a row and, independently of the valence, maximal two old or new pictures may occur one after another. One group (8 male, 11 female; mean age: 24.0 years; one left-handed) of participants was instructed to decide if each picture has been presented earlier in the study, and, at picture offset, to press either a “yes” or “no” button. A second group (11 male, 8 female; mean age: 24.5 years; all right-handed) was instructed to decide if the picture depicted one person or more than one person, and, at picture offset, to press a button marked either "one" or "more". The assignment of left and right button presses to both “yes”/”no” and “one”/”more” responses was counterbalanced

across participants. After recognition or categorization, participants were asked to rate all previously seen pictures for their subjective hedonic valence and arousal using the SAM rating procedure (Bradley & Lang, 1994).

5.2.4 EEG-recording

The EEG signal was 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 band-pass filtered on-line from 0.1 to 100 Hz. Offline analyses were performed using ElectroMagnetoEncephalography Software (EMEGS; Peyk, De Cesarei & Junghöfer, 2011), including low-pass 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). Extracted epochs were corrected for eye movement and blink artifacts using the MATLAB-based toolbox BioSig (Vidaurre, Sander, & Schlögl, 2011).

5.2.5 Data analysis

ERPs were computed for each sensor and participant. Based on both visual inspection of the waveforms and previous findings (e.g., Rugg & Curran, 2007; Weymar et al., 2009), mean ERP amplitudes were analyzed in a 300-500 ms and 500-800 ms window over frontal and centro-parietal brain sensors (cf., Figures 3 or 4, middle insets), where the overall difference between old and new conditions was expected to be maximal. ERP data were analyzed in a mixed analysis of variance (ANOVA) involving Task (recognition vs. categorization) as a between subject factor and Hedonic Content (unpleasant vs. neutral vs. pleasant), and Memory (old vs. new) as repeated measures.

Hit rate (H), false alarm rate (FA) and recognition accuracy (Pr = H – FA) were analyzed in the recognition task. For ERPs in the explicit memory group, only trials with correct behavioral responses (hits and correct rejections) were used in the averaged ERP

data[11]. For effects involving repeated measures, the Greenhouse-Geisser procedure was used to correct violations of sphericity, where relevant.

Spontaneous remembering of emotional pictures was recently associated with a centro-parietal ERP old-new difference when old and new pictures where presented immediately after encoding in a context where the task did not explicitly probed memory. Based on these ERP findings and evidence from autobiographical memory studies (Berntsen, 2010), suggesting that involuntary retrieval of personal long-term memories are a common retrieval mechanism in everyday life we expected to find spontaneous retrieval (as indexed by ERP old-new differences) on a delayed test. Specifically, we hypothesized that if implicit memory for emotional stimuli was based on familiarity, it might result in an old/new effect for emotional pictures on frontal electrodes. In contrast, differences found over parietal brain regions in the categorization task would indicate recollection processes mediating the spontaneous retrieval of emotional events. We accepted an alpha level at 0.1 as sufficient for triple interactions (Hedonic Content × Memory × Task) to conduct follow-up analyses separately for each group.

5.3 Results

5.3.1 Memory performance

Replicating previous findings, correct recognition of emotional pictures was better than for neutral pictures, Hit rate (H): $F(2,36) = 4.50$, $p < .05$. False alarms (FA) were low but slightly higher for emotional than neutral pictures [$F(2,36) = 18.27$, $p < .001$]. Correct discrimination (Pr = H – FAs) did not differ for emotional and neutral pictures. Memory performance values are summarized in Table 2. The accuracy in the categorization task was near to perfect (98,7%), suggesting that the participants correctly performed the

[11] Because ERP analysis in the explicit recognition group included trials associated with correct behavioral performance (e.g., hits and correct rejections), we also analyzed ERPs for this group using old and new trials (irrespective of the performance) to ensure that differences were not because of the averaging. The results were identical to the differences found using correct trials only. Again, the Memory × Task interaction was significant, $F(1,36) = 4.89$, $p < .05$, in the late time window (500-800 ms) over centro-parietal electrodes, the Hedonic Content × Memory × Task interaction was not significant. However, planned comparisons showed that only emotional pictures, $t(18) = 1.58$, $p = .06$, showed enhanced (trend towards significance) ERP old-new differences during recognition, but not neutral pictures, $t(18) = -0.8$, $p = .47$, both one-tailed.

task[12].

Table 2. Memory performance data.

Picture type	H	FA	Pr
Emotional	.80 (.09)	.12 (.08)	.68 (.11)
unpleasant	.82 (.11)	.15 (.09)	.67 (.14)
pleasant	.78 (.12)	.09 (.06)	.69 (.13)
Neutral	.73 (.13)	.06 (.08)	.66 (.13)

Note: Depicted are mean hit rate (H), false alarm rate (FA), and discrimination index (Pr) for each picture type. Standard deviations are given in parentheses.

5.3.2 Event-related potentials

Figure 4 and 5 illustrate the grand averages of the ERP waveforms for old and new pictures during recognition and categorization, respectively. Figure 6 depicts scalp voltage distributions for old minus new waveforms for both time-windows over all experimental conditions.

Early old/new effect (300-500 ms). Analysis of the frontal electrode cluster revealed that old pictures prompted a larger ERP positivity than new pictures, $F(1,36) = 5.36$, $p < .05$. This ERP old-new difference was not significantly modulated by emotional content, Hedonic Content × Memory: $F(2,72) = 3.13$, $p = .06$. The early frontal old-new difference was similar during recognition and categorization, Memory × Task, $F(1,36) = 1.78$, $p = .19$, Hedonic Content × Memory × Task: $F(2,72) < 1$. No significant memory effects or interactions were observed for the centro-parietal cluster.

Late old/new effect (500-800 ms). For the later time window, the ERP old-new difference remained significant at frontal electrodes, Memory: $F(1,36) = 15.02$, $p < .001$. The interaction between hedonic content, memory and task was significant (at an alpha level of 0.1), $F(2,72) = 2.42$, $p = .09$. Separate analysis (cf., section 5.2.5) for each group confirmed that during explicit recognition, both neutral and emotional pictures elicited pronounced old-new differences, Memory: $F(1,18) = 12.49$, $p < .01$; Hedonic Content × Memory: $F(2,36) < 1$. On the other hand, only emotional pictures showed an ERP old-new difference during categorization,

[12] Recognition and categorization button responses were delayed until the offset of the 3 s picture presentation in order to avoid contamination by motor potentials. Reaction times are therefore not informative and were not analyzed.

Hedonic Content × Memory: $F(2,36) = 7.10$, $p<.01$.

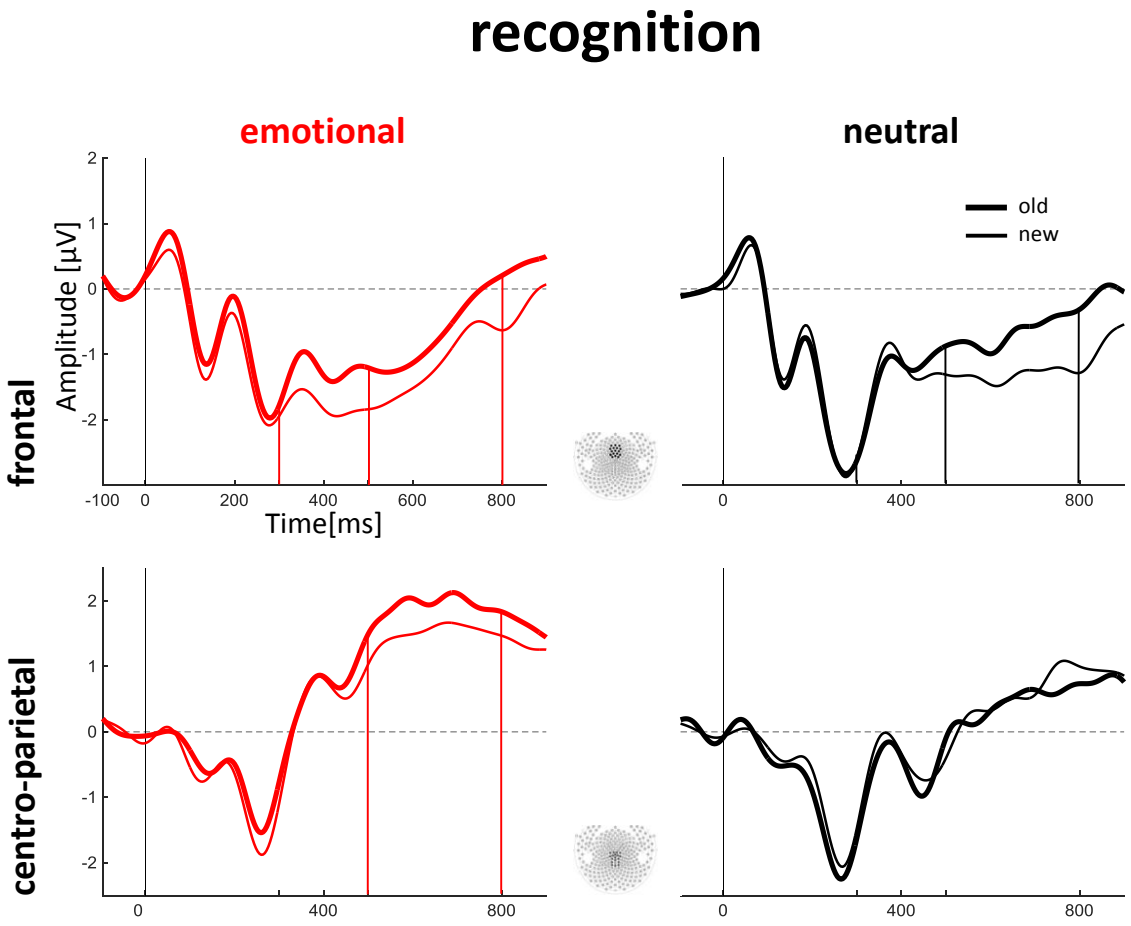

Figure 4. Grand average ERP waveforms for correctly recognized old (thick line) and correctly rejected new (thin line) pictures at representative sensor groups (averaged within both clusters, depicted in the middle insets) over frontal (upper panel) and centro-parietal brain regions during explicit recognition. Emotional pictures are depicted in red, neutral in black. The early and late frontal old/new effects for both picture categories as well as the late centro-parietal old/new effect are all indicated by vertical lines within the respective time-windows.

Over centro-parietal sites, larger ERP positivity was observed for remembered old pictures, relative to new, during explicit recognition, but not during categorization, Memory × Task: $F(1,36) = 5.87$, $p < .05$. Overall, this late parietal old/new effect was modulated by emotional content, Hedonic Content × Memory $F(2,72) = 3.52$, $p < .05$, replicating previous studies (e.g., Weymar & Hamm, 2013). Emotional stimuli elicited larger old-new differences than neutral stimuli [old minus new emotional vs. neutral: $F(1,36) = 5.48$, $p < .05$, pleasant vs. unpleasant: $F(1,36) < 1$].

The Hedonic Content × Memory × Task interaction was not significant, $F(2,72) < 1$[13].

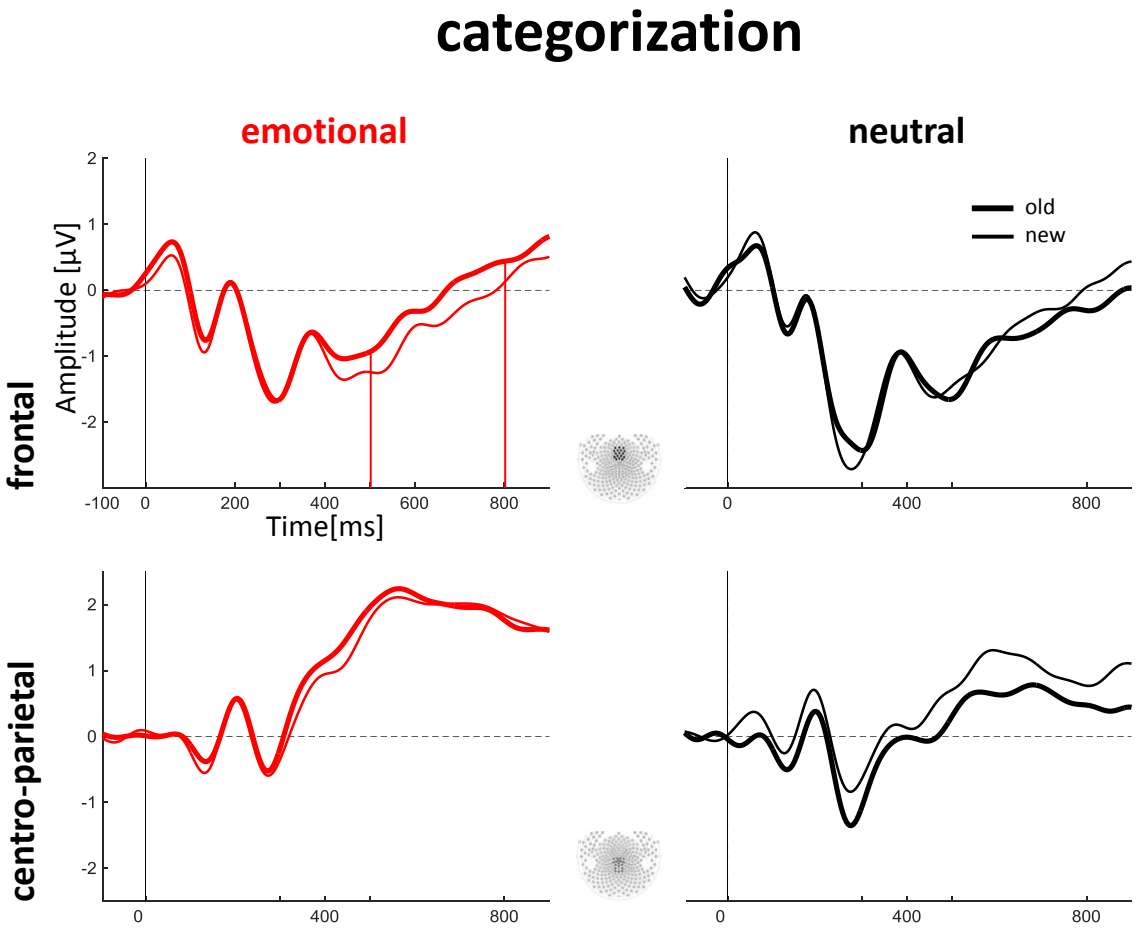

Figure 5. Grand average ERP waveforms for old (thick line) and new (thin line) pictures at representative sensor groups (averaged within both clusters, depicted in the middle insets) over frontal (upper panel) and centro-parietal brain regions during categorization. Emotional pictures are depicted in red, neutral in black. The late frontal old/new effect for emotional stimuli is indicated by vertical lines within the 500-800 ms time-window.

[13] Although the three-way interaction was not significant, brain activity between the tasks differed in a considerable way: a typical old/new difference ("old" more positive-going than "new") for emotional stimuli in the recognition task, and an inverted old/new effect ("new" more positive going than "old") for neutral stimuli in the categorization task. Because the difference of the differences *("emotional old" minus "emotional new") minus ("neutral old" minus "neutral new")* was the same in both tasks (i.e. emotional pictures elicited numerically greater old/new effects in both tasks), the computed interaction was not informative. Pairwise comparisons for each group revealed larger ERP positivity for old emotional than new pictures [$t(18) = 2.15$, $p < .05$, one-tailed; pleasant vs. unpleasant: $t(18) = 0.87$, $p = .20$, two-tailed], but not neutral [$t(18) = -0.41$, $p = .35$, one-tailed] pictures during explicit remembering. During categorization, however, only neutral pictures showed ERP old/new differences, *but in opposite direction* (greater positivity for new relative to old; neutral: $t(18) = -3.00$, $p < .05$, one-tailed; emotional: $t(18) = 0.21$, $p = .42$, one-tailed; pleasant vs. unpleasant: $t(18) = .44$, $p = .66$, two-tailed).

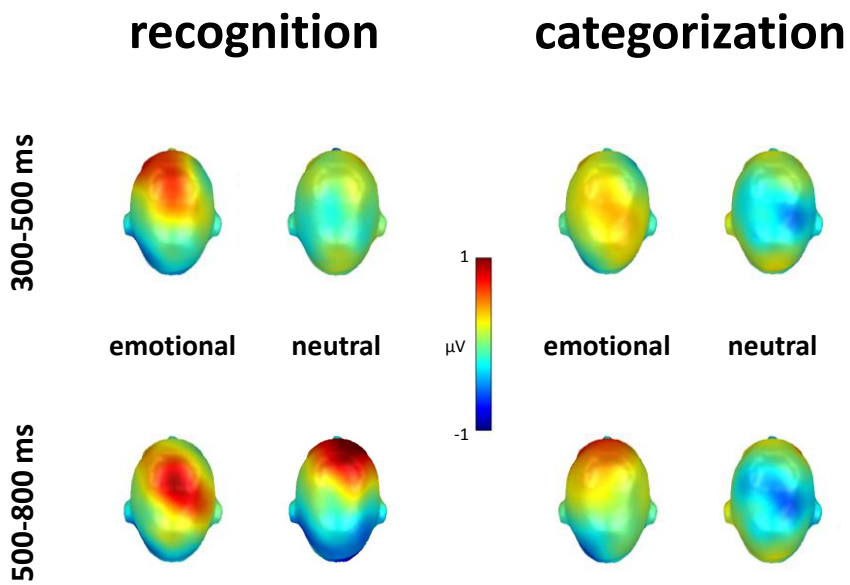

Figure 6. Mean scalp voltage differences between correctly recognized and correctly rejected stimuli (left panel), and old and new stimuli (right panel) in two analyzed time windows for both picture categories

5.4 Discussion

The goal of the present ERP study was to examine explicit and spontaneous retrieval of emotional and neutral stimuli following a delayed retention interval. We found that explicit recognition of both emotional and neutral scenes were associated with an early starting ERP old-new difference over frontal electrodes. Furthermore, retrieval of emotional scenes was associated with a late ERP old-new difference over centro-parietal electrodes, particularly during the explicit recognition task. In contrast, during categorization, enhanced old-new differences were only observed for emotional scenes over frontal brain regions. The current data may indicate that emotionally evocative cues spontaneously trigger familiarity-based episodic retrieval on a delayed implicit retrieval test, when no memory search is required.

As in previous studies using explicit memory tasks emotionally arousing stimuli were better remembered than low arousing neutral stimuli (LaBar & Cabeza, 2006; Weymar & Hamm, 2013), as indicated by the hit rate. However, false alarm rates were higher for emotional, compared to neutral, scenes leading to comparable discrimination (Pr) between old and new pictures for emotional and neutral pictures. Previous studies

reported that remembering emotional events is sometimes associated with a more liberal response bias (e.g., Dougal & Rotello, 2007) and enhanced recollective experience (e.g., Phelps & Sharot, 2008; Weymar et al., 2010a), and not necessarily associated with better memory accuracy for emotional material. It is discussed that this phenomenon serves an evolutionary function (Phelps & Sharot, 2008).

In ERPs, enhanced old-new differences were observed for emotional and neutral pictures starting around 300 ms over frontal regions (cf., Weymar et al., 2009). In addition, a late centro-parietal old/new effect was found for emotional, relative to neutral pictures, in the time window between 500 and 800 ms, replicating previous studies (cf., Langeslag & Van Strien, 2008; Newsome, Dulas, & Duarte, 2012; Schaefer et al., 2011; Weymar et al., 2011; Weymar et al., 2010a, 2010b; Wirkner et al., 2013). Because the frontal old/new effect is often interpreted as an electrophysiological signature of memory based on familiarity (e.g., Rugg & Curran, 2007), and the late parietal old/new effect point to successful episodic recollection (e.g., Voss & Paller, 2008) in line with dual-process models of memory (Yonelinas, 2001), the present data suggest, that correct picture recognition after a delay is mediated by both familiarity and – especially in case of emotional stimuli – recollection in the context of an explicit recognition memory task (cf., Ochsner, 2000).

During semantic categorization task, where episodic memory was not directly probed, the ERP old/new effect was also reliable for emotional pictures (similar as in Ferrari et al., 2012; Weymar et al., 2013), but, in this experiment, most prominent over frontal electrodes. Old-new differences over frontal regions (cf., FN400) have been related to familiarity-based recognition, which might be mediated by perceptual (Curran & Doyle, 2011) or conceptual fluency (Voss, Schendan, & Paller, 2010). Irrespective of the specific mechanism mediating the frontal old/new effect, the present data might indicate that spontaneous remembering of emotional scenes after delays are more driven by familiarity than recollection (Rugg & Curran, 2007). On the other hand, following a delay, an active semantic categorization decision during viewing old and new stimuli might overshadow with implicit retrieval, thus reducing the centro-parietal characteristic of the old-new difference. Because long-term memory studies with explicit recognition tasks suggested that enhanced emotional memory is driven by recollection (e.g., Dolcos et al., 2005; Sterpenich et al., 2009; Weymar & Hamm, 2013;

Weymar et al., 2011) and associated with a centro-parietal old-new difference in the ERP, it is possible that spontaneous remembering of emotional events also involves recollection-based memory processes. This aspect, however, needs further investigation.

Involuntary or spontaneous memories are assumed to occur about as frequent in daily life as intended or voluntary memories (Berntsen, 2010). According to Hintzman (2011), "reminding" (e.g., spontaneous retrieval) is a common phenomenon in which cues spontaneously activate memory representations associated with them in a bottom-up fashion. This finds its function in providing information about environment and regulating behavior according to its requirements and promoting survival. Such common functional "reminding" can become maladaptive and clinically relevant, by turning into distressing intrusive memories of an experienced trauma, as it is in post-traumatic stress disorder (PTSD; Ehlers & Clark, 2000). In PTSD, one of three clusters of symptoms involves a specific disturbance of long-term episodic memory processes, which manifests in re-experiencing of the trauma event. This includes, among others, intrusive thoughts and memories, nightmares, flashbacks – phenomena that are often cued by reminders of the traumatic event (Parsons & Ressler, 2013). As reported recently, the proportion of concern-related memories recalled in both voluntary and involuntary way correlates with the severity of PTSD (and depression) symptoms (Johannessen & Berntsen, 2010). Knowing the neural correlates of involuntary retrieval in healthy populations might help to translate basic findings to clinical populations to understanding involuntary retrieval. Our ERP data suggest that spontaneous remembering of emotional events following longer delays might be driven by familiarity rather than recollection. This mechanism might be mediated by elaborative processing (Bradley, 2009; Weymar et al., 2013) at the time of the event, degree of rehearsal (Ferree & Cahill, 2009) or by increased generalization as would be suggested by recent data from a study with anxiety disorder patients (Lissek, Rabin, Heller, Lukenbaugh, Geraci, Pine, & Grillon, 2010).

6 Study 2: Reliability of a crossover design in emotional memory recognition study. Testing for pharmacological studies

6.1 Research objectives

Several studies have yet been conducted exploring the influences of the adrenergic transmitter system on emotional memory consolidation (e.g., Cahill et al., 1994; Maheu et al., 2004; O'Carroll, Drysdale, Cahill, Shajahan, & Ebmeier, 1999; Strange & Dolan, 2004; van Stegeren et al., 2005; Weymar et al., 2010b) and reconsolidation (Kindt, Soeter, & Vervliet, 2009; Kroes, Strange, & Dolan, 2010; Schwabe et al., 2012; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009a, 2009b; see also Chamberlain et al., 2006; Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013 for reviews).

Pharmacological studies of emotional episodic memory are usually conducted in a parallel group design (for exceptions see Moor, Mundorff, Bohringer, Philippsen, Langewitz, Reino, & Schachinger, 2005; van Stegeren et al., 2005), i.e., where two groups of participants are formed: one group receives the study medication and the other one a placebo. The effects of the medications are then compared between groups (Fitzpatrick, 2006). The advantage of this design is its simplicity: one has to randomly assign the participants to the groups. Furthermore, possible dropouts are just replaced in one group. Nevertheless, two main disadvantages characterize the parallel design: the inter-subject variability between two groups is relatively high and, in order to achieve desired power, one needs to conduct studies with a relatively large number of subjects. In contrary, the crossover design yields a more efficient comparison of treatments. It has the advantage of a relatively low inter-subject variability and, consequently, an improved power. In this case, every participant is his own matched control and the number of subjects needed to achieve the sufficient power is lower than in a parallel group design (Chow & Liu, 2008). There are also disadvantages: once a participant drops out, he simultaneously drops out from two treatment groups (placebo and the target medication). Another possible consequence of the crossover design is the carry over effect (Machin & Fayers, 2010). Effects from the first treatment can influence those from the second one. Thus, the fundamental requirement is, that the changes induced by a

particular treatment must return to baseline before the next (comparative) treatment is being administered (Richens, 2001). For this purpose, in pharmacological trials the wash-out period of the drug has to be considered.

In a typical memory recognition paradigm, a memory retrieval test follows an incidental encoding session. During the encoding phase, the participants are either instructed to passively watch the stimuli (e.g., Weymar et al., 2009) or to complete a task which is not directly associated with the retrieval task (e.g., Schaefer et al., 2011). In both cases, the subjects are not supposed to be aware of a future memory test. This manipulation is employed to prevent subjects from using learning strategies and to maximize the comparability of encoding conditions between subjects.

If applying the recognition paradigm with an incidental encoding to a pharmacological study in a crossover design, one possibility is to introduce two incidental encoding sessions, during which two medications are randomly administered, e.g.:

Subject 1: 1st encoding – study medication, 2nd encoding – comparator
Subject 2: 1st encoding – comparator, 2nd encoding – study medication
…

In this case, when applying a crossover design, one should consider the risk of carry-over effects originating in both the administered drug and the memory formation principles. The carry over effects mutual to the administered drug are easily to control, i.e., the second encoding session takes place after the drug has been "washed-out" from the system (i.e., after the wash-out period). The second group of artifacts may arise from sequential presenting of two sets of stimuli. In this case priming (e.g., some type of stimuli might be primed during the first encoding session, and are thus easier to process during the second encoding session and, as a consequence, better retrieved) or sequence effects (e.g., stimuli learned during the second session are better remembered because they constitute newer memory) that can be carried over from the first to the second encoding session and finally influence the memory results.

The possible effects arising from presentation of two sets of stimuli in two consecutive incidental encoding sessions are which we concentrate on. By planning a clinical trial in cross-over design, we were interested whether implementing two incidental encoding sessions into a classical recognition memory paradigm will change

the typical pattern of brain activity in ERPs and behavioral performance. For this purpose, we conducted an experiment, in which participants passively watched two different stimuli sets on two consecutive days and completed a recognition test one week later. Our goal was to examine whether there are some differences in brain responses (LPPs) between the first and second incidental encoding session and whether we find any differences during retrieval, when comparing brain responses (ERP old/new effects) and behavioral responses (memory performance as well as valence and arousal ratings) to stimuli learned during the first and second encoding session.

Importantly, in order to assure reliability of the crossover design in the episodic recognition memory paradigm, we needed to answer questions concerning differences not present between experimental conditions. In this case, our main research question corresponded to the H_0: two encoding sessions have no effect on encoding and recognition memory. Therefore, where possible, we conducted post-hoc power analyses to test whether the possible missing differences between the parameters of interest were due to insufficient power.

6.2 Method

6.2.1 Participants

Participants were 18 healthy male students of the University of Greifswald in age between 19 and 33 years (mean: 24.8). All were right-handed and had normal or corrected-to-normal vision. The sex of the participants was predefined in order to correspond with inclusion criteria in the planned clinical trial[14]. The participants provided written informed consent approved by the Review Board of the University of Greifswald and received financial compensation or course credits for the participation in the study.

6.2.2 Stimulus material

Overall, 444 photographs[15] were selected from the International Affective Picture System (IAPS; Lang et al., 2008), the Emotional Picture Set (EmoPicS;

[14] For a detailed explanation for including only men in the clinical trial please refer to section 7.3.3.2
[15] For numbers of pictures from all three picture sets see Appendix II.

Wessa et al., 2010) and the picture set from Stark et al. (Stark, Schienle, Girod, Walter, Kirsch, Blecker, Ott, Schäfer, Sammer, Zimmermann, & Vaitl, 2005). The stimulus material consisted of 180 unpleasant (including mutilation, contamination, accidents, threat and attack), 180 pleasant (erotic couples, attractive and erotic females, families and children, romance, adventure and sport) and 180 neutral scenes (people, nature, objects and buildings). The stimuli were divided into four sets, with 30 pictures from every emotional category each, carefully matched based on the normative valence and arousal ratings as well as semantic content. Additionally, four sets of buffer pictures were created, with 21 stimuli (7 unpleasant, 7 pleasant, 7 neutral) each. Table 3 depicts normative valence and arousal ratings as well as ratings obtained in the study.

Table 3. Normative and individual valence and arousal ratings.

	Normative ratings		Individual ratings	
Emotional content	Valence	Arousal	Valence	Arousal
Unpleasant	2.66 (.08)	5.78 (.11)	2.58 (.14)	5.88 (.32)
Pleasant	7.01 (.07)	5.83 (.18)	6.84 (.14)	5.19 (.37)
Neutral	4.99 (.04)	3.03 (.95)	5.49 (.25)	2.57 (.25)

Note: Normative ratings calculated for stimuli values for IAPS, EmoPics and erotic pictures from Stark et al. picture set (for references see section 6.2.2). Individual ratings were obtained in the study. Standard errors are given in parentheses.

According to normative data, the three emotional categories differed in valence ratings, $F(2,58) = 1050.08$, $p < .001$. Normative arousal ratings were higher for both unpleasant and pleasant than for neutral pictures [$F(2,56) = 190.76$, $p < .001$] and did not differ between unpleasant and pleasant pictures, $F(1,29) < 1$.

Individual hedonic valence and arousal ratings of all pictures in our sample were obtained to check for correspondence with the normative ratings of the IAPS, the EmoPicS and the Stark et al. (2005) set. As expected, unpleasant pictures were rated as less pleasant than neutral and pleasant pictures, neutral as less pleasant than pictures of positive valence, $F(2,34) = 238.25$, $p < .001$. Emotional pictures were rated as more arousing than neutral pictures, $F(2,34) = 79.56$, $p < .001$. Contrary to normative ratings, unpleasant stimuli were rated as more arousing than pleasant stimuli, $F(1,17) = 4.39$, $p = .05$.

6.2.3 Procedure

The subjects were seated in a reclining chair in a sound-attenuated, electrically shielded, dimly lit room. The stimuli were displayed on a 21 in computer screen and the EEG-signal was recorded. During the tasks, the participants were instructed to fixate the center of the screen and to reduce eye-blinks and avoid body movements. Figure 7 schematically displays the experimental procedure.

On the first and second day, the participants were instructed to attentively view the presented pictures. No mention of a memory test was made. In both incidental encoding sessions two different sets of 90 target photographs were displayed together with 21 buffer pictures at the beginning and 21 pictures at the end of the presentation (to avoid serial position effects, i.e., primacy and recency effects). Each stimulus was displayed for 3000 ms with a random intertrial interval (ITI) of 3500, 4000, or 4500 ms. A 500 ms fixation cross preceded each picture to ensure that participants fixated the center of the screen. Within every session, the pictures were presented in pseudorandom order with the restrictions that no picture of the same valence was displayed twice in a row and every picture triplet contained all three valence categories. The order of picture presentation was fully randomized across participants. Set (set 1–4) to session (encoding 1 vs. encoding 2) assignments were counterbalanced.

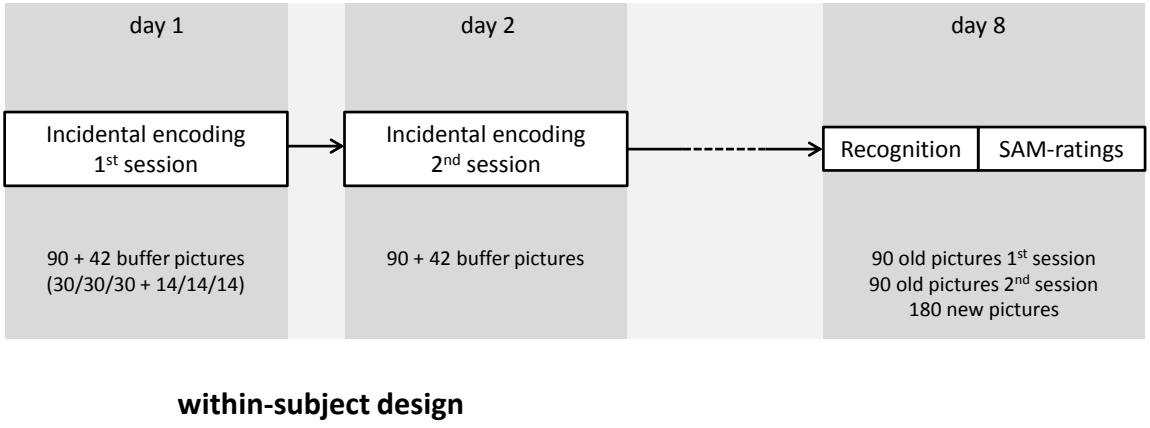

Figure 7. Scheme of the experimental procedure introduced in the study. Description in text. The two incidental encoding sessions were conducted in one group of participants. Pictures presented in the first and second encoding session differed. Abbreviations: SAM – Self-Assessment Manikin.

One week after the second encoding session, 90 old pictures from the first session, 90 old from the second session and 180 new pictures were each displayed for 3000 ms, preceded by a 500 ms fixation cross. The buffer pictures were no longer presented. At picture offset, the participants were prompted to decide whether they had seen the picture before by pressing either a "yes-" or a "no-" marked button on a computer keyboard. The assignment of left and right button presses to "yes"/"no" responses was counterbalanced across participants. After completing the recognition task, participants were asked to rate all previously seen pictures for their subjective hedonic valence and arousal using the SAM rating procedure (Bradley & Lang, 1994). For this purpose, the participants rated each picture on two standardized 9-point Likert scales of emotional arousal (1, lowest; 9, highest) and emotional valence (1, most unpleasant; 5, neutral; 9, most pleasant), by pressing the appropriate key on a computer keyboard. There was no time limit for making a decision. In both tasks the stimuli were presented in a pseudorandom order with the following restrictions: (1) pictures of the same valence were displayed at most twice in a row, (2) old and new pictures were displayed at most twice in a row, (3) every picture sextet contained old and new pictures from all three valence categories. The order of pictures presentation was fully randomized across participants.

6.2.4 EEG-recording

The continuous EEG signal was 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 band-pass filtered online from 0.1 to 100 Hz. Offline analyses were performed using ElectroMagnetoEncephalography Software (EMEGS; Peyk et al., 2011). The analysis steps were as follows: (1) low-pass filtering at 40 Hz; (2) segmentation of continuous signal in 1300 ms long epochs starting 100 ms before stimulus onset; (3) automatized detection and rejection of artifactual trials and channels based on recorded reference for average reference computing; (4) artifact detection and rejection (based on statistical editing parameters: maximum absolute value over time, standard deviation over time and maximum gradient over time) with an

average reference and spherical sensor interpolation; (5) baseline correction (100 ms epoch before trigger stimulus) and averaging (Junghöfer et al., 2000).

6.2.5 Data analysis

ERPs were computed for each sensor and participant. Based on both visual inspection of the waveforms and previous findings (e.g., Rugg & Curran, 2007; Weymar et al., 2009), we selected ERP data for further analyses. Cluster-averaged mean ERP amplitudes were analyzed in a 300-500 ms time-window over frontal brain regions (cf., Figure 12, right upper inset) for recognition data and in a 500-850 ms time-window over centro-parietal brain regions for encoding and recognition data (cf., Figure 13, right upper inset).

The ERP data was analyzed in two ways using repeated measures ANOVA. The first analysis aimed at assessing the emotional modulation of the ERPs during encoding and retrieval. For encoding data, the analyses were performed with factor Hedonic Content (unpleasant vs. neutral vs. pleasant). For recognition data, the analyses were conducted with factors Hedonic Content (unpleasant vs. neutral vs. pleasant), and Memory (old vs. new; "olds" are merged old stimuli from the first and the second encoding session). These waveform analyses were conducted at an alpha level of 10%. The purpose of the second analysis was to examine the possible effects of two encoding sessions on encoding and recognition ERPs. Here, the analyses were conducted with factors Hedonic Content (unpleasant vs. neutral vs. pleasant) and Session (encoding 1 vs. encoding 2) for encoding data; and factors Hedonic Content (unpleasant vs. neutral vs. pleasant) and Session (old 1^{st} session vs. old 2^{nd} session vs. new). For ERPs, only trials with correct behavioral responses (hits and correct rejections) were used in the averaged ERP data.

For memory performance, Hit rate (H), False Alarm rate (FA) and discrimination index (Pr = H – FA) were analyzed. Similarly to ERPs, we conducted two separate analyses: the first to obtain the effects of the emotional modulation on memory performance (for this purpose "hit" responses associated with stimuli from both first and second encoding session were merged), and the second to examine the effects of encoding order on these memory parameters.

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

6.2.5.1 Power analyses

In order to assure reliability of the crossover design in the episodic recognition memory paradigm, we needed to answer the question whether introducing two subsequent incidental encoding sessions would have any effect on encoding itself and recognition memory. In this case, our research question corresponds to the null-hypothesis: two encoding sessions have no effect on encoding and recognition memory.

For this purpose, where possible, we conducted post-hoc power analyses to test whether the missing differences between the parameters of interest were due to insufficient power[16].

To explore the differences between ERPs for old pictures from the first and second encoding session during recognition we conducted post-hoc power analyses with an alpha criterion of 10% and a population effect size estimated from data reported in previous studies. Because this is the first study employing two consecutive incidental encoding sessions and explicitly comparing ERPs for old stimuli learned in two different sessions, the reports about the empirical effect sizes (or the population effect size) are not available. Thus, we decided that, for purposes of our research question (and in context of possible application of these data in clinical trials in crossover design), it is sufficient that the difference in ERPs for stimuli encoded during the first and the second session is neither equal nor greater than the typical difference between old and new stimuli. We analyzed the data from ERP studies which used neutral and emotional natural scenes as stimuli and tested memory after a retention period from one day to one week. Here, we were mainly interested in the late parietal old/new effect (although the frontal effect is analyzed too), as it reflects recollection-based recognition memory processes. The parietal effect was shown to be affected by a pharmacological beta-blockade (Weymar et al., 2010b), and was in focus of the next (reported in section 7) study.

[16] Where possible, we included population effect sizes instead of empirical effect sizes in the computation of achieved power. The use of empirically obtained effect sizes for computing power for a non-significant test is not informative, because the only possible conclusion of the analysis is a non-sufficient power (<50%) (Sedlmeier & Renkewitz, 2013). Additionally, to achieve required power (e.g., of 90%) with the empirically obtained effect sizes, we would have needed extremely large sample sizes.

For calculating the empirical effect sizes we used the following formula:

$$\eta_p^2 = \frac{F \cdot df_{(k-1)}}{F \cdot df_{(k-1)} + df_{(k-1)\cdot(n-1)}}$$, where:

η_p^2 is partial eta squared, $F \cdot df_{(k-1)}$ is the sum of squares of the effect, and $df_{(k-1)\cdot(n-1)}$ is the residual sum of squares.

Table 4 lists the analyzed reports and the relevant *F* and *df* values used in the calculations for the early frontal and late parietal old/new effects.

Table 4. Partial eta squared calculations for early frontal and late parietal ERP old/new effects.

Report	*F* value ef	*F* value lp	*df* (k – 1)	*df* (n – 1)	η_p^2 ef	η_p^2 lp
König & Mecklinger, 2008	31.09	81.36	1	19	.62	.81
Schaefer et al., 2009	7.3	10.5	1	16	.31	.40
Schaefer et al., 2011	23.7‡ 6.2*	21.81†	1	26	.48‡ .19*	.46†
van Strien et al., 2009	36.55	32.77	1	20	.65	.62
Weymar et al., 2009	10.50	68.19	1	28	.27	.71
Weymar et al., 2010a	n.a.	107.38†	1	11	n.a.	.91†
Weymar et al., 2010b	n.a.	29.33	1	44	n.a.	.39
Weymar et al., 2011	n.a.	40.03	1	20	n.a.	.67
Wirkner et al., 2013	39.30	19.45	1	50	.44	.28
				mean	**.44**	**.58**

Note: Given are reported F values and degrees of freedom from available studies which used neutral and emotional natural scenes as stimuli and tested memory after a retention period from one day to one week. Abbreviations for *F* values: "ef" – early frontal, "lp" – late parietal ERP old/new effect. In studies assessing recollection and familiarity directly, † and ‡ are reported values for "remember minus new", * for "know minus new" comparisons only. Values from Schaefer et al., 2011 were averaged (.36) for the main η_p^2 mean calculation. N.a. – not analyzed in the report.

The empirical effect sizes of the ERP old/new effects for parietal electrodes calculated from the available reports are typically extremely large (see Table 4) which indicates very large population effects. Therefore, we set the effect sizes (η_p^2) for power calculations for the early frontal old/new effect at 0.4 and for the late parietal at 0.5. All power calculations were conducted with G*Power v.3.9.1[17] (Faul, Erdfelder, Lang, & Buchner, 2007).

In the available literature we did not find comparable research reports which we could have used for estimating the population sizes of encoding session effects on LPPs and behavioral data. Therefore, in these cases we only report the *F*, *p* and empirical

[17] Calculations were conducted with the most conservative effect size specification by Cohen, recommended by *GPower.

η_p^2 (where relevant) values for main effects and interactions as a material for further research.

6.3 Results

6.3.1 Subjective valence and arousal ratings

The subjective valence and arousal ratings of single stimulus sets are reported in the stimulus material section (cf., section 6.2.2). Figure 8 shows rated valence (upper panel) and arousal (lower panel) of experimental stimuli with respect to stimulus status (old stimuli from the first and second encoding session, and new stimuli).

Pleasant pictures were associated with highest pleasantness ratings, followed by neutral and unpleasant stimuli, $F(2,34) = 234.17$, $p < .001$. There were no differences between old pictures encoded during the first and the second encoding session, $F(1,17) = 1.31$, $p = .268$, $\eta_p^2 = .07$, and no significant interaction between valence and the status of the stimulus, $F(4,68) = 1.00$, $p = .41.$, $\eta_p^2 = .06$. According to arousal, similarly, the ratings were affected by the emotional content of the stimulus, $F(2,34) = 81.12$, $p < .001$, yielding the pattern of emotional pictures being more arousing than neutral pictures. Similar as in Study 1, unpleasant stimuli were rated as more arousing than pleasant ones, $F(1,17) = 4.39$, $p = .05$. There were no differences between two old picture types, $F(1,17) = .19$, $p = .89$, $\eta_p^2 = .001$ and no significant interaction between rated arousal and the status of the stimulus, $F(4,68) = .59$, $p = .67$, $\eta_p^2 = .03$.

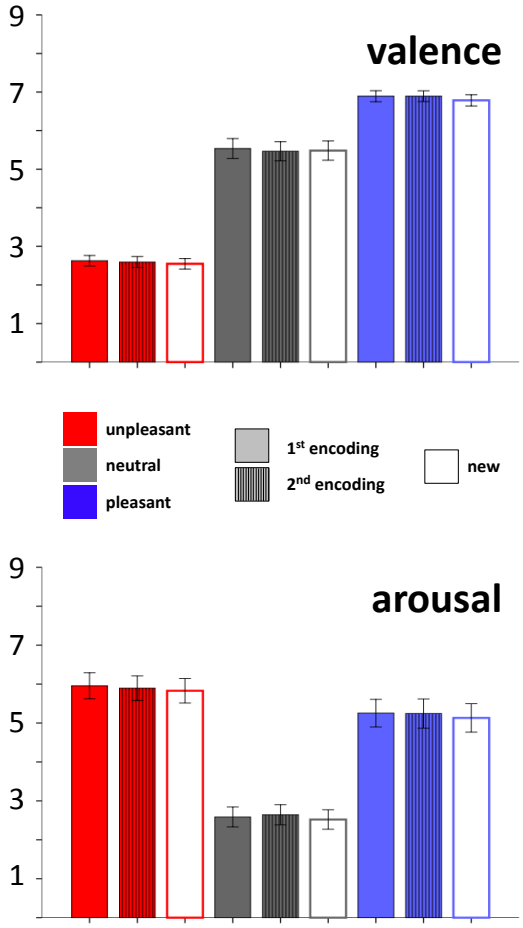

Figure 8. Mean rated valence and arousal of the experimental stimuli. Mean values for unpleasant pictures are depicted in red, for neutral in grey, and for pleasant in blue. Solid bars represent old stimuli from first encoding session, striped bars old stimuli from second encoding session; new stimuli are depicted by bars with no filling. Error bars represent standard errors.

6.3.2 Memory performance

Correct recognition of emotional pictures was higher for both emotional pictures categories than neutral pictures, $F(2.34) = 28.37$, $p < .001$. Hit rates for unpleasant and pleasant pictures did not differ, $F(1,17) = 1.99$, $p = .18$. There was neither a difference in hit rates between old pictures encoded during first and second encoding session [$F(1,17) = .39$, $p = .54$, $\eta_p^2 = .02$] nor an interaction between hedonic content and the status of the stimulus, $F(2,34) = .46$, $p = .64$, $\eta_p^2 = .03$. False alarm rates for pleasant were higher than for unpleasant and neutral pictures, $F(2,34) = 3.55$, $p < .05$. Correct discrimination (Pr = H – FA) was highest for unpleasant pictures and lowest for neutral stimuli, $F(2,34) = 22.19$, $p < .001$. The discrimination of pleasant pictures was attenuated by the high false alarm rate (cf., Figure 9). Parallel to hit rates, there was no difference in correct discrimination between old pictures encoded during first and second encoding session [$F(1,17) = .39$, $p = .54$, $\eta_p^2 = .02$] as well as no interaction between hedonic content and

the status of the stimulus, $F(2,34) = .46$, $p = .64$, $\eta_p^2 = .03$.

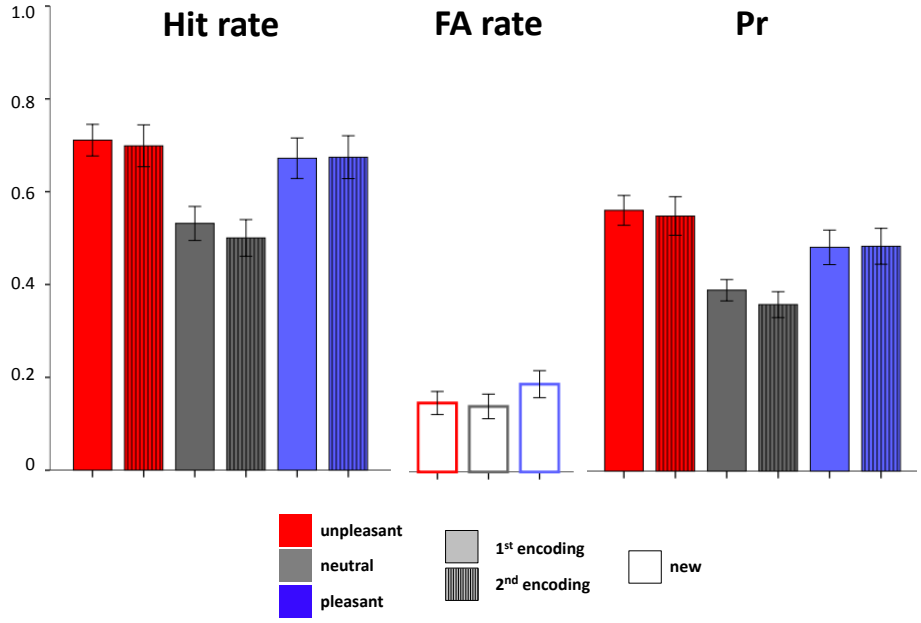

Figure 9.. Memory performance as represented by mean hit rates, false alarm (FA) rates and correct discrimination (Pr, hit minus FA) for unpleasant (red), neutral (black) and pleasant stimuli. Solid bars represent old stimuli from the first encoding session and striped bars old stimuli from the second encoding session; new stimuli are depicted by bars with no filling. Error bars represent standard errors.

6.3.3 Event-related potentials

6.3.3.1 Encoding

Figure 10 illustrates grand averages of the ERP waveforms for pictures in the first and second encoding session in the analyzed centro-parietal sensor cluster as well as scalp voltage distributions for emotional minus neutral waveforms in the 500-850 ms time-window.

The averaged ERPs during encoding were computed for the same sensors' cluster as the ERPs during retrieval. As expected, during encoding, the late positive potentials observed over central sites were enhanced for emotional pictures when compared to neutral pictures, $F(2,34) = 63.78$, $p < .001$. Additionally, pleasant pictures elicited more positive-going waveforms than unpleasant pictures, $F(1,17) = 38.75$, $p < .001$.

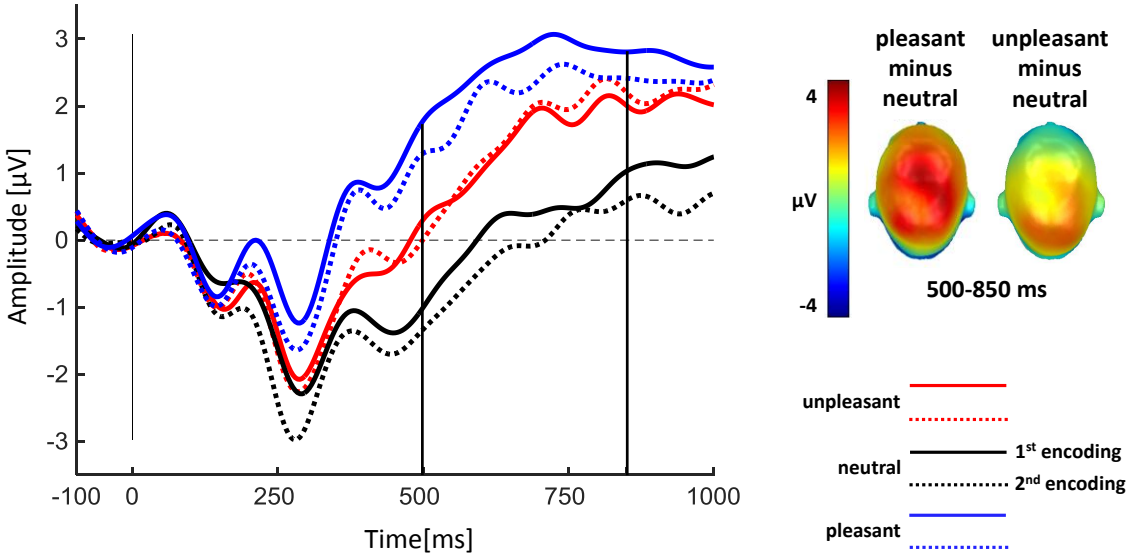

Figure 9. Left panel: Grand average ERP waveforms for stimuli presented in the first (solid line) and the second (dotted line) encoding session, averaged within the analyzed centro-parietal cluster. Unpleasant pictures are depicted in red, neutral in black, and pleasant in blue. The analyzed time-window 500-850 ms is indicated by vertical lines. Right panel: Mean overall scalp voltage differences between ERP waveforms (averaged waveforms for stimuli from the first and second encoding session) for pleasant and neutral (left), and unpleasant and neutral (right) in the 500-850 ms time-window.

The amplitude of ERP waveforms for pictures encoded during the first and the second encoding session did not differ significantly, $F(1,17) = 2.19$, $p = .16$, $\eta_p^2 = .11$. The interaction between the hedonic content of the pictures and the day of encoding was also not significant, $F(2,34) = 1.49$, $p = .24$, $\eta_p^2 = .08$.

6.3.3.2 Recognition

Figure 11 depicts scalp voltage distributions for old minus new ERP waveforms for both time-windows over all experimental conditions. Figures 12 and 13 illustrate grand averaged ERP waveforms for old and new pictures (lower panel) as well as old pictures encoded during first and second encoding session and new pictures (upper panel) during recognition, over frontal and centro-parietal brain regions, respectively. Figure 14 depicts main ERP old/new effect over centro-parietal sensor sites.

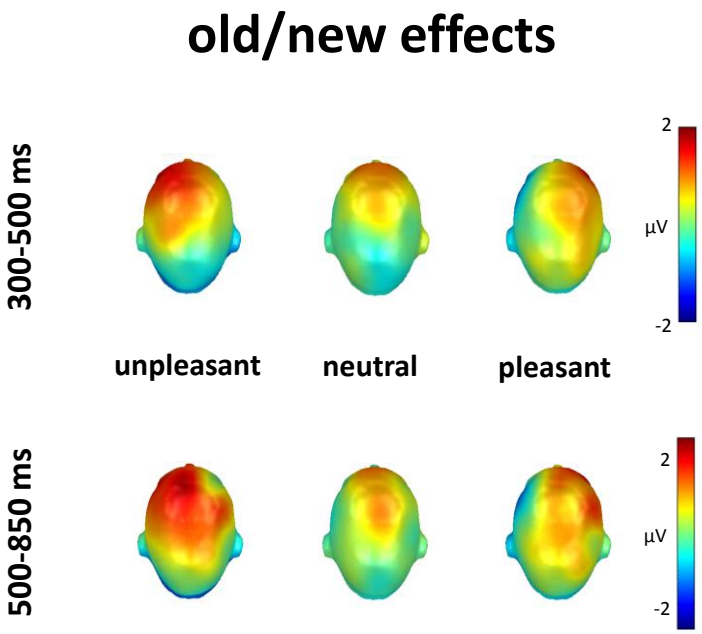

Figure 11. Mean scalp voltage differences between ERP waveforms for correctly recognized (averaged "olds" from both encoding sessions) and correctly rejected (new) stimuli in two analyzed time windows for all picture categories.

Early old/new effect (300-500 ms). Analysis of the frontal sensors' cluster revealed a main effect of memory, $F(1,17) = 19.59$, $p < .001$. Overall, correctly recognized old pictures were associated with a more positive-going ERP curve than correctly rejected (new) pictures. This effect was not modulated by emotion, Hedonic Content × Memory: $F(2,34) < 1$.

Over frontal sites, old pictures from the first and second encoding session both elicited an enhanced ERP waveform as compared to new stimuli, $F(2,34) = 13.95$, $p < .001$, $\eta_p^2 = .45$. There were no differences between old stimuli from the first and second encoding session, $F(1,17) = 2.7$, $p = .12$, $1\text{-}\beta = .91$ (encoding 1 vs. new: $F(1,17) = 9.84$, $p < .01$, $\eta_p^2 = .38$; encoding 2 vs. new: $F(1,17) = 28.83$, $p < .001$, $\eta_p^2 = .63$). Interestingly, old neutral stimuli from the second encoding session seem to have elicited more positive-going ERPs than old stimuli from the first encoding session (cf., Figure 12, upper panel), however this difference was not validated, when directly

comparing old stimuli from the first and second encoding session, $F(1,17) = 2.70$, $p = .12$, empirical $\eta_p^2 = .14$.

Neither a memory effect nor interactions were observed over central sites. We did not pursue further analyses for this time interval.

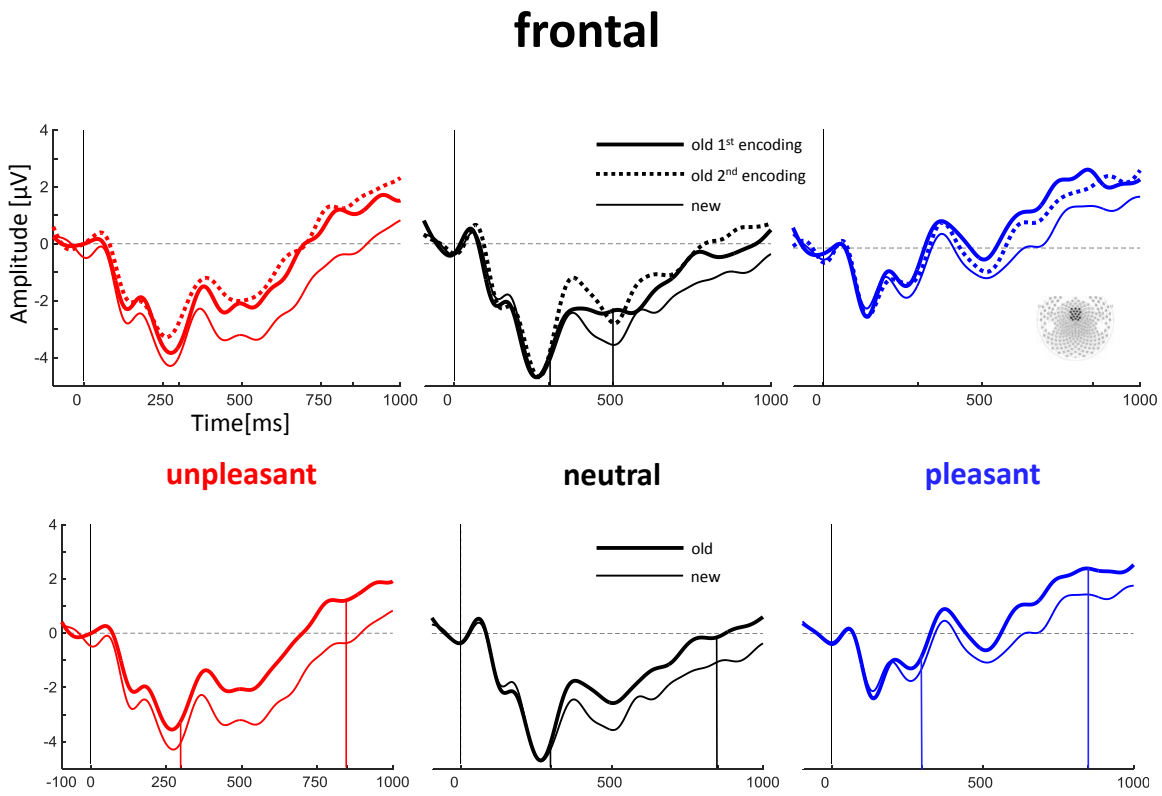

Figure 102. Lower panel: Grand average ERP waveforms for correctly recognized old (thick line) and correctly rejected new (thin line) pictures averaged within the frontal cluster (depicted by the inset to the right in the upper panel) during retrieval task. Unpleasant pictures are depicted in red, neutral in black, and pleasant in blue. The early and late old/new effects for all picture categories are indicated by vertical lines within the respective time-windows (300-500 and 500-850 ms). Upper panel: Grand average ERP waveforms for correctly recognized old pictures from the first encoding session (thick, solid line), old pictures from the second encoding session (thick, dotted line), and correctly rejected new (thin line) pictures averaged within the analyzed cluster during retrieval. The early difference between neutral old pictures from first and second encoding session is indicated by vertical lines within the 300-500 time-window.

Late old/new effect (500-850 ms). The old/new effect observed in the early time window over frontal sites remained significant in the later time interval [$F(1,17) = 15.25$, $p = .001$] and, similarly, its magnitude was comparable for all emotional conditions, Hedonic Content × Memory: $F(2,34) < 1$.

Over frontal sites, larger ERP positivities were also observed for both types of old pictures than new pictures, $F(2,34) = 8.50$, $p = .001$, $\eta_p^2 = .33$. Similar to the earlier time-course of the waveforms, we did not observe now any differences between the two old picture types, $F(1,17) = .11$, $p = .75$, $1\text{-}\beta = .91$ (encoding 1 vs. new: $F(1,17) = 9.46$, $p < .01$, $\eta_p^2 = .36$; encoding 2 vs. new: $F(1,17) = 15.67$, $p < .001$, $\eta_p^2 = .48$).

Over central sites, larger ERP positivity was observed for old than new pictures, $F(1,17) = 15.41$, $p = .001$. Replicating previous findings (e.g., Weymar et al., 2009), this old-new difference was modulated by the emotional content of the pictures, $F(2,34) = 2.54$, $p < .10$. Post-hoc comparisons revealed that the old-new difference was greater for unpleasant than neutral stimuli, $F(1,17) = 6.67$, $p < .05$ (cf., Figure 13, lower panel).

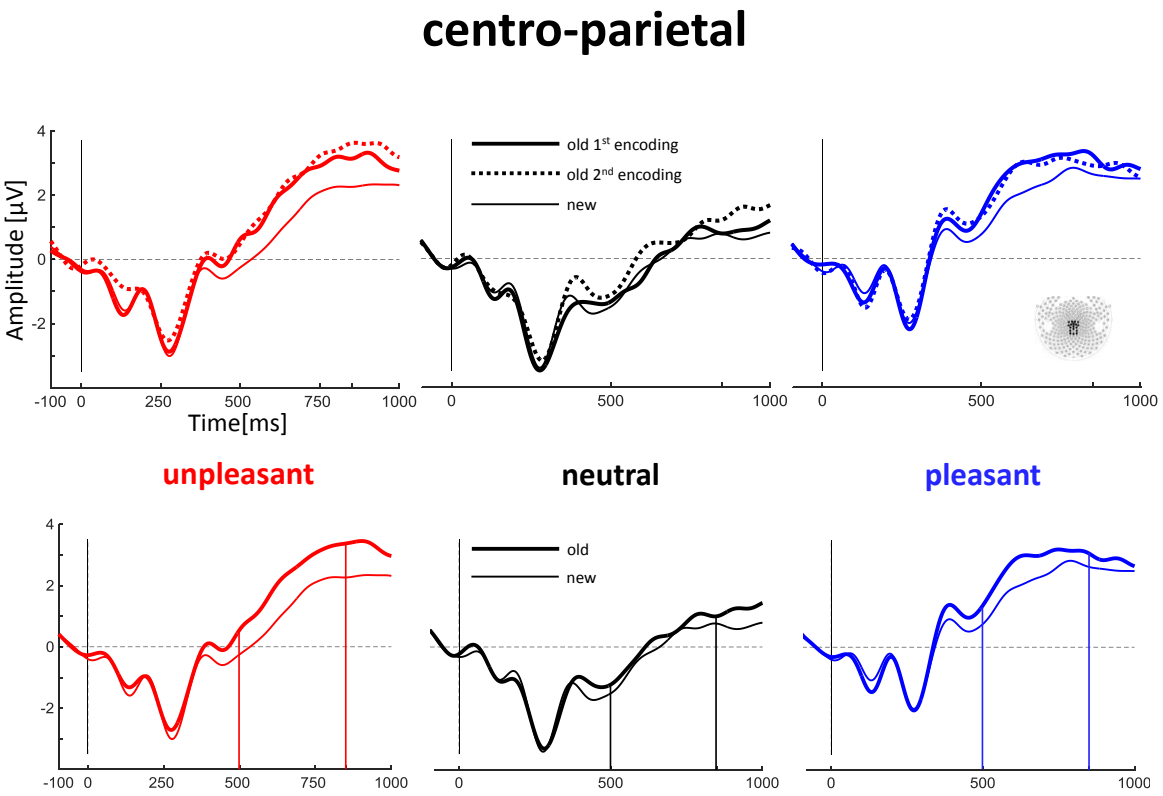

Figure 113. Lower panel: Grand average ERP waveforms for correctly recognized old (thick line) and correctly rejected new (thin line) pictures averaged within the centro-parietal cluster (depicted by the inset to the right in the upper panel) during retrieval task. Unpleasant pictures are depicted in red, neutral in black, and pleasant in blue. The late 500-850 time-window, where the old-new differences among picture categories differed, is indicated by vertical lines. Upper panel: Grand average ERP waveforms for correctly recognized old pictures from the first encoding session (thick, solid line), old pictures from the second encoding session (thick, dotted line), and correctly rejected new (thin line) pictures averaged within the analyzed cluster during retrieval.

Over central sites, 500-850 ms post-stimulus, both categories of old stimuli (first and second encoding session) were associated with more positive-going waveforms than new pictures, $F(2,34) = 7.78$, $p < .01$, empirical $\eta_p^2 = .31$ (cf., Figure 14). We did not find any differences between two old stimulus types, $F(1,17) = .48$, $p = .50$, $1\text{-}\beta = .98$ (encoding 1 vs. new: $F(1,17) = 8.52$, $p = .01$, $\eta_p^2 = .33$; encoding 2 vs. new: $F(1,17) = 15.01$, $p = .001$, $\eta_p^2 = .47$).

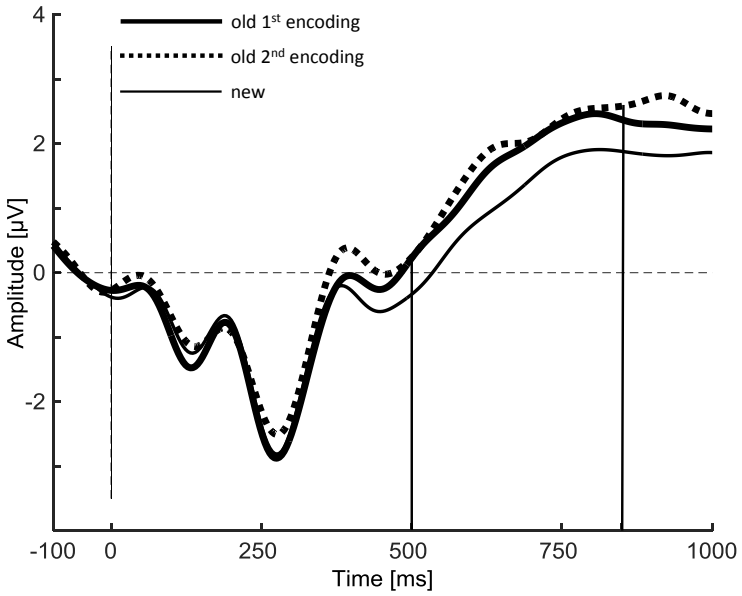

Figure 14. Grand average ERP waveforms for correctly recognized old from first (solid, thick line) and second encoding session (dotted, thick line), and correctly rejected new (thin line) pictures averaged within the centro-parietal cluster during retrieval task. The late 500-850 time-window, where the overall old/new effects were pronounced, is indicated by vertical lines.

6.4 Discussion

In the present ERP study, we examined the influences of order of the encoding session on the retrieval performance and ERP brain activity during encoding and recognition of emotional and neutral pictures as well as the modulation of these processes by the hedonic content of the stimulus material. In examining the influences of the day of encoding on memory processes the null-hypothesis was our hypothesis of interest.

Therefore, where possible, we conducted power analyses to verify that the missing differences were not due to insufficient power of the test. Firstly, we found that ERPs associated with correct recognition of old pictures were not modulated by the day of encoding. We also did not observe any encoding session's effect on behavioral performance during retrieval. Additionally, ERPs during encoding did not differ significantly, when compared between first and second session. Finally, as in previous studies, the correct recognition of emotional and neutral stimuli was associated with pronounced old/new effects over frontal brain regions and these effects were not modulated by emotion. Over central brain regions, the ERP old/new effect for emotionally arousing stimuli was enhanced as compared to neutral stimuli.

As in previous findings, hedonic content of the pictures modulated the amplitude of late ERP components during encoding of the stimuli in a way that emotional scenes prompted more positive going waveforms than neutral pictures. This typical enhancement of the LPP is thought to reflect the process of stimulus categorization (Codispoti, Ferrari, De Cesarei, & Cardinale, 2006) – the ability of the organism to organize the environment into meaningful groupings, or, in other words, to select and evaluate the motivationally relevant input (Lang, Bradley & Cuthbert, 1997), in order to be able to react properly and adaptively to relevant stimuli in various situations. Emotional stimuli are motivationally significant and more likely to sustain attention than affectively neutral cues. This evolutionary inherited, natural selective attention is highly adaptive for survival and reflects engagement of primary motivational – appetitive and aversive – systems (Bradley, 2009). The motivational meaningfulness of emotional natural scenes seems to automatically and rapidly direct attentional resources onto these stimuli. Here, the selective processing is reflected by enhanced LPPs for pleasant and unpleasant scenes starting roughly 300 ms after stimulus onset.

Interestingly, in our study, pleasant stimuli at encoding were associated with more positive-going ERP waveforms than unpleasant stimuli. Studies examining impact of emotion on the variation of the LPPs consistently find enhanced ERP waveforms for highly emotional stimuli, irrespective of valence, during picture viewing. In most cases, pleasant and unpleasant pictures elicit LPPs comparable in amplitude (Hajcak, Dunning, & Foti, 2007; Weinberg & Hajcak, 2011). When amplitude differences arise, unpleasant stimuli are associated with enhanced LPP amplitudes as compared to pleasant ones

(Hajcak & Olvet, 2008), which is in line with the notion that LPPs vary as a function of emotional arousal and reflect increased attention to motivationally relevant stimuli. However, several studies using emotional and neutral words have shown that particularly pleasant words are associated with higher LPP amplitudes than unpleasant words (Herbert, Junghofer, & Kissler, 2008; Herbert, Kissler, Junghöfer, Peyk, & Rockstroh, 2006; Schapkin, Gusev, & Kuhl, 2000) which might reflect enhanced evaluative processing of pleasant stimuli and indicate that LPPs may be specifically driven not only by emotional arousal but by emotional valence as well. In respect to our data, the results obtained by Herbert et al. (2006, 2008) might suggest that pleasant, especially erotic, stimuli used in the encoding sessions attracted more attention in our male sample and underwent a more profound, evaluative encoding than unpleasant items from the used photographs sets. In addition, the pleasant stimulus sets used in the study were particularly rich in erotic, sexually arousing pictures. Several studies (Schupp et al., 2004, 2007) have shown that large LPPs arise when people view erotic scenes. The amplitudes of these LPPs are comparable to those associated with viewing mutilation scenes. It has been discussed (Bradley, 2009) that the magnitude of the LPP reflects an activation of motivational systems (either defensive or appetitive system), which have developed to react on evolutionary significant features of the environment. In our study, the observed selective enhancement of the LPP for pleasant pictures (which in a great part consisted of naked women and sexual intercourse scenes) might suggest that this kind of stimulation is of high motivational significance for the male sample, even if it was not reflected by explicit arousal ratings. Moreover, Schaefer et al. (Schaefer et al., 2009) showed that stimuli evaluated at encoding as moderately arousing elicit the greatest ERP old-new differences at retrieval. Although in that study the authors did not analyze the encoding ERPs, it would be interesting to see whether the ERPs at encoding were modulated by the level of arousal as well. This finding opens a new area of questions, since, by now, not many studies directly compared encoding and retrieval ERPs and codependences between arousal at encoding, the amplitude of LPP and retrieval components.

As in previous studies, emotionally arousing (both unpleasant and pleasant) stimuli were better remembered than neutral, low arousing stimuli, as indicated by hit rates. Discrimination performance (Pr), however, was enhanced only for highly arousing, unpleasant pictures. The reduction of correct discrimination for pleasant pictures resulted

from a relatively high false alarm rate associated with this type of stimuli. The reduction of Pr values for emotional stimuli is sometimes observed in recognition studies (Jaworek et al., 2014; Johansson, Mecklinger, & Treese, 2004; Maratos, Dolan, Morris, Henson, & Rugg, 2001; Windmann & Kutas, 2001) and might be referred to as an "emotion-induced recognition bias" (Dougal & Rotello, 2007). This phenomenon reflects the biological (or evolutionary) significance of emotional stimuli, causing an attentional bias towards them. The involuntary process guarantees that in an evolutionary sense relevant stimuli are not ignored or neglected (Koenig & Mecklinger, 2008). In our study, however, only pleasant stimuli were associated with high false alarm rates. This response bias might be associated with increased subjective feeling of remembering (Phelps & Sharot, 2008), familiarity processes underlying the recognition of pleasant pictures (e.g., Ochsner, 2000) or perceptual similarity that is characteristic for erotic pictures with sexual scenes.

In line with previous studies (Jaworek et al., 2014, see also Study 1; Weymar et al., 2009) the early frontal old/new effect was not modulated by emotional content of the pictures. This result is in line with the notion that familiarity processes, as manifested in the frontal memory effect, arise from the global similarity between the features of the stimulus and the contents of the memory trace (Mecklinger, 2006). This global study-to-test matching seems not to be affected by the hedonic content of stimulus material. First in the later retrieval stages this emotional effect gains in importance. In our study, unpleasant pictures were associated with an enhanced ERP old/new effect over centro-parietal sites. This finding implicates, similarly as the previous reports (Jaworek et al., 2014; Schaefer et al., 2011; Weymar et al., 2009; Weymar et al., 2010a), that remembering of arousing emotional events is (after the initial familiarity-based recognition) preferentially based on recollection, i.e., these events are remembered more vividly and with more details.

Within-subject designs have the characteristic of a relatively low inter-subject variability (as compared to parallel-group designs) which enables to run studies with relatively small samples. For some objectives, keeping the sample as small as possible is extremely desirable. For example, in a study conducted in our department (see Study 3), we were interested in β-receptor genotype dependent propranolol effects on ERPs

associated with recognition of emotionally arousing stimuli. In this study, we planned to analyze the effects four different genotype combinations, some of which are extremely rare. Therefore, in order to be able to fill all genotype cells we wanted to set a crossover design for this study. Here, we wanted to clarify, whether an introduction of two incidental encoding sessions in one study would have any effects on typical memory performance and brain response patterns, which are usually found in studies using a simple recognition memory paradigm with one encoding session. If we succeeded in showing that repeated encoding does not influence the typical results, we would be able to make use of the advantages of the crossover design in a clinical trial, where study medication and the comparator are administered to the same group of participants, simultaneously minimizing any confounding carry-over/sequence effects originating from two encoding sessions.

The overall frontal and centro-parietal old/new effects, as well as the old/new effects for separate emotional conditions were comparable for old stimuli encoded during the first and second encoding session. Thus, the introduction of two consecutive incidental encoding sessions did not influence the recognition ERPs. The same pattern of results seems to be reflected by memory performance data. The LPPs during first and second encoding did not show significant differences, however, the visual inspection of the waveforms indicates some numerical differences between two encoding sessions.

In two ERP studies Codispoti and colleagues (Codispoti et al., 2007) as well as Ferrari et al. (Ferrari, Codispoti, & Bradley, 2005) examined the influences of stimulus repetition on the affective modulation of the LPP. They found that although the amplitude of the LPPs decreased as a function of stimulus repetition (within one session), the preferential processing of emotional stimuli did not habituate. This finding suggests that a repetition of stimuli does not change the strength of involvement of the motivational system during later stages of affective stimulus processing. Despite robust differences in design, there is a clear parallel between our LPP results and the finding reported by Codispoti et al. (2007) and Ferrari et al. (2005). It is plausible that the repetition of the encoding context might have resulted in a general tendency towards a decrease of the LPP amplitudes, but the strong involvement of the motivational system in the affective modulation left the pattern of ERP waveforms associated with emotional and neutral stimuli unchanged.

Our results clearly show that it is possible to test emotional memory recognition when two incidental encoding sessions are implemented in a within-subject design. In the study, the statistically proven absent differences in ERPs (old/new effects) between old stimuli learned on the first and the second encoding session during retrieval of one-week old emotional and neutral stimuli were accompanied by missing differences in other tested parameters: LPPs and memory performance measures. As explained earlier, we were not able to conduct power analyses on these results: since this study is the first to compare the effects of two encoding sessions on retrieval measures, there is no reference in the literature that we could have relied on while estimating the appropriate effect size parameters. Nevertheless, alongside the statistical analyses, the visual analyses of the presented data, especially the behavioral measures (i.e., memory performance and individual SAM-ratings), suggest no session-dependent differences in tested parameters. Only the LPPs and the frontal old/new effect for neutral stimuli showed not significant but considerable differences, which seemed to depend on the day of encoding. In future research, the question whether there are some differences in these measures should be addressed.

In sum, the data replicated the emotional modulation of ERPs and behavioral measures, and demonstrated no or negligibly small effects of two encoding sessions on the encoding and recognition data. Consequently, introducing a crossover design in emotional recognition memory studies is a reliable solution for clinical trials.

7 Study 3: Beta-blocker influences on formation and retrieval of emotional memories. Preliminary data

This study was conducted as a registered clinical trial under the EudraCT number: 2012-005806-23 and the title "*Effects of propranolol (vs. placebo) on information processing during presentation of emotionally arousing pictures after single dose (80 mg) administration and relationships between ß$_1$- and ß$_2$-adrenoreceptors genotype and both the information processing and the propranolol effects in 64 healthy male subjects*" at the study site in the Department of Clinical Pharmacology, C_DAT, Felix-Hausdorff-Str. 3, D-17487 Greifswald. The cooperating partners were the Department of Clinical Pharmacology, University Medicine Greifswald and the Department of Biological and Clinical Psychology, University of Greifswald. The study was approved by both German national competent authority (Bundesinstitut für Arzneimittel und Medizinprodukte) and the local ethics committee (Ethikkommission der Universitätsmedizin Greifswald).

As the title of the trial indicates, the study was primarily planned to investigate both the influences of propranolol on behavioral memory performance and its electrophysiological correlates, and propranolol interaction with the β-adrenoreceptor genotype variations. Because the number of participants in a predefined sample who were available and willing to participate was too low, we managed to assess only 8 participants in the study. The distribution of the desired genotype variations in the assessed sample was not sufficient to proceed with further analyses focusing on genotype effects. Thus, the results reported here only comprise analyses of propranolol influences on peripheral measures, memory performance, and memory-related ERPs independently of β-adrenoreceptor genotype. As being not further relevant for the following analyses, all information referring to genotype, like background information and primary objectives, planned sample, and genotype groups are to be found in the Appendix III.

Moreover, the small number of participants did not enable us to interpret the obtained data in a scientifically justifiable manner. Thus, all findings are to be held as preliminary results.

7.1 Background information

In human, the modulatory effects of noradrenergic activation on memory have been predominantly explored with propranolol – the lipophylic $\beta_1\beta_2$-adrenergic receptor blocker which easily crosses the blood-brain barrier (van Stengeren, 2008). Chamberlain and colleagues (2006) reviewed the available research effects of norepinephrine drugs (e.g.: propranolol, nadolol, metoprolol, yohimbine, reboxetine and epinephrine) on emotional memory in healthy human and reported consistent findings that the centrally acting beta-blocker propranolol generally reduced memory for emotional content. Van Stegeren et al. (1998) compared in one study the effects of centrally acting propranolol and nadolol, a water soluble drug which crosses the blood-brain barrier to a considerably lesser extent. In the study, only propranolol showed impact on reduction of emotional memory. Therefore, propranolol administration seems to be a reliable experimental manipulation if a modulation of emotional processing is desired.

There is strong evidence for propranolol to selectively impair the human emotional episodic memory, without affecting neutral memory, when given prior to learning (Cahill et al., 1994, Strange et al., 2003). The memory becomes weaker, at least in part, due to the beta-receptors blockade in the amygdala. The decreased amygdala activation during encoding after propranolol administration results in diminished memory effects for emotional stimuli (Strange & Dolan, 2004). Thus, the amygdala plays a crucial role in emotional arousal, mediating, via the noradrenergic system, emotional memory processes; and propranolol, blocking the β-receptors in the amygdala disrupts the encoding and consolidation processes in healthy men (McGaugh, 2000; van Stegeren et al., 2005) and anxiety patients (Reist, Duffy, Fujimoto, & Cahill, 2001; see also Lonergan et al., 2013 for a review).

A recent study conducted in our department (Weymar et al., 2010b) found propranolol's reducing effects on the ERP old-new difference for unpleasant pictures. However, medication did not influence the behavioral recognition of stimuli, i.e., the emotional stimuli were still better remembered than the neutral ones. Additionally, even though propranolol was active during learning, it did not influence encoding processes, as reflected by enhanced LPPs for emotionally arousing stimuli after propranolol administration. It can be concluded that propranolol partially blocked the development of

a neural signature for retrieval of emotional memories, by targeting the amygdala, partially disrupting the consolidation process for emotional material.

7.2 Research objectives

The main objective of the present study was to combine two lines of research, investigating the interaction between emotional processing and memory performance (on both behavioral and electrophysiological levels) and its modulation by beta-blockade.

The current investigation has been designed to replicate, in a crossover design study, former results (from a parallel-group study, Weymar et al., 2010b) in which ERP correlates of recognition memory for emotional pictures were reduced by beta-blocker propranolol. For this purpose, we implemented the previously tested (cf., Study 2) modified recognition memory paradigm with two incidental encoding sessions in a randomized clinical trial with propranolol and placebo administered in a crossover design.

We hypothesized, that emotionally arousing stimuli would be better remembered as neutral stimuli. Moreover, we expected that the prioritized processing of emotional stimuli would be associated with enhanced LPPs and ERP old/new effects for these stimuli. Finally, we expected that propranolol administered at encoding would cause a reduction of electrocortical correlates of emotional recognition memory.

7.3 Method

7.3.1 Design

The study was performed as a randomized, double-blind, single-dose, placebo-controlled crossover study. Participants received either 80 mg propranolol or placebo in either first or second treatment, in a random order. The block-randomization was performed in sizes of two[18]. A wash-out period of 48 h was held.

[18] For detailed randomization rules see Appendix IV.

7.3.2 Study medication

As target medication we used 80 mg beta-blocker propranolol (trade name: Propanolol-CT 80 mg Filmtabletten, international name: propanololhydrochloride, marketing authorization (MA) number: 6740.01.00, MA holder: CT Arzneimittel GmbH, Lengeder Str. 42a, 13407 Berlin, Germany) which was compared with placebo. Manufacturer of placebo and encapsulation was the University Pharmacy of the University of Mainz. The encapsulation of the study medication did not influence the ingestion and absorption of medication but only served the assurance of blinding.

Propranolol was administered as a single oral dose and alternated with placebo (both together with 240 ml tap water) on both encoding sessions, with 48 h wash-out period between the treatments. The treatments were set as follows: study day 1 – propranolol or placebo, study day 3 – placebo or propranolol. The participants were assigned to the treatment patterns according to a randomization list.

As a surrogate for adrenergic activity, a salivary sample to measure activity of the alpha-amylase was employed (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Saliva samples were collected at baseline, i.e., before drug administration, and shortly before encoding (approx. 90 minutes after drug administration) using a commercially available collection device (Salivette®, Sarstedt, Germany). Peripheral measures (heart rate, blood pressure) served as control parameters of propranolol influence on the sympathetic arousal. Systolic and diastolic arterial blood pressure, and heart were measured rate using IntelliVue MP2 (Philips Medizin Systeme GmbH Böblingen, Deutschland).

7.3.2.1 Safety

Recently, a collapse was reported after administration 80 mg propranolol in a healthy young participant (de Rover, van Noorden, Nieuwenhuis, & van der Wee, 2010). In the present study, we introduced safety and emergency procedures to prevent and correctly react to serious adverse events. On day one and three of the study, on which study medication was administered, the participants were monitored for heart beat (continuously) and blood pressure and were interviewed for adverse events in a non-leading manner, until the measured values came to the baseline levels. Additionally, an indwelling cannula was placed to the forearm of the participants for the purpose of a safe access in emergency case. During the whole study, a physician was present at the study

site and emergency equipment was available in the rooms in which the examinations were carried out. In case of serious adverse events, the Emergency Service was to be called immediately.

7.3.3 Participants

7.3.3.1 Planned sample

The study was planned to be conducted in 46 healthy, male, university students, genotyped for β-adrenoreceptor single nucleotide polymorphisms (cf., Appendix III). All subjects were to be selected from the clientele of healthy subjects of the Department of Clinical Pharmacology, University Medicine Greifswald, depending on whether they are in reach and willing to participate.

7.3.3.2 Inclusion criteria

First, only male participants were included in the study. Females were excluded in order to avoid possible confounding effects of changing hormonal status or contraceptives on memory performance and propranolol metabolism (Ertman, Andreano, & Cahill, 2011; Kendall, Jack, Quarterman, Smith, & Zaman, 1984; Nielsen, Ertman, Lakhani, & Cahill, 2011). Further inclusion criteria for the study were: age in range of 18 - 35 years, Caucasian ethnic origin, body mass index ranging from 19 to 27 kg/m², and one of the relevant genotype variations. In addition, participants were to be in good health as evidenced by the results of the clinical examination, electrocardiography (ECG), ergometry and the laboratory check-up[19]. The systolic and diastolic blood pressure must not be lower than 110 and 70 mm; and heart frequency not falling below 50 bpm (guidelines of the World Health Organization). Eventually, all subjects were to give a written informed consent. For exclusion criteria please refer to Appendix V.

7.3.3.3 Participants

After primary selection and extensive acquisition and pre-screening of the participants, we eventually were able to invite eight subjects for the initial screening session.

[19] For a listing of laboratory parameters please refer to Appendix VI.

All pre-screened male subjects participated in this clinical trial. One participant was excluded from further analyses because of poor EEG-data quality. The analyzed sample consisted of seven healthy subjects in age between 22 and 33 (mean = 25.9) years. All participants were right handed and had normal or corrected-to-normal vision. All participants fulfilled the study inclusion criteria.

7.3.4 Stimulus material

Stimulus material used in this was the same as in Study 2 (cf., section 6.2.2). Four different sets of stimuli were counterbalanced across the encoding sessions as well as according to the old/new status[20].

Individual hedonic valence and arousal ratings of pictures were obtained to check for correspondence with the normative ratings (reported in section 6.2.2, see also Table 3) of the IAPS, the EmoPicS and the Stark et al. (2005) set. As expected, unpleasant pictures were rated as less pleasant than neutral and pleasant pictures, neutral as less pleasant than pictures of positive valence, $F(2,12) = 175.53$, $p < .001$. Emotional pictures were rated as more arousing than neutral pictures, $F(2,12) = 85.69$, $p < .001$. Contrary to normative ratings, unpleasant stimuli were rated as more arousing than pleasant stimuli, $F(1,6) = 15.56$, $p < .01$, see Table 5.

Table 5. Individual valence and arousal ratings.

Emotional content	Valence	Arousal
Unpleasant	2.97 (.18)	5.48 (.32)
Pleasnt	6.38 (.07)	4.17 (.40)
Neutral	5.06 (.13)	1.96 (.20)

Note: Standard errors are given in parentheses.

7.3.5 Procedures

The whole study consisted of a pre-study examination, a psychophysiological experiment and pharmacokinetics measurements, and a follow-up examination. Before the start of the study written informed consent was obtained from all participants. All sessions (including the pre-study and the follow-up examination) and study procedures

[20] For detailed counterbalancing rules see Appendix IV.

took place at the study site of the Department of Clinical Pharmacology, University Medicine Greifswald.

7.3.5.1 Pre-study (inclusion) and follow-up examination

Within four weeks prior to the first pharmacokinetic study, the volunteers were subjected to a pre-study (inclusion) examination during which compliance with the inclusion criteria was tested. The follow-up examination was conducted within 1 week after the last psychophysiological examination.

7.3.5.2 Psychophysiological study protocol

The admission of participants to the study site took place in the evening one day before the experiment and was followed by a short medical examination, a questioning for state of health, and drug and alcohol screening. During the time the participants were at the study site, several additional measurements were conducted, aiming at examining the pharmacokinetics of propranolol. Intake of food and beverages was standardized for the first and third study day as well as the evenings before.

The research reported in the following sections covers the psychophysiological part of the clinical trial conducted in cooperation with the Department of Clinical Pharmacology. A detailed study protocol is to find in Appendix VII.

The experiment consisted of two encoding sessions with propranolol or placebo administration and one retrieval session off-medication. Figure 15 depicts a summary of the study procedures.

7.3.5.3 Baseline measures and drug administration

The administration of the study medication took place in the early morning. Before administration of the study medication, blood pressure (BP) and heart rate (HR) were determined and salivary α-amylase (sAA) was collected for baseline values, and questioning for state of health was conducted. Then, participants randomly received either placebo or propranolol. During the time needed (90 min) for propranolol to reach the plasma peak levels, HR and BP were controlled. Shortly before stimulus presentation BP, HR and sAA[21] were determined again as markers of adrenergic activity. In addition, adverse events were checked. Stimulus presentation started 90 min after drug

[21] sAA was collected once again after the stimulus presentation, but was not further analyzed.

administration. Starting with the end of the stimulus presentation, heart rate and blood pressure were being determined continuously until return to baseline values. Adverse events (AE)-questioning was conducted every 2 h until leaving the department. The same procedures were implemented during the first and second encoding session. According to the crossover design, participants who were administered with propranolol on the first session received placebo on the second session, and participants who received placebo on the first, underwent the propranolol treatment on the second session.

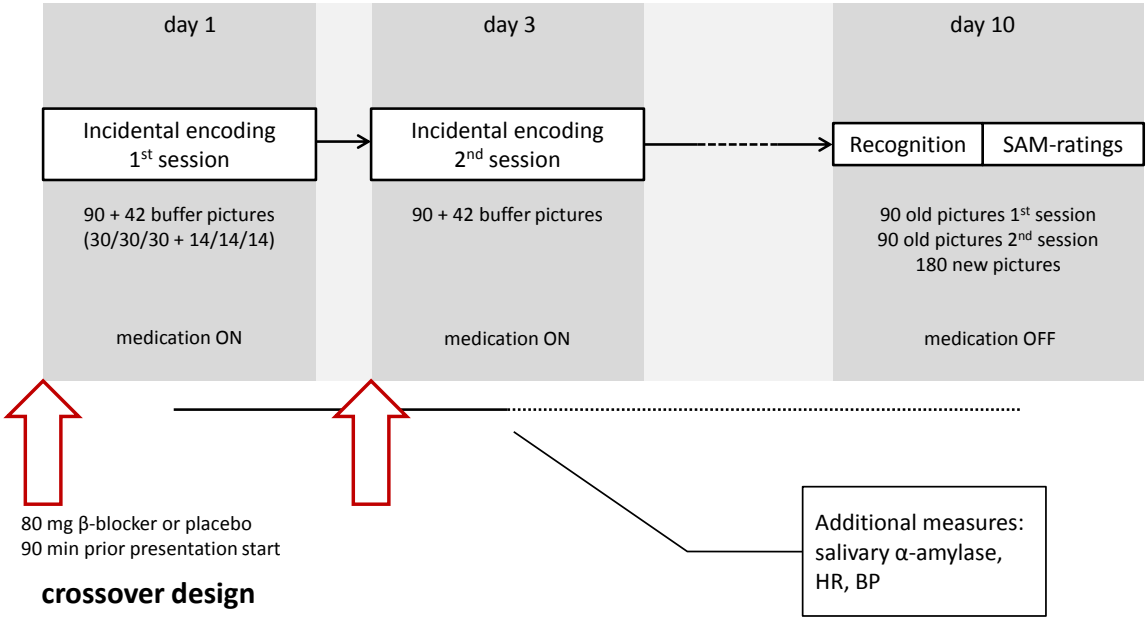

Figure 12. Scheme of the experimental procedure introduced in the study. Description in text. Medication was administered 90 minutes before the beginning of picture presentation on days 1 and 3 in crossover design during two incidental encoding sessions, to one group of participants. Pictures presented in the first and second encoding session differed. The recognition task, where all old pictures intermixed with new pictures were presented, was conducted without any medication. On days 1 and 3 additional peripheral measures were collected. Abbreviations: SAM – Self-Assessment Manikin, HR – heart rate, BP – blood pressure.

7.3.5.4 Experimental procedure

The subjects were seated in a reclining chair in a sound-attenuated, dimly lit room. The stimuli were displayed on a 20 in computer screen and the EEG-signal was recorded. During the tasks, the participants were instructed to fixate the center of the screen and to reduce eye-blinks and avoid body movements. The participants were told to break the experiment immediately by pressing the alarm button if they experienced any physical inconvenience.

Both stimulus presentation and experimental tasks were the same as in Study 2 and are in detail described in 6.2.3.

On the first study day (day 1), 90 min after drug administration participants viewed series of emotional and neutral pictures. During the presentation, EEG was recorded. The subjects were instructed to attend the stimuli carefully. No mention of the future memory test was made to avoid cognitive effects on emotional memory (e.g., subjective strategies). They were instructed that the experiment is designed to test the impact of β-blockers on electrocortical and peripheral physiological activity during processing of emotional pictures. An explanation of the study goals was given at the end of the third laboratory session 10 days later.

Procedures in the second encoding session (day 3) were the same as in the first encoding session

In the third session (day 10) the participants returned to the laboratory. This session contained a stimulus recognition task and a stimuli rating task and in all cases took place before noon.

7.3.6 EEG-recording

The EEG signal was continuously recorded from 129 electrodes using an Electrical Geodesics® (EGI) Geodesic Sensor Net 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 50 kΩ, as recommended by the manufacturer. All channels were band-pass filtered online from 0.1 to 30 Hz in order to eliminate slow shifts in the voltage as well as the line-frequency noise (40 Hz) received in the recording room. Offline, the data was analyzed in the same way as in Study 2 (cf., section 6.2.4). Although the original data was low-pass filtered at 30 Hz online, the data was additionally offline low-pass filtered at 40 Hz in order to ensure that the high-frequency components of the signal are sufficiently attenuated and the data can be compared across studies conducted in our laboratory.

7.3.7 Data analysis

ERPs were computed for each sensor and participant. Based on visual inspection of the scalp voltage distributions and ERP waveforms, sensors' clusters and time intervals were selected where the differences between conditions in the ERP data were most pronounced for further analyses. Cluster-averaged mean ERP amplitudes were analyzed in 550-700 ms time-windows for encoding and recognition, and additionally 550-1000 ms for encoding over central (cf., Figure 21) brain regions[22].

The ERP data were analyzed using repeated measures ANOVA. The analysis aimed at assessing the study medication effects on emotional modulation of the ERPs during encoding and retrieval. For encoding data, the analyses were performed with factors Hedonic Content (unpleasant vs. neutral vs. pleasant) and Drug (propranolol vs. placebo). For recognition data, the analyses were conducted with factors Hedonic Content (unpleasant vs. neutral vs. pleasant), and Memory/Drug (old propranolol vs. old placebo vs. new). For ERPs, only trials with correct behavioral responses (hits and correct rejections) were used in the averaged ERP data.

For memory performance, Hit rate (H), false alarm rate (FA) and recognition accuracy (Pr = H – FA) were analyzed. Interactions with study medication were tested, where applicable.

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

7.4 Results

The preliminary results reported below originate from a small sample and therefore are to be regarded highly explorative. Although in such a small sample one cannot reliably interpret the obtained results, for readability reasons, I use the derivates of words "modulation", "influences", "effects" or "differences", etc. when reporting the

[22] The somewhat longer analysis interval for LPPs (500-1000ms) was dictated mainly by the visual inspection of the waveforms. Although not often analyzed and reported in the literature, the LPPs usually last for several milliseconds after stimulus onset (Cuthbert et al., 2000).

preliminary results. Additionally, I indicate the calculated statistical test and *p*-values to quantify tendencies in data.

7.4.1 Subjective valence and arousal ratings

The subjective valence and arousal ratings of single stimulus sets are reported in the stimulus material section (cf., section 7.2.4). Figure 16 shows rated valence (upper panel) and arousal (lower panel) of experimental stimuli in respect to experimental condition (old stimuli in propranolol and placebo condition, and new stimuli).

Overall, unpleasant stimuli were rated as more unpleasant as neutral and pleasant stimuli, and pleasant stimuli as more pleasant as neutral ones, Emotional Content: $F(2,12) = 152.26$, $p < .001$. The rated valence was not influenced by drug administration, Emotional Content × Drug: $F(4,24) = 1.81$, $p = .17$.

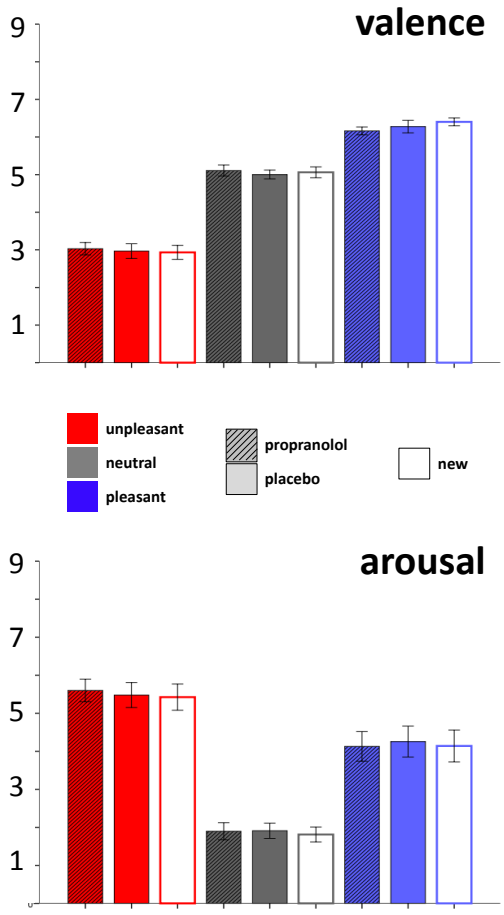

Figure 13. Mean rated valence and arousal of the experimental stimuli. Mean values for unpleasant pictures are depicted in red, for neutral in grey, and for pleasant in blue. Striped bars represent old stimuli encoded after propranolol administration, solid bars old stimuli encoded after placebo administration; new stimuli are depicted by bars with no filling. Error bars represent standard errors.

According to arousal, emotional pictures (both unpleasant and pleasant) were rated as more arousing than neutral pictures, $F(2,12) = 87.84$, $p < .001$, and unpleasant as more arousing as pleasant $F(1,6) = 16.42$, $p < .01$. There rated arousal was not influenced by drug administration, Emotional Content × Drug: $F < 1$.

7.4.2 Memory performance

Figure 17 shows hit rates and discrimination indexes for both old stimulus types encoded after propranolol and placebo administration as well as false alarm rates. Overall, the emotional content modulated the hit rates for the stimuli, Emotional Content: $F(2,12) = 10.71$, $p < .01$. Unpleasant pictures were better recognized (hit rates) as pleasant [$F(1,6) = 8.56$, $p < .05$] and neutral [$F(1,6) = 18.59$, $p < .01$] stimuli. Pleasant stimuli were associated with slightly higher hit rates than neutral stimuli, $F(1,6) = 4.54$, $p < .08$. False alarm rates differed slightly between the emotional categories, Emotional Content: $F(2,12) = 3.46$, $p = .07$, and were higher for both neutral and pleasant than for unpleasant stimuli. Correct discrimination was also modulated by the emotional content of the stimuli, $F(2,12) = 20.03$, $p < .001$, yielding the previously reported pattern of results (cf., section 6.3.2) with better discrimination performance for unpleasant than for both pleasant and neutral pictures. In addition, visual inspection of Figure 17 (but not statistical values) indicates that memory performance for emotional stimuli, as indexed by both hit rate and discrimination index, tended to be lower after propranolol administration, Emotional Content × Drug: $F(4,24) = 1.21$, $p = .30$, both.

7.4.3 Salivary α-amylase

The levels of sAA at baseline did not differ[23] between drug conditions, $t(6) = .01$, $p = .99$. The sAA level at the time of encoding was modulated by drug administration, $F(1,6) = 76.04$, $p < .001$. At the time of encoding, sAA was lowered as compared to baseline at a trend level after propranolol administration, $t(6) = 2.28$, $p = .06$. Interestingly, in placebo condition at the same protocol time-point, peripheral enzyme level was heightened in comparison to baseline, $t(6) = 4.45$, $p < .01$. Additionally, the levels differed between conditions at the time of encoding, $t(6) = 7.51$, $p < .001$. The results are depicted in Figure 18 and exact values summarized in Table 6.

[23] In this section all *t*-tests were computed for dependant samples. Reported are two-way significances.

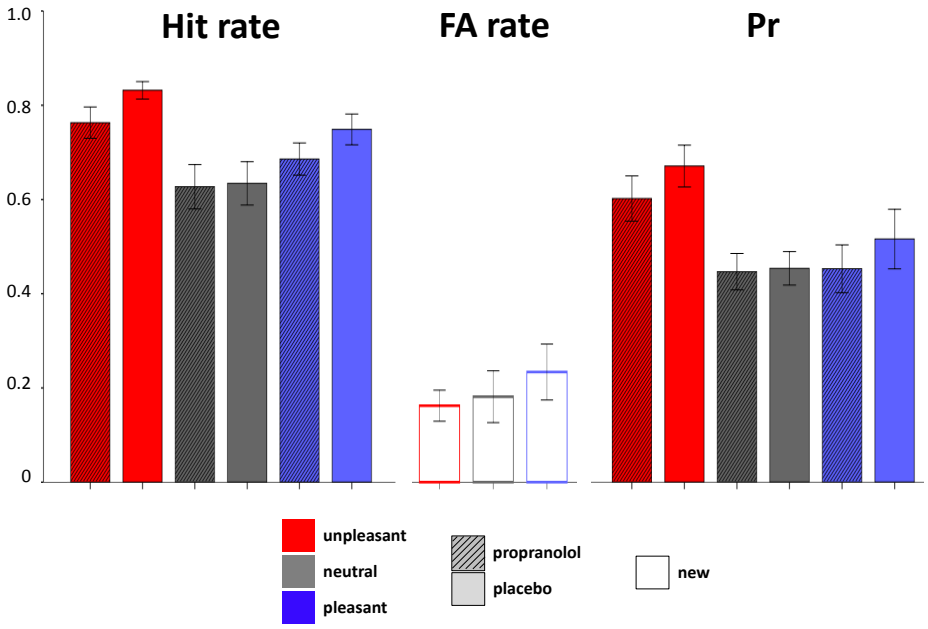

Figure 17.. Memory performance as represented by mean hit rates, false alarm (FA) rates and correct discrimination (Pr, hit minus FA) for unpleasant (red), neutral (black) and pleasant stimuli. Striped bars represent old stimuli encoded after propranolol administration, solid bars old stimuli encoded after placebo administration; new stimuli are depicted by bars with no filling. Error bars represent standard errors.

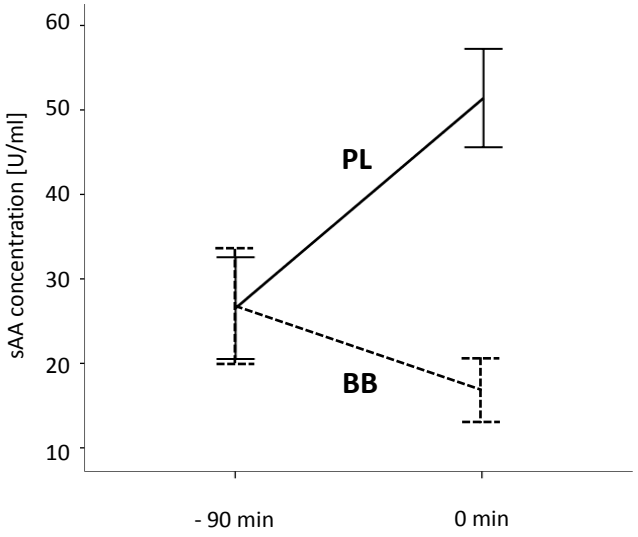

Figure 14. Mean salivary α-amylase concentrations at baseline, before drug administration (-90 min) and shortly before stimulus presentation (0 min) at encoding sessions. The values are given in units per milliliter (U/ml). Used abbreviations: PL – placebo, BB – propranolol. Error bars depict standard errors.

7.4.4 Heart rate and blood pressure

The results for blood pressure and heart beat values are summarized in Table 6.

Table 6. Blood pressure, heart frequency, and salivary α-amylase values.

Measure type	PL (-90 min)	PL (0 min)	BB (-90 min)	BB (0 min)
Sys [mmHg]	124.3 (11.8)	129.1 (12.2)	119.9 (14.2)	117.3 (7.6)
Dias [mmHg]	74.3 (5.1)	80.6 (7.4)	75 (6.5)	76.9 (4.5)
HR [bpm]	62.7 (7.9)	62.6 (7.1)	60.4 (3.6)	54.7 (6.6)
sAA [U/ml]	26.6 (18.2)	51.4 (15.4)	26.5 (15.93)	16.6 (10.0)

Note: All measures were collected during first and second encoding session. Mean values are depicted for both placebo (PL) and propranolol (BB) conditions at two time points: at baseline, before drug administration (-90 min) and shortly before picture presentation (0 min). Used abbreviations: Sys – systolic blood pressure, Dias – diastolic blood pressure, HR – heart rate, sAA – salivary α-amylase. Standard deviations are given in parentheses.

The blood pressure was comparable[24] in both drug conditions at baseline, systolic: $t(6) = .53$, $p = .62$; diastolic: $t(6) = .28$, $p = .79$. The systolic blood pressure was not further affected by the administered drug, $F(1,6) = .69$, $p = .44$. In contrast, the diastolic blood pressure was modulated in dependence of drug at the time of encoding, $F(1,6) = 8.61$, $p < .05$. As compared to baseline, diastolic blood pressure was heightened at the time of encoding, $t(6) = 4.46$, $p < .01$, in the placebo condition. No further differences were observed.

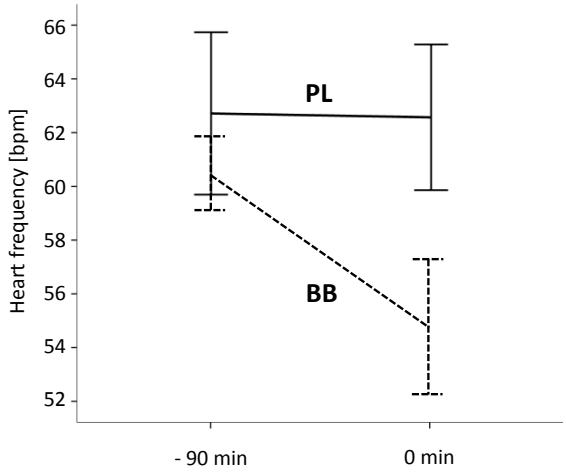

Figure 15. Mean heart frequency at baseline, before drug administration (-90 min) and shortly before stimulus presentation (0 min) at encoding sessions. The values are given in beats per minute (bpm). Used abbreviations: PL – placebo, BB – propranolol. Error bars depict standard errors.

Similarly, heart rate at baseline was the same in both conditions, $t(6) = 1.03$, $p = .34$. The administered drug influenced the heart measures, Time × Drug:

[24] In this section all *t*-tests were computed for dependant samples. Reported are two-way significances.

$F(1,6) = 3.79$, $p > .1$., lowering the frequency of heart beat at encoding after propranolol administration, propranolol baseline vs. encoding: $t(6) = 2.80$, $p < .05$. In addition, the heart rate at encoding was greater after placebo than propranolol administration, $t(6) = 4.88$, $p < .01$ (cf., Figure 19).

7.4.5 Event-related potentials

7.4.5.1 Encoding

At central sensor sites, in the 550-1000 ms time-window, overall, the ERP amplitudes were enhanced for emotional pictures in comparison to neutral pictures, Emotional Content: $F(2,12) = 7.71$, $p < .01$, cf., Figure 20). The administration of propranolol seemed to have enhanced the emotional minus neutral differences, especially for pleasant stimuli (cf., Figure 20, inset), Emotional Content × Drug: $F(2,12) = 2.04$, $p = .17$. Analyses over a shorter time-interval (550-700 ms) revealed a similar pattern of results.

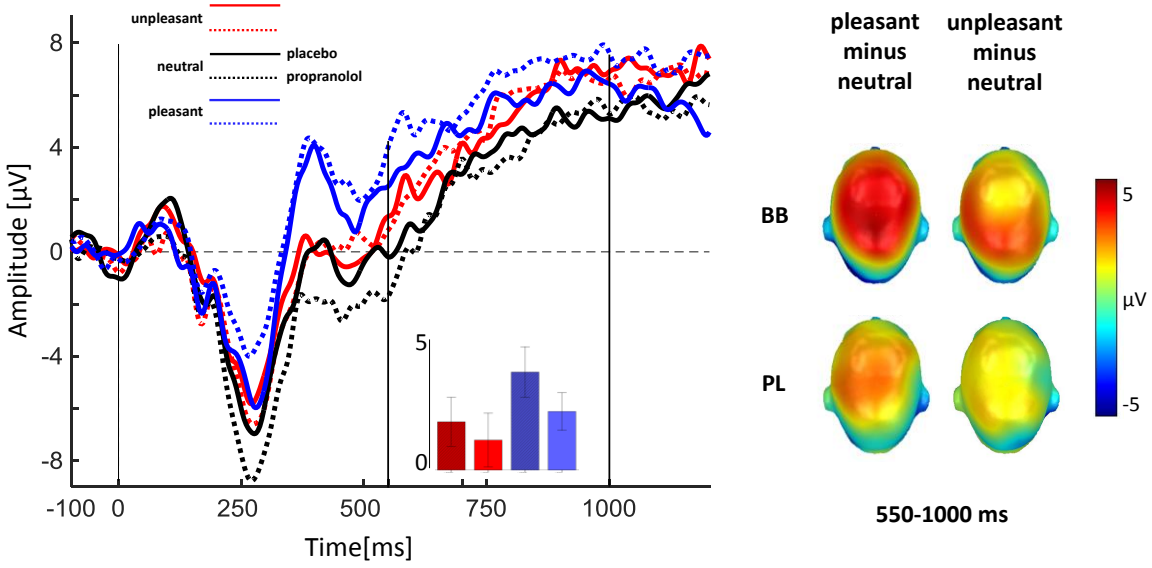

Figure 16. Left panel: Grand average ERP waveforms for stimuli presented after placebo (solid line) and propranolol (dashed line) administration, averaged within the analyzed central cluster. Unpleasant pictures are depicted in red, neutral in black, and pleasant in blue. The analyzed time-window 550-1000 ms is indicated by vertical lines. The inset represents mean difference values between unpleasant and neutral stimuli in propranolol (dark red) and placebo (light red) condition, and pleasant and neutral stimuli in propranolol (dark blue) and placebo (light blue) condition. Right panel: Mean overall scalp voltage differences between ERP waveforms for pleasant and neutral (left), and unpleasant and neutral (right) in propranolol (BB) and placebo (PL) condition in the 550-1000 ms time-window.

7.4.5.2 Recognition

Figure 21 displays recognition memory ERPs for the analyzed sensors' cluster and scalp voltage distributions for old minus new ERP waveforms in the recognition task.

In the time-window 550-700 ms over central brain regions, overall, the ERP waveforms seemed to be modulated by drug administration, Memory/Drug: $F(2,12) = 2.50$, $p = .12$. ERP amplitudes for old pictures after propranolol administration did not differ from those for new pictures, whereas waveforms for old pictures learned under placebo condition showed a difference as compared to new pictures, old placebo vs. new: $F(1,6) = 5.71$, $p = .05$; old propranolol vs. new: $F(1,6) = .94$, $p = .37$. This effect was probably due to selective attenuation of the old/new effects for emotional stimuli, Emotional Content × Drug/Memory: $F(4,24) = 1.20$, $p = .34$ (see Figure 21, lower panel).

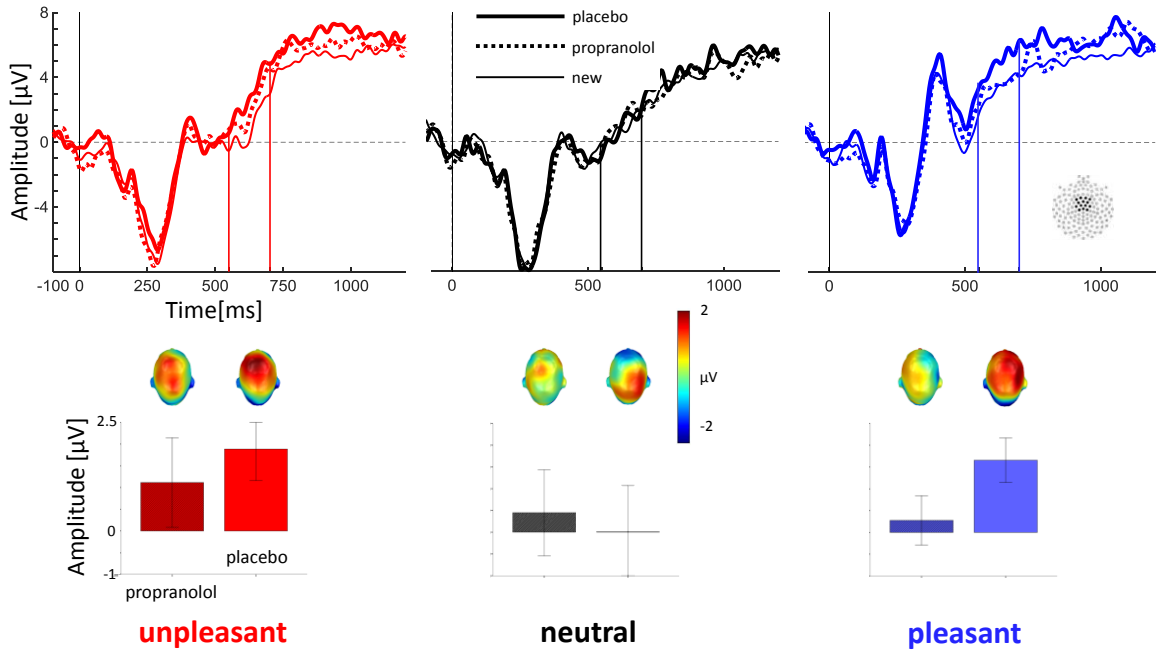

Figure 17. Upper panel: Grand average ERP waveforms for correctly recognized (old) pictures after placebo administration (thick line), correctly recognized (old) pictures after propranolol administration (dashed line), and correctly rejected (new) pictures averaged within the central cluster (depicted by the inset to the right) during retrieval task. Unpleasant pictures are depicted in red, neutral in black, and pleasant in blue. The late 550-700 ms time-window, where the old-new differences were mostly pronounced, is indicated by vertical lines. Lower panel: The bars represent mean difference values between old and new stimuli in propranolol (striped) and placebo (solid) condition for all three picture categories. Error bars represent standard errors. Middle panel: Mean scalp voltage differences between ERP waveforms for correctly recognized (old) and correctly rejected (new) stimuli (old/new effects) in the analyzed time window in propranolol (left) and placebo (right) condition for all picture categories.

7.5 Discussion

The goal of this clinical, double blind, crossover trial was to determine influences of a beta-blockade on emotional memory formation and retrieval, as well as the interplay between the β-adrenergic receptor polymorphisms, emotional memory processes and the beta-blockade. The sample in this study was in most cases too small to obtain significant and reliable results. Especially, for the reasons explicated in section 7 we waived considering the genotype data in further analyses. The remaining preliminary data were analyzed in an explorative manner and should be regarded as revealing directions of beta-blocker influences on emotional memory processes. However, the reported data are by large coherent with previous findings concerning emotional modulation of episodic memory and beta-blocker influences on it.

In general, emotional pictures seemed to be better remembered than neutral ones, replicating previous findings (e.g., Bradley et al., 1992; Weymar et al., 2009). During encoding, the late positive potentials seemed to be enhanced for emotional stimuli, as in previous findings (Schupp et al., 2006). Interestingly (and similar to Study 2), pleasant pictures were associated with numerically highest LPP amplitudes[25]. Similar to previous studies (e.g., Weymar et al., 2009, 2013; Schaefer et al., 2009, 2011; as well as studies reported here: Study 1 and 2) the ERP old/new effects for emotional pictures seemed to be enhanced in comparison to neutral pictures.

At the time of encoding, the beta-blockade was probably active after propranolol administration, as indexed by peripheral measures[26]. At recognition, both the enhanced

[25] The LPPs, similar as in Study 2, seemed to be enhanced for pleasant as compared to unpleasant stimuli. This uncommon pattern of results has already been discussed earlier (cf., section 6.4). Interestingly, the pattern emerges for the second time when using the same set of pleasant stimuli (see Study 2).

[26] The beta-blockade that was probably active at the time of encoding lowered the heightened in the placebo condition diastolic blood pressure. Moreover, it attenuated the increase of the activity of the peripheral marker of the noradrenergic system activation (salivary α-amylase) observable in the placebo condition. Interestingly, unlike in Weymar et al. (2010b), the salivary enzyme levels and blood pressure did numerically increase in comparison to baseline after drug administration, as demonstrated in the placebo condition. This might be explained by a general, non-specific arousal increase solely affected by study medication administration. It would be plausible that participants reacted with heightened psychological stress to the experimental situation. The 50% chance of getting the target medication might have caused a certain excitement state in which the participants awaited the possible propranolol effects to set in. In addition, it is also probable that the subjects might have taken the briefed adverse effects of propranolol very seriously, which might have been strengthened by the study trial context. Unlike in other propranolol studies (Weymar et al., 2010b; de Rover et al., 2012), where the here reported effects were not observed, the physical environment of this trial reminded of a hospital environment. The study site is a partially

memory performance for emotional stimuli and the ERP old/new effects, especially the memory effect for pleasant stimuli, seemed to be selectively blocked by propranolol.

The memory performance for emotional events, mostly hit rates, seemed to be reduced after propranolol administration, reaching the memory performance level of neutral material used in the study. This preliminary result is in line with a great amount of findings revealing emotional memory performance decrement caused by beta-blocker (Cahill et al., 1994; Maheu et al., 2004; O'Carroll et al., 1999; Schwabe et al., 2013; Strange et al., 2003; van Stegeren et al., 2005, 1998).

In line with previous studies (Schaefer et al., 2011; Weymar et al., 2009; Wirkner et al., 2013) and similar to the studies reported here (Study 1 and 2), the general old/new effect during recognition showed some modulation by emotional content of the pictures after a one week retention interval. It has been proposed, that the enhancing effect of emotion is stronger after longer retention intervals, because of the influences of the noradrenergic system on consolidation processes (McGaugh, 2004). Similarly as in Weymar et al. (2010b) the active beta-blockade during encoding might have had a selective impact on memory-related ERPs. The emotional memory advantage in retrieval, as suggested by enhanced ERP old/new effects, seemed to be attenuated after propranolol administration at encoding, as compared to placebo condition, which is in line with the results reported by Weymar et al. (2010b). The data suggest that propranolol probably selectively targeted the consolidation processes, which, in line with the modulation hypothesis (cf., section 3.1), prioritizes the processing and storage of emotional events in memory.

In contrast to ERP and memory performance measures, the beta-blockade did not change the participants' subjective evaluation of valence and intensity of the experimental

restricted area, specially designed for pharmacological studies and visibly equipped with ER-apparatus, life-sustaining devices, and other medical accessories making the impression of a hospital department rather than a typical study site one usually encounters in psychological departments. Additionally, the medical staff involved in the study wore doctor's gowns, the vital signals were continuously monitored and the participants underwent mild invasive medical procedures (e.g., blood withdrawal) several times during the day. Taken together, all these factors might have confusingly affected the participants' perception of the probability of adverse events and their severity, resulting in heightened arousal measures after study medication administration. Similar results in respect to salivary α-amylase were reported by van Stegeren et al. (2006). The authors observed an increase in sAA levels in the placebo condition, as compared to baseline and beta-blocker, shortly before the participants entered an MRI scanner. The authors interpreted these changes in sAA levels as changes in sympathetic activation in reaction to psychological stress.

stimuli. The data fit the results pattern reported by Weymar and colleagues (2010b) and are in line with previous results (reviewed by Lonergan et al., 2013).

Interestingly, unlike in Weymar et al. (2010b), LPPs elicited by emotional pictures, especially pleasant, were slightly increased by the blockade of the noradrenergic system. The numerical increase of LPPs might be, of course, on the one hand, an artifactual modulation in this small group of participants. On the other hand, however, we may also hypothesize that the enhancement of the LPPs observable here might be associated with greater arousal at the time of encoding. Recent results reported by de Rover et al. (2012) demonstrate that increased LPPs for emotional stimuli after propranolol administration are associated with higher anxiety and arousal levels in the investigated sample. Indications for heightened arousal levels at the time of encoding in our study, as the peripheral measures in the placebo condition showed, together with heightened LPPs after propranolol administration fit in the response pattern reported by de Rover and colleagues (2012)[27]. This interesting issue should become a subject of further investigations.

This study, although its results are explorative, has shown a tendency that a pharmacological beta-blockade influences the emotional memory-related processes, as indexed by diminished retrieval ERPs. Based on a previous study conducted in our department (Weymar et al., 2010b), we were able to replicate the existing data. The crossover design of this study gave us the advantage that each participant was his own perfectly matched control, which may very well explain the missing differences at baseline (prior study medication administration) in physiological data (heart rate, blood pressure, salivary α-amylase) and stress the preliminary found differences in other data in

[27] This rather unexpected finding could find explanation in results reported recently by de Rover and colleagues (2012). When examining the influence of a beta-blockade on encoding ERPs they found, similar as Weymar et al., (2010b), no attenuation of emotional LPPs after propranolol administration, in the examined sample. However, after the sample was split according to basal anxiety levels, a numerical raise of LPPs associated with emotional stimuli after propranolol treatment in the high-anxiety group arose. In our experiment, we did not collect any anxiety measures. On the one hand, in our sample there were only men, who generally report less fear and anxiety than women (McLean & Anderson, 2009). This may suggest that our participants were more similar to the low-anxiety sub-sample in the de Rover et al. (2012). However, the physiological data at the time of encoding might suggest that the participants might have been aroused before (and probably during) stimulus presentation, probably making the basal arousal level in this sample similar as in the high anxiety group from the de Rover et al. study.

such small sample. Here, we showed once again that propranolol probably selectively targeted the consolidation of emotional memories.

These preliminary data might serve an important impulse for further investigations on propranolol influences on memory formation, especially on beta-blocker impact on encoding processes dependent on the basal level of arousal. A recent review paper on propranolol effects on consolidation and reconsolidation of long-term emotional memory (Lonergan et al., 2013) has demonstrated, that propranolol is an effective agent in reducing memory or its physiological manifestations for emotionally arousing material in healthy population. Nevertheless, comprehensive research on beta-blocker effects on emotional responding and memory in clinical populations is missing.

Post-traumatic stress disorder (PTSD) might develop after an exposure to a life-threatening event. One of the features of the disorder is a continuous occurrence of intrusive memories that cause an arousing re-living of the experienced trauma. The disturbing traumatic memories are commonly explained as being the consequence of an enhancement in memory encoding induced by the traumatic event (Pitman, 1989). There is also evidence for heightened arousal, as indexed by physiological function (heart rate, blood pressure) as well as elevated levels of norepinephrine in the cerebrospinal fluid in PTSD patients and their co-occurrence with the severity of the disorder, suggesting noradrenergic activity's involvement in the maintenance of PTSD symptoms (Geracioti, Baker, Ekhator, West, Hill, Bruce …, & Kasckow, 2001). There is only one study which demonstrated that emotional memory reduction is possible in a PTSD sample (Reist et al., 2001), but this memory involved emotionally narrated slides (as in Cahill et al., 1994) and did not refer to highly arousing traumatic memories. Whether propranolol would be a promising solution in treatment of trauma memory, still remains unclear. On the one hand, there are some results showing a certain reduction of physiological responding and PTSD symptoms in anxiety patients after propranolol treatment (Brunet, Orr, Tremblay, Robertson, Nader, & Pitman, 2008; Pitman, Sanders, Zusan, Healy, Cheema, Lasko…, & Orr, 2002), but on the other hand studies testing propranolol's preventive role in PTSD development directly after trauma exposure have shown conflicting results (Pitman, Miland, Igoe, Vangel, Orr, Tsareva…, & Nader, 2011). Moreover, to my best knowledge, there are no studies showing a reduction of trauma memories caused by propranolol. In addition, the data about genetic underpinnings of

PTSD development and their interactions with noradrenergic agents is spare (de Quervain, Kolassa, Ertl, Onyut, Neuner, Elbert, & Papassotiropoulus, 2007). Therefore, seeing the possible application of beta-blockers in the clinical context of treatment of disorders having as their core a disrupted emotional memory (e.g., anxiety disorders as PTSD, and addictions), it is highly desirable to further investigate the emotional memory modulation as well as the genetic influences on emotional processing and memory formation in dependence on noradrenergic system in both healthy and clinical samples.

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8 Summary and final discussion

In this dissertation, I reported the results from three ERP studies that I conducted during my doctoral studies at the Department of Biological and Clinical Psychology at the University of Greifswald. The impulse for conducting these studies came two influential concepts of emotional impact on memory formation: the concept of natural selective attention, introduced and over years elaborated by Margaret Bradley and Peter Lang, and numerous collaborators, and the neuromodulation hypothesis elaborated by James McGaugh. According to the first concept, emotionally significant stimuli engage one of the motivational systems, appetitive or aversive. The activation of one of the systems results in a cascade of perceptual and motor processes that facilitate the selection of behaviors that have developed to support survival. The activation is observable in different phenomena, like for example ERPs, indexing enhanced perceptual processing of emotionally significant stimuli. The neuromodulation hypothesis stresses the central role of the amygdala, stress hormones and neurotransmitters, and their interactions with other brain regions in formation of long lasting (i.e., consolidation of) emotional memories. Additionally, a strong impact on the form of the conducted experiments originates from the work of Mathias Weymar on the influences of emotional contents on memory-related ERPs. Next to interpreting the results in the light of hitherto existing findings and mentioned concepts, I also put the here obtained results in a broader context of emotional episodic long-term memory and discuss the possible implications for clinical research.

In the reported studies I explored emotion-related influences on episodic memory. Before I proceed with the emotional effects and single studies separately, I briefly focus on episodic memory itself.

In section 2.1, a summary proposed by Rudy (2014) of episodic memory characteristics was provided. With the studies I demonstrated that all results reported here satisfy these characteristics. First, the successful memory search was initiated either intentionally (Study 1-3) or incidentally (categorization task in Study 1) and it might be assumed that the retrieval from episodic memory was either based on familiarity (categorization task in study 1) or recollection (Study 1-3), as indirectly indicated by

related ERPs. Second, the information was probably stored without any learning effort in incidental encoding sessions in all studies, where the participants did not expect the following recognition test. Moreover, this fact is additionally validated by data from an unpublished study with an incidental encoding session, which I conducted, but which is not reported here. In this study, no participant, when explicitly asked after finishing the study, was expecting a following memory test. Third, the high memory performance in all studies proves that most of the presented stimuli were stored as single events (with exception of some emotional stimuli) and eventually successfully retrieved. Finally, the learned stimuli were stored in long-term memory, as indicated by successful retrieval one week after encoding.

The reported results from three studies demonstrate that both memory effect and emotional influences are strong and stable. Over all studies, the memory for learned stimuli persisted over a one-week retention interval. Moreover, these memory effects were continuously enhanced by emotional content of the stimuli, independently on the retrieval mode, i.e., explicit or implicit. This effect was probably mediated by noradrenergic activation (within the amygdala), as the preliminary results from the last study suggest. Here, further proof is provided for the broadly accepted opinion that emotional events are better remembered than neutral ones. In addition, these effects are accompanied by electrophysiological measures, as observed in enhanced ERP old/new effects for emotional stimuli.

Particularly, the first study showed that emotional events tend to elicit spontaneously arising memories, as it has been demonstrated for autobiographical memory, even one week after encoding, and that this phenomenon is probably mediated by familiarity processes. Although we did not control for that, it is probable that participants in the categorization task did explicitly recognize the presented emotional stimuli. According to the Memory As Reinstatement model, described in section 2.1.1, retrieval cues are first processed in the hippocampus, from where they activate the cortical representations of the event. When the cortical representations are activated, a feeling of remembering emerges, mediating the conscious recognition in episodic memory. Moreover, the stimuli were encoded incidentally. The process of encoding of information into episodic memory does not require any intention. The episodic memory

system has the ability to automatically capture information about the events, simply while exploring and experiencing (Rudy, 2014). It might have been the case in our participants that they were able to access the episodic representation of the event together with contextual information, e.g., where and when they have seen the stimuli before. The concurring task, i.e., categorization, however, probably inhibited the full recollection, as it was demonstrated by an absent parietal ERP old/new effect, allowing recognition based on familiarity processes (as indexed by frontal old/new effect), accompanied by a certain feeling of remembering. As mentioned in section 2.1.3, the familiarity process runs automatically and, as opposed to recollection, is not prone to distractions during retrieval. Recollection, as more elaborated, controlled processes seemed to be distracted by a concurring task at retrieval after a one-week retention interval. Familiarity, however, remained unaffected by the categorization task and took place automatically and in parallel to it. Moreover, the effect of spontaneous remembering was only present for emotional pictures, which were processed in a more elaborative manner during the incidental encoding. This let us conclude that the categorization task has overshadowed recognition of weaker encoded neutral stimuli. Moreover, it once again provides an argument for the special role of emotional stimuli in emotional processing and memory, for which memory traces were so strong, that a single recognition cue in a primarily different task was able to automatically elicit spontaneous recognition processes.

Tulving (1985) proposed that familiarity processes depend on semantic memory (abstract knowledge that an event had happened) and are accompanied by noetic consciousness (cf., sections 2 and 2.3.1). He introduced the term of episodic memory to describe a specific state of remembering, which is characterized by the ability of the "mental time travel" (Tulving, 2002) and recollecting the facts around an event. According to the spontaneous remembering data, it is possible that at retrieval the participants were not able to go on the "mental time travel", for the trivial reason of insufficient time and resources. They were occupied by a different task, and the task was changing every few seconds. This probably enabled the participants to shortly recognize the stimuli as seen before, without any additional information. This interpretation is additionally supported by assumptions included in dual-process models, which propose that familiarity and recollection processes are independent and take place in parallel:

familiarity processes are automatic and depend on memory strength whereas recollection needs a conscious and time-consuming search in memory (Yonelinas, 2002).

In the second study, the memory one week after encoding was assessed in a direct way only, where the recognition performance was tested explicitly. There were several reasons for doing so. First, the second study was thought to test a modified recognition memory paradigm for a clinical trial with a pharmacological beta-blockade. The stronger episodic memory for emotional events, which is at least in part mediated by the noradrenergic system, rather relies – according to numerous research findings – on recollection than familiarity. Second, the first study demonstrated that spontaneous remembering of emotional events (as tested indirectly) after one week is mediated by familiarity processes. In contrast to recent studies using a very short retention interval (e.g., Weymar et al., 2013), it was not possible to engage recollection processes with indirect memory assessment after one week. Third, the longer retention interval used here, enabled the consolidation process to happen and to strengthen the memory traces for motivationally significant stimuli.

This study demonstrated that the stable memory for emotional events does not change, even if the tested items are learned in two consecutive, separate encoding sessions. This is the first time this recognition memory paradigm was tested, although similar procedures have already been introduced earlier in two propranolol studies (de Rover et al., 2012; van Stegeren et al., 2005).

In reviewing the effects of noradrenergic modulation on episodic memory, Chamberlain and colleagues (2006) raised the already mentioned problem of practice effects in neuropsychological tests which are possible when testing memory in within-subjects designs. They proposed that a method addressing the issue of practice effects is to train participants up to a certain level of baseline performance before proceeding with the study tasks (*Ibid.*). Here, an alternative method is proposed, which bypasses the additional training session prior the proper experiment. The results from the second study validated the possibility of testing the recognition memory in a new paradigm with two separate encoding sessions, without having any confounding practice or sequence effects. Any possible sequence effects, which were indicated in this study, or further unexpected effects, are easy to minimize through appropriate randomization and counterbalancing means. Consequently, the study opened the possibility to test the effects

of a pharmacological beta-blockade on recognition memory in a crossover design. The application of the crossover design helps to significantly reduce the sample sizes, which might be of great interest, e.g., when exploring the genotype influences on memory processes in pharmacological studies.

For the purpose of the third study, the results from the second study were utilized and the modified paradigm in testing the recognition memory with two consecutive encoding sessions in a within-subject design was introduced. Moreover, based on experiences from the former study, elegant counterbalancing and randomization means were used in order to minimize the potential sequence effects that could not have been definitely excluded before.

The preliminary results from the third, pharmacological crossover study demonstrated that a pharmacological beta-blockade at the time of encoding probably affects the memory consolidation processes for emotional stimuli, and in acting so, it reduces the emotional memory enhancement and memory-related ERP old/new effects. These results are in line with findings stressing the role of the noradrenergic system and the amygdala in emotional memory formation (McGaugh, 2004). In addition, the results from this crossover study overlap with those presented by Weymar and colleagues (2010b), who collected the data in a parallel group study. By giving some insights into directions for further research on the effects of propranolol on encoding and memory formation of emotional material, the preliminary results complement the hitherto existing body of knowledge that might be utilized in search for proper treatment of PTSD.

The great disadvantage of the study is the absence of ERP results in dependence of β-adrenoreceptors genotype. The crossover design in this study was purposely applied in order to analyze four genotype combinations within one study and using a sample as small as possible. Unfortunately, the number of available and willing to participate young men with the appropriate genotype in the available database was too small to achieve this goal and to be able to test the primary objectives of the study. These tight inclusion criteria have additionally negatively influenced the total number of participants who participated in the study.

For we were not able to recruit a sufficient number of participants for the study, we failed to answer the primary questions about effects of β_1- and β_2-adrenergic receptor

polymorphisms on emotional memory formation and on the demonstrated propranolol influences on emotional memory. The variations of ADRB1 have been shown to modulate the LPPs at encoding (de Rover et al., 2012). By now, the ADRB2 has not been explored in context of emotional processing or memory, however, since propranolol is a non-selective $\beta_1\beta_2$-adrenergic receptor blocker, it would be interesting to see whether functional changes at the receptor associated with variants of the ADRB2 rs1042713 and rs1042714 (cf., Appendix III) would have any impact on propranolol effects on emotional memory. This aspect remains an open issue worth further explorations, especially in the context of findings about deletion variant of α-adrenergic receptor polymorphism. This polymorphism has been associated with increased amygdala activity during encoding of emotional stimuli (Rasch et al., 2009), seems to mediate enhancement of emotional memory in healthy populations and might play a role in development of symptoms in PTSD populations (de Quervain et al., 2007).

Different types of interfering and strengthening effects on memory indicate the presence of multiple phases or processes that build the temporal development of memory formation (Inda, Muravieva, & Alberini, 2011). The presented studies focus on consolidation processes only. The existing and stabile memory traces (i.e., consolidated memory) may once again become labile as a consequence of retrieval or reactivation. This process has been termed reconsolidation, suggesting a repeated consolidation process. The differences and similarities of both processes are significant subjects of current research and a widely explored topic in the ongoing debate (Nader & Einarsson, 2010). Nevertheless, reconsolidation, similar to consolidation, can also undergo manipulations, resulting in changes of the present memory trace.

The existing human studies exploring the modulation of non-declarative forms of memory after reactivation of consolidated memory have demonstrated reconsolidation impairments in fear conditioning (Schiller, Monfils, Raio, Johnson, LeDoux, & Phelps, 2010) fear potentiated startle (Kindt et al., 2009), and motor sequence learning (Walker, Brakefield, & Hobson, 2003). Moreover, within declarative memory, it has been shown that stress (Schwabe & Wolf, 2009) and new learning (Schwabe & Wolf, 2009; Wichert, Wolf, & Schwabe, 2013; Wirkner, Löw, Hamm, & Weymar, 2015) impair reconsolidation of episodic memories and that aversive stimuli presented directly after

reactivation of established memories cause their targeted forgetting (Strange, Kroes, Fan, & Dolan, 2010). The impairing effects of propranolol on reconsolidation of emotional memories have been shown in lowered recognition and recall performance (Kroes et al., 2010; Schwabe et al., 2012) in healthy subjects.

Reconsolidation is a phenomenon common across species (Davis, Renaudineau, Piorier, Pucet, Save, & Laroche, 2010) and there are some crucial prerequisites for reconsolidation to be induced. First, Nader et al. (Nader, Schafe, & LeDoux, 2000) argue that stored (consolidated) memory can be changed only if the manipulation occurs after the reactivation of the memory. In case of absence of the reactivation, the memory remains unchanged (Dębiec & LeDoux, 2004). Second, the memory can be modulated only within a specific period of time after being reactivated. The reconsolidation time-window lasts up to six hours for conditioned fear in rats (Monfils, Cowansage, Klann, & LeDoux, 2009) and in humans (Schiller et al., 2010). Third, reconsolidation requires synaptic protein synthesis and changes in gene expression (Tronson & Taylor, 2007). Reconsolidation also depends on the age of memory, the strength of former learning, the duration of stimuli during reactivation, the degree of similarity between encoding and retrieval conditions (reviewed by Davis et al., 2010).

Lonergan et al. (2013) have recently reviewed studies in which propranolol was administered in context of emotional memory. They showed that a pharmacological beta-blockade was effective in disrupting consolidation of emotional memories. Data about reconsolidation disruption are less clear. The reconsolidation blockade by beta-blocker does not seem to be relatively stable phenomenon in healthy participants (cf., Lonergan et al., 2013). Moreover, there is not much known about the modulation of consolidation and reconsolidation processes in the population group that would profit most from this research, the PTSD patients. As shown before, the emotional memory formation can be disrupted at all stages of processing: encoding, consolidation and reconsolidation. In PTSD patients, preventive administration of beta-blocker before a traumatic event occurs would target the encoding process. This procedure, although possibly very effective, would raise a lot of ethical concerns. Targeting the consolidation process, with an exception of some groups, like soldiers or accident victims, would be hard to implement in reality. First after the trauma memory has become a stable memory trace, it could be reactivated and hopefully changed, e.g., when PTSD patients come to

counseling. By now, there are two preliminary studies with trauma patients in which the investigators attempted to target the memory at two latter levels. In a clinical study with PTSD patients, administration of propranolol reduced physiological responding to a personal script driven imagery when administered after the reactivation of traumatic memories (Brunet et al., 2008) in the same manner as the drug given shortly after occurrence of traumatic event in healthy subjects (Pitman et al., 2002). These studies, however, have been conducted in small samples. Moreover, the propranolol effects were only restricted to physiological responding. Although one study demonstrated a reduction of mildly arousing emotional episodic memory in PTSD patients (Reist et al., 2001), reports about manipulations aiming at attenuation of trauma memory itself are missing.

"*Considering the pivotal role of negative emotional experiences in the development and persistence of mental disorders, interfering with the consolidation/reconsolidation of such experience would open a novel treatment approach in psychiatry*" (Lonergan et al., 2013, pg. 222). The role of consolidation is forming new memory. As it has been proposed (Alberini, 2005; Hupbach, Gomez, Hardt, & Nadel, 2007), the functional role of reconsolidation may be both strengthening and updating of memory, where post-reactivation manipulations can modulate or alter the original memory. An extensive further research on modulation of reconsolidation and consolidation processes in healthy participants and possibilities of changing the trauma memory in PTSD patients, by both targeting the consolidation and reconsolidation, is necessary. In doing so, the main characteristics must be observed: the process of reconsolidation must be properly induced (i.e., by reactivating the aimed memories) and the manipulations should take place within the sensitive time-windows, when the processes are sensitive to changes. As these variables are relatively easy to control, other, like the age of trauma memory and the depth of processing at encoding, i.e., during the trauma event, might be a serious challenge. Additionally, as it has been shown in the first study, spontaneous retrieval of emotional events takes place, when participants are presented with encountered stimuli, even if recognition is not the actual task. This finding might indirectly stress the role of memory cues in the development of strong trauma memories in PTSD. As the emotional memories can be spontaneously triggered by single cues, the returning intrusive memories might repeatedly strengthen the trauma memory, making it even more stable and less prone changes.

Elaborated processing at encoding and deep encoding of emotional information enables profound consolidation and results in strong memory traces. Selectivity of reconsolidation (i.e., only the reactivated memories undergo changes during reconsolidation) seems to protect the global integrity of memory. In PTSD, traumatic memories are deeply encoded, hard to change and the associated arousal not easy to alleviate. Additionally, the trauma memory trace is reactivated with every reoccurrence of the trauma event (e.g., through intrusive memories or trauma event cues), resulting in stronger memory for the traumatic event with each reactivation. In PTSD, the consolidation process strengthens the traumatic memories and the reconsolidation process seems to protect the global integrity of trauma memory – the probably most intensive form of emotional memory.

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Acknowledgements

First of all, I would like to thank Professor Alfons Hamm for his expert supervision, wise advices, and patience; for allowing me to develop and work autonomously, for encouraging me to learn "by doing", and for being on my side each time we met major obstacles in the project.

Furthermore, I thank our department's heart and secretary, Sylvia Scholz, without whom every single administrative process, especially during the preparation of clinical trials, would have lasted much longer.

I thank my colleague and co-author, Dr. Mathias Weymar, for introducing me to our laboratory, helping at designing the first studies, for collaboration even from abroad, as well as for the deadlines and extreme motivational input during the preparation of manuscripts.

I would also like to thank Dr. Julia Wendt, as a colleague for her professional guidance and insightful discussions, and as a friend for the wonderful and unforgettable time in Greifswald.

Many thanks to Dr. Heino Mohrmann and Dr. Andreas Löw for their excellent technical support, help in programming and taking care of the research equipment.

I thank my office mate, Katja Lindner, for her wisdom, readiness to help and a really great, cheerful and open atmosphere during work.

I thank my friends (and partially colleagues), Dr. Anke Limberg, Kristin Zielinski, Dr. Alexander Lischke, Dr. Dirk Schultze, Dr. Marcin Jakubowski and Carlos Ventura-Bort for being there and making the time at the Baltic coast the time of my life.

I also want to thank all my colleagues not mentioned before: Dr. Jarosław Michałowski Katharina Holtz, Jan Philipp Stender, Janine Wirkner, Jörg Neubert, Frank Herzer, Dr. Christiane Pané-Farré, Manuela Alius, Kristin Fenske, Elisa Steinfurth, Dr. Jan Richter, Dr. Thomas Reininger, Dr. Carmen Hamm, Katrin Schlapmann, Dr. Susan Richter, and Anett Hoffmann for welcoming me to the team as a newcomer. It was a pleasure to work with experts.

A special „Thank You" to Dr. Izabela Wilkosz, who has been supporting me since my arrival in Munich.

Last but not least, my partner, aerospace engineer, Tim Fricke for his remote technical assistance and aid in computing questions, for proof-reading this thesis, and for motivating me and holding my hand in moments of despair.

Most of all, I thank my parents for giving me support all the time and allowing me the luxury of post-graduate studies.

Kochani Rodzice, dziękuje Wam za nieustające wsparcie przez cały ten czas i za luksus kształcenia się.

Ania

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Appendices

Appendix I

IAPS and EmoPics numbers for Study 1

IAPS (n=120) numbers: 2034, 2102, 2190, 2191, 2200, 2210, 2214, 2305, 2383, 2390, 2393, 2396, 2397, 2435, 2495, 2516, 2518, 2570, 2580, 2595, 2700, 2890, 3001, 3010, 3015, 3016, 3051, 3059, 3060, 3061, 3063, 3068, 3069, 3071, 3100, 3120, 3130, 3140.1, 3160, 3180, 3181, 3190, 3191, 3195, 3225, 3261, 3500, 3530, 4002, 4007, 4008, 4085, 4142, 4180, 4210, 4220, 4232, 4233, 4290, 4300, 4310, 4460, 4470, 4490, 4503, 4505, 4520, 4525, 4530, 4542, 4550, 4575, 4597, 4599, 4604, 4623, 4640, 4647, 4658, 4659, 4660, 4668, 4680, 4687, 4689, 4693, 4694, 6231, 6243, 6250, 6312, 6313, 6315, 6350, 6360, 6510, 6520, 6530, 6540, 6550, 6560, 6561, 6831, 8001, 8065, 8117, 8158, 8179, 8180, 8186, 8192, 8205, 8206, 8220, 8467, 8540, 9254, 9410, 9414, 9433.

EmoPicS (n=48) numbers: 050, 064, 070, 076, 082, 084, 087, 090, 093, 094, 096, 099, 101, 105, 109, 110, 112, 113, 114, 124, 129, 130, 133, 136, 137, 146, 148, 153, 161, 163, 173, 174, 177, 179, 181, 182, 184, 199, 200, 217, 218, 219, 220, 222, 239, 240, 242, 246

Appendix II

IAPS, EmoPics and erotic pictures (Stark et al., 2005) numbers for study 2 and 3 (without primacy-recency pictures)

IAPS (n=274) numbers: 1050, 1220, 1271, 1274, 1275, 1304, 1410, 1525, 1595, 1630, 1650, 1720, 1721, 1722, 1810, 2104, 2107, 2190, 2191, 2200, 2210, 2211, 2214, 2215, 2221, 2273, 2280, 2372, 2377, 2383, 2393, 2396, 2411, 2440, 2480, 2485, 2495, 2512, 2516, 2518, 2520, 2570, 2580, 2840, 2890, 3000, 3001, 3015, 3016, 3019, 3030, 3053, 3059, 3060, 3062, 3063, 3064, 3068, 3069, 3071, 3100, 3102, 3110, 3131, 3140, 3150, 3168, 3170, 3181, 3185, 3191, 3195, 3213, 3225, 3250, 3261, 3266, 3400, 3530, 4001, 4002, 4003, 4005, 4007, 4008, 4071, 4085, 4090, 4141, 4142, 4180, 4210, 4220, 4225, 4255, 4290, 4300, 4310, 4325, 4597, 4599, 4606, 4607, 4608, 4612, 4619, 4623, 4624, 4625, 4640, 4641, 4643, 4647, 4652, 4658, 4659, 4668, 4670, 4672, 4689, 4692, 4693, 4694, 4695, 4697, 4800, 4810, 5120, 5130, 5395, 5740, 6150, 6212, 6230, 6231, 6244, 6260, 6312, 6313, 6315, 6350, 6520, 6530, 6540, 6550, 6560, 6563, 6571, 6571.1, 6821, 6825, 6834, 7000, 7001, 7002, 7004, 7006, 7009, 7010, 7012, 7014, 7016, 7018, 7019, 7020, 7021, 7023, 7025, 7033, 7034, 7036, 7037, 7041, 7050, 7059, 7060, 7081, 7090, 7096, 7100, 7130, 7140, 7150, 7160, 7161, 7175, 7179, 7180, 7184, 7185, 7186, 7187, 7207, 7217, 7224, 7234, 7235, 7242, 7300, 7354, 7491, 7500, 7504, 7546, 7547, 7590, 7595, 7700, 7705, 7710, 7950, 8065, 8117, 8158, 8161, 8163, 8178, 8179, 8180, 8185, 8186, 8192, 8232, 8260, 8341, 8400, 9031, 9042, 9050, 9140, 9180, 9181, 9182, 9183, 9185, 9210, 9252, 9253, 9254, 9280, 9301, 9320, 9322, 9326, 9340, 9341, 9342, 9360, 9405, 9414, 9428, 9500, 9520, 9530, 9560, 9561, 9570, 9571, 9610, 9611, 9621, 9622, 9700, 9830, 9901, 9903, 9904, 9905, 9909, 9910, 9911, 9912, 9920, 9921

EmoPics (n=72) numbers: 11, 12, 13, 15, 17, 19, 21, 22, 23, 25, 26, 28, 43, 44, 45, 50, 51, 52, 56, 57, 58, 59, 60, 61, 63, 64, 66, 67, 68, 70, 71, 74, 75, 77, 78, 165, 167, 172, 176, 178, 185, 190, 194, 195, 196, 201, 204, 216, 227, 232, 233, 234, 236, 238, 239, 240, 242, 248, 325, 326, 327, 334, 338, 340, 348, 352, 354, 358, 363, 375, 377, 378

Erotic pictures (n=14) numbers: er_18, er_23, er_40, er_15, er_24, er_39, er_02, er_14, er_20, er_26, er_28, er_32, er_36, er_37

Appendix III

Background information: Genotype, emotional processing and memory

Although numerous findings from animal and human studies have provided general underpinnings for genetic determinants of emotional processing, little is known about the interplay between β_1- and β_2-adrenoreceptor polymorphisms and the episodic emotional memory formation.

In the mammalian brain, most β-adrenergic receptors are of the β_1 type (Zill, Baghai, Engel et al., 2003) and in the amygdala they are most frequent (Tiong & Richardson, 1990). Recently, de Rover et al. (de Rover, Brown, Boot et al., 2012) demonstrated a LPP amplitude dependence on allelic variation in the β_1-receptor gene polymorphism. The polymorphism that they focused on was β_1-adrenergic receptor (ADRB1) gene polymorphism rs1801253. The C-alelle of the polymorphism is associated with an enhanced coupling to the stimulatory G protein and an increased response to the receptor agonists (Mason, Moore, Green, & Liggett, 1999). In the study (de Rover et al., 2012), the authors found that subjects who were C/C homozygotes showed enhanced LPP amplitudes while viewing emotional stimuli as compared to heterozygotes G/G and homozygotes G/G. These results suggest that the generation of the LPP is at least in part mediated by the activation of β_1-receptors in the amygdala.

To our best knowledge, the individual differences in emotional processing and memory formation depending on the β_2-adrenergic receptor (ADRB2) genotype are so far not known. However, the internal data of the Department of Clinical Pharmacology, suggest that this genotype variation might play a role in modulation of the ERP old/new effect. Therefore, in addition to the ADRB1 gene polymorphism, in the present study we planned to focus on the β_2-receptor polymorphisms: rs1042713 and rs1042714, which have been associated with functional changes of the β_2-adrenoreceptor. In rs1042713, the reference allele is A and the malfunction allele G. In the case of rs1042714, the reference allele is C and the malfunction allele is G.

We have decided to investigate the genetic variations of two types of β-adrenergic receptors since the study medication to be utilized is a non-selective $\beta_1\beta_2$-recptor antagonist.

Objectives

The main objective of the present study was to combine two lines of research, investigating the interaction between emotional processing and memory performance (on both behavioral and electrophysiological levels) and its modulation by beta-blockade.

In addition, recent study conducted by our working group (Weymar, Löw, Modess et al., 2010) revealed some hints about beta-blocker influences on ADRB2-dependent formation of emotional memory (unpublished data). Here, we planned to examine these effects in dependence on ADRB1 and ADRB2 genotype in a pre-selected, genotyped sample of healthy young male participants. Concerning pharmacological manipulations with beta-blockers, there are no studies, which investigated the effects of propranolol on electrophysiological (ERPs) and behavioral measures of recognition memory along with their codependence on individual variations of adrenergic receptors' polymorphisms. Moreover, the findings about genetic influences of ADRB2 on recognition memory for emotional contents are lacking as well.

Therefore, the current investigation has been designed to replicate, in a study in a crossover design, former results in which ERP correlates of recognition memory for emotional pictures were reduced by beta-blocker propranolol. Furthermore, our goal was to test, whether there are any differences between carriers of genetic variants of the ADRB1 and ADRB2 in memory performance and/or changes in event-related potentials and in propranolol influences on the above mentioned processes. For this purpose, we implemented the formerly tested modified recognition memory paradigm with two incidental encoding sessions in a randomized clinical trial with propranolol and placebo administered in a crossover design.

In addition to the objectives introduced in the main text, we hypothesized a potential impact of genetic variants of the ADRB1 and ADRB2 on the emotional information processing and memory formation alone, and on the propranolol modulation of those processes.

Genotyping (excerpt from the Clinical Trial Protocol – BBEmoMem_G; changed)

Healthy volunteers were genotyped for polymorphisms in the coding regions of the $ß_1$- and β_2-adrenoreceptors (ADRB1 and ADRB2, respectively).

Two polymorphisms, namely rs1042713 (c.46A>G; p 16 Arg>Gly), and rs1042714 (c.318 C>G; p.27 Gln>Glu), have been associated with functional changes of the β2-adrenoreceptor. Importantly, in case of rs1042713 the reference allele A is less frequent (allele frequency 0.39) in a Caucasian population compared to the malfunction allele G. In the case of rs1042714 the reference allele C (allele frequency 0.53) is more frequent than the malfunction allele G encoding for Glu.

These two polymorphisms of the β_2-adrenoreceptor have been summarized in four different haplotypes (cf., Table I). Individuals were selected according to their haplotype. Individuals homozygote for haplotype 2 or haplotype 4 were assigned to two study groups.

Table I. Haplotypes of ADRB2

Haplotype	rs1042713	rs1042714
1	A (Arg)	G (Glu)
2	G (Gly)	C (Gln)
3	G (Gly)	G (Glu)
4	A (Arg)	C (Gln)

Source: http://www.pharmgkb.org/gene/PA39#tabview=tab4&subtab=33

Those study groups are further divided into two subgroups genotyped for the polymorphism ADRB1 rs1801253 (c.1165G>C; p.Arg389Gly). The major allele in this polymorphism is the C-allele encoding for Arg in position 389, with a major frequency in the Caucasian population of 0.7. The G allele encoding for Glycin has the minor frequency of 0.3 (Eisenach & Wittwer, 2010).

Eventually, individuals homozygote for ADRB2 haplotype 2 and homozygote for ADRB1 c.1165 (CC), or homozygote/heterozygote for ADRB1 c.1165 (GG/CG) are selected, forming the first two groups. Individuals homozygote for ADRB2 haplotype 4 and homozygote for ADRB1 c.1165 (CC), or homozygote/heterozygote for ADRB1 c.1165 (GG/CG), form another two groups.

Planned sample

The study was planned to be conducted in healthy, male, university students, genotyped for the ADRB1 and ADRB2 SNP-polymorphisms, as mentioned earlier (cf., 7.1.1). All subjects were to be selected from the clientele of healthy subjects of the

Department of Clinical Pharmacology, University Medicine Greifswald, depending on whether they are in reach and willing to participate.

The planned genotype groups included in the study were as follows:

- homozygote for ADRB2 haplotype 2 (GG/CC) and homozygote for ADRB1 (CC)
- homozygote for ADRB2 haplotype 2 and homozygote for ADRB1 (GG) or heterozygote for ADRB1 (CG)
- homozygote for ADRB2 haplotype 4 (AA/CC) and homozygote for ADRB1 (CC)
- homozygote for ADRB2 haplotype 4 and homozygote for ADRB1 (GG) or heterozygote for ADRB1 (CG)

The original analysis of the provided data base resulted in a possible maximal sample of 120 subjects. According to power analyses, the aimed sample was N=64, with 16 participant within every genotype group. The sample sizes of single genotype groups are depicted in Table II.

Table II. Genotype groups planned in the study.

	Group A	Group B	Group C	Group D
ADRB2	Haplotype 2/2	Haplotype 2/2	Haplotype 4/4	Haplotype 4/4
ADRB1	CC	GG and CG	CC	GG and CG
N	13	3	63	41

Note: ADRB2 – β_2-adrenergic receptor gene, ADRB1 – β_1-adrenergic receptor gene. Description in the text.

Because of the small number of participants in the haplotype 2/2 group (A and B), we decided, within this genotype group, to merge participants with all variations of the ADRB1 genotype.

After this primary selection and extensive acquisition and pre-screening of the participants, we eventually were able to invite eight subjects for the initial screening session.

In the study, we managed to include only three participants homozygotous for haplotype 2/2 (genotype group A+B), one of which we excluded from further analyses due to bad EEG signal. Thus, the remaining sample of N=2 was too small even for explorative, comparative analyses, especially in the case of EEG.

References

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Appendix IV

Randomization and counterbalancing scheme

Table III presents a template for counterbalancing and randomization scheme which has been used in Study 3.

Table III. Counterbalancing and randomization scheme for a clinical trial in crossover design.

Subject	old / encoding I	old / encoding II	new / recognition	medication order
001	set 1	set 2	set3, set 4	A
002	set 1	set 2	set3, set 4	B
003	set 2	set 1	set 3, set 4	B
004	set 2	set 1	set 3, set 4	A
005	set 3	set 4	set 1, set 2	A
006	set 3	set 4	set 1, set 2	B
007	set 4	set 3	set 1, set 2	A
008	set 4	set 3	set 1, set 2	B

Note: Sets to be used in the experiment: 1-4. The clinical trial consists of two incidental encoding sessions (encoding I and encoding II) and a recognition session. Status of each stimulus set (old from first encoding session, old from second encoding session, new) changes according to the counterbalancing scheme. Medication orders implemented: A – 1. here beta-blocker, 2. placebo; B – 1. placebo, 2. beta-blocker.

For subjects 001 and 002 picture sets 1 and 2 are assigned for the first and the second encoding session, respectively. In the recognition task, these sets have an "old" status and are intermixed with "new" stimuli from sets 3 and 4. For participants 003 and 004 the old/new status of the sets is the same. These participants, however, get the set 2 presented in the first encoding session, and the set 1 in the second session. The old/new status of stimuli is inverted for participants 005-008, i.e. the stimuli from sets 3 and 4 are displayed during the encoding sessions and count as "old" stimuli in the memory test; in the contrary, sets 1 and 2 become now "new" pictures. Moreover, analogous to the set pattern for the subjects 001-004, the participants 005 and 006 encode the set 3 in the first and the set 4 in the second session; for participants 007 and 008 these sets are presented in a reversed manner.

The right column in the Table 4 shows an exemplary drug assignment order for every participant. The orders of medication are coded A (beta-blocker first) and B (placebo first). The drug assignments are randomized in blocks of two consecutive subjects, so that the randomization blocks match the counterbalancing pattern. By these means, it is assured that within every set-to-session assignment one participant gets the

beta-blocker in the first session (and placebo in the second session) and the other one gets the drug in the second session (and placebo in the first one). In our example (see Table 4), the subject 001 gets the beta-blocker (highlighted in grey in the 2nd and 3rd column) in first session when the set 1 is presented (serves as "old" from the first session), whereas the subject 002 gets the beta-blocker in the second session when the set 2 is presented ("old" second session). Further, the 003 gets the beta-blocker during the second session with set 1, and 004 – during first session with set 2. This pattern continues over the next participants for whom the old/new status of the stimuli changes (005 – 008). When we consider the highlighted fields in the 2nd and 3rd column we notice that the beta-blocker administration takes place in the context of any possible stimulus set and session combination. Applying this procedure for the whole sample we assure that a specific drug administration covers every available set-session combination an equal number of times.

In the way described above, one can assure that any possible effects originating from differences between the sets, the set-session interactions, as well as session-set combinations to study medication order interactions cancel out.

Appendix V

Exclusion criteria (excerpt from the Clinical Trial Protocol – BBEmoMem_G):

- sex: female
- hepatic and renal diseases and/or pathological findings, which might interfere with pharmacokinetics and pharmacodynamics of the study medication
- existing cardiac or hematological diseases and/or pathological findings, which might interfere with the drug's safety, tolerability and/or pharmacokinetics (e.g. bradycardia, hypotonia, av- block I°)
- volunteers liable to orthostatic dysregulation, fainting, or blackouts
- peripheral circulatory disturbances
- gastrointestinal diseases and/or pathological findings (e.g. stenoses), which might interfere with pharmacokinetics and pharmacodynamics of the study medication
- obstructive disorder of breathing (e. g. asthma bronchiale)
- known allergic reactions to the active ingredients used or to constituents of the study medication
- known allergic reactions to any drug therapy in the anamnesis or actual de-allergisation
- psoriasis
- diabetes mellitus
- addiction to hypoglycemia
- pheochromocytoma
- myasthenia gravis
- drug or alcohol dependence
- positive drug or alcohol screening
- smokers of 10 or more cigarettes per day
- positive results in HIV, HBV and HCV screenings
- volunteers who are on a diet which could affect the pharmacokinetics of the drug (e. g. vegetarian
- heavy tea or coffee drinkers (more than 1L per day)

- volunteers suspected or known not to follow instructions of the clinical investigators
- volunteers who are unable to understand the written and verbal instructions, in particular regarding the risks and inconveniences they will be exposed to as a result of their participation in the study
- less than 14 days after last acute disease
- any medication within 4 weeks prior to the intended first administration of the study medication which might influence functions of the gastrointestinal tract (e.g. laxatives, metoclopramide, loperamide, antacids, H_2-receptor antagonists, proton pump inhibitors, anticholinergics)
- any other medication within two weeks prior to the first administration of the study medication, but at least 10-time the half-live of the respective drug (except oral contraceptives)
- intake of grapefruit containing food or beverages within 14 days prior to administration of the study medication
- intake of poppy seed containing food or beverages within 14 days prior to administration of the study medication

Appendix VI

Laboratory parameters obtained in the inclusion (and follow-up) examination (excerpt from the Clinical Trial Protocol – BBEmoMem_G):

Clinical-chemical laboratory

sodium	ASAT (SGOT)
potassium	ALAT (SGPT)
calcium	alkaline phosphatase
chloride	γ-GT
creatinine	α-amylase
Urea	thromboplastin time
glucose	partial thrombin time
cholesterol	triglyceride

Haematology

hemoglobin	erythrocytes
hematocrit	thrombocytes
leukocytes	

Urine analysis

leukocytes	nitrite
urobilinogen	protein
bilirubin	glucose
ketones	blood/erythrocytes

Other screenings

HBV, HCV, HIV

drugs (amphetamine, metamphetamine, benzodiazepine, cannabinoids, opiates, methadone, tricyclic antidepressants, barbiturates, cocaine, ecstasy)

alcohol screening (only in suspicious cases)

Appendix VII

Psychophysiological, pharmacokinetic and pharmacodynamic study protocol (excerpt from the Clinical Trial Protocol – BBEmoMem_G; changed)

Study days – 1 and 3 (1st and 2nd session)

The time of drug administration represents the point zero on the time axis. The protocol for study day 1 and 3 is the same with the restriction to the study medication. The sessions will start with the in-house-confinement in the evening before the pharmacokinetic study day.

Time pre/post drug administration	Procedure
Day -1 and 2	
>-10 h	Admission at the department, questioning for state of health (AE on day 2, respectively), keeping the restrictions, physical examination, drug and (only in case of suspicion) alcohol screening Standard dinner
Day 1 and 3	
>-10 min	Urine sampling, placing an indwelling cannula, blood sampling, spirometry, skin conductance measurement
-10 min	Measurement of blood pressure, heart rate, pulse wave analysis and collection of salivary α-amylase,
0 min	Oral administration of 80 mg propranolol or placebo with 240 ml table water, mouth checking, beginning of the urine sampling period
5 min	Start of the continuous monitoring of heart rate and measurements of blood pressure and pulse wave analysis
15 min	Blood sampling
20 min	Measurement of blood pressure, heart rate, pulse wave analysis
30 min	Blood sampling
40 min	Measurement of blood pressure, heart rate, pulse wave analysis
45min	Blood sampling
60 min	Blood sampling ,100 ml table water
70 min	Preparation for EEG
80 min	Measurement of blood pressure, heart rate, pulse wave analysis and saliva sampling, AE-questioning
90 min	Stimulus presentation with EEG-recording (without measurements of blood pressure, heart rate and pulse wave analysis), skin conductance measurement
110 min	Start of the second period of heart rate – monitoring and measurement of blood pressure and pulse wave analysis until return to the initial baseline values or clinically not relevant deviations; AE-questioning
2 h	Blood sampling, 100 ml table water, spirometry, ergometry
3 h	Blood sampling, 100 ml table water, measurement of heart rate, blood pressure and pulse wave analysis

4 h	Blood sampling, 100 ml table water, AE-questioning, skin conductance measurement
5 h	Measurement of blood pressure, heart rate, pulse wave analysis
6 h	Blood sampling, 100 ml table water, AE-questioning
7 h	Measurement of blood pressure, heart rate, pulse wave analysis
8 h	Blood sampling, 100 ml table water
10 h	Blood sampling, 100 ml table water,
11 h	Measurement of blood pressure, heart rate, pulse wave analysis
12 h	Blood sampling, 100 ml table water, AE-questioning, dismissal
Day 2 and 4	
24 h	Urine sampling

Study day 10 (3rd session)

The time of stimuli presentation represents the point zero on the time axis.

Time pre/post stimulus presentation	Procedure
- 40 min	Admission at the department, preparation for EEG, AE-questioning
0 min	Recognition memory task with EEG-recording, skin conductance measurement
40 min	SAM – rating task (no EEG)
90 min	Clarification of study goals, end of the session, leaving the department