From the Institute of Neuroendocrinology

at the University of Lübeck

Director: Prof. Dr. Jan Born

Effects of enhancing GABAergic transmission on sleep-associated memory consolidation

Thesis

for

the acquisition of a medical doctorate

at the University of Lübeck

- Medical Faculty -

Presented by

Ying Ma

From Hangzhou, China

Lübeck 2010

1. Referee: Prof. Dr. rer. soc. Jan Born
2. Referee: Priv.-Doz. Dr. med. Johannes Hagenah

Day of oral examination: 11.11.2011
Approved for printing. Lübeck，11.11.2011

Index

Abbreviations

REM - Rapid Eye Movement

NREM - non- Rapid Eye Movement

EEG - Electroencephalogram

EMG- Electromyogram

EOG- Electrooculogram

S1 - Stage 1 Sleep

S2 - Stage 2 Sleep

S3 - Stage 3 Sleep

S4 - Stage 4 Sleep

SWS - Slow Wave Sleep

SWA - Slow Wave Activity

PAL - Paired Associate Learning

IAPS - International Affective Picture System

Ach - Acetylcholine

NA - Noradrenalin

GABA - Gamma-AminoButyric Acid

IEG - Immediate Early Gene

LTP - Long-Term Potentiation

LC - Locus Coeruleus

HPA - Hypothalamo-Pituitary-Adrenal axis

GAT-1 - GABA Transporter 1

PVT - Psychomotor Vigilance Task

SSS - Stanford Sleepiness Scale

SEM - Standard Error of Mean

1. Introduction

Sleep is a fundamental biological process with consequences on human health. Loss of behavioral control and consciousness is probably the core characteristic that separates sleep from wakefulness. Yet, although the organism is cut-off from the outside world or at least seems to be, the brain never really stops working.

Although the functions of sleep remain largely unknown, one of the most exciting hypotheses is that sleep contributes importantly to the processes of memory and brain plasticity (Maquet, 2001; Stickgold, 2005; Born et al., 2006). Over the past decades, a large body of work, spanning most of the neurosciences, has provided a substantive body of evidence supporting this role of sleep in what is becoming known as sleep-dependent memory processing (Wilson and McNaughton, 1994; Sirota et al., 2003; Huber et al., 2004; Marshall et al., 2006; Gais et al., 2007).

1.1 Sleep stages

Following the discovery of rapid eye movement (REM) sleep and non-REM (NREM) sleep (Aserinsky and Kleitman, 1953), Rechtschaffen and Kales (1968) distinguished 5 different sleep stages in sleep architecture that cyclically succeed each other during the course of nocturnal sleep (Fig.1A). Each stage of sleep is characterized by a particular pattern of brain waves in electroencephalogram (EEG). Stage 1 is a transition between wakefulness and sleep, and is characterized by the presence of slow eye movements and mixed frequency waves (alpha and theta waves). Stage 2 is defined by the presence of

sleep spindles and/or K complexes and the absence of sufficient high amplitude, slow activity. The deepest stage, stages 3 and 4 together are known as slow wave sleep (SWS), and are characterized by very high (>75μv) and slow (<2 Hz) delta waves. The final stage is REM sleep. During this stage, breathing becomes rapid and shallow, and the eyes move back and forth quickly. The first period of REM sleep usually begins about 70-90 minutes after falling asleep. Stages 1-4 are also referred to as NREM sleep. In humans, NREM and REM sleep alternate or "cycle" throughout the night every 90 minutes (Fig. 1A). Due to an underlying circadian rhythm, early nocturnal sleep in humans is dominated by SWS and contains only little amounts of REM sleep, whereas this relationship is reversed during the late part of sleep when REM sleep is predominant.

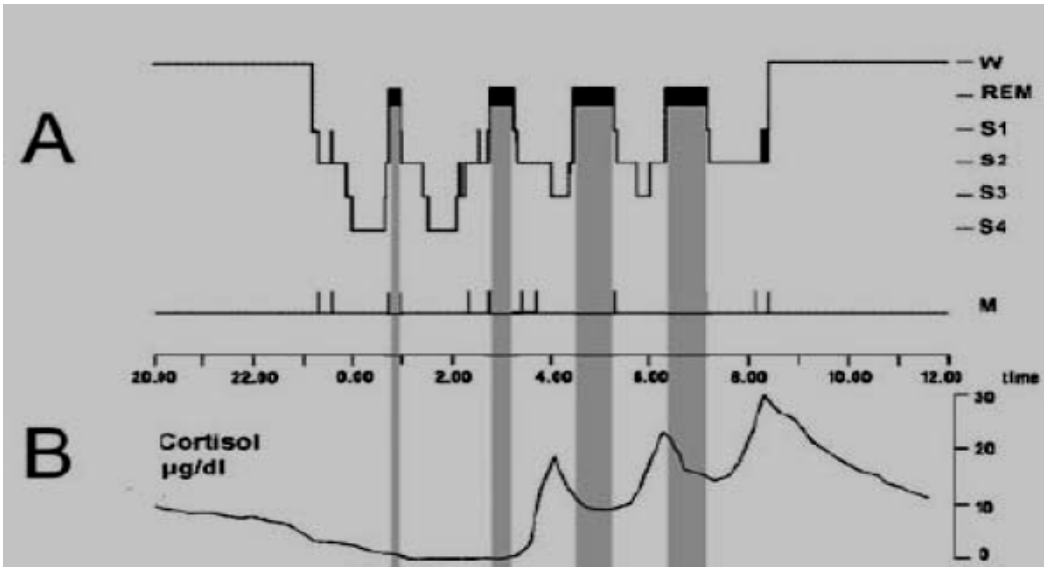

Figure 1 (A) Sleep hypnogram for a typical night of sleep, showing the sleep stages (REM sleep and NREM sleep stages S1–S4). The deepest sleep stages S3 and S4 indicate SWS. While sleep in the first half of the night is dominated by SWS, sleep in the second half of the night contains high amounts of REM sleep. W, wake; M, movement activity. (B) Cortisol release is strongly suppressed during early sleep, but increases distinctly during late sleep, reaching a maximum in the early morning hours. Accordingly, SWS-dominated early sleep is accompanied by minimal levels of circulating cortisol, while basal cortisol levels are high during REM sleep-dominated late sleep. Figure is modified from Wagner and Born (2008).

1.2 Memory processes

Memory differentiates three fundamental processes: acquisition, consolidation, and retrieval. Acquisition refers to the learning process whereby the new information is encoded into a neuronal trace, which is fragile and remains vulnerable to disruption (from interference). Consolidation is the focus of this study, which is conceptualized as a process triggered by a learning experience whereby the newly encoded representations transform into a robust and enduring form (McGaugh, 2000). Thus, consolidation counteracts forgetting due to the decay of the fresh trace or retroactive interference from subsequently encoded material (Wixted, 2004). Retrieval refers to the behavioral utilization of previously stored memory traces.

1.3 Categorization of memory systems

Memory is not a unitary system (Squire, 1986). There is more than one type of memory. For example, the capital of France, what you had for dinner last night and how to ride a bicycle. All three of these recollections require information that you have learned and stored, but they are very different types of memory. Multiple memory systems store different classes of memory in different brain regions, and quite probably, in different formats. Memories are most commonly divided into declarative memory, which is defined by memories accessible to conscious recollection (for example, the capital of France or last night's dinner), and non-declarative memory, which includes a heterogeneous collection of abilities resulting from experiences that are not necessarily available for conscious

recollection (for example, how to ride a bicycle or play the piano, Fig. 2).

Declarative memories are further divided into episodic memories, that is, memories of specific events (such as what you had for dinner last night), and semantic memories, in other words, memories of general information (such as the capital of France). The verbal paired associate learning (PAL) task has been most often employed experimentally to examine declarative memory consolidation during sleep, which requires the subject to learn a list of associated word-pairs and after sleeping or staying awake for several hours, cued recall is assessed. Non-declarative memories are also divided into several subcategories, usually including procedural skills, priming as well as conditioning, and are also generally encountered in literature as unconscious or implicit forms of memory. Procedural skills is the type of non-declarative memory most thoroughly studied with regard to the effects of sleep. It has been often assessed by the finger sequence tapping task which requires the subject to tap a certain sequence of finger taps on a key board as fast and as accurately as possible.

In recent years, there has been an increasing amount of literature demonstrating that memory process is modulated by the emotional strength of the material being learned (Cahill, 2000; McGaugh, 2004; Phelps, 2004). In general, emotional events are remembered with greater accuracy and vividness than neutral ones (Labar and Cabeza, 2006). Emotional texts and the pictures of International Affective Picture System (IAPS) are often used to test the consolidation of emotional memory.

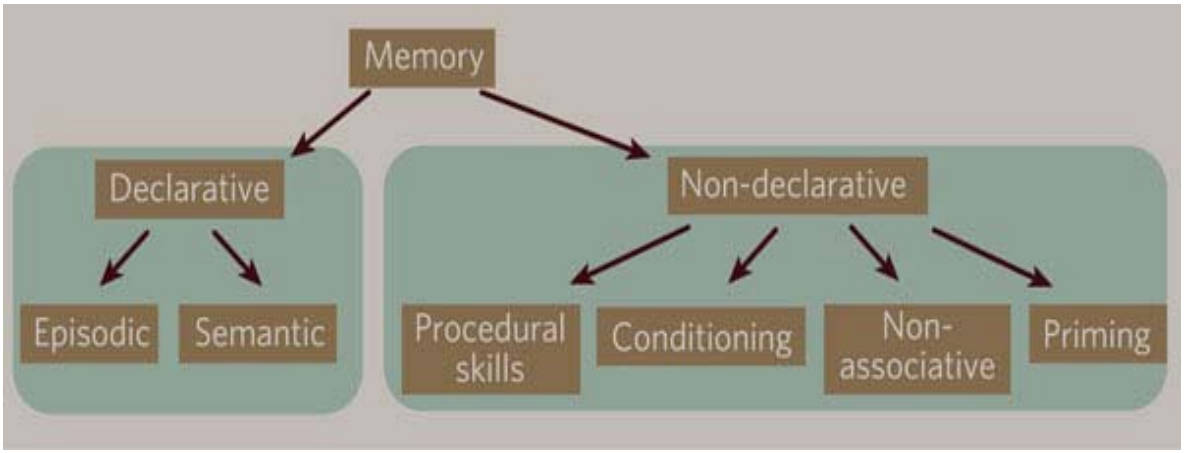

Figure 2 Categorization of memory systems. Figure is modified from Stickgold (2005).

The declarative and non-declarative memory systems involve different brain structures. Declarative memories essentially rely on the hippocampus whereas procedural memories strongly rely on striatal and cerebellar functions besides neocortical contributions (Squire, 1992; Doyon and Benali, 2005). Moreover, the emotional enhancement reflects primarily a modulating influence of the amygdala on hippocampal memory function (Akirav and Richter- Levin, 2002; Dolcos et al., 2004; Phelps, 2004). However, there is increasing evidence that these memory systems are not as independent as originally assumed. Learning a task does not lead to isolated activation of only one of the memory systems (Poldrack et al., 2001; Schendan et al., 2003). For example, particularly in the initial stages of learning the hippocampus is also activated during procedural tasks (Forkstam and Petersson, 2005).

1.4 Beneficial effects of sleep on memory consolidation

In recent years, the hypothesis that sleep facilitates memory consolidation has received

substantial supports from a rapidly growing number of studies performed in various species and at different levels of analysis, comprising behavioral, cellular and molecular experiments (Gais and Born, 2004a; Born et al., 2006; Frank and Benington, 2006; Joiner et al., 2006; Tononi and Cirelli, 2006; Hennevin et al., 2007). Consolidation of memory during sleep can produce a strengthening of associations as well as qualitative changes in memory representations. Strengthening a memory behaviorally expresses itself as resistance to interference from another similar task ("stabilization") and as an improvement of performance ("enhancement") that occurs at re-testing, in the absence of additional practice during the retention interval.

Up to now, the relatively consistent view is that declarative memories, which critically depend on the integrity of the hippocampus (Squire, 1992), benefit specifically, but not exclusively, from early nocturnal sleep, in which SWS predominates (Fowler et al., 1973; Plihal and Born, 1997), whereas memories not relying on the hippocampus, i.e. procedural memories and amygdala-dependent emotional memories benefit to a greater extent from late REM-dominated nocturnal sleep (Plihal and Born, 1997; Plihal and Born, 1999a; Wagner et al., 2001). However, memory tasks are never purely declarative or procedural, and both systems interact during sleep-dependent consolidation. Thus, declarative aspects of explicitly training a skill might subsequently contribute to consolidation during sleep. Conversely, the strengthening of procedural associations can impact declarative task aspects (Peigneux et al., 2001; Poldrack and Rodriguez, 2004; Born and Wagner, 2004; Fischer et al., 2006). Additionally, intermediate sleep stages (non-REM sleep stage 2 in

humans, transitory sleep in rats) can also contribute to memory consolidation (Datta, 2000; Fogel and Smith, 2006).

1.5 Models of sleep-dependent memory process

1.5.1 Model of declarative memory consolidation during sleep

Based on the evidence that acquired memories are reactivated during sleep (Wilson and McNaughton, 1994, Sutherland and McNaughton, 2000; Peigneux et al., 2004), the process of consolidation for hippocampus-dependent memories can be conceptualized as an interaction between neocortical and hippocampal networks (Buzsáki 1996; Gais and Born 2004a). Based on this concept, Born et al (2006) proposed the model of declarative memory consolidation during sleep (Fig. 3). This model suggests that new materials are encoded predominantly into hippocampal networks, where they are stored only temporarily, then during subsequent periods of SWS, driven by the slow oscillations, the newly encoded representations are repeatedly reactivated in the hippocampus. These reactivations, which are accompanied by sharp wave–ripple activity and occur in association with thalamocortical spindle activity, stimulate a transfer of information from hippocampal to neocortical networks. Together, these concerted activations are able to achieve a transfer of information as well as a strengthening of weak memory traces by providing repeated activation of memory traces without further training.

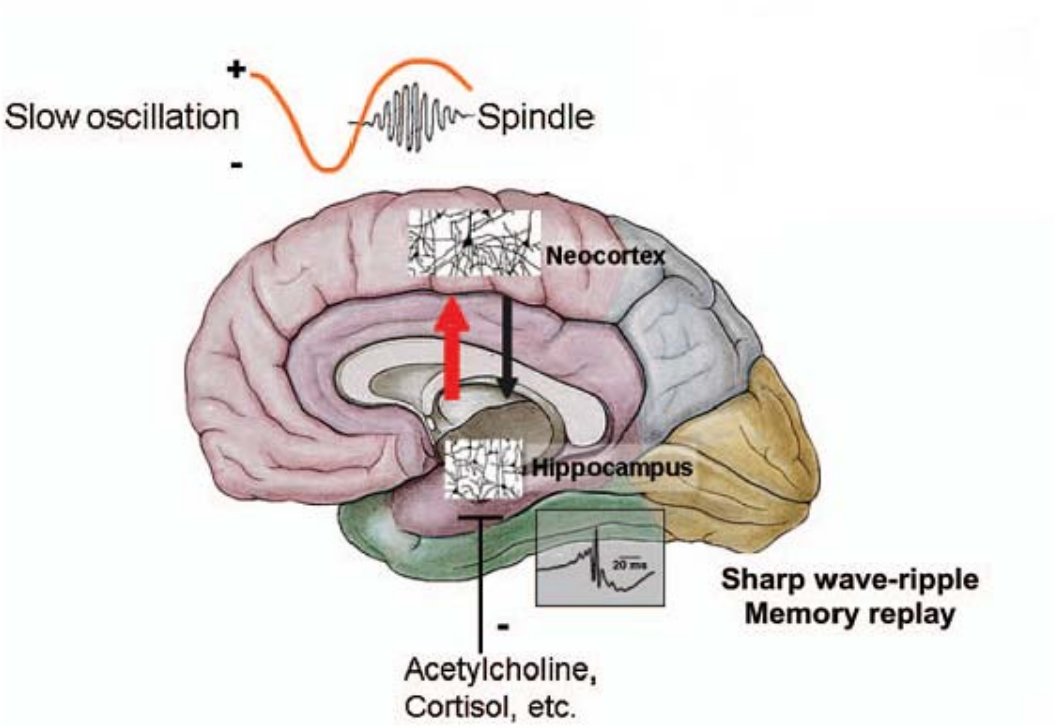

Figure 3 Model of declarative memory consolidation during sleep. During wakefulness, the information to be stored is encoded into neocortical networks and parts of it, simultaneously, in hippocampal networks serving as an intermediate buffer in this system (black arrow). During slow-wave sleep, the newly encoded information in the hippocampus is repeatedly reactivated. Reactivations are associated with sharp wave–ripple activity in the hippocampus. They are driven by slow oscillations, which originate in neocortical networks and synchronize hippocampal memory reactivation with the occurrence of spindle activity in thalamocortical circuitry. Hippocampal reactivations stimulate a transfer of the newly encoded information back to neocortical networks (red arrow). The hippocampal input arriving in synchrony with spindle input at the neocortical circuitry leads to long-term plastic changes selectively at those synapses previously used for encoding, that is, a long-term memory of the information in neocortical networks. High levels of acetylcholine or cortisol activity in the hippocampus suppress reactivations of memories in hippocampal networks and flow of information to the neocortex. Figure is modified from Born et al. (2006).

1.5.2 Model of sleep-dependent emotional memory process

Retention of declarative memory has been shown to benefit specifically from early nocturnal SWS-predominant sleep, however, when declarative memory for highly emotional rather than neutral material is assessed, the data point to a more beneficial influence of REM sleep (Greenberg et al., 1983; McGaugh, 2004; Wagner et al., 2001,

2006; Hu et al., 2006). Recently, Walker (2009) proposed a hypothesis for sleep-dependent emotional memory processing that we sleep to forget the emotional tone, and yet we sleep to remember the tagged memory of that episode (Fig. 4). This hypothesis supposes that the neuroanatomical, neurophysiological and neurochemical conditions of REM sleep offer a unique biological theatre in which a balanced neural potentiation of the informational core of emotional experiences (the memory) is achieved, yet the affective tone originally acquired at the time of learning (the emotion) is gradually ameliorated.

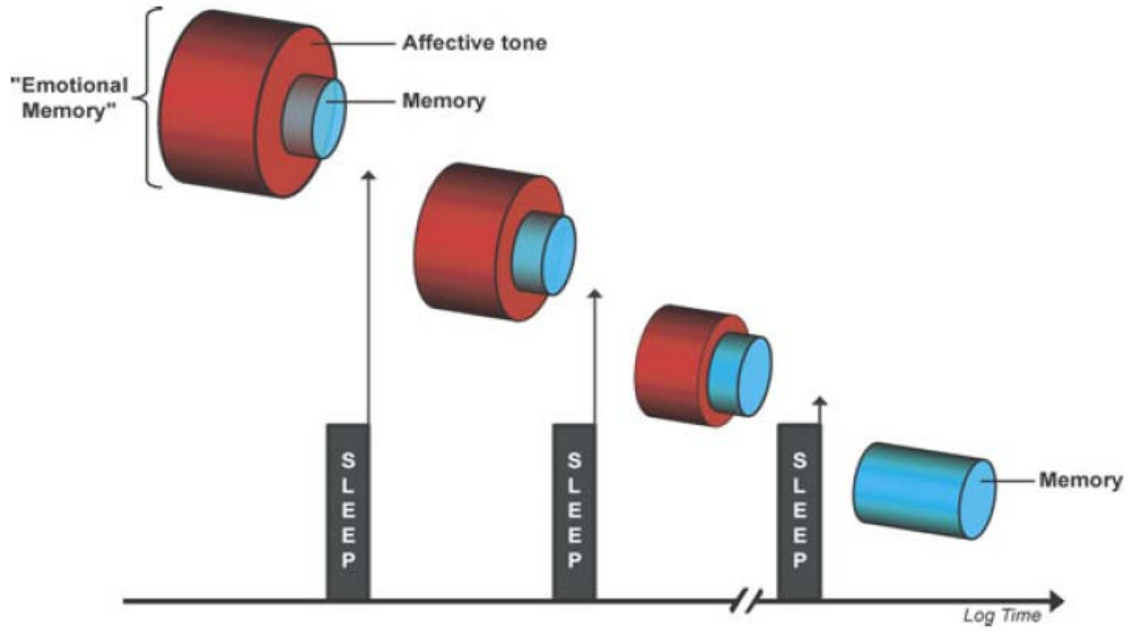

Figure 4 Model of sleep-dependent emotional–memory process: A sleep to forget and sleep to remember hypothesis. When formed, a newly encoded “emotional–memory” is created in a milieu of high adrenergic tone, resulting an associated affective “blanket.” With multiple iterations of sleep, particularly REM, not only is the informational core (memory) contained within that affective experience strengthened overnight(s), resulting in improved memory for that event, the autonomic tone “enveloped” around the memory becomes gradually ameliorated (emotional forgetting). Over time, the stored information of the original experience ultimately becomes decoupled and freed of its autonomic “charge,” leaving just the salient memory of that emotional experience, but without the affective tone previously associated at the time of learning. Figure is modified from Walker (2009).

1.6 Impact of different neuromodulators on memory consolidation during sleep

The specific neurochemical milieu of neurotransmitters and hormones differs strongly between SWS and REM sleep. Some of these neuromodulators contribute to memory consolidation.

1.6.1 Neurotransmitters and hormones

Many neurotransmitters, such as acetylcholine (Ach), noradrenalin (NA), serotonin, glutamate, and gamma-aminobutyric acid (GABA) are involved in the sleep processes (Pace-Schott and Hobson, 2002), in which cholinergic and noradrenergic activity are essential for sleep-associated memory consolidation (Hasselmo, 1999; Gais and Born, 2004b; Rasch et al., 2009b; Walker, 2009).

Ach is supposed to play a key role in the regulation of the dialogue between the neocortex and hippocampus (Buzsáki, 1989，1996; Hasselmo, 1999; Born et al., 2006). Cholinergic activity is at a minimum during SWS, which is thought to enable the spontaneous re-activation of hippocampal memory traces and information transfer to the neocortex by reducing the tonic inhibition of hippocampal CA3 and CA1 feedback neurons (Hasselmo, 1999; Marshall and Born, 2007). Hasselmo (1999, 2004) suggested that Ach serves as a switch between two modes of brain activity, from encoding during wakefulness to consolidation during SWS. On the contrary, cholinergic activity during REM sleep is similar or higher than during waking. This high cholinergic activity might promote synaptic consolidation by supporting plasticity-related immediate early gene (IEG) activity (Teber et

al., 2004) and the maintenance of long-term potentiation (LTP, Lopes Aguiar et al., 2008). Accordingly, blocking muscarinic receptors in rats by scopolamine during REM sleep impaired memory in a radial arm maze task (Legault et al., 2006). In humans, blocking cholinergic transmission during late nocturnal REM-predominant sleep prevented gains in finger motor skill (Rasch et al., 2009b). Conversely, enhancing cholinergic tone during post-training REM-rich sleep improved consolidation of a visuo-motor skill (Hornung et al., 2007).

Pharmacological studies have demonstrated that NA interacts with other neurotransmitters and stress hormones in the amygdala or the hippocampus to promote long-term memory formation (McGaugh and Roozendaal, 2009). There is evidence that noradrenergic activity is at an intermediate level during SWS and reaches a minimum during REM sleep (Aston-Jones and Bloom, 1981; Rasch et al., 2007). In rats, phasic burst firing in the locus coeruleus (LC, the brain's main source of NA) can be entrained by slow oscillations in the frontal cortex (Lestienne et al, 1997). It is possible that such bursts enforce plasticity-related IEG activity in the neocortex (Cirelli and Tononi, 2000; Cirelli et al., 2004), and thereby support the stabilization of newly formed memory representations at the synaptic level. In humans, the consolidation of odor memories was impaired after pharmacological suppression of noradrenergic activity during SWS-rich sleep and improved after increasing noradrenaline availability (Gais S, Rasch B, Dahmen JC, Sara SJ, and Born J, unpublished observations). Although it is unclear whether the low noradrenergic activity during REM sleep contributes to memory consolidation, it has been

proposed that the release from inhibitory noradrenergic activity during REM sleep enables the re-activation of procedural and emotional aspects of memory (in cortico-striatal and amygdala networks, respectively), thus supporting memory consolidation (Hasselmo, 1999; Walker, 2009). However, enhancing noradrenergic activity during post-learning REM sleep in humans failed to impair procedural memory consolidation (Rasch et al., 2009a).

The electrophysiological dynamics in sleep architecture is paralleled by distinct temporal changes in endocrine activity throughout the night embracing almost all hormonal systems (Steiger, 2003). In humans, hypothalamo-pituitary–adrenal axis (HPA) activity is distinctly suppressed in the first half of the night, but disinhibited in the second half of the night (Born and Fehm, 1998). Consequently, early SWS-dominated sleep is associated with very low levels of circulating cortisol, while late REM-dominated sleep is associated with a high basal cortisol level, which reaches its circadian maximum around the time of morning awakening (Fig. 1B).

Glucocorticoids (cortisol in humans) are a potent modulator of memory functions (Lupien and McEwen 1997; Roozendaal 2000). The suppression of cortisol during early SWS-rich sleep was shown to be a critical factor for the formation of hippocampus-dependent declarative memory during sleep (Plihal and Born, 1999b; Plihal et al., 1999). Since glucocorticoids block the hippocampal information flow to the neocortex (de Kloet et al, 1998), if the level of glucocorticoids is artificially increased during SWS, the consolidation of declarative memories is impaired (Wagner and Born, 2008). There is evidence that procedural memory function relying on cortico-striatal circuitry is generally much less

sensitive to effects of corticosteroids (Buske-Kirschbaum et al., 1996; Plihal et al., 1999), whereas the increase in cortisol concentration during late REM-predominant sleep enhances amygdala-dependent emotional memory formation (Wagner et al., 2005).

1.6.2 GABA

As mentioned above, Ach and NE are two most essential neurotransmitters related to sleep-associated memory consolidation. Another important neurotransmitter GABA is also involved in the sleep processes (Pace-Schott and Hobson, 2002), and it has been proposed to have effects on the memory consolidation (Shahidi et al., 2008; Carbo Tano et al., 2009; Fogel et al., 2010), although no study has investigated such effects in humans.

Five decades ago, GABA was identified as a normal constituent of the mammalian brain. Subsequent research demonstrated that GABA is one of the most prevalent neurotransmitters in the CNS (Fahn and Côté, 1968; Perry et al., 1971). Depending on the brain region, approximately 20% to 50% of all synapses use GABA as their neurotransmitter (Roberts and Frankel, 1974), which indicates that a large percentage of the central neurons are under GABA control. Synaptically released GABA exerts its effects via the specific interaction with three types of membrane-bound GABA receptors, classified as the functionally and pharmacologically differing ionotropic $GABA_A$, $GABA_C$ and metabotropic $GABA_B$ receptors (Barnard et al., 1998; Bowery, 2006). In nearly all brain areas, $GABA_A$ receptors are the most abundant (Young and Chu, 1990).

GABA-containing neurons in the basal forebrain and preoptic area have been known to

play an important role in promoting sleep by tonic inhibition of Ach-containing and NA-containing wake-promoting neurons in the hypothalamus and brainstem (Jones, 2005; McGinty and Szymusiak, 2005; Murillo-Rodríguez et al., 2009). Opening Cl^- channels through $GABA_A$ receptors and opening K^+ channels through $GABA_B$ receptors can hyperpolarize the wake-promoting neurons (Shefner and Osmanovic, 1991), thus directly or indirectly promoting sleep with slow-wave oscillations. Pharmacological studies have demonstrated that the agonists of $GABA_A$ receptors as well as $GABA_B$ receptors can enhance slow-wave activity (SWA) and SWS (Williams et al., 1995; Krogsgaard-Larsen et al., 2004; Walsh et al., 2006a, b).

The reciprocal-interaction model (Hobson et al, 1975；McCarley and Hobson, 1975) proposed that the onset and maintenance of REM sleep is due to reciprocal inhibitory interactions between cholinergic REM-on neurons and monaminergic REM-off neurons. As the inhibitory neurotransmitter GABA is involved in NREM sleep, it also relates to REM sleep regulation (Steriade and McCarley, 1990). Inhibition by GABA both facilitates and inhibits REM sleep. For example, GABA inputs to the dorsal raphe nucleus and LC could be the final synaptic step responsible for shutting down REM-off serotonergic and noradrenergic cells, thereby disinhibiting pontine cholinergic REM-on networks (Gervasoni et al., 2000; Maloney et al., 1999; Nitz and Siegel, 1997), probably through both $GABA_A$ and $GABA_B$ receptors (Varga et al., 2002). But GABA also directly inhibits the mesopontine cholinergic REM-on neurons (Datta, 1999). For example, the $GABA_B$ agonist baclofen significantly reduced the duration of REM sleep on account of inhibitting the activity of

mesopontine REM-on cells (Ulloor et al., 2004), and these cells are thought to be cholinergic (Jia et al, 2003), projecting widely throughout the brainstem and forebrain (Datta, 1995; Rye, 1997).

Although GABAergic mechanism is involved in the sleep processes, it is still not very clear what its effects are on sleep-associated memory consolidation. Some animal evidence has shown that GABAergic transmission is involved in memory consolidation (Salado-Castillo et al., 1996; Shahidi et al., 2008; Carbo Tano et al., 2009; Fogel et al., 2010), though the results are inconsistent. Shahidi's study (2008) indicated a post-training blockage of $GABA_A$ receptors in the dentate gyrus of hippocampus impaired memory consolidation of inhibitory avoidance task in rats, whereas Fogel et al. (2010) found that inhibition of REM sleep by injection of a $GABA_B$ agonist into the region of the pedunculopontine nucleus and deep mesencephalic reticular nucleus was associated with impaired subsequent consolidation of inhibitory avoidance task. Moreover, considerable evidence indicates that GABAergic influences on memory consolidation act upstream from noradrenergic activation by modulating NE release within the basolateral amygdala (Castellano et al., 1989; Introini-Collison et al., 1994; Gervasoni et al., 1998; Berlau and McGaugh, 2006). For example, the study using systemically post-training administration found that β-adrenergic antagonist propranolol blocked the memory-enhancing effects of GABAergic antagonist bicuculline and that norepinephrine blocked the memory-impairing effects of the GABAergic agonist muscimol (Introini-Collison et al., 1994). However as far as we know, no study has focused on the effects of the GABAergic mechanism on memory

consolidation in humans.

The agonists of GABA receptors were used in most of the pharmacological studies which explored the role of enhancing GABAergic transmission on sleep processes and sleep-associated memory consolidation. However, as both $GABA_A$ and $GABA_B$ receptor agonists are poor substrates for uptake, they probably produce more tonic and widespread effects than the endogenous liberated GABA (Lancel et al., 1998). In line with this postulate, Tiagabine, which on the one hand steadily elevates the level of GABA in the synaptic cleft and on the other hand perhaps prolongs and enhances the action of endogenous GABA through selective inhibition of the GABA transporter 1 (GAT-1) (Fink-Jensen et al., 1992; Borden et al., 1994), is probably more appropriate to investigate the effects of enhancing GABAergic activity on sleep-associated memory consolidation. Some pharmacological studies have shown that Tiagabine increases sleep continuity and the duration of SWS in humans, yet reduces the percentage of REM sleep following an increase in dosage (Mathias et al., 2001; Walsh et al., 2006a, b; Roth et al., 2006).

1.7 Aim of the study

As we know, SWS benefits the consolidation of hippocampus-dependent declarative memories, whereas REM sleep improves procedural memories involving striato-cortical circuitry and amygdala-dependent emotional memories. Based on the evidence that enhancement of GABAergic activity significantly increases SWS (Lancel,1999; Mathias et al., 2001; Roth et al., 2006) and reduces the duration of REM sleep (Ulloor et al., 2004;

Walsh et al., 2006a,b), as well as the animal evidence that GABAergic mechanisms are involved in memory consolidation (Shahidi et al., 2008; Carbo Tano et al., 2009; Fogel et al., 2010), we speculate that the GABAergic transmission is concerned with the human's sleep-associated memory consolidation.

Therefore, the purpose of the study is to investigate the role of enhancing GABAergic transmission on different types of memory consolidation during sleep. We expect that post-learning Tiagabine administration will benefit declarative memory consolidation because of the enhancement of SWS, whereas it will impair procedural memory because of the reduction of REM sleep.

2. Materials and Methods

2.1 Participants

Sixteen healthy, non-smoking, drug-free, native German speaking men (mean ± SD age: 24.3 ±3.9 years, range 19-31 years, body mass index: 20.3-24.8) were recruited to participate in the experiment and received a money reward for their participation. The subjects orally reported to habitually sleep 7-9 hours per night, and not to have had any major disruption of the sleep-wake cycle during 6 weeks before the experiment. All subjects had no any history of somatic and/or psychiatric diseases, and were proven to be medically healthy as determined by a physical examination as well as laboratory tests to rule out any medical conditions before their participation. The subjects were accustomed to the experimental sleep condition by spending an adaptation night in the sleep laboratory.

On the two experimental days the participants were instructed not to take any naps during the day and not to ingest alcohol or caffeine containing drinks (after 03:00 PM). The study was approved by the ethics committee of the University of Luebeck. All subjects gave written informed consent before participation.

2.2 Design and procedure

The study followed a randomized, double-blind, placebo-controlled within-subject crossover design. To accustom subjects to sleeping under laboratory conditions, all subjects spent an adaptation night in the sleep laboratory, including the placement of electrodes and the saline infusion via a catheter before participating in the experiment. Each subject participated in two experimental sessions (Tiagabine vs placebo) separated by an interval of at least 2 weeks according to a double-blind crossover design. One session served to assess effects of GABAergic function and the other as placebo control condition. Different versions of memory tasks were used in two sessions. The order of conditions was balanced across subjects.

Each condition started at 07:30 PM with the placement of a venous catheter for blood collection into the forearm and subsequent attachment of electrodes for polysomnographic recordings. The subjects then performed three memory tasks (learning phase, 09:00–10:20 PM; see below). The order of tasks was held constant in all conditions (first a learning task of IAPS pictures, then a declarative PAL task, and at last a procedural finger sequence tapping task). After learning, the subjects were administered a capsule

containing either placebo or Tiagabine (10mg) at 10:30 PM. Lights were turned off at 11:00 PM to enable post-learning sleep, and the subjects were allowed to sleep for 8 hours (11:00 PM to 07:00 AM). In the morning, the subjects went home and during the daytime followed their usual activities. Retrieval on the three memory tasks was tested 22.5 hours after learning, starting at 07:30 PM the following night, with the order - finger sequence tapping, PAL and IPAS.

Additionally, psychomotor vigilance and the degree of sleepiness were assessed before learning and retrieval testing. Blood pressure, heart rate and subjective symptoms were measured before learning and retrieval as well as in the morning after the subjects were woken up. Blood for determination of plasma cortisol concentrations was sampled before learning and after retrieval testing as well as hourly during sleep via thin plastic tubes from an adjacent room without disturbing the subject's sleep, beginning after the lights were turned off. Blood for determination of Tiagabine concentration was also sampled 30 minutes, 90 minutes and 22.5 hours after substance administration. A summary of the procedure is given in Fig. 5.

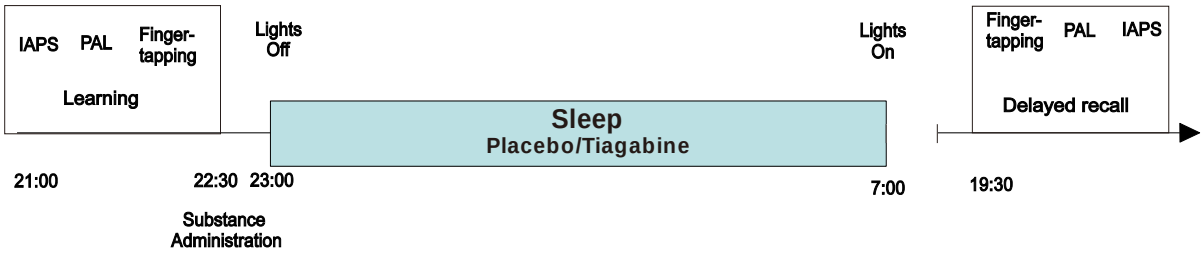

Figure 5 Experiment procedure

2.3 Memory tasks

50 neutral and 50 negative emotional pictures were selected from the International Affective Picture System (IAPS, Lang et al., 1993) to measure the emotional declarative memory. This pool of pictures was divided into two sets each consisting of 25 neutral and 25 emotional pictures for two learning sessions. Pictures were presented in pseudo-randomized order (at maximum three subsequent pictures with the same valence), with each subject receiving the same order. Each picture was presented for 4 seconds, followed by the computer-based version of the Self- Assessment- Manikin (SAM) rating system on a 9-point scale for valence and arousal (Bradley and Lang, 1994). Subjects were instructed to watch each picture for the entire presentation time. Immediately after picture presentation they had to rate their subjective feelings of valence and arousal. Time for ratings was not limited but subjects were instructed to respond spontaneously and as quickly as possible. At retrieval testing, subjects were instructed to write down description of all previously presented pictures they remembered as detailed as necessary to allow clear-cut identification (delayed free recall test).

A declarative word paired associate learning task was employed requiring the learning of a list of 40 pairs of semantically related words (e.g., clock-church). Different word-lists were used on the subject's two experimental sessions. During the learning phase, the word-pairs were presented sequentially on a computer screen, each for 5 seconds, separated by interstimulus intervals of 100 ms. After presentation of the entire list, performance was tested using a cued recall procedure, that is, the first word (cue) of each pair was presented and the subject had to name the associated second word (response). The

correct response word was then displayed for 2 seconds, regardless of whether the response was correct or not, to allow re-encoding of the correct word-pair. The cued recall procedure was repeated until the subject reached a criterion of 60% correct responses. In retrieval, subject was tested using the same cued recall procedure as during the learning phase. As dependent variable, we used the number of correctly recalled words at retrieval relative to the number of correctly recalled words during the learning period. Note that this measure can result in the values higher than 100% if subjects recall more words during the retrieval as compared to the learning period.

The finger sequence tapping task was adopted from previous studies indicating also most robust sleep-dependent improvements on this task (Walker et al., 2003). It requires the subject to tap repeatedly one of two five-element sequences (4–1–3–2–4 or 4–2–3–1–4) on a keyboard with the fingers of his non-dominant hand as fast and as accurately as possible for 30-s epochs interrupted by 30-s breaks. The numeric sequence was displayed on the screen at all times to keep working memory demands at a minimum. A key press resulted in a white dot in the center of the screen. After each 30-s trial, feedback was given about the number of correctly completed sequences and error rate. At learning, subjects trained on twelve 30-s trials. The average score for the last three of these trials was used to indicate learning performance. At retrieval, subjects were tested on another three trials. Overnight changes in performance were calculated as absolute and relative differences in the number of correctly completed sequences between the three trails at retrieval and the last three trials at learning.

2.4 Substance administration

The subjects were administered with either placebo or the GABA uptake inhibitor Tiagabine (10 mg). Substances were enclosed in a capsule and administered orally immediately after the learning phase, 30 minutes before bedtime.

Tiagabine [(R-)-N- (4, 4-di (3-methylthien-2-yl) but-3-enyl) nipecotic acidhydrochloride] is a nipecotic acid analogue, into which a lipophilic anchor has been incorporated to facilitate crossing of the blood-brain barrier after oral administration (Fig. 6, Leach and Brodie, 1998). It is rapidly absorbed, with a Tmax of about 45 minutes in the fasting state. The rate, but not the extent, of absorption is reduced when ingested with food. It has an elimination half-life of 7 to 9 hours, with dose to be titrated starting at 4 mg per day. The maximum recommended daily dose is 56 mg per day for adults taking hepatic enzyme-inducing drugs. The primary metabolic pathway for Tiagabine is CYP3A4, and the P450 system is neither induced nor inhibited (Walsh et al., 2006a).

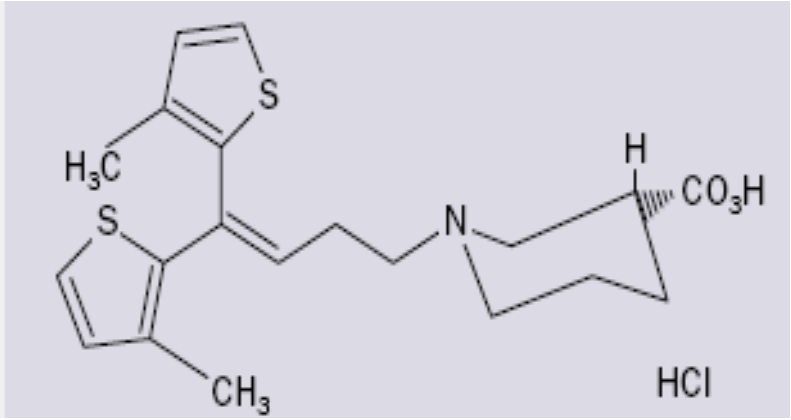

Figure 6 Tiagabine hydrochloride. figure is modified from Leach and Brodie (1998).

2.5 EEG recordings and sleep analysis

The EEG was recorded continuously using a SynAmps amplifier (NeuroScan Laboratories, Sterling, VA) or a BrainAmp amplifier (Brain Products). EEG signals were filtered between 0.16–35 Hz and sampled with 250 Hz. Twelve Ag-AgCl electrodes were placed according to the international 10–20 System, referenced to an electrode attached to the nose (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, Z, A1, A2). Additionally, horizontal and vertical electrooculogram (HEOG, VEOG), chin electromyogram (EMG) and electrocardiogram (ECG) were recorded for standard polysomnography.

Sleep architecture was determined visually according to standard polysomnographic criteria using EEG recordings from C3 and C4. Every offline 30-s epoch was scored independently by two experienced technicians who were blind to the assigned treatment according to the criteria by Rechtschaffen and Kales (1968). In case of disagreement, a third expert was consulted. For each night, total sleep time, sleep onset latency, SWS latency, REM sleep latency, and absolute as well as relative time spent awake and in sleep stages 1, 2, 3, 4, and REM sleep were determined. The duration of SWS was defined by the sum of time spent in sleep stages 3 and 4. Sleep onset latency was defined by the first period in stage 1 sleep followed immediately by stage 2 sleep. Sleep onset latency was determined with reference to the time lights were turned off. REM sleep latency was determined with reference to sleep onset.

2.6 Vigilance, sleepiness and symptom checklist

Reaction times were assessed as a measure of vigilance by a standardized Psychomotor Vigilance Test (PVT) that required subjects pressing a button as fast as possible whenever a big red disc appeared on a computer screen (Little et al., 1998). On 40 trials the subject fixated his gaze on a centrally located cross, displayed for 500–1000 ms on a white screen. Then, in 35 trials, a red disc appeared and, in five random no-go trails, the screen remained white.

To assess how sleepy the subjects were when they were in the learning and retrieval phase, the Stanford Sleepiness Scale (SSS) was evaluated before learning and before recalling. This simple seven-point rating scale has descriptors ranging from “feeling active, vital alert, or wide awake” (score = 1) to “no longer fighting sleep, sleep onset soon and having dream-like thoughts” (score = 7).

To evaluate the possible side effects of Tiagabine, subjects were instructed to rate their subjective symptoms by a 5-point rating scale including 18 different items (Symptoms Checklist) before learning and before retrieval, as well as in the morning after the subjects were woken up.

2.7 Cardiovascular measures, concentration of cortisol and Tiagabine

Blood pressure and heart rate were measured with a digital blood pressure meter (Boso-Medicus, Bosch & Sohn GmbH, Jungingen, Germany) both before learning and before retrieval testing, as well as in the morning after the subjects were woken up.

To control for the changes in blood cortisol concentrations between the two conditions

possibly affecting memory consolidation (Wagner and Born, 2008), blood samples of every subject in two sessions were collected at 11 time points, which were immediately centrifuged and then stored at –20°C until they were analyzed by chemiluminescent immunoassay (Immulite system, DPC Biermann, Bad Nauheim, Germany) for determination of serum cortisol (sensitivity, 0.2 µg/dl; inter- and intraassay coefficient of variation <10%).

To ensure whether Tiagabine was absorbed into the blood circulation, as well as to detect whether the concentrations in different time points reached the effective concentration which possibly affects the memory consolidation and retrieval processes, blood samples of every subject in two sessions were collected 30 minutes, 90 minutes and 22.5 hours (after retrieval testing) after substance administration, which were immediately centrifuged and then stored at –20°C until they were analyzed.

2.8 Statistical analysis

One subject was excluded from the study because an epileptic seizure was identified in his first experimental night according to EEG, although no behavioral seizure was observed during sleep. The data from another subject were excluded from analyses because he was administered the same substance in two sessions. The data from two further subjects were excluded from the analyses because their concentrations 90 minutes after Tiagabine administration were too low to reach the effective concentration, which meant they could not be used to study the GABAergic effect on sleep-associated memory consolidation. The

final data from 12 men in two conditions were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows, version 16.0). Sleep parameters and memory performance as well as other psychological, physiological and pharmacological indices were analyzed with paired-samples T-tests. Chi-square analyses were used for comparing the incidence rate of every reported symptom in two conditions. A two-tailed *P*-value < 0.05 is considered significant.

3. Results

3.1 Sleep parameters

Total sleep time during the retention sleep period was closely comparable between the two conditions (441.4±12.2min vs 458.0±5.4min; P=0.220), as well as the proportion of wakefulness (2.3±1.1% vs 1.1±0.5%, P=0.097). Significant decrease in the proportion of stage 1 was found in the Tiagabine condition (9.9±1.0% vs 6.4±1.1%, P=0.004), however, there was no significant difference in stage 2 between the two conditions (52.9±1.7% vs 53.2±1.8%, P=0.784). Compared with those in the placebo condition, the proportion of both stage 3 (9.3±1.0% vs 13.3±1.8%, P=0.021) and stage 4 (6.1±1.5% vs 11.2±1.8%, P=0.005) significantly increased in the Tiagabine condition. The increase of SWS sleep was accompanied by a significant suppression of REM sleep after Tiagabine administration. In the placebo condition, REM sleep averaged 18.6±1.2% of total sleep time and occurred with a latency of 118.3±11.9min after sleep onset, whereas in the Tiagabine condition, REM sleep was reduced to only 14.1±1.9% of total sleep time (P=0.021), and occurred

with a latency of 145.4±36.6min (P=0.453). There was no significant difference in the sleep latency (45.5±12.9min vs 29.4±5.7min, P=0.253) and SWS (20.0±2.2min vs 15.5±2.4min, P=0.095) latency between the two conditions, see detailed results in Table 1.

Table 1 Sleep parameters after the administration of either placebo or Tiagabine

	Placebo(Mean±SEM)	**Tiagabine(Mean±SEM)**	**P**
Wake %	2.3±1.1	1.1±0.5	0.097
Stage 1 %	9.9±1.0	6.4±1..1	0.004
Stage 2 %	52.9±1.7	53.2±1.8	0.784
Stage 3 %	9.3±1.0	13.3±1.8	0.021
Stage 4 %	6.1±1.5	11.2±1.8	0.005
REM sleep %	18.6±1.2	14.1±1.9	0.021
Total sleep time (min)	441.4±12.2	458.0±5.4	0.220
Sleep latency (min)	45.5±12.9	29.4±5.7	0.253
SWS latency (min)	20.0±2.2	15.5±2.4	0.095
REM latency (min)	118.3±11.9	145.4±36.6	0.453

REM, rapid eye movement; SEM. standard error of mean; SWS, slow wave sleep.
Time periods of intermittent wakefulness (wake), stages 1, 2, 3 and 4 sleep and REM sleep are indicated as percentages of total sleep time. SWS refers to stages 3 and 4 together. Total sleep time, sleep latency (with reference to lights off), SWS latency and REM sleep latency (with reference to sleep onset) are indicated in minutes. Mean values, SEM, and P-values for paired-samples T-tests are indicated. $P > 0.05$ is no significance.

3.2 Performance of memory tasks

3.2.1 PAL

In the learning period, neither the number of correctly recalled word-pairs in the training trial (28.3±1.1 vs 27.5±1.0, P=0.539) nor the number of trials to reach the criterion (1.83±0.24 vs 1.75±0.22, P=0.723) differed between the two conditions. When tested the next evening, the subjects had equally forgotten word-pairs in the two conditions (1.8±0.8 vs 1.2±1.0, P=0.692). It was inconsistent with our hypothesis that GABAergic

enhancement after learning improves declarative memory consolidation. See detailed results in Fig. 7 and Table 2.

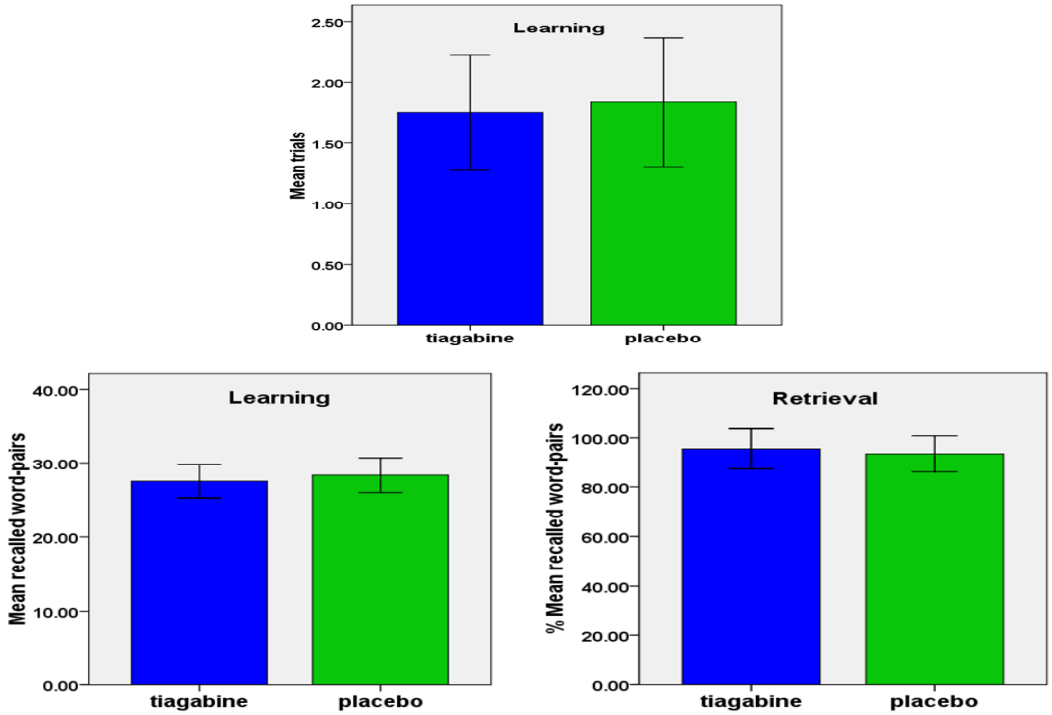

Figure 7 Performance of PAL task in learning and retrieval. Relative values are indicated in retrieval (the number of correctly recalled word-pairs in retrieval relative to the number of correctly recalled word-pairs in learning).

3.2.2 Finger sequence tapping

During learning, the performance of the finger sequence tapping task indicated by the number of correctly tapped sequences was unexpectedly smaller in the placebo condition than in the Tiagabine condition (16.9±1.3 vs 19.3±1.5, P=0.024). In retrieval, although there was no significant difference in the absolute number of correctly tapped sequences between the two conditions, the relative values (137.7±7.6% vs 114.2±2.6%, P=0.006) and the overnight gains (5.8±1.1 vs 2.7±0.5, P=0.013) were significantly smaller in the

Tiagabine condition. See detailed results in Fig. 8 and Table 2.

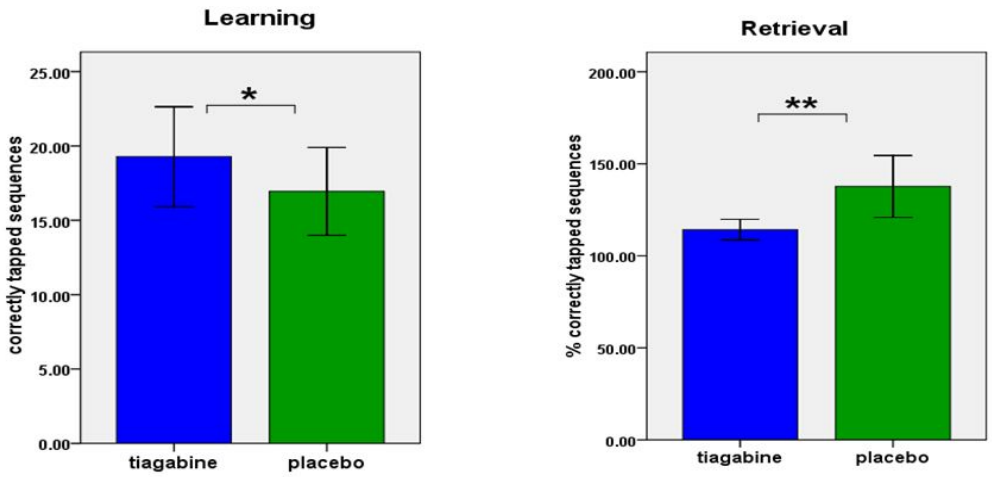

Figure 8 Performance of finger sequence tapping task in learning and retrieval. Relative values are indicated in retrieval (the number of correctly tapped sequences in retrieval relative to the number of correctly tapped sequences in learning). *p < 0.05, **p < 0.01.

3.2.3 Free recall of IAPS pictures

The delayed free recall performance of IAPS pictures was better for emotional than for neutral pictures in the Tiagabine condition (P=0.039), rather than in the placebo condition (P=0.856). Moreover, the emotional delayed free recalled pictures in the Tiagabine condition were significantly more than that in the placebo condition (P=0.027), whereas there was no significant difference in the neutral recalled pictures between the two conditions (P=0.468). See detailed results in Fig. 9 and Table 2.

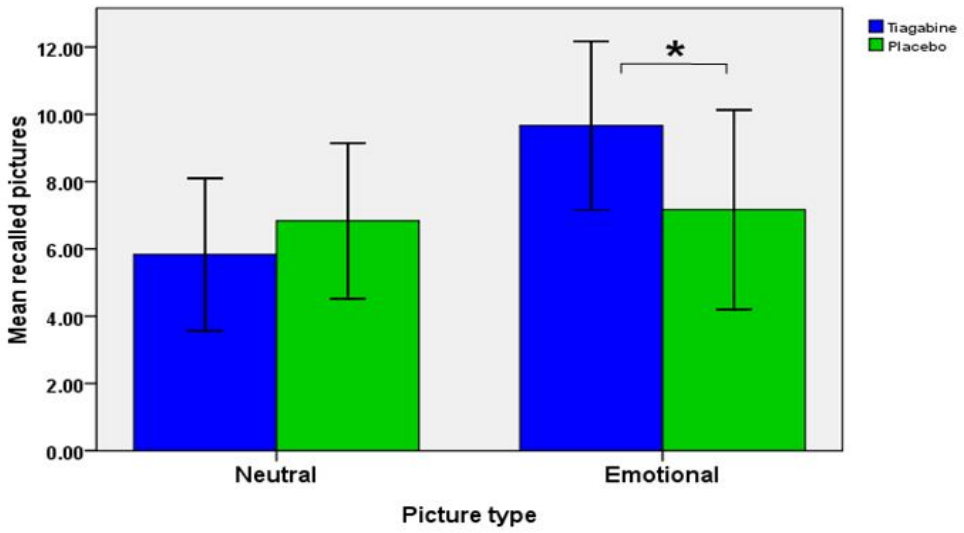

Figure 9 delayed free recall performance of IAPS pictures. *p < 0.05.

Table 2 Memory performance in two conditions (placebo or Tiagabine)

		Placebo (Mean±SEM)	Tiagabine (Mean±SEM)	P
PAL				
-Number of trials to criterion	Learning	1.8±0.2	1.8±0.2	0.723
-Number of recalled	Learning	28.3±1.1	27.5±1.0	0.539
Word-pairs	Retrieval	26.6±1.5	26.3±1.5	0.897
	Change	-1.8±0.8	-1.2±1.0	0.692
	Change%	93.5±3.3	95.5±3.7	0.697
Finger sequence tapping				
-Number of correctly	Early learning	14.2±1.2	14.5±1.7	0.783
tapped sequences	Late learning	16.9±1.3	19.3±1.5	0.024
	Retrieval	22.7±1.7	22.0±1.8	0.514
	Change	5.8±1.1	2.7±0.5	0.013
	Change%	137.7±7.6	114.2±2.6	0.006
Free recalled pictures of IAPS				
	Neutral	6.8±1.1	5.8±1.0	0.468
	Emotional	7.2±1.3	9.7±1.1	0.027

SEM. standard error of mean; PAL, paired associate learning; IAPS, international affective picture system.
For the PAL, the number of trials needed to reach a learning criterion of 60% correct responses is indicated. The absolute and relative changes of the number of correctly recalled words at retrieval are indicated. For the Finger sequence tapping, the number of correctly tapped sequences of early Learning refers to the total sequence number of the first three learning trials, while that of late learning refers to the total sequence number of the last three learning trials. The absolute and relative changes of the number of correctly tapped sequences are indicated. P＞ 0.05 is no significance.

3.3 Reaction times, degree of sleepiness and symptom checklist

Reaction times on the PVT were well comparable between the two conditions both at learning (301.0±11.8 vs 287.3±4.4, p=0.185) and at retrieval testing (285.3±4.6 vs 289.2±6.5, p=0.553), see detailed results in Table 3.

The degree of sleepiness which was reflected by the scores of SSS between the two conditions did not have a significant difference both before learning (3.3±0.4 vs 3.0±0.4, P=0.394) and before retrieval testing (2.3±0.3 vs 2.8±0.3, P=0.214), see detailed results in Table 3.

Table 3 Reaction times, degree of sleepiness and concentrations of Tiagabine

	Placebo (Mean±SEM)	**Tiagabine (Mean±SEM)**	**P**
RT (ms)			
Learning	301.0±11.8	287.3±4.4	0.185
Retrieval	285.3±4.6	289.2±6.5	0.553
SSS			
Learning	3.3±0.4	3.0±0.4	0.394
Retrieval	2.3±0.3	2.8±0.3	0.214
Concentration of Tiagabine (ng/dl)			
30min	2.0±0.0	69.3±37.4	0.105
90min	2.0±0.0	131.7±16.5	0.000
22.5h	2.0±0.0	22.3±4.3	0.001

SEM, standard error of mean; RT, reaction time; SSS, Stanford Sleepiness Scale.

The concentrations of Tiagabine measured 30 minutes, 90 minutes and 22.5 hours after substance administration are indicated. P＞ 0.05 is no significance.

All the symptoms the subjects reported in the symptom checklist were listed in Table 4. The most common symptoms were tiredness, drowsiness and listlessness with the minor to moderate degree of severity. However, both the incidence rate and severity of all

reported symptoms showed no significant difference between the two conditions not only in the morning after substance administration but also in the retrieval evening (all $P > 0.05$). This indicates that Tiagabine (10 mg) did not cause distinct side effects except in the excluded subject who had an epileptic seizure according to his EEG.

Table 4 Reported symptoms

Symptom	Placebo (morning)	Tiagabine (morning)	Placebo (night)	Tiagabine (night)
Headache	2	0	2	1
Listlessness	3	5	3	2
Tremor	1	0	0	0
Dizziness	0	0	1	0
Feeling of heaviness	1	0	1	2
Sweating	2	2	2	3
Numbness	3	2	0	1
Tiredness	10	9	8	7
Nervousness	2	0	5	5
Agitation	2	1	4	2
Drowsiness	6	8	4	5

Data are presented as the number of subjects who reported the symptoms in the respective conditions.

3.4 Cardiovascular measures, concentration of cortisol and Tiagabine

No significant difference in blood pressure and heart rate existed before substance administration, as well as in the next morning and at retrieval testing (all $P > 0.05$).

Serum levels of cortisol were compared between the two conditions before learning and after retrieval testing, as well as hourly during the retention sleep. There was no significant difference in all 11 time points. See detailed results in Fig. 10.

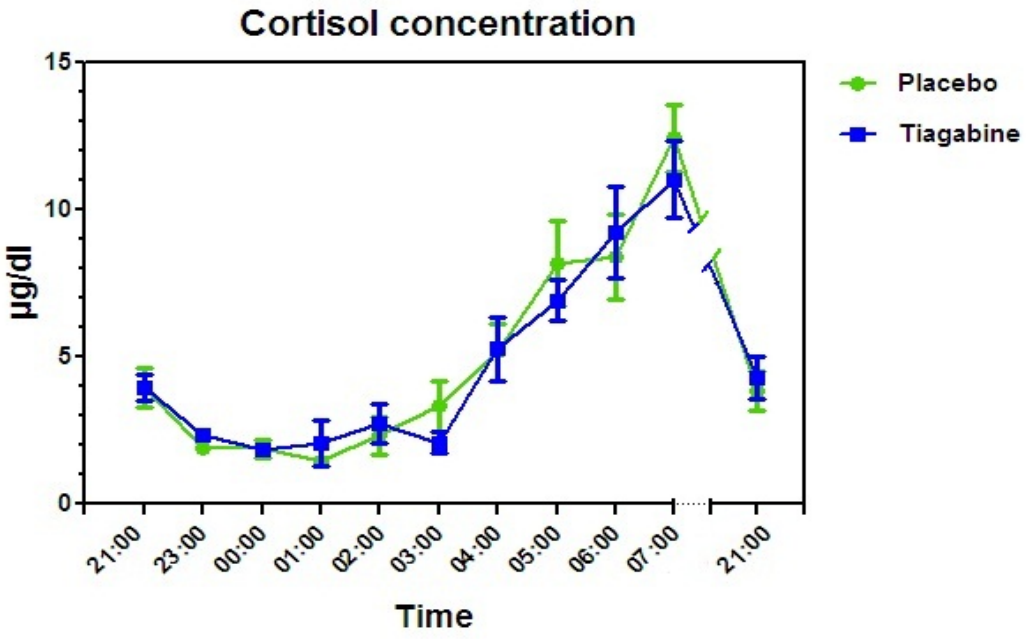

Figure 10 Serum concentration of cortisol

The difference of Tiagabine concentration 30 minutes after substance administration between the two conditions did not reach the significance (2.0±0.0 vs 69.3±37.4, P=0.105), whereas the concentration 90 minutes after Tiagabine administration was significantly higher than that in the placebo condition (2.0±0.0 vs 131.7±16.5, P=0.000). The concentration 22.5h after substance administration in the Tiagabine condition was still higher than in the placebo condition (P=0.001), although this concentration of Tiagabine (22.3±4.3 ng/dl) might be low enough not to influence the retrieval process. See detailed results in Table 3.

4. Discussion

In the present study, Tiagabine (10 mg) significantly increased the percentage of SWS, whereas it reduced the percentage of REM sleep. The latency of sleep onset, SWS and

REM sleep were not influenced. The GABAergic enhancement did not affect the consolidation of neutral declarative memory for word paired associates, but improved the consolidation of emotional declarative memory for pictures from the IAPS. The consolidation of procedural memory for finger sequence tapping was impaired by Tiagabine.

GABAergic uptake inhibitor Tiagabine may hyperpolarize Ach-containing and NA-containing arousal neurons, and then directly or indirectly promoting slow oscillation. In our study Tiagabine increased the duration of SWS sleep, which is reasonably consistent with previous pharmacological studies in humans (Mathias et al., 2001; Walsh et al., 2006a, b; Roth et al., 2006). Tiagabine also significantly reduced the duration of REM sleep, possibly by decreasing the activity of mesopontine cholinergic REM-on cells. It is inconsistent with the results reported by Mathias et al (2001), which showed that REM sleep was not influenced by Tiagabine (5 mg). However, according to the results of other studies (Walsh et al., 2006a, b; Roth et al., 2006), which the percentage of REM sleep was reduced after an increase in the dosage of Tiagabine, we speculate that the outcome in our study is on account of the relatively high dosage of Tiagabine (10 mg).

A large number of studies support the assumption that cholinergic activity at a minimum level during SWS benefits the consolidation of hippocampus-dependent declarative memory. Low Ach level is thought to enable the spontaneous re-activation of hippocampal memory traces and information transfer to the neocortex by reducing the tonic inhibition of hippocampal CA3 and CA1 feedback neurons (Buzsáki, 1989, 1996; Hasselmo, 1999；

Gais and Born, 2004b; Marshall and Born, 2007). Based on this hypothesis, we supposed the retrieval performance of PAL task would be improved after Tiagabine administration compared to placebo. But the result was inconsistent.

A recent study discovered that there is a transient increase in LC activity in trained rats during SWS two hours after learning (Eschenko and Sara, 2008). Given the ubiquitous projections of LC neurons (Jones 1991), the increase in LC activity may release NE in key target regions where reactivation is taking place. If such neuronal firing bursts occur at the the right time, they possibly enforce plasticity-related IEG activity in the neocortex (Cirelli and Tononi, 2000; Cirelli et al, 2004) and thereby support the stabilization of newly formed memory representations at the synaptic level. Therefore, Eschenko and Sara (2008) proposed an intriguing possibility that the hippocampal-cortical replay activity during SWS may act to ‘‘wake-up’’ the LC, with consequent long-lasting reinforcement of the reactivated networks. In humans, the consolidation of odor memories was impaired after pharmacological suppression of noradrenergic activity during SWS-rich sleep and improved after increasing noradrenalin availability (Gais S, Rasch B, Dahmen JC, Sara SJ, and Born J, unpublished observations). Consequently, we speculate that relatively high noradrenergic activity as well as low cholinergic activity is critical for hippocampus -dependent memory consolidation during the early SWS-dominated nocturnal sleep.

We suppose that, during the early SWS-dominated sleep, Tiagabine inhibited the noradrenergic activity by enhancing GABAergic input, which may be unfavorable to declarative memory consolidation, while it suppressed the cholinergic activity, which

facilitated the declarative memory consolidation. Accordingly, the retrieval performance of PAL in this study was not improved after Tiagabine administration despite the increase of SWS.

It is common knowledge that emotional memories are usually remembered better than neutral ones (Payne et al., 2008), and it is associated with an enhanced activity in the amygdala (Dolcos et al., 2004). Declarative memory for emotional events, although still hippocampus-dependent, becomes specifically enhanced by amygdala modulation of hippocampal functioning (Akirav and Richter-Levin 2002; Packard and Cahill 2001; Phelps, 2004). Retention of declarative memory has been shown to benefit specifically from early SWS-dominated nocturnal sleep. However, declarative memory with emotional tone has been proven to get more beneficial influence from REM sleep (Greenberg et al, 1983; McGaugh, 2004; Wagner et al, 2001, 2006; Hu et al., 2006). Moreover, high cholinergic activity during REM sleep may promote synaptic consolidation by supporting plasticity-related IEG activity (Teber et al., 2004) and the maintenance of LTP (Lopes Aguiar et al., 2008). Based on the above viewpoints, we speculated that the administration of Tiagabine before sleep would impair emotional declarative memory, by decreasing REM sleep and persistently suppressing cholinergic tone in late REM-predominant nocturnal sleep.

But unexpectedly, the amount of free recalled emotional pictures from the IAPS in the Tiagabine condition was higher than that in the placebo condition. We consider there are three possible explanations: (1) According to the “sleep to forget and sleep to remember”

hypothesis formulated by Walker (2009), some interactions for forgetting the emotional tone during REM sleep critically, and perhaps most importantly, take place within a brain that is devoid of aminergic neurochemical concentration, particularly noradrenergic input from the LC (Pace-Schott & Hobson 2002; Walker, 2009). Effects of persistent GABAergic inhibition on noradrenergic neurons in late nocturnal sleep may happen to provide such a biological theatre, and thereby benefiting emotional memory consolidation. (2) Although REM sleep was reduced, some unidentified processes which are also critical for emotional memory consolidation, such as the expression of plasticity-related IEG, the spindle activity, may still persist during the late nocturnal sleep after Tiagabine administration. (3) Inconsistent with the general view that emotional memories are preferentially remembered over neutral ones, in our study, delayed free recall performance of IAPS pictures was better for emotional than neutral pictures just in the Tiagabine condition, but not in the placebo condition. This inconsistency between the two conditions may influence the results.

Neurophysiologically, Tiagabine restrained REM sleep. Neurochemically, cholinergic activity could not reach its normal elevated level during the late REM-predominant sleep due to the persistent GABAergic inhibitory input. This elevated cholinergic level is thought to benefit the consolidation of procedural memory (Legault et al., 2006; Hornung et al., 2007; Rasch et al., 2009b). Consequently, as we expected for procedural memory, the overnight gains of correctly tapped sequences in the finger sequence tapping task were significantly decreased after Tiagabine administration. However, possibly owing to the

small sample size, the number of correctly tapped sequences in the learning stage was unexpectedly smaller in the placebo condition than in the Tiagabine condition. This baseline difference may influence the result of overnight gains, thereby lowering the reliability of this result.

Our results show that the enhancement of GABAergic activity did not affect the hippocampus-dependent declarative memory despite an increase in SWS, whereas it facilitated the amygdala-dependent emotional declarative memory despite a decrease in REM sleep. At first glance, it seems to be in contrast with the classical “dual-process hypothesis”, which assumes that SWS facilitates declarative, hippocampus-dependent memory and REM sleep supports non-declarative, hippocampus-independent memory (Maquet, 2001). In fact, our findings highlight the facts that (1) GABAergic transmission essentially relates to the off-line memory consolidation both during early SWS-dominated period of retention sleep and during late REM-predominant period of retention sleep. (2) The GABAergic mechanisms are involved in different types of sleep-associated memory consolidation possibly by regulating cholinergic and noradrenergic activity. (3) It is not a particular sleep stage that separately mediates memory consolidation, but rather the neurophysiological and neurochemical mechanisms associated with those sleep stages, and that some of these mechanisms are shared by different sleep stages.

There is evidence that $GABA_A$ receptors are critically involved in the generation of sleep spindles (Lancel, 1999; Pardi and Black, 2006). However, we did not detect any change concerning sleep spindle activity which is thought to also contribute to memory

consolidation (Schabus et al., 2004; Clemens et al., 2005; Mölle et al., 2009). We also did not separate the retention sleep into two halves (early and late half nocturnal sleep) when analyzing the changes of SWS and REM sleep, therefore we do not know whether SWS increased and REM sleep decreased only during part of the night. Additionally, on account of the small sample size, some of the independent variables were not completely balanced between the two conditions, which may lower the reliability of our results. For example, in the placebo condition, the number of correctly tapped sequences was smaller during learning. And delayed free recall performance of IAPS pictures was not better for emotional than for neutral pictures in the placebo condition.

In conclusion, the findings of the present study indicate that the GABAergic mechanisms are involved in sleep processes as well as in sleep-associated memory consolidation possibly by regulating the activity of other neurotransmitters such as acetylcholine and noradrenalin. In future studies, we will enlarge sample size and further investigate whether the sleep spindle activity is increased by the enhancement of GABAergic activity, which may benefit memory consolidation. And we also will respectively analyze the changes of sleep architecture after Tiagabine administration during the two halves of the nocturnal retention sleep.

5. Summary

Slow wave sleep (SWS) benefits the consolidation of hippocampus-dependent declarative memories, whereas rapid eye movement (REM) sleep improves procedural memories involving striato-cortical circuitry and amygdala-dependent emotional memories. Based on the evidence that enhancing GABAergic activity increases SWS and reduces REM sleep, as well as the evidence from animal studies that GABAergic transmission is involved in memory consolidation, we designed the present study to investigate the role of GABAergic transmission on sleep-associated memory consolidation in humans. In a randomized, double-blind, within-subject crossover study, 12 men learned three different types of memory tasks in the evening before an overnight retention sleep. The subjects were administered with either placebo or the GABA uptake inhibitor Tiagabine (10 mg) after learning. Memory retrieval performance was assessed 22.5 hours after learning. Each subject participated in two experimental sessions (placebo or Tiagabine) separated by an interval of at least 2 weeks. The results show that Tiagabine significantly increased SWS, whereas it reduced REM sleep. The enhancement of GABAergic activity did not affect the consolidation of neutral declarative memory for word paired associates, but improved the consolidation of emotional declarative memory for pictures from the International Affective Picture System. The consolidation of procedural memory for finger sequence tapping was impaired by Tiagabine. The findings indicate that GABAergic mechanisms are involved in sleep processes as well as in sleep-associated memory consolidation possibly by regulating the activity of other neurotransmitters such as acetylcholine and noradrenalin.

6. Reference List

Akirav I, Richter-Levin G: Mechanisms of amygdale modulation of hippocampal plasticity. J Neurosci 22, 9912-9921 (2002)

Aserinsky E, Kleitman N: Regularly occurring periods of eye motility and concurrent phenomena during sleep. Science 118, 273-274 (1953)

Aston-Jones G, Bloom FE: Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1, 876-886 (1981)

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ: International Union of Pharmacology. XV. Subtypes of GABAA receptors: classification on the basis of subunit structure and receptor function. Pharmacol. Rev 50, 291-313 (1998)

Berlau DJ, McGaugh JL: Enhancement of extinction memory consolidation: The role of the noradrenergic and GABAergic systems within the basolateral amygdale. Neurobiol Learn Mem 86, 123-132 (2006)

Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C: Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. Eur J Pharmacol 269, 219-224 (1994)

Born J, Fehm HL: Hypothalamus–pituitary–adrenal activity during human sleep: A coordinating role for the limbic hippocampal system. Exp Clin Endocrinol Diabetes 106,153-163 (1998)

Born J, Wagner U: Awareness in memory: being explicit about the role of sleep. Trends Cogn Sci 8, 242-244 (2004)

Born J, Rasch B, Gais S: Sleep to remember. Neuroscientist 12, 410-424 (2006)

Bowery NG: GABAB receptor: a site of therapeutic benefit. Curr. Opin. Pharmacol 6, 37-43 (2006)

Bradley MM, Lang PJ: Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. J.Behav.Ther.Exp.Psychiatry 25, 49-59 (1994)

Buske-Kirschbaum A, Grota L, Kirschbaum C, Bienen T, Moynihan J, Ader R, Blair ML, Hellhammer DH, Felten DL: Conditioned increase in peripheral blood mononuclear cell (PBMC) number and corticosterone secretion in the rat. Pharmacol Biochem Behav 55, 27-32 (1996)

Buzsáki G: Two-stage model of memory trace formation: a role for “noisy” brain states. Neuroscience 31, 551-570 (1989)

Buzsáki G: The hippocampo-neocortical dialogue. Cereb Cortex 6, 81-92 (1996)

Cahill L: Neurobiological mechanisms of emotionally influenced, long-term memory. Prog Brain Res. 126, 29-37 (2000)

Carbó Tano M, Molina VA, Maldonado H, Pedreira ME: Memory consolidation and reconsolidation in an invertebrate model: the role of the GABAergic system. Neuroscience 158, 387-401 (2009)

Castellano C, Brioni JD, Nagahara AH, McGaugh JL: Post-training systemic and intra-amygdala administration of the GABA-B agonist baclofen impairs retention. Behav Neural Biol 52, 170-179 (1989)

Cirelli C, Tononi G: Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. J. Neurosci 20, 9187-9194 (2000)

Cirelli C, Gutierrez CM, Tononi G: Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron 41, 35-43 (2004)

Clemens Z, Fabo´ D, Hala´sz P: Overnight verbal memory retention correlates with the number of sleep spindles. Neuroscience 132, 529-535 (2005)

Datta S: Neuronal activity in the peribrachial area: Relationship to behavioral state control. Neurosci Biobehav Rev 19, 67-84 (1995)

Datta S: in Rapid Eye Movement Sleep (eds Mallick, B. N. & Inoue, S.) 91-106 (Marcel Dekker, New York, 1999).

Datta S: Avoidance task training potentiates phasic pontine-wave density in the rat: A mechanism for sleep-dependent plasticity. J. Neurosci 20, 8607-8613 (2000)

de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M: Brain corticosteroid receptor balance in health and disease. Endocr. Rev 19, 269-301 (1998)

Dolcos F, LaBar KS, Cabeza R: Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. Neuron 42, 855-863 (2004)

Doyon J, Benali H: Reorganization and plasticity in the adult brain during learning of motor skills. Curr Opin Neurobiol 15, 161-167 (2005)

Eschenko O, Sara SJ: Learning-dependent, transient increase of activity in noradrenergic neurons of locus coeruleus during slow wave sleep in the rat: brain stem-cortex interplay for memory consolidation? Cereb.Cortex 18, 2596-2603 (2008)

Fahn S, Côté LJ: Regional distribution of γ-aminobutyric acid (GABA) in brain of the Rhesus

monkey. J Neurochem 15, 209-213 (1968)

Fink-Jensen A, Suzdak PD, Swedberg MD, Judge ME, Hansen L, Nielsen PG: The gamma-aminobutyric acid (GABA) uptake inhibitor, Tiagabine, increases extracellular brain levels of GABA in awake rats. Eur J Pharmacol 220, 197-201 (1992)

Fischer S, Drosopoulos S, Tsen J, Born J: Implicit learning— explicit knowing: a role for sleep in memory system interaction. J Cogn Neurosci 18, 311-319 (2006)

Fogel S, Smith CT: Learning-dependent changes in sleep spindles and Stage 2 sleep. Journal of Sleep Research 15, 250-255 (2006)

Fogel SM, Smith CT, Beninger RJ: Increased GABAergic activity in the region of the pedunculopontine and deep mesencephalic reticular nuclei reduces REM sleep and impairs learning in rats. Behav Neurosci 124, 79-86 (2010)

Forkstam C, Petersson KM: Towards an explicit account of implicit learning. Curr Opin Neurol 18, 435-441 (2005)

Fowler MJ, Sullivan MJ, Ekstrand BR: Sleep and memory. Science 179, 302-304 (1973)

Frank MG, Benington JH: The role of sleep in memory consolidation and brain plasticity: Dream or reality? Neuroscientist 12, 477-488 (2006)

Gais S, Born J: Declarative memory consolidation: mechanisms acting during human sleep. Learn Mem 11, 679-685 (2004a)

Gais S, Born J: Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. Proc Natl Acad Sci U S A 101, 2140-2144 (2004b)

Gais S, Albouy G, Boly M, Dang-Vu TT, Darsaud A, Desseilles M, Rauchs G, Schabus M, Sterpenich V, Vandewalle G, Maquet P, Peigneux P: Sleep transforms the cerebral trace of declarative memories. *Proc. Natl Acad. Sci. USA* 104, 18778-18783 (2007)

Gervasoni D, Darracq L, Fort P, Soulière F, Chouvet G, Luppi PH: Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. Eur J Neurosci 10, 964-970 (1998)

Gervasoni D, Peyron C, Rampon C, Barbagli B, Chouvet G, Urbain N, Fort P, Luppi PH: Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. J. Neurosci 20, 4217-4225 (2000)

Greenberg R, Pearlman C, Schwartz WR, Grossman HY: Memory, emotion, and REM sleep. J Abnorm Psychol 92, 378-381 (1983)

Hennevin E, Huetz C, Edeline JM: Neural representations during sleep: From sensory processing to memory traces. Neurobiol Learn Mem 87, 416-440 (2007)

Hasselmo ME: Neuromodulation: acetylcholine and memory consolidation. Trends Cogn. Sci 3, 351-359 (1999)

Hasselmo ME, McGaughy J: High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. Prog. Brain Res 145, 207-231 (2004)

Hobson JA, McCarley RW, Wyzinki PW, Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. Science 189, 55-58 (1975)

Hu P, Stylos-Allen M, Walker MP: Sleep facilitates consolidation of emotionally arousing declarative memory. Psychol Sci 17, 891-898 (2006)

Huber R, Ghilardi MF, Massimini M, Tononi G: Local sleep and learning. Nature 430, 78-81 (2004)

Hornung OP, Regen F, Danker-Hopfe H, Schredl M, Heuser I: The relationship between REM sleep and memory consolidation in old age and effects of cholinergic medication. Biol. Psychiatry 61, 750-757 (2007)

Introini-Collison IB, Castellano C, McGaugh JL: Interaction of GABAergic and beta-noradrenergic drugs in the regulation of memory storage. Behav Neural Biol 61, 150-155 (1994)

Jia HG, Yamuy J, Sampogna S, Morales FR, Chase MH: Colocalization of gamma-aminobutyric acid and acetylcholine neurons in the laterodorsal and pedunculopontine tegmental nuclei in the cat: A light and electron microscopic study. Brain Research 992, 205-219 (2003)

Joiner WJ, Crocker A, White BH, Sehgal A: Sleep in Drosophila is regulated by adult mushroom bodies. Nature 441, 757-760 (2006)

Jones BE: Noradrenergic locus coeruleus neurons: their distant connections and their relationship to neighboring (including cholinergic and GABAergic) neurons of the central gray and reticular formation. Prog Brain Res 88, 15-30 (1991)

Jones BE: From waking to sleeping: neuronal and chemical substrates. Trends Pharmacol Sci 26, 578-586 (2005)

Krogsgaard-Larsen P, Frølund B, Liljefors T, Ebert B: GABA(A) agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. Biochem. Pharmacol 68, 1573-1580 (2004)

Labar KS, Cabeza R: Cognitive neuroscience of emotional memory. Nat Rev Neurosci 7, 54-64

(2006)

Lancel M, Faulhaber J, Deisz RA: Effect of the GABA uptake inhibitor Tiagabine on sleep and EEG power spectra in the rat. Br J Pharmacol 23, 1471-1477 (1998)

Lancel M: Role of GABAA Receptors in the Regulation of Sleep: Initial Sleep Responses to Peripherally Administered Modulators and Agonists. Sleep 22, 33-42 (1999)

Lang PJ, Greenwald MK, Bradley MM, Hamm AO: Looking at pictures: affective, facial, visceral, and behavioral reactions. Psychophysiology 30, 261-273 (1993)

Leach JP, Brodie MJ: Tiagabine. Lancet 351, 203-207 (1998)

Legault G, Smith C T Beninger, RJ: Post-training intra-striatal scopolamine or flupenthixol impairs radial maze learning in rats. Behav. Brain Res 170, 148-155 (2006)

Lestienne R, Herve-Minvielle A, Robinson D, Briois L, Sara SJ: Slow oscillations as a probe of the dynamics of the locus coeruleus-frontal cortex interaction in anesthetized rats. J. Physiol. Paris 91, 273-284 (1997)

Little JT, Johnson DN, Minichiello M, Weingartner H, Sunderland T: Combined nicotinic and muscarinic blockade in elderly normal volunteers: cognitive, behavioral, and physiologic responses. Neuropsychopharmacology 19, 60-69 (1998)

Lopes Aguiar C, Romcy-Pereira RN, Escorsim Szawka R, Galvis-Alonso OY, Anselmo-Franci JA, Pereira Leite J: Muscarinic acetylcholine neurotransmission enhances the late-phase of longterm potentiation in the hippocampal-prefrontal cortex pathway of rats in vivo: a possible involvement of monoaminergic systems. Neuroscience 153, 1309-1319 (2008)

Lupien SJ, McEwen BS: The acute effects of corticosteroids on cognition: Integration of animal and human model studies. Brain Res Brain Res Rev 24, 1-27 (1997)

Maloney KJ, Mainville L, Jones BE: Differential c-fos expression in cholinergic, aminergic and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. J. Neurosci 19, 3057-3072 (1999)

Maquet P: The role of sleep in learning and memory. Science 294, 1048-1052 (2001)

Marshall L, Helgadottir H, Molle M, Born J: Boosting slow oscillations during sleep potentiates memory. Nature 444, 610-613 (2006)

Marshall L, Born J: The contribution of sleep to hippocampus-dependent memory consolidation. Trends Cogn. Sci 11, 442-450 (2007)

Mathias S, Wetter TC, Steiger A, Lancel M: The GABA uptake inhibitor Tiagabine promotes slow wave sleep in normal elderly subjects. Neurobiol Aging 22, 247-253 (2001)

McCarley RW, Hobson JA: Neuronal excitability modulation over the sleep cycle: a structural andmathematical model. Science 189, 58-60 (1975)

McGaugh JL: Memory--a century of consolidation. Science 287, 248-251 (2000)

McGaugh JL: The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27, 1-28 (2004)

McGaugh JL, Roozendaal B: Drug enhancement of memory consolidation: historical perspective and neurobiological implications. Psychopharmacology (Berl.) 202, 3-14 (2009)

McGinty D, Szymusiak R: Sleep-promoting mechanisms in mammals. In Principles and Practice of Sleep Medicine (Kryger, M.H. et al., eds), Elsevier Saunders (2005)

Mölle M, Eschenko O, Gais S, Sara SJ, Born J: The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. Eur J Neurosci 29, 1071-1081 (2009)

Murillo-Rodríguez E, Arias-Carrión O, Sanguino-Rodríguez K, González-Arias M, Haro R: Mechanisms of sleep-wake cycle modulation. CNS Neurol Disord Drug Targets 8, 245-253 (2009)

Nitz D, Siegel JM: GABA release in the dorsal raphe nucleus: role in the control of REM sleep. Am. J. Physiol 273, R451-R455 (1997)

Pace-Schott EF, Hobson JA: The neurobiology of sleep: genetics, cellular physiology and subcortical networks. Nature Rev. Neurosci 3, 591-605 (2002)

Packard MG, Cahill L: Affective modulation of multiple memory systems. Curr Opin Neurobiol 11, 752-756 (2001)

Pardi D, Black J: gamma-Hydroxybutyrate/sodium oxybate: neurobiology, and impact on sleep and wakefulness. CNS Drugs 20, 993-1018 (2006)

Payne JD, Stickgold R, Swanberg K, Kensinger EA: Sleep preferentially enhances memory for emotional components of scenes. Psychological Science 19, 781-788 (2008)

Peigneux P, Laureys S, Delbeuck X, Maquet P: Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport 12, A111-124 (2001)

Peigneux P, Laureys S, Fuchs S, Collette F, Perrin F, Reggers J, Phillips C, Degueldre C, Del Fiore G, Aerts J, Luxen A, Maquet P: Are spatial memories strengthened in the human hippocampus during slow wave sleep? Neuron 44, 535-545 (2004)

Perry TL, Berry K, Hansen S, Diamond S, Mok C: Regional distribution of amino acids in human brain obtained at autopsy. J Neurochem 18, 513-519 (1971)

Phelps EA: Human emotion andmemory: interactions of the amygdale and hippocampal complex. Curr Opin Neurobiol 14, 198-202 (2004)

Plihal W, Born J: Effects of early and late nocturnal sleep on declarative and procedural memory. J Cogn Neurosci 9, 534-547 (1997)

Plihal W, Born J: Effects of early and late nocturnal sleep on priming and spatial memory. Psychophysiology 36, 571-582 (1999a)

Plihal W, Born J: Memory consolidation in human sleep depends on inhibition of glucocorticoid release. Neuroreport 10, 2741-2747 (1999b)

Plihal W, Pietrowsky R, Born J: Dexamethasone blocks sleep induced improvement of declarative memory. Psychoneuroendocrinology 24:313-331 (1999)

Poldrack RA, Clark J, Paré-Blagoev EJ, Shohamy D, Creso Moyano J, Myers C, Gluck MA: Interactive memory systems in the human brain. Nature 414, 546-550 (2001)

Poldrack RA, Rodriguez P: How do memory systems interact? Evidence from human classification learning. Neurobiol Learn Mem 82, 324-329 (2004)

Rasch B, Dodt C, Molle M, Born J: Sleep-stage-specific regulation of plasma catecholamine concentration. Psychoneuroendocrinology 32, 884-891 (2007)

Rasch B, Pommer J, Diekelmann S, Born J: Pharmacological REM sleep suppression paradoxically improves rather than impairs skill memory. Nature Neurosci 12, 396–397 (2009a)

Rasch B, Gais S, Born J: Impaired off-line consolidation of motor memories after combined blockade of cholinergic receptors during REM sleeprich sleep. Neuropsychopharmacology 34, 1843-1853 (2009b)

Rechtschaffen A, Kales A: A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects, N.I.H. Publication No. 204, Maryland. (1968)

Roberts E, Frankel S: Gamma-aminobutyric acid in brain: its formation from glutamic acid. J Biol Chem 187, 55-63 (1974)

Roozendaal B: Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology 25, 213-238 (2000)

Roth T, Wright KP, Walsh J: Effect of Tiagabine on sleep in elderly subjects with primary insomnia: a

randomized, double-blind, placebo-controlled study. Sleep 29, 335-341 (2006)

Rye DB: Contributions of the pedunculopontine region to normal and altered REM sleep. Sleep 20, 757-788 (1997)

Salado-Castillo R, Díaz del Guante MA, Alvarado R, Quirarte GL, Prado-Alcalá RA: Effects of regional GABAergic blockade of the striatum on memory consolidation.Neurobiol Learn Mem 66, 102-108 (1996)

Schabus M, Gruber G, Parapatics S, Sauter C, Klösch G, Anderer P, Klimesch W, Saletu B, Zeitlhofer J: Sleep spindles and their significance for declarative memory consolidation. Sleep 27, 1479-1485 (2004)

Schendan HE, Searl MM, Melrose RJ, Stern CE: An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. Neuron 37, 1013-1025 (2003)

Shahidi S, Komaki A, Mahmoodi M, Lashgari R: The role of GABAergic transmission in the dentate gyrus on acquisition, consolidation and retrieval of an inhibitory avoidance learning and memory task in the rat. Brain Res 1204, 87-93 (2008)

Shefner SA, Osmanovic SS: GABAA and GABAB receptors and the ionic mechanisms mediating their effects on locus coeruleus neurons. Prog. Brain Res 88, 187-195 (1991)

Sirota A, Csicsvari J, Buhl D, BuzsakiG: Communication between neocortex and hippocampus during sleep in rodents. Proc. Natl Acad. Sci. USA 100, 2065-2069 (2003)

Squire LR: Mechanisms of memory. Science 232, 1612-1619 (1986)

Squire LR: Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. Psychol Rev 99,195-231 (1992)

Steiger A: Sleep and endocrine regulation. Front Biosci 8:s358-s376. (2003)

Steriade M, McCarley RW: Brain stem control of wakefulness and sleep. New York: Plenum Press. (1990)

Stickgold R: Sleep-dependent memory consolidation. Nature 437, 1272-1278 (2005)

Sutherland GR, McNaughton B: Memory trace reactivation in hippocampal and neocortical neuronal ensembles. Curr Opin Neurobiol 10, 180-186 (2000)

Teber I, Kohling R, Speckmann EJ, Barnekow A, Kremerskothen J: Muscarinic acetylcholine receptor stimulation induces expression of the activityregulated cytoskeleton-associated gene (ARC). Brain Res. Mol. Brain Res 121, 131-136 (2004)

Tononi G, Cirelli C: Sleep function and synaptic homeostasis. Sleep Med Rev 10, 49-62 (2006)

Ulloor J, Mavanji V, Saha S, Siwek D F, Datta S: Spontaneous REM sleep is modulated by the activation of the pedunculopontine tegmental GABAB receptors in the freely moving rat. J Neurophysiol 91, 1822-1831 (2004)

Varga V, Sik A, Freund TF, Kocsis B: GABAB receptors in the median raphe nucleus: distribution and role in the serotonergic control of hippocampal activity. Neuroscience 109, 119-132 (2002)

Wagner U, Gais S, Born J: Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. Learn Mem 8, 112-119 (2001)

Wagner U, Degirmenci M, Drosopoulos S, Perras B, Born J: Effects of cortisol suppression on sleep-associated consolidation of neutral and emotional memory. Biol Psychiatry 58, 885-93 (2005)

Wagner U, Hallschmid M, Rasch B, Born J: Brief sleep after learning keeps emotional memories alive for years. Biol Psychiatry 60, 788-790 (2006)

Wagner U, Born J: Memory consolidation during sleep: interactive effects of sleep stages and HPA regulation. Stress11, 28-41 (2008)

Walker MP, Brakefield T, Hobson JA, Stickgold R: Dissociable stages of human memory consolidation and reconsolidation. Nature 425, 616-620 (2003)

Walker MP: The role of sleep in cognition and emotion. Ann. N. Y. Acad. Sci 1156, 168-197 (2009)

Walsh JK, Perlis M, Rosenthal M, Krystal A, Jiang J, Roth T: Tiagabine increases slow-wave sleep in a dose-dependent fashion without affecting traditional efficacy measures in adults with primary insomnia. J Clin Sleep Med 2, 35-41 (2006a)

Walsh JK, Zammit G, Schweitzer PK, Ondrasik J, Roth T: Tiagabine enhances slow wave sleep and sleep maintenance in primary insomnia. Sleep Med 7, 155-161 (2006b)

Williams SR, Turner JP, Crunelli V: Gamma-hydroxybutyrate promotes oscillatory activity of rat and cat thalamocortical neurons by a tonic GABAB receptor-mediated hyperpolarization. Neuroscience 66, 135-141 (1995)

Wilson MA, McNaughton BL: Reactivation of hippocampal ensemble memories during sleep. Science 265, 676-679 (1994)

Wixted JT: The psychology and neuroscience of forgetting. Annu Rev Psychol 55, 235-269 (2004)

Young AB, Chu D: Distribution of GABAA and GABAB receptors in mammalian brain: potential targets for drug development. Drug Dev Res 21,161-167 (1990)

7. Attachment

Attachment 1 experiment procedure

Versuchablauf Subject_____

Date_____

Ereignis	Dauer	Geplante Uhrzeit	Uhrzeit	
Ankunft Schlaflabor		19:30		
Probandenblatt ausfüllen				
Venenverweilkanüle legen				
Elektrodenkleben				
PANAS				
SSS				
Symptom-Checkliste **(BP-HR)**				
Blutentnahme 1				
Instruktion PVT	1 min	21:00		
PVT *Instruktion*	5 min			
Instruktion: Im Folgenden wirst Du 3 Aufgaben durchführen, in denen Du unterschiedliche Dinge lernen musst. Die Inhalte der Aufgaben werden morgen Abend nochmal abgefragt.				
1. IAPS lernen	12 min	21:10		
Pause (Snood)	15 min			
2. PAL lernen	25 min	21:40		
Pause (Snood)	10 min	22:05		
3. Fingertapping	12 min	22:10		
Instruktion: Keine Merkstrategien				
Medikamentengabe		22:30		
Elektroden mit Verstärker verbinden, Signaltest	2 min	22:45		
Blutentnahme 2		23:00		
Licht aus				
Blutentnahme 3		00:00		
Blutentnahme 4		01:00		

Blutentnahme 5		02:00		
Blutentnahme 6		03:00		
Blutentnahme 7		04:00		
Blutentnahme 8		05:00		
Blutentnahme 9		06:00		
Blutentnahme 10		07:00		
Licht an (in W, S1 oder S2)		07:00		
Elektroden abziehen				
Kanüle abziehen				
Symptom-Checkliste **(BP-HR)**				
SF A -R				
Ankunft Proband		19:30		
Probandenblatt_recall				
SSS				
PANAS				
Symptom-Checkliste **(BP-HR)**				
PVT	5 min			
Fingertapping Abruf	3 min	19:45		
Fingertapping Kontrollsequenz	3 min			
PAL Abruf	10 min	19:55		
IAPS_FR Abruf	30 min	20:00		
IAPS_Rec Abruf	22 min	20:30		
Nachbefragung 1				
Nur nach EXP2: Nachbefragung 2				
Blutabnahme		21:00		

Attachment 2 Stanford Sleepiness Scale

Stanford-Schläfrigkeits-Skala

Probanden-Nr.: Datum: vor

Im folgenden soll der Grad der Schläfrigkeit (wie wach fühlen Sie sich?) erhoben werden:
Kreuzen Sie bitte das entsprechende Kästchen an.

Schläfrigkeitsgrad	Punktwert
Ich fühle mich aktiv, lebhaft, aufmerksam oder sehr wach	1
Ich kann konzentriert arbeiten, habe aber kein Leistungshoch	2
Ich fühle mich wach, entspannt und aufnahmefähig aber nicht voll konzentriert	3
Ich fühle mich irgendwie träge	4
Ich fühle mich träge, verlangsamt, und könnte mich hinlegen	5
Ich fühle mich schläfrig, benebelt, kämpfe gegen die Müdigkeit und würde mich lieber hinlegen	6
Ich bin kurz vor dem Einschlafen und habe bereits Traumdeutungen	7
Ich schlafe	8

Attachment 3 Symptom checklist

Fragebogen zur Befindlichkeit

Bitte kreuzen Sie an inwiefern nachfolgende Symptome im Moment bei Ihnen vorliegen:

	Gar nicht	etwas	mäßig	stark	sehr stark
Hautveränderungen	0	1	2	3	4
Juckreiz	0	1	2	3	4
Kopfschmerzen	0	1	2	3	4
Antriebsschwäche	0	1	2	3	4
Herzrasen, Herzstolpern	0	1	2	3	4
Verstopfung	0	1	2	3	4
Zittern	0	1	2	3	4
Magen-Darm-Beschwerden	0	1	2	3	4
Schwindelgefühl	0	1	2	3	4
Übelkeit	0	1	2	3	4
Völlegefühl	0	1	2	3	4
Schwitzen	0	1	2	3	4
Benommenheit	0	1	2	3	4
Müdigkeit	0	1	2	3	4
Nervosität	0	1	2	3	4
Erregtheit	0	1	2	3	4
Schläfrigkeit	0	1	2	3	4
Halluzinationen	0	1	2	3	4

Sonstiges:

W*ird vom Versuchsleiter ausgefüllt:*

Blutdruck:
Herzrate:

8. Acknowledgements

I would like to take this opportunity to express my gratitude to the following people who have made the completion of the present thesis possible.

My heartfelt thanks first go to my supervisor Professor Dr. Jan Born, who guided me from the basics until the end of this study. His advanced research thoughts were the premise of my study. His support and encouragement were of great value and motivation for my research work.

I want to give my special thanks to Ines Wilhelm, who helped me organizing the whole studying procedure and taught me everything. She offered many valuable suggestions and comments at every stage of my research work and thesis writing.

I am also very grateful to Maren Janotta, who provided language help to make the experiment going on wheels. Her enthusiasm and friendly help were full of the whole experiment course.

I also warmly thank to Sabine Groch, who was always helpful whenever I need technical assistance or academic advice.

I also want to say thank you to Lisa Marshall, Gordon Feld, Susanne Diekelmann, and other colleagues, who gave their unselfish help and make me staying in the institute pleasantly.

I would also like to extend my gratitude to Ms. Marin Maass and Ms. Bernadette Sagel in the international office and my friends who accompanied me during my stay in Luebeck. They took trouble in helping me and kept me staying in Germany pleasantly.

Last but not least, I would like to thank my family and relatives, especially my husband Zhijun Hu and my son Yi Hu. Their persistent support and encouragement ensured the accomplishment of my study and this thesis.

9. Curriculum Vitae

Name：Ying Ma

Sex: Female

Date of Birth: Nov 11, 1972

Place of Birth：Haining, Zhejiang Province, P. R. China

Education:

Sep.1979-Jul.1984 Primary School, Zhejiang Province, P.R.China

Sep.1984-Jul.1987 Junior High School, Zhejiang Province, P.R.China

Sep.1987-Jul.1990 Senior High School, Zhejiang Province, P.R.China

Sep.1990-Jul.1995 Bachelor Degree from Wenzhou Medical College, P.R.China

Sep.1998-Jul.2001 Master Degree in Neurology from School of Medicine, Zhejiang University, P.R.China

Oct.2009-Sep.2010 Visiting scholar and Medical Doctor Candidate, University of Luebeck, Germany

Working experience：

Aug.1995-Nov.2000 resident, Psychiatry department in the Second Affiliated Hospital, School of Medicine, Zhejiang University

Dec.2000-Nov.2006 attending physician, Psychiatry department in the Second Affiliated Hospital, School of Medicine, Zhejiang University

Dec.2006-Sep.2009 associate chief physician, Psychiatry department in the Second

Affiliated Hospital, School of Medicine, Zhejiang University

Publications：

1. Ma Y, LI HC, Zheng LL, Yu HL: Hemodynamic changes in depressive patients，J Zhejiang Univ Sci B. 7, 133-137 (2006)

2. Li HC, Chen QZ, Ma Y, Zhou JF: Imbalanced free radicals and antioxidant defense systems in schizophrenia: a comparative study. J Zhejiang Univ Sci B. 7, 981-986 (2006)