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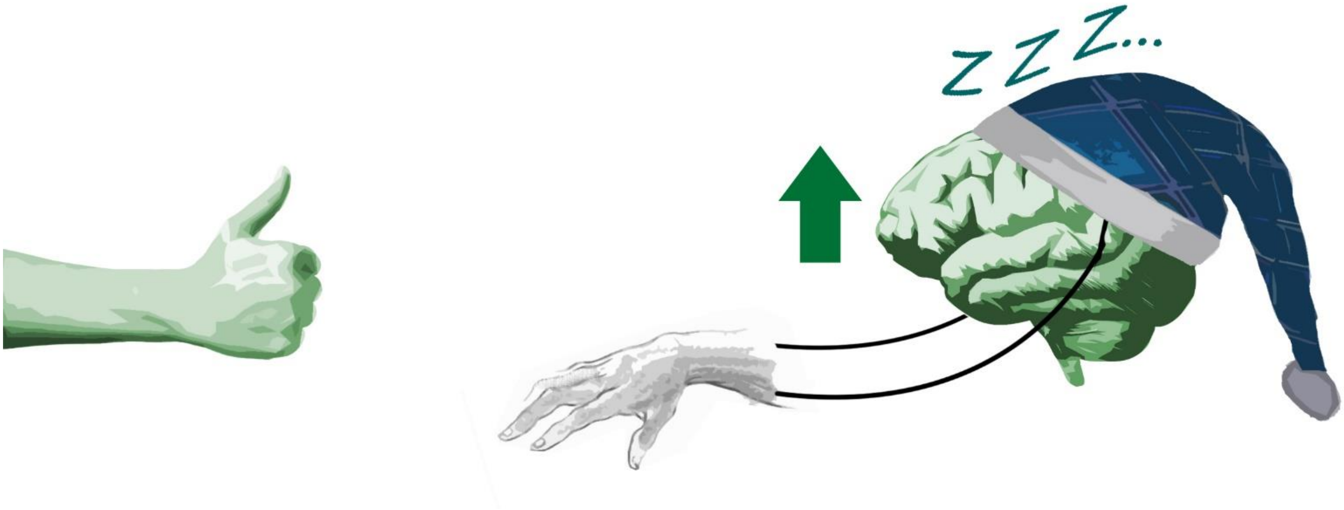

Improved motoric memory consolidation

The effect of positive feedback on motoric memory

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Foreword:

I would like to thank Christian Benedict and Frida Rångtell for letting me be a part of the neuroscience department in Uppsala and for giving me the opportunity to work with this exciting project. I would also like to thank Xiao Tan for getting me started and helping me in times of need. Lastly, I salute all the participants that have been participating in this lengthy study, without them this would never have been achievable.

Abstract:
Motor memory is an essential part of everyday life and essential for learning and retaining motor skills such as playing computer games, piano, driving a car and dancing. Difficulties executing motor movements can have a significant impact on daily functioning. The exact mechanisms underlying the formation of new motor memory is not entirely clear, and with this study, we want to further develop our knowledge of how the motor memories are created. Converging evidence that sleep plays a significant role in motor memory learning is already widely accepted, but there are still questions on which aspects of motor learning that are influenced by sleep, and what aspects of learning that could improve motor performance. Therefore, by using a motor sequence test for finger skills, we aimed to examine how positive feedback and sleep affect motoric memory consolidation compared to wakefulness. In addition, we studied if there were any gender differences in motor learning, as this has not been vastly researched. Usually, memory studies only carry out retrieval tests after a shorter period, a few hours to a day after initial learning. Retention of motor memories for a longer period which is highly relevant in daily life has rarely been studied, and therefore this study also examined how stable the learned motor memories were after four weeks.

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1. Introduction

This study will be carried out over a longer period than what this thesis captures. The thesis covers about eight weeks of data gathering and includes about half of the estimated number of participants needed for the full execution of the study. Therefore, this thesis can be seen as an initial analysis of a larger project.

1.1. Aim and structure

The purpose of this study is to investigate if positive feedback improves memory consolidation, and therefore gives a guideline for how the educational system and teachers should give feedback to pupils. The educational system is one of the most important pillars that builds the foundation of a working society, and due to cultural differences, divergent approaches of teachers and authorities arise toward students learning and performance (Hofstede, 1986). If positive feedback has a profound effect on memory consolidation and procedural memory, it would be wise for universities around the globe to embrace a more positively oriented way of teaching.

This study aims to further contribute to the knowledge of how procedural memories are developed and acquired.

Aspects of this project are:

- The effect of positive feedback on procedural memory learning.
- The importance of sleep on memory consolidation in comparison to an awake state in learning and memory recall.
- Gender differences in motoric memory learning.
- The stability of these memories after four weeks.

1.2. A Historical Introduction to the research of memory consolidation

Around 1900 Müller and Pilzecker did pioneering work on human subjects which resulted in a preservation-consolidation hypothesis of memory. The studies showed that newly learned knowledge was disrupting other newly learned knowledge after the initial learning phase. A shorter interval between learning phase and newly acquired knowledge lead to greater memory deficit of the initial learning. Thus, they concluded that memories are fragile throughout a time after initial learning and facilitates interference with the stabilizing process of memory retention (Lechner, Squire, & Byrne, 1999 1999). Later in 1920, the interference theory shed light upon other aspects of memories with less focus on the time-dependent consolidation processes and instead focused on encoding and retrieval processes of memory (Anderson, 2003). The interest in consolidation did not re-emerge until 1960 when several patients underwent a dissection of the medial temporal lobe in which the widely-known patient Henry Molaison (H.M.) was recognized for (Scoville & Milner, 1957).

The reason H.M.'s lesion was remarkable was because of his severe amnesia that followed, which compared with the amnesia of other patients, was far less extensive. His working memory seemed to be intact, but he could not form new memories which resulted in him only remembering ten minutes of his past. Since the lesion involved the hippocampus, it was believed that the hippocampus played a significant role in acquiring new memories. It is because of this memory deficit, which induced realizations of separate memory systems and which later reintroduced the focus of a new discussion about memory and memory consolidation (Schmolck, Kensinger, Corkin, & Squire, 2002 & Squire, 2002).

1.3. What is memory consolidation and why does it occur during sleep?

Memory consolidation is a process in which memories strengthens and solidifies. Memory functions consist of three processes; encoding, consolidation, and retrieval (Brosch, Scherer, Grandjean, & Sander, 2013 & Sander, 2013). During encoding, perceived stimuli are creating new memory traces which are very sensitive to surrounding influences, decay and forgetting. Consolidation, which this study is investigating, is the process of which memory traces and existing knowledge is merged with preexisting networks of knowledge (James L McGaugh, 2000), while retrieval is the process of accessing already stored knowledge. Countless studies have conclusively shown that these processes are operating in different situations, and consolidation has for example been assumed to play a bigger part during sleep (Rasch & Born, 2013).

During sleep, an individual undergoes a series of different sleep stages (**Figure A**). At first, an individual is drifting between sleep and wakefulness, eye movements slow down and a sudden muscle contraction might occur which very often is described as a falling sensation. Once asleep, in the first sleep stage (N1), eye movements disappear and slower brainwaves can be measured. In the succeeding two stages, N2 and N3, the brainwaves are characterized by slow high-amplitude oscillations which under normal circumstances dominate the first half of nocturnal sleep. This sleep stage is also referred to as slow-wave sleep (SWS). Lastly, an individual enters the rapid eye movement (REM) sleep stage, which is categorized by rapid eye movements and low-amplitude oscillations of brainwaves, very similar to conditions during an awake state (Boostani, Karimzadeh, & Nami, 2017). The transition between sleep stages reoccurs in sleep cycles during the night, as seen in Figure A, but the duration spent in each stage changes during the night. The SWS sleep stage dominates the first half of the sleep period and has been seen to play a significant role for integrating memories into long-term memory, also referred to as consolidation and is widely accepted in the standard model of consolidation. However, the processes that occur in the brain is a highly complex phenomenon and the understanding of them is rapidly changing. For instance, recent findings in memory consolidation among rats has shown the importance of REM sleep regarding specific circumstances (Durrant, Cairney, McDermott, & Lewis, 2015). If a rat is to rapidly encode information into memory in an environment that is already known, schema conformant memories, REM sleep plays a bigger part in consolidating them. This suggests that REM sleep might be a less demanding and simpler process which rapidly consolidates small quantities of information into already existing knowledge schemas. As seen in Figure A, REM sleep dominates the second half of the nocturnal sleep.

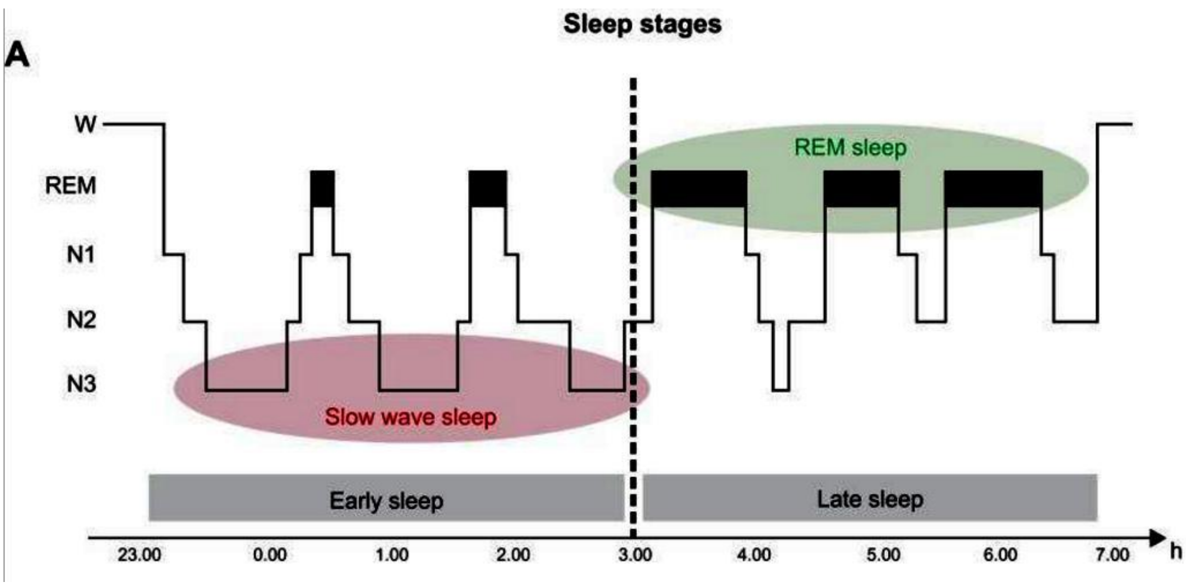


Figure A

The Y-axis represents the different sleep stages with “W” as an awake state. The X-axis represents the time period of nocturnal sleep (Rasch & Born, 2013).

1.4. The Dual Process Hypothesis

This hypothesis is assuming that the different sleep stages are improving diverse kinds of memory formations (Rauchs, Desgranges, Foret, & Eustache, 2005). Consolidation is thought to take place during sleep because of substantial evidence supporting the standard two-stage memory system theory, also referred to as the dual process hypothesis proposed by Marr (Marr, 1971). In brief, it assumes that newly acquired knowledge initially gets stored by a rapid learning process, often involving hippocampal activity in which it is also vulnerable to retroactive interferences from other newly learned knowledge. Over time it is gradually integrated into long-term memory in neocortex by a slower storage process involving repeated activation of neuronal patterns, without overwriting old knowledge. This progression materializes continuously across the day but is enhanced during sleep when external stimuli no longer are present. In this way, knowledge becomes solidified into long term memory and can eventually lead to very robust neuronal patterns. If many patterns are representing similar knowledge, one will eventually become stronger or more easily activated than the competing neuronal patterns and this is, among other things, due to the consolidation process. Thus, in a sense, knowledge has been exchanged (Marr, 1971).

With this in mind, an awake state is believed to be optimal for encoding and retrieval processes and not for consolidation, simply due to the conflict of mutually overlapping neuronal structures with consolidation processes. Thus, when external stimuli no longer are present, a dormant state is optimal for consolidation, integrating newly acquired information to preexisting memory without retrograde interference. Recent evidence shows that consolidation transpires in waves across a lengthy period, days after the initial learning has occurred, also referred to as reconsolidation (Nader & Hardt, 2009). Reverberating activity in neurons builds up two different types of consolidation, "systems consolidation" and "synaptic consolidation" (Dudai, 2004). The latter promotes remodeling of synapses contributing to the distinct memory and eventually produces lasting changes in the efficiency of the partaking synapses (Redondo & Morris, 2011). With a concrete example, this could manifest as learning how to change gear while driving, or promoting the neural pattern in charge of changing the gear. System consolidation builds upon synaptic consolidation but refers to the process in which newly acquired memories connect to other neurons and memories for long term storage such as associations. This could manifest as the procedural neural pattern of changing gear connecting to declarative memories of how one should drive to consume less petrol.

Interestingly and yet highly controversial, consolidation of procedural memories, mainly seems to take place during REM sleep (Rasch & Born, 2013). Many of the experiments showing support for this assumption have come from studies of REM sleep-deprived rats and cats which have shown an immensely decreased ability to learn complex tasks such as two-way shuttle box avoidance and intricate mazes. Rasch and Born (2013) also points out that these experiments have been criticized for putting a lot of stress on the rats when depriving them of REM sleep. The flower pot strategy in which rats stand on a platform, a flower pot surrounded by water, are deprived from REM sleep when they fall into the water due to REM atonia, an almost complete paralysis of the body while entering REM sleep. This method inevitably causes a lot of stress to the rats and is the cause of the valid criticism. It has however, also been shown in other milder and less stressful versions of REM deprivation such as head lifting and nudging the rats before entering REM sleep, an impairing effect on procedural memory formation (Rasch & Born, 2013).

Studies from 1971 were the first to demonstrate the beneficial effect SWS had on declarative memories such as word pairs (Yaroush, Sullivan, & Ekstrand, 1971) while later studies demonstrated the effect REM sleep had on emotional and procedural memories (Plihal & Born, 1997). Furthermore, the beneficial effect REM sleep has on implicit memories such as procedural memories were replicated in face and masked stimuli tests (Wagner, Hallschmid, Verleger, & Born, 2003). These results were achieved by studies in night-half paradigm, meaning that one group slept three hours before the learning phase in which they immediately afterwards went back to sleep while the other group only got to sleep after the learning phase. In this way, SWS was thought to solely be limited to one group of participants during post learning sleep, leaving the other group to exclusively consolidate their learning with REM sleep instead.

Studies of procedural memories on human and bird infants shows that they benefit considerably less than adults from sleep. In the case of bird infants, they actually mimic their parents song melody worse after nocturnal sleep while improving during wakeful time (Deregnaucourt, Mitra, Feher, Pytte, & Tchernichovski, 2005). Human children also showed this phenomenon, which is surprising considering that riding a bike, writing and speaking are major challenges during that period of time. Peculiarly, children with ADHD with difficulties performing procedural tasks improved towards normal motor functioning after nocturnal sleep (Rasch & Born, 2013).

The reason why infants do not improve in motor skills during nocturnal sleep has been explained by the prolonged SWS period which they go through, hampering the formation of implicit memories. But additional explanations refer to children's decreased ability to learn procedural skills which generally are much slower and inaccurate. Thus, children’s and adult’s motor skills are not comparable because children need longer time to improve. Undeniably, children that received prolonged training in motor skills did indicate an improvement over nocturnal sleep (I. Wilhelm, Metzkow-Meszaros, Knapp, & Born, 2012). These finding clearly support the theory of a dual processing hypothesis in which motoric and procedural memories are enhanced from REM sleep (Verleger, Schuknecht, Jaskowski, & Wagner, 2008). However, this conclusion is not accepted by all sleep and memory researchers. For example, critics have pointed out a drawback of the night-half paradigm research, referring to the lack of consideration of stage one sleep (“N1” in Figure A) when depriving participants from SWS sleep (Fogel & Smith, 2006). This sleep stage has been proven to contribute to memory formation of declarative memories and has been shown to play an important role in developing finger tapping skills (Rasch & Born, 2013).

1.5. Future relevance

It is important to understand that not every event or learning phase during the wake period is consolidated and solidified into long term memory (LTM). This is because a global strengthening of newly acquired memories inevitably would result in a system overflow. Interestingly, before the brain transfers temporary knowledge into LTM, it seems to undergo a process of selective consolidation. Especially emotional memories and memories that are of relevance due to future expectations are widely known to be enhanced (Goschke & Kuhl, 1993). Furthermore, a study made by Wilhelm et al. (2011) demonstrates that both declarative and procedural memories are improved when expectation of later use is present. This is evident regardless of if it was induced by the experimenter or spontaneously generated by the participants themselves, suspecting retesting in a later moment (Ines Wilhelm et al., 2011).

1.6. Positive feedback and Emotions

Not only does expected and relevant information for future use enhance the consolidation of memories, other influences such as arousal has been proven to enhance the consolidation process. In a study in which participants got to watch arousing movie clips after a learning phase performed better than the control group (Esmaeili, Karimi, Tabatabaie, & Moradi, 2011). The results indicate that arousal after learning enhances memory consolidation. Interestingly, women's performance in this study was generally better than men’s. Evidence of positive affect and arousal due to receiving a gift or watching a pleasant film has later revealed that it likewise promotes creative problem solving and overall cognitive performance (Estrada, Isen, & Young, 1994). The reason for this is that pleasant experiences tap into the dopaminergic system releasing dopamine which in turn affects and facilitates learning and memory recall of declarative memory (J. L. McGaugh, 2004). Emotions in general can modulate consolidation of memory traces by inducing a physiological change by which the amygdala, a part concerned with automatic emotional regulations, modulates hippocampal activity leading to an augmentation of specific memory traces (Dolcos, LaBar, & Cabeza, 2004). This self-regulatory memory effect has been further confirmed with participants doing face and name associations, receiving both negative and positive feedback on their performance. Over time, a declining performance was observed in groups of negative and no feedback while it was stable for those receiving positive feedback (Strickland-Hughes, West, Smith, & Ebner, 2016). To clarify, previous mentioned studies only concerns declarative memories, the effect of positive feedback on procedural memories has not yet been examined.

1.7. Decay of memory

To answer how long a memory lasts, many factors need to be regarded. Retention studies on medical students in the past show that much of the declarative knowledge learned during their education (e.g. anatomy, biochemistry and physiology) are forgotten (Custers, 2010). After one year in college, one third of what is learned in school is already forgotten, and after the preparation years for clinical work, medical students have almost forgotten half of what has been taught. The rate of forgetting that the students manifest, follows an exponentially downward retention curve, also called Ebbinghaus curve (**Figure B**). The timeframe and the amount of knowledge lost is very different from study to study due to the divergent nature of the experiments, but all results align with the exponential shape of Ebbinghaus curve (Custers, 2010). Not so surprisingly, the slope of forgetting is very similar for both motoric and declarative memory (Nilsson, Cohen, & Nyberg, 1989).

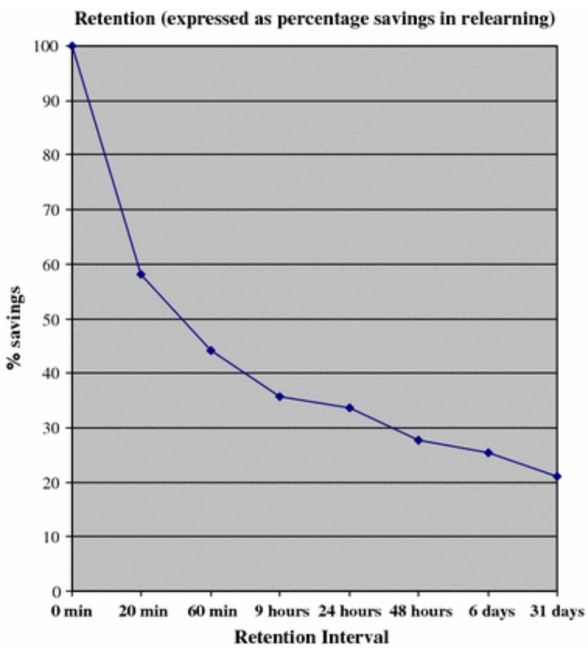


Figure B

The decay of memory in combination with strong emotional associations to it, may lead to a delusional sense of recollection. After the terrorist attack on World Trade Center in 2001, observers of the scene were to transcribe their experience. A few months later, they were to retell their story with the same amount of detail as the transcription. Even though they claimed to be able to recollect this event better than other normal events evidence indicated the opposite. The deterioration of the emotional memory seemed to have suffered the same amount of decay as any other memory, reflecting an increased and delusional sense of recollection (Talarico & Rubin, 2003).

1.8. Summary

The process of transformation between newly acquired information from temporary to long-term memory storage, has through converging evidence been shown to be facilitated by neurological processes during sleep (Durrant et al., 2015; Fischer, Hallschmid, Elsner, & Born, 2002; Walker, Brakefield, Hobson, & Stickgold, 2003). These processes reside in the brain, and are explicitly dependent on the hippocampus, referring to declarative memories such as fact based, spatial and semantic information (Lin & Yang, 2014). The strengthening process of memories (consolidation) majorly takes place during Slow-Wave-Sleep (SWS), a sleep stage that predominates during the first 2–3 h after sleep onset. Interestingly, implicit memory such as procedural memory (riding a bicycle) is believed to be consolidated during another type of sleep stage called Rapid-Eye-Movement (REM) sleep, which dominates the second half of the nocturnal sleep (Karni, Tanne, Rubenstein, Askenasy, & Sagi, 1994; Rasch & Born, 2013). Notably, the expectation of information to be useful in the future has been shown to increase the effect of nocturnal sleep consolidation (Ines Wilhelm et al., 2011) along with other studies suggesting that arousal has a similar improvement on memory recall for declarative memories (Esmaeili et al., 2011). These results indicate that sleep promotes the memory retrieval that is of relevance and of pleasant experiences. Nonetheless, no current studies have been investigating whether providing a positive feedback after training of procedural memories, irrespective of the subject's actual performance, increases the effect of sleep-dependent memory consolidation in humans. Neither has the effect of positive feedback on procedural memories been tested after a prolonged interval of one month after initial learning.

Thus, due to the lack of knowledge in this area, a study of healthy young students was conducted to investigate possible independent and additive effects of sleep and wakefulness, positive reinforcement and no reinforcement on procedural memory consolidation twelve hours after initial learning, and one month later.

2. Hypothesis

A greater score in the finger tapping test is to be expected in participants receiving positive feedback and/or with nocturnal sleep in relation to offline gain. These participants are also expected to perform better than the control group one month later, even though both groups are bound to the Ebensburg’s curve of forgetting.

As previous studies of memory consolidation have shown a generally better performance in declarative memory recall among women (Maitland, Herlitz, Nyberg, Bäckman, & Nilsson, 2004), no studies up to date have shown any gender differences in consolidation processes regarding the formation of procedural memories. Therefore, it would be interesting to see if there are any gender differences for procedural memories when positive feedback is applied to participants.

3. Method

The study consisted of two parts. In one part, we examined how the positive feedback in learning situations is affecting motor skill. In the second part, we compared the effect of memory consolidation on learning during the day (wakefulness) and during the night (sleep).

3.1. Participants

Forty-four healthy young volunteering students (18–30 years old; mean ± SD: 23.4 ± 3.2 years) who fulfilled criteria of having generally good health status (BMI ± SD: 22.3 ± 2.2; mean), being nonsmokers, good sight, and no history of sleep disturbances or drug usage participated in the experiment [Appendix 1]. To minimize the effect of sex hormones as confounding factor, female participants had to take monophasic birth control pills and only attended during days in which they took the active pills. All subjects regularly obtained 7–8 h of sleep per night and were most of them abstained from caffeine, nicotine and alcohol two days before the experimental session. They were instructed to go to bed between 22:00 - 00:00 and sleep 7-9 hours. Participants in the wake group were instructed to not take any naps during the day nor to do any heavy exercise on the day of the sessions.

3.1.1. Groups

Group A: ♂ & Positive feedback & Awake
Group B: ♀ & Positive feedback & Awake
Group C: ♂ & Positive feedback & Sleep
Group D: ♀ & Positive feedback & Sleep
Group E: ♂ & No feedback & Awake
Group F: ♀ & No feedback & Awake
Group G: ♂ & No feedback & Sleep
Group H: ♀ & No feedback & Sleep

BMI mean: 22,80888 **Age mean:** 22,74583
BMI mean: 22,83111 **Age mean:** 23,65952
BMI mean: 21,94775 **Age mean:** 23,51667
BMI mean: 21,96998 **Age mean:** 24,43036

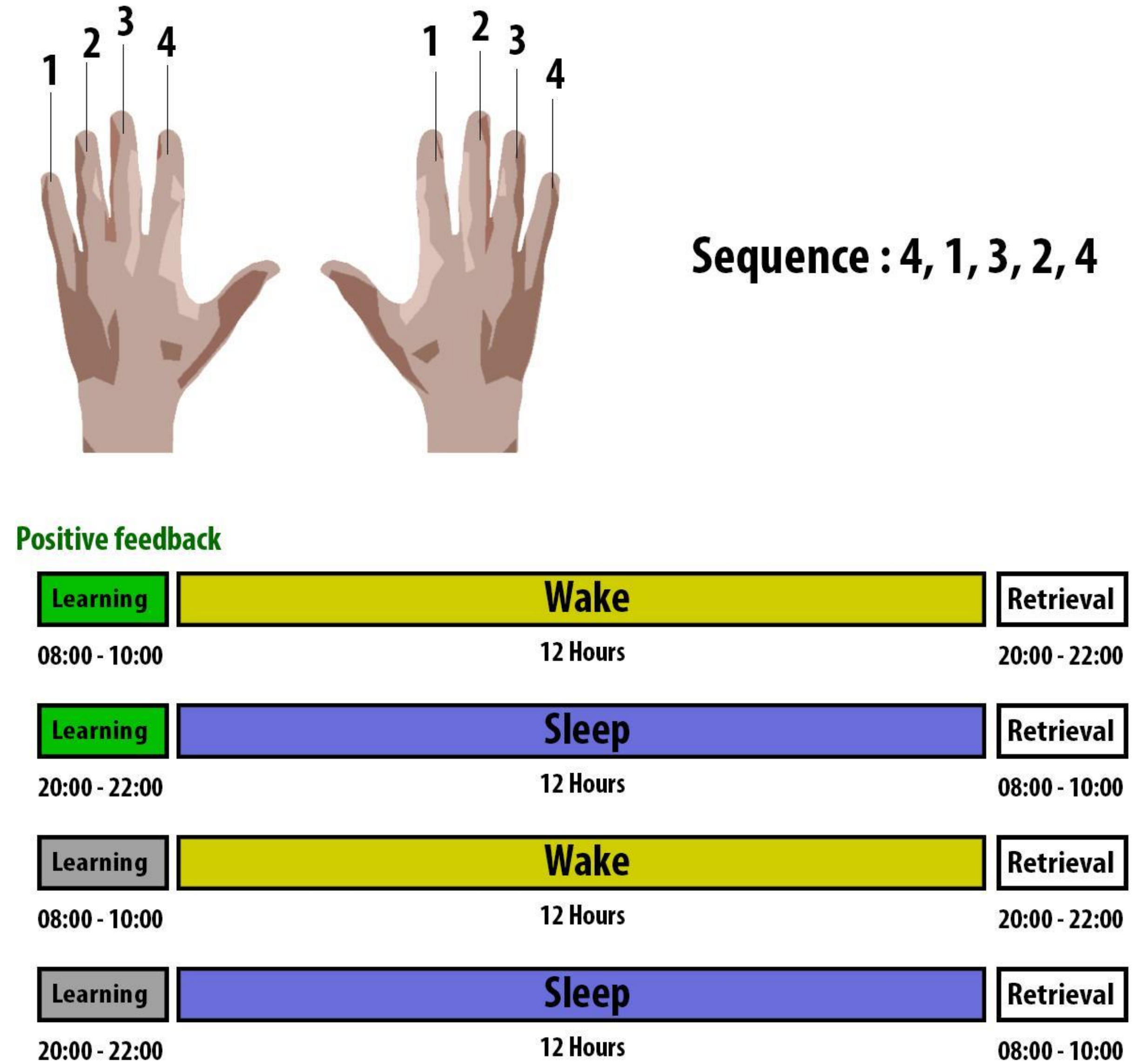


Figure C

The green color indicates that the first and the second group received positive feedback after the learning phase, while the grey color indicates no feedback. Twelve hours after the learning phase, all participants were retested. During this time, participants of the wake group were not allowed to take any naps during the day, and participants of the sleep group were told to at least sleep seven hours.

3.2. Learning Task

Right handed subjects were to place their nondominant hand over the upper numerical numpad on the keyboard starting with the little finger on number 1 and vice versa for left handed people (**Figure C**). Subjects were then to habituate with the task by doing an exercise of four trials consisting of the numbers (1-1-2-3-4) without looking at the hand. After pre-initialization, they were presented with feedback on the amount of correctly tapped sequences and were given the opportunity to retry the exercise once more. They were told to do the actual consecutive test as rapidly and accurately as possible. Following test consisted of the numbers (e.g. 4-1-3-2-4) in twelve trials, each lasting thirty seconds. Between each trial was a pause of thirty seconds. The same procedure was done for retrieval testing after the retention interval of twelve hours.

After the task was completed, participants in the feedback group were shown the text "calculating" followed by the positive feedback saying "Congratulation, you have achieved among the best results of all participants so far ". During the whole procedure participants sat in a silent and lightened room. All instructions were presented on a 21.5-inch monitor.

3.3. Design and procedure

3.3.1. Before the first session

Ads of the study were handed out at universities around Uppsala with contact information and a brief introduction to the study. Interested students were assigned an anonymous code and received a questionnaire on Survey Monkey, a free online survey tool, in our case consisting of background information [Appendix D], The Pittsburgh Sleep Quality [Appendix E] Index and The Epworth Sleepiness [Appendix F]. All responses underwent a screening process in which only participants fulfilling criteria of participation [Appendix A] were included. Furthermore, they had to answer a Competence Based Self-esteem Scale questionnaire [Appendix G]. An appointment for the first and second session was set, which either was in the morning respectively twelve hours later in the evening the same day or in the evening respectively twelve hour later in the morning the day after. Participants were given a sleep diary [Appendix C] three days before the first session together with the location of the rendezvous and lastly some restrictions the participant had to follow the day before the first session [Appendix B].

At the beginning of the study participants were free to choose a time during morning and respectively during the evening the same day, or a time during the evening and respectively a time during the morning the next day. Later, they were given time due to balancing the groups regarding BMI, education, and age.

3.3.2. The first session: Learning phase

A laptop was in advance put into the testing room with the software already set up. Participants were greeted and shown to the testing room which varied between two different rooms depending on the availability of them. They were introduced to the experiment, informed that they could leave at any time during the study and asked to hand in the sleeping diary of the three previous nights. They were then requested to sign a form of consent, filling out contact details and a questionnaire about previous day's activity, mood and alcohol, nicotine and food consumption. Meanwhile the experimenter read through the sleeping diary to check if it had been correctly filled out and if participants had followed the restrictions. If not, the participants were asked to carry out the first session another time. The experimenter gave instructions for the finger tapping experiment and left the room. When the participants of the groups A, B, C or D completed the test they were presented with a screen saying "calculating" in three seconds followed by a positive feedback notifying them that their performance was among the best ones so far. The control groups E, F, G or H were only presented with a screen thanking them for their participation (No feedback). The participants were then asked to fill in a test evaluation questionnaire together with the STOP-bang questionnaire [Appendix H]. The STOP-bang questionnaire was meant to function as an aid in determining if a participant might suffer from sleep apnea, which would not be detectable by the ACTi graph device. Lastly, they were given a reminder of the next session. All participants received an ACTi sleep device to monitor their arm movements in purpose of controlling that they had a regular night sleep and that they did not take any naps during the day. Participants from the sleep group C, D, G or H also received a sleeping diary for the succeeding night.

3.3.3. The second session: Recall phase

All participants handed in the ACTi sleep device and the sleep group handed in the sleep diary of the previous night. The same procedure as in the first session followed with exception to the STOP BANG questionnaire not being handed out, and feedback not received. Lastly, they were told that they would be notified for the third session two weeks before the appointment. It was not necessary to carry out the third session exactly thirty days after the second session if the participant could not arrive on that date.

3.3.4. The last session; Recall phase, one month later

The same questionnaires and procedures as in the second session were carried out. When completed, the experimenter handed out the two promised cinema tickets to the participants and were later sent a voluntary study evaluation survey [Appendix I].

3.3.5. Ethics, data protection and archiving

The study was approved by the Regional Ethical Review Board in Uppsala and conducted according to the Declaration of Helsinki. All participants provided written informed consent. Collected data during the study was coded according to a consecutively numbered code list.

3.4. Analysis of the results:

Once the collected data from the finger tapping test was archived and imported into SPSS for analysis, a Univariate test between the sleep, wake, positive feedback and no feedback groups was carried out once the groups distribution was balanced. The significance level, p value, was set to $p < 0.05$, two-tailed. Competence-Based Self-Esteem (CBSE) scores were summed and has a range of 12 to 60 points, a lower score means a higher self-esteem. Participants were categorized as having high self-esteem if the summed score was lesser than the median value of the CBSE scale 36, and vice versa.

Learning performance was measured by the mean value of the three last correctly typed sequences during learning phase to represent a more accurate learning value since block one to nine was regarded as an adaptation period. Recall performance was measured by the mean value of the three first correctly typed sequences during recall to represent a more accurate recall performance, excluding the latter blocks due to training effect.

Offline gain was measured by dividing the recall performance with the learning performance and was represented in terms of percentage where value 100 is the baseline value. An offline gain value of 120 therefore means a 20% increase in performance during the interval of testing and retesting.

No analysis was made for the monthly recall due to lack of participants (N=19), unevenly spread among the groups. Results of this will be presented in the continued study once a meaningful amount of data have been collected.

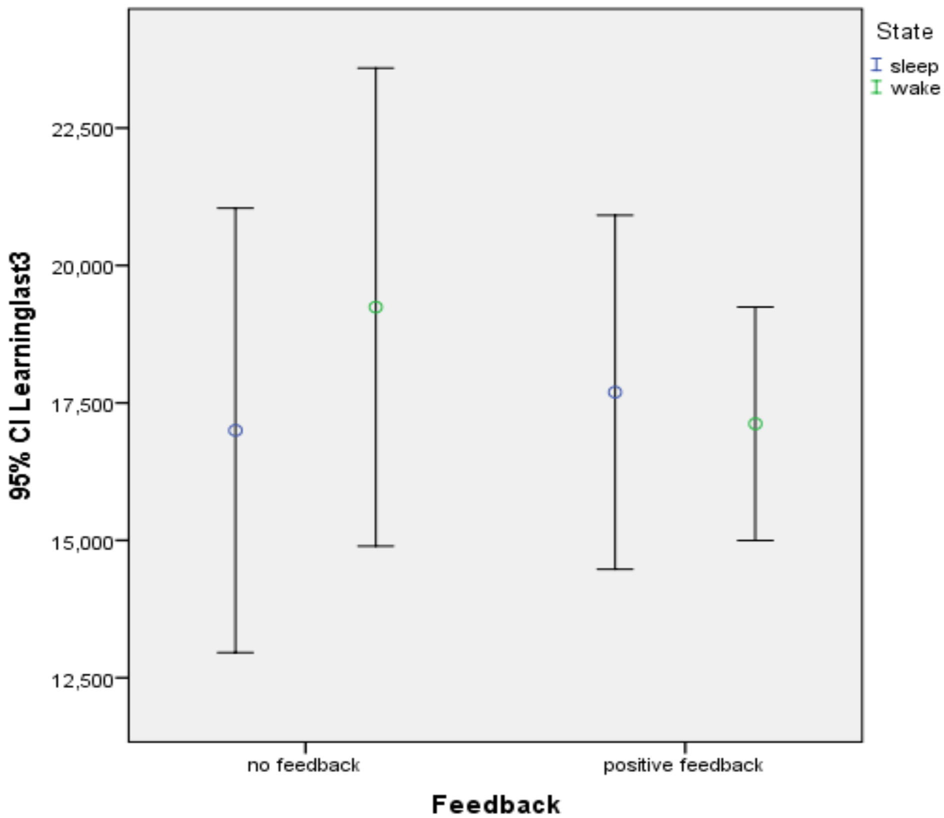
3.5. Outliers

Subjects whose learning performance, recall performance or offline gain from learning to recall differed from the group mean (sleep and wake group, separately) by more than two standard deviations were identified as outliers and excluded from analysis (N=1, i.e. the initial sample consisted of 44 subjects).

4. Results

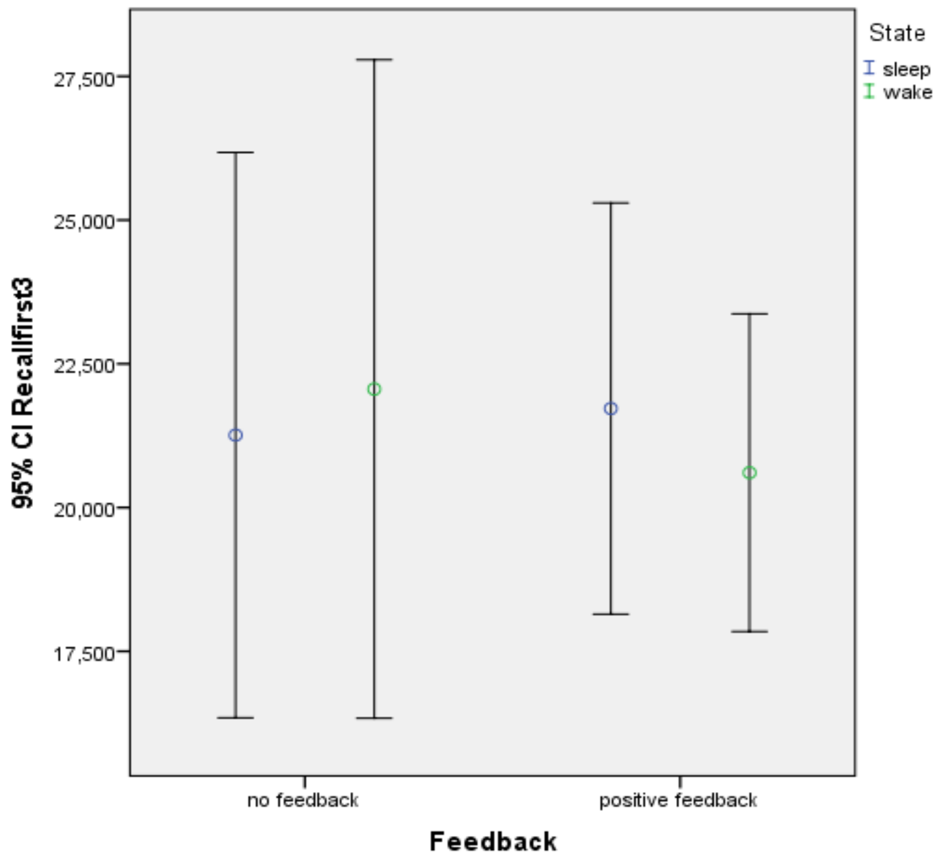
Judging from Table 1 (**Appendix J**) each group of the independent variables were evenly distributed among all conditions and were balanced in regard to gender, BMI and age. Therefore, all groups were comparable with each other. None of the participants scored highly on the STOP-BANG questionnaire and females scored five points greater in the CBSE questionnaire. This CBSE difference was not significant judging from an independent samples T-test of females ($M=36.83$, $SD=7.93$) and males ($M=31.32$, $SD=7.77$), $t(38)= -2.21$, $p = 0.90$.

To examine the differences between the independent variables and performance a One-way ANOVA was carried out both for the learning phase and the recall phase, Table 2 (**Appendix J**). The Initial learning before the recall interval was comparable for state and reinforcement conditions regarding the non-significant value of reinforcement [$F(1,41) = 0.28$, $p = .60$] and state [$F(1,41) = 0.26$, $p = .61$]. The same can be concluded for the recall performance in relation to state [$F(1,41) = 0.01$, $p = .92$] or reinforcement [$F(1,41) = 0.07$, $p = .79$]. The only significant effect detected was detected between gender and performance during learning [$F(1,41) = 9.26$, $p = .004$] and recall [$F(1,41) = 10.01$, $p = .003$]. A Three-way ANOVA, comparing the independent factors in relation to offline gain did not show any significance, hence no interaction effect could be concluded, Table 4 (**Appendix J**). A trend of correlation between state and offline gain might however be present [$F(1,41) = 3.11$, $p = 0.085$]. Notably, the result of an interaction effect between gender and reinforcement on offline gain is [$F(1,41) = 1,64$ $p = 0.21$]. To examine if self-esteem is a predictor for how susceptible participants are to the reinforcement a Two-way ANOVA between the reinforcement group and participants categorized as having low and high self-esteem was carried out. It did not show any significant effect on offline gain [$F(1,40) = 1.18$, $p = 0.26$], not did a One-way ANOVA between self-esteem and offline gain [$F(1,40) = 2.67$, $p = 0.11$] show any significance.



Plot 1 - Learning performance

The Wake group without reinforcement (n=11), even if not significant, had a slightly higher mean score (Mean = 19.3) than the other groups. Wake & reinforcement (n = 11, Mean = 17), Sleep & reinforcement (N = 12, Mean = 17.7) and Sleep & No reinforcement (N = 9, Mean = 17.4). Y-axis represents the amount of correctly typed sequences; X-axis represents the reinforcements conditions.



Plot 2 - Recall Performance

No observable difference in performance between the independent groups can be seen in the plot. Wake & No Reinforcement group (n=11, Mean = 22), Wake & Reinforcement (n =11, Mean = 20.6), Sleep & reinforcement (n = 12, Mean = 21.7) and Sleep & No reinforcement (n = 9, Mean = 21.3). Y-axis represents the amount of correctly typed sequences; X-axis represents the reinforcements conditions.

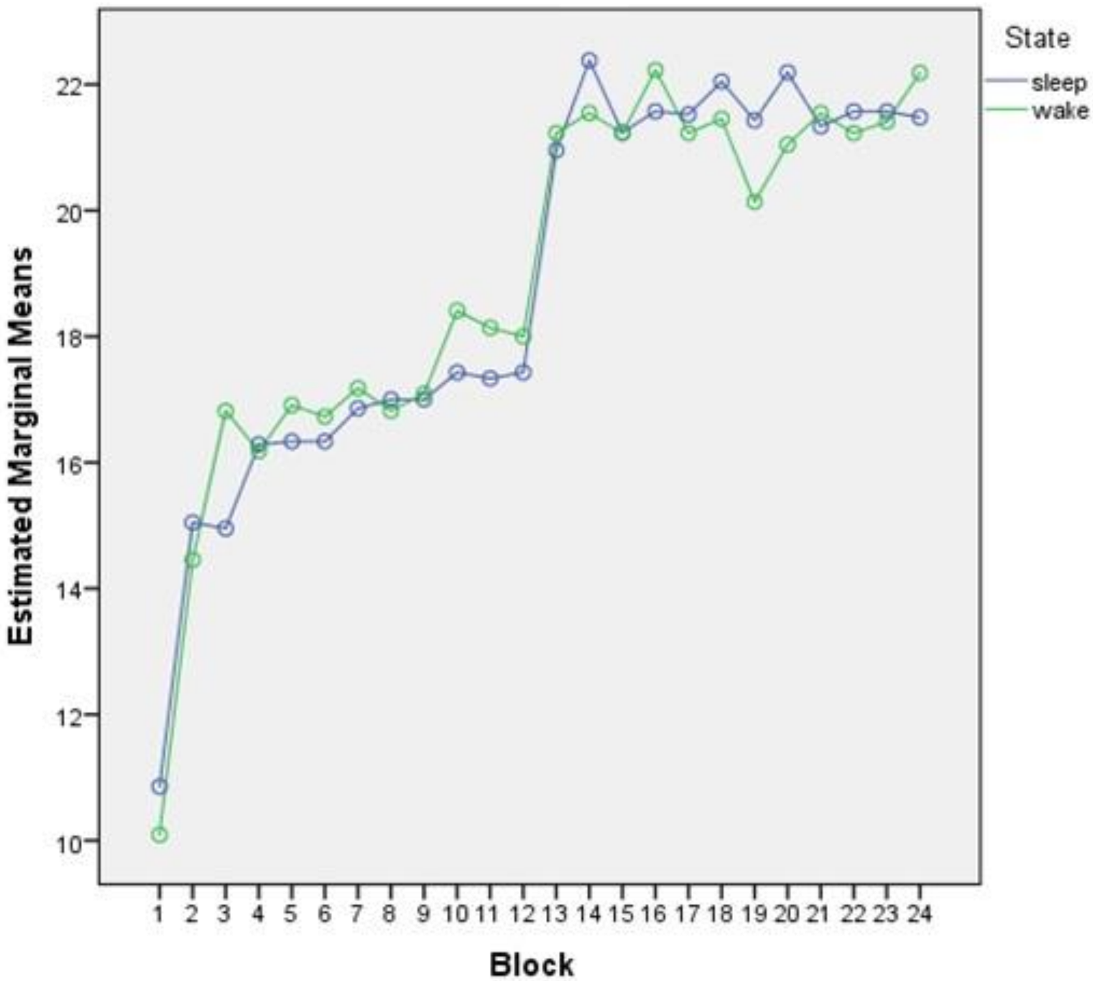


Figure 1 - Finger tapping sequence

A figure illustrating the amount of correctly typed sequences for each block for learning session (block one to twelve) and recall session (block thirteen to twenty-four). The consolidation effect can be seen in between block twelve and thirteen for both the participants that slept and for participants that stayed awake before recall phase.

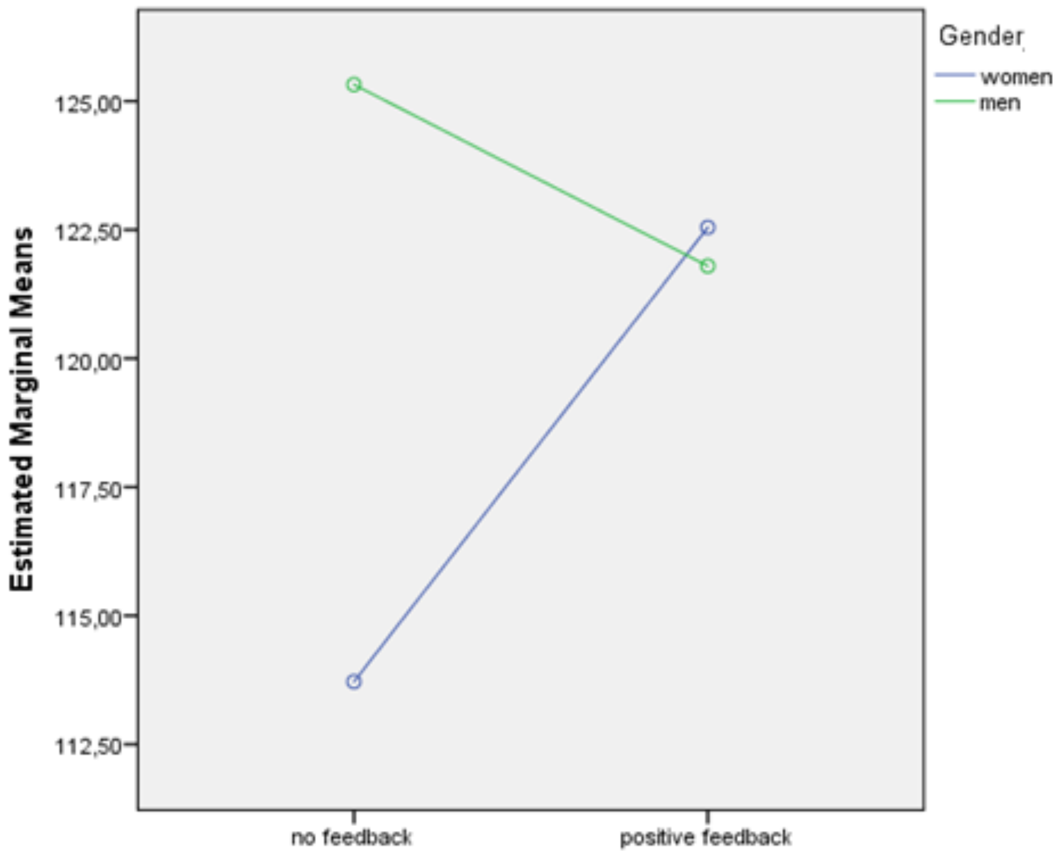


Figure 2 - Gender and Reinforcement regarding offline gain

A figure indicating an offline gain difference between gender and reinforcement. Y-axis represents a percentage gain compared to baseline value (100) for the number of correctly typed sequences during the learning phase in comparison to the number of correctly typed sequences during the recall phase.

5. Discussion

This study did not find any significant effect of sleep or reinforcement on memory consolidation, it did however find a difference between genders in finger tapping performance. Men had a significantly higher performance compared to women and did on average 4 to 5 correct sequences more than females during both learning and recall which contradicts previous research that often points out females outperforming males in fine motoric skills. It has later been shown that these differences disappear when finger thickness is regarded as a covariation factor (Peters, Servos, & Day, 1990). The results from this study have not taken finger thickness into account but regardless indicate that men outperform women. After further analysis, using prior computer and instrument playing habits as a covariate factor, men still were significantly better than women. It can therefore be concluded for the results of this study that men are better at finger tapping tasks.

Furthermore there seems to be a trend of correlation between the state condition and offline gain shown in Table 4, which probably would show a significant effect with a larger sample size, judging from similar finger tapping studies (Fischer et al., 2002). The slightly better performance of the wake control group (two correct sequences) during learning phase should be considered as an indication that this group might be biased or skewed and therefore might have obscured the presumably significant effect of sleep. Unfortunately, confounding factors such as fatigue, induced by a possible sleep deprivation of the sleep group, could not be ruled out due to an expired license of the ACTI sleep devices, making it impossible to analyze the sleep quality prior to recall. The sleep diaries did however give an indication that participants slept well with a subjectively evaluated sleep quality mean of 80 out of 100, but this cannot be confirmed without the data from the ACTI sleep device. Therefore, a significant effect might have been obtained if the sample size was increased and if participants with a low amount of sleep previous night were considered as covariates in subsequent Univariate tests.

From Figure 1, it can be concluded that a consolidation effect is taking place for both the wake and sleep group. To investigate if participants which received positive feedback improved the consolidation effect, a Univariate test was made to analyze the interaction effect between conditions regarding offline gain. No significant effects or interaction effects between conditions were detected, however, some possible trends are emerging. Due to the small number of participants only a trend of the sleep effect on offline gain can be detected, which would confirm research done in previous finger tapping studies(Fischer et al., 2002). Participants that slept therefore seems to have improved more than participants that stayed awake, but this conclusion is too early to make this early in the study.

Furthermore, the continuation of the study might reveal an interaction effect between gender and reinforcement. Judging from the visualization of Figure 2, it seems that men and women could be differently affected by positive feedback. Yet again, this conclusion cannot be made since the p value is too high, but with a persistent continuation of the study and an improvement to the positive feedback received by participants, a drastic change to these results might follow and a significance might be found.

5.1. Why did not reinforcement have the expected impact on memory consolidation?

Usually studies test their subject right after the influence of reinforcement, which is to prefer if subtle effects like positive effect are to be measured. In this study, the reinforcement might be intercepted by other reinforcements throughout the day, especially since the reinforcement was very subtle in text form, possibly only having a very weak impact on the participants. If the reinforcement would be given by a social mean, by the experimenter, the impact could be greater since the reinforcement would be multimodal, transferred through both sound, sight and possibly touch if the experimenter would give an eager and congratulating handshake as positive feedback instead. However, putting more emphasis on the experimenter's part is in general not a good idea. Studies show that subjects of opposite sex to the experimenter performs much better in serial-trigram memory learning tests than subjects of same sex (Archer, Cejka, & Thompson, 1961). This effect has also been replicated in sorting tasks (Stevenson & Allen, 1964). Whit this in mind, the small possible effect on offline gain for females, seen in Figure 2, might be due to the experimenter of this study being a male.

Self-esteem might also interfere with the impact of reinforcement as shown by the comparison between self-esteem group and reinforcement groups on offline gain ($P > 0.285$) under Table 4. This should not be considered as any clear sign, of course, but could show significance with a larger sample size since the number of participants for this Univariate test is very small. Prior studies on self-esteem have revealed that participants with poor learning of motor functioning also manifested lower self-esteem, vice versa for high esteem participants (Poole et al., 2017). This might explain why women scored five points higher on the CBSE questionnaire and performed worse on learning and recall performance of this study. If the experimenter effect is ignored, a complementary explanation to the gender difference in offline gain might be due to lower self-esteem individuals, in this case females, being more prone to be manipulated by positive feedback.

5.2. Limitations and confounding factors

Due to trying to minimize the effects of sex hormones on memory consolidation, only women that took monophasic birth control pills could participate in this study. Women having a different level of estrogen at testing, undoubtedly influence fine motor skills (Bayer & Hausmann, 2012). However, with this elimination comes a drawback, namely that the oscillating hormone levels which are a feature of the female menstrual cycle, is being neutralized. Therefore, the question whether the female results represents actual gender differences is problematic.

Of all the students that applied for the study almost two thirds got excluded due to not fulfilling the criteria of participation. In some borderline cases, it was unclear if the participant should be excluded or included, but in lack of candidates some of them were included and not thought to influence the results too much. Most of these borderline cases were participants without optimal sleep, but also participants using asthma medication and a very small dosage of nicotine days before testing were included.

Difficulty deciding the amount of compensation participants would receive after completed study might have influenced the motivation of participants. In the beginning of the study, it was written on the ads for the study that participants would receive three cinema tickets for their participation, one for each session. Due to resources changes of the department this amount got changed to one cinema ticket and further changed to two cinema tickets. These sudden shifts in the ads for the study around universities could have affected the experience and expectations of participants, which in turn might have influenced the measured performance.

Since many projects were ongoing and due to the departments limited space, two very different rooms were used as testing rooms. One was a sleeping room in the basement containing a bed and a table without any windows while the other was a small regular office room with a window and belonging office material. The effect the different rooms had on the participants might have been subtle, but could have had an impact on their stress levels and therefore on their cognition. However, participants were always retested in the same room as the initial finger tapping test and only a very small number of participants were tested in the office room since it was not meant to be used in the progression of this study.

5.3. Further research and improvements

Except from eliminating the confounding mentioned above, further studies need to take the experimenter effect into account. This could be solved by introducing a gender-neutral avatar instructing and interacting with subjects. The positive feedback needs to become stronger and be transferred through other perceptual senses to maximize the influence of the reinforcement. Since subjects might be differently susceptible to reinforcement, such as people with low self-esteem, it could be of interest to do a personality test in the screening process of participants and balance the groups accordingly.

Further research can also introduce negative feedback as a condition to investigate its effect on procedural memory consolidation. Since the continued study might show more convincing results, it could be of interest to examine what happens if positive feedback gets normalized, if it is continuously exposed to participants during procedural tasks. It could be so, that the supposedly positive impact wears off with time and ceases to exist once it becomes a norm during learning.

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7. Appendix

A - Criteria for participation

Description	Exclude based on the question? Yes/No	Desirable	Absolute limit
Age	Yes	18-26	18-30
BMI	Yes	20-25	19-26
Gender	Yes	50/50	50/50
Education	No	High school	
Swedish speaking	Yes	Able to speak & read Swedish	
Uncorrected eyesight problem	Yes	No problems with eyesight	
Alcohol 1 standard glass = 33cl beer (5,2%) 4cl spirit (40%) 15cl wine (12%)	Yes	0-6 standard glasses	9 standard glasses
Nicotine	Yes	None	Must be able to stay away from nicotine, without experiencing withdrawal symptoms during the tests.

Drugs	Yes	None	None
Caffeine 1 mug = 1,5 dl of coffee	Yes	max 3 mugs per day	No dependence symptoms (feeling bad) without caffeine
Abroad last month	No	Book so that it does not affect (determines how much impact it would have; 1-2 hours is ok)	
Abroad next month	No	Book so that it does not affect (determines how much impact it would have; 1-2 hours is ok)	
Severe sickness	Yes	Determine / consult	
Psychological diagnosis	Yes	Determine / consult	
Recent disease	Yes	Determine / consult	
Takes medicine	Yes	Determine / consult	
Birth control pills	Yes	Monophasic birth control pills	Monophasic birth control pills
Sleep questions	Yes	Go to bed at 21-01, waking up around 06-10, sleep 7-9 hours, feeling refreshed, Naps (1 day/week), have a good sleep = “3” or more	

B - Restrictions before the session

Please follow the below instructions prior to your visit.

· Women: visits should be planned so that they are in the active phase of the prevention pill schema, i.e. not during the days that you do not take any pills or taking sugar pills

· 3 evenings before participating: go to bed between 22 and 24, slept 7-9 hours

· 24 hours before your visit: abstain from alcohol

· 12 hours before visiting: maximum caffeine intake corresponding 1 coffee (1.5 dl)

· 12 hours before your visit: Avoid naps

· 12 hours before your visit: Avoid intense exercise

· 6 hours before your visit: avoid caffeine

· 3 hours before your visit: Avoid large meals

· 1 hour before your visit: eat nothing

· You feel healthy when participating

C - Sleep diary (3 days before first session)

Please fill out this sleep diary for the three nights before your visit. You can answer on your computer or print and fill it in by hand. For the information to be as accurate as possible, please fill in the details as soon as possible after you have woken up.

It is important that you remember to bring your paper to your visit, or send it to our mail, minnestestneuro@gmail.com before the visit.

	Night, two days before the time of the study	Night one day before the time of the study	The night before
Date			
The time when you put out to sleep			
Estimated time before falling asleep (min)			
Time of Awakening			
Estimated sleep quality (0-100, 100 = excellent quality of sleep)			
Did you nap / s yesterday? If so, detail total nap time			
How rested did you feel when you woke up? (1 = not at all, 7 = completely refreshed)			

How many times did you wake up during the period of sleep? How long were you awake?			
Did you set an alarm to wake up? What woke you up?			
Consider how a typical night's sleep, look out for you. To what extent was this night's sleep like a typical night's sleep for you? (1 = very poor, 7 = much better)			

D - Background information

ID	
Age	
Length	
Weight	
Gender	
Education	
Can you write and understand Swedish?	
Do you have any eye sight problems?	
Handedness	
Do you play any instrument? If yes, how many hours per week?	
Do you play computer games? If yes, how many hours per week?	
How much alcohol do you consume per week? (1 standard glass equals 33cl strong beer, 4cl 40% spirit, 15cl wine)	
Do you use nicotine?	
Do you use drugs?	
How much caffeine do you consume per day? (One cup equals 1.5 dl)	
Have you ever had any sleep related disease?	
Do you currently have any disease?	
Do you take any medication?	
Do you take any medication?	
For how long do you snooze in the morning?	
How well do you sleep on weekdays? 1 - 5	
How well do you sleep on weekends? 1 - 5	
How often are you very tired during weekends?	
How often do you take naps?	
What kind of studies/experiments have you attended before?	

E - THE PITTSBURGH SLEEP QUALITY INDEX (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? ____

2. How long (in minutes) has it taken you to fall asleep each night? _________________________

3. When have you usually gotten up in the morning? ____________________________________

4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed)____

5. During the past month, how often have you had trouble sleeping because you…	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
Wake up in the middle of the night or early morning				
Have to get up to use the bathroom				
Cannot breathe comfortably				
Cough or snore loudly				
Feel too hot				
Have bad dreams				
Have pain				
ther reason(s), please describe, ncluding how often you have				

ad trouble sleeping because of this reason(s)				
6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
ıring the past month, ›w much of a problem s it been for you to ep up enthusiasm get things done?				
During the past month, how ›uld you rate your sleep quality erall?	Very good (0)	Fairly good (1)	Fairly Bad (2)	Very Bad (3)

F - The Epworth Sleepiness Scale (ESS)

The Epworth Sleepiness Scale is widely used in the field of sleep medicine as a subjective measure of a patient's sleepiness. The test is a list of eight situations in which you rate your tendency to become sleepy on a scale of 0, no chance of dozing, to 3, high chance of dozing. When you finish the test, add up the values of your responses. Your total score is based on a scale of 0 to 24. The scale estimates whether you are experiencing excessive sleepiness that possibly requires medical attention.

How Sleepy Are You?

How likely are you to doze off or fall asleep in the following situations? You should rate your chances of dozing off, not just feeling tired. Even if you have not done some of these things recently try to determine how they would have affected you. For each situation, decide whether you would have:

- No chance of dozing =0
- Slight chance of dozing =1
- Moderate chance of dozing =2
- High chance of dozing =3

Write down the number corresponding to your choice in the right-hand column.

Situation	Chance of Dozing
Sitting and reading	
Watching TV	
Sitting inactive in a public place (e.g., a theater or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	

Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in traffic	

G - Competence Based Self-esteem Scale (CBSE)

Johnson, M. & Blom, V. (2007). Development and validation of two measures of contingent self-esteem. Individual Differences Research, 5, 300-328.

1. I feel worthwhile only when I have performed well
2. I think my worth as a person is determined by how well I succeed
3. It is not, who I am but what I can accomplish that matters
4. I sometimes try to prove my value by achievements
5. My self-esteem is highly dependent upon the results of my daily actions
6. I experience other people's success as threatening
7. Other people's success makes me push myself even harder
8. I easily get restless if I have nothing at hand to accomplish
9. No matter how well I have done a task, there is always a nagging feeling that I should have done better
10. When I have failed in an exam or in another context performed worse than I expected it has made me doubt my self-worth
11. It is hard for me to forgive myself when I fail in an important task
12. My feeling is that no matter how hard I work I'll never reach my best performance goals.

Response options are 1-5, where
1. I think that I sometimes try to prove my worth by being competent
2. My self-esteem is far too dependent on my daily achievements
3. At times, I must be better than others to be good enough myself
4. Occasionally I feel obsessed with accomplishing something of value

H - STOP-Bang Questionnaire

Snoring?

Do you Snore Loudly (loud enough to be heard through closed doors or your bed-partner elbows you for snoring at night)?

Tired?

Do you often feel tired, fatigued, or Sleepy during the daytime (tired enough that you could fall asleep while driving)?

Observed?

Has anyone observed you stop breathing or choking/gasping during your sleep?

Pressure?

Do you have or are you being treated for High Blood Pressure? Yes No Body Mass Index more than 35 kg/m2?

Age older than 50 years old?

Neck size: Is it large? (Measured around Adams apple)

For male, is your shirt collar 17 inches/43 cm or larger?

For female, is your shirt collar 16 inches/41 cm or larger?

Gender: Male?

Response options are Yes/No

I - Study evaluation

Thank you for participating in the study on the learning of motor memories!

We want participation in our studies should be an enjoyable experience where you as a volunteer feel appreciated and get the information you need. If you want to share with us your experiences from your participation, we would be very grateful. The evaluation consists of four questions, three yes / no questions and one multiple-choice question, and a field where you can leave a comment. Of course, you are completely anonymous and no one can associate your responses with you.

It is optional to complete the evaluation, so it does not affect your participation.

Thanks!

The study leaders
Department of Neuroscience
Uppsala university

Did you get a good reception when you visited us?
Yes/No/A bit

Would you recommend to your friends and acquaintances to participate in the study?
Yes/Maybe/No

Were you assigned enough information to easily understand what the study entailed and what was expected of you as a student?
Yes/Barely/No

How did you hear about this study there?

Ads/Handouts/Studentkaninen.se

A friend/Directly from examiner

Social platforms

Somewhere else __________

Do you have any other comments and feedback you would like to share with us?

J – Results

Table 1 - Descriptive Conditions							
				Gender Ratio			
Gender	N	BMI Mean	Age Mean	Positive Feedback	No Feedback	Sleep	Wake
♂	23	22.7	23.4	52%	40%	48%	46%
♀	20	21.9	23.4	48%	60%	52%	54%
				State Ratio			
State	N	BMI Mean	Age Mean	Positive Feedback	No Feedback	Men	Female
Sleep	21	21.7	23.5	52%	45%	48%	50%
Wake	22	22.9	23.4	48%	55%	52%	50%
				Reinforcement Ratio			
Reinforcement	N	BMI Mean	Age Mean	Male	Female	Sleep	Wake
Positive Feedback	23	22.0	24.5	52%	48%	52%	45%
No Feedback	20	22.6	23.4	40%	60%	48%	55%

Table - 1, a descriptive table indicating the ratio of BMI, Age, number of participants for each independent variable.

Table 2 - Univariate test - Performance						
	Learning			**Recall**		
Gender	Mean	Std	P	Mean	Std	P
♀	15.51	3.67	0.004	18.53	5.20	0.003
♂	19.78	5.28		23.94	5.87	
Reinforcement	Mean	Std	P	Mean	Std	P
Positive feedback	18.23	5.92	0.602	21.19	7.46	0.789
No feedback	17.42	4.18		21.70	4.88	
State	Mean	Std	P	Mean	Std	P
Sleep	17.39	1.09	0.614	21.52	1.27	0.920
Wake	18.18	1.08		21.33	1.40	

Table -2, A one-way ANOVA test for each independent variable with the dependent variable performance was carried out for both the learning phase and the recall phase.

Table 3 - Univariate test – Offline gain					
Effects and Interaction effects					
Source	Type III Sum of Squares	df	Mean Square	F	P
Gender	305,401	1	305,401	1,261	0,269
Reinforcement	72,880	1	72,880	0,301	0,587
State	685,249	1	685,249	2,830	0,085
Gender * Reinforcement	395,764	1	395,764	1,635	0,209
Gender * State	205,161	1	205,161	0,847	0,364
Reinforcement* State	178,489	1	178,489	0,737	0,396
Gender * Reinforcement * State	170,203	1	170,203	0,703	0,407
Error	8473,929	35	242,112		
Total	640527,050	43			
Corrected Total	10396,512	42			

A univariate test comparing the independent factors in relation to offline gain together with the interaction effects between the independent variables.

	♀ Mean	♂ Mean	♀.M/♂.M
Sum score	37.2	31.3	1.2

Table 4 - Descriptive Competence Based Self-esteem Scale

A higher score indicates a lower self-esteem and vice versa.