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Master Thesis

Auditory evoked potentials elicited by closed-loop stimulation during sleep in children and adults

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Abstract

Sleep plays a crucial role in brain development and cognitive function, with auditory evoked potentials (AEPs) recorded during sleep offering valuable insights into neural processing. The present study examines AEPs elicited by closed-loop down-phase targeted auditory stimulation (down-PTAS) during NREM sleep in a pediatric sample and compares these responses to those observed in adults. We focused on the P200, N550, and P900 components to investigate developmental differences in sleep-dependent auditory processing.

We conducted the overnight high-density EEG (hd-EEG) recordings in two experimental conditions: a stimulation night (STIM) and a control night (SHAM). During the STIM night, auditory stimuli were presented bilaterally through headphones, and during the SHAM night stimulation flags were saved without actual stimuli have been presented. The study included 8 healthy children and 14 healthy adults, allowing for a comparative analysis of AEPs across two age groups.

Notably, a significant correlation between age and N550 latency was observed in children, with younger participants exhibiting faster neural responses. Furthermore, predictive relationships between early and late AEP components were robust in adults but not present in children, suggesting developmental differences in the neural mechanisms underlying these responses. Additionally, a significant correlation between N550 amplitude and overnight change in reaction time (RT) was found in the pediatric group, indicating a link between sleep-related neural activity and cognitive performance. These findings highlight the role of developmental factors in the neural processing of auditory stimuli during sleep and suggest implications for sleep-dependent cognitive functions.

Abbreviations

EEG: electroencephalography

NREM: non-rapid eye movement sleep

REM: rapid eye movement sleep

SWA: slow wave activity (EEG power between 1 and 4.5 Hz)

KC: K-Complex

low-SWA: low slow wave activity (EEG power between 1 and 2 Hz)

hd: high-density

EOG: electrooculography

EMG: electromyography

SHAM: Condition with no acoustic stimulation

STIM: Condition with acoustic stimulation

UP: acoustic stimulation time-locked to the up-phase of slow waves

DOWN: acoustic stimulation time-locked to the down-phase of slow waves

ERP: event-related potential

AEP: auditory evoked potential

SHY: synaptic homeostasis hypothesis

WPMT: word-pairs memory task

TAP: test of attentional performance

RT: reaction time

1. Introduction

1.1 Electrophysiological measurements

1.1.1 Electroencephalography (EEG)

Electroencephalography (EEG) is a non-invasive neuroimaging method that measures the electrical activity from the scalp surface generated primarily by the cortical pyramidal cells. Deeper brain structures will not contribute directly to the EEG signal (Rosenberg, 1994). The basis of EEG measurements is the difference in the excitatory and inhibitory postsynaptic potentials, generated by the flow of ions through membrane channels of neural cells (Nunez & Srinivasan, 2006). The EEG is determined by the synchronous firing of large populations of cortical neurons rather than single-cell activity. That is why this neuroimaging method allows a high temporal resolution but cannot provide a precise special image (Buzsáki et al., 2012). In sleep research, EEG became a valuable tool providing insights into brain activity during different sleep stages.

1.1.2 Electromyography (EMG)

Electromyography (EMG) allows the recording of the electrical activity produced by skeletal muscles from the skin surface, which originates from the cumulative effect of non-synchronous potentials generated by numerous muscle fibers (Merletti & Farina, 2016). Surface EMG can detect low-level muscle activity, or passive partial muscle contraction, which occurs even when the muscle is not actively engaged. In sleep studies, EMG electrodes are often placed on the chin muscles, and the muscle tone variations are used to distinguish sleep stages. For instance, REM sleep is characterized by low muscle activity, meanwhile during non-REM sleep higher muscle activity is present (Michael H. Silber et al., 2077).

1.1.3 Electrooculography

Electrooculography (EOG) is a technique used to measure the corneo-retinal standing potential between the front and the back of the human eye. Electrodes placed around the eyes

allow the recording of eye movements by detecting changes in the electrical potential caused by the rotation of the eyeball (Leigh & Zee, 2015). EOG is crucial in sleep research for identifying different sleep stages, especially rapid eye movement (REM) sleep (Abbott Memorial et al., 2003).

1.2 Sleep characteristics

1.2.1 Electrophysiological patterns of sleep

Three main categories of vigilance can be found in humans: wakefulness, rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. Wakefulness is characterised by complex and diverse patterns of neural activity. In wake EEG, sinusoidal 8–13 Hz alpha rhythm can be detected over occipital regions of the brain, as well as rapid, slow and reading eye movements, blinks and variable high amplitude EMG (Berry et al., 2017).

NREM sleep is commonly divided into three subsequent substages (N1, N2 and N3) based on relative factors such as density of spindles (sigma frequency band, 11–18 Hz) and amount of slow-wave activity (SWA, delta frequency band, 0.5–4 Hz) registered (Fig. 1.1. A-D). The N1 stage is present during the transition from wakefulness to sleep or during transitions from deeper sleep stages to REM sleep. The main features of N1 are the presence of mixed-frequency low-amplitude (4–7 Hz) activity, vertex sharp waves and slow eye movements. N2 can be distinguished from other stages by the appearance of sleep spindles (trains of sinusoidal waves of 11–16 Hz frequencies) or K-complexes (KC, isolated sharp slow-wave, prominent in frontal derivations). The N3 stage is defined by the predominance of continuous slow wave activity in the delta band (0.5–2 Hz) (Aeschbach & Borbély, 1993).

REM sleep, also known as paradoxical sleep, is characterised by rapid eye movements and muscle atonia, as well as sawtooth waves of 2 – 6 Hz frequency (Fig. 1.1. E). In healthy adults, sleep occurs in approximately 90-minute long cycles, where NREM sleep is followed by REM sleep. Over the night the proportion of REM sleep gradually increases and NREM stages become shorter (Fig. 1.1. F) (Feinberg & Floyd, 1979).

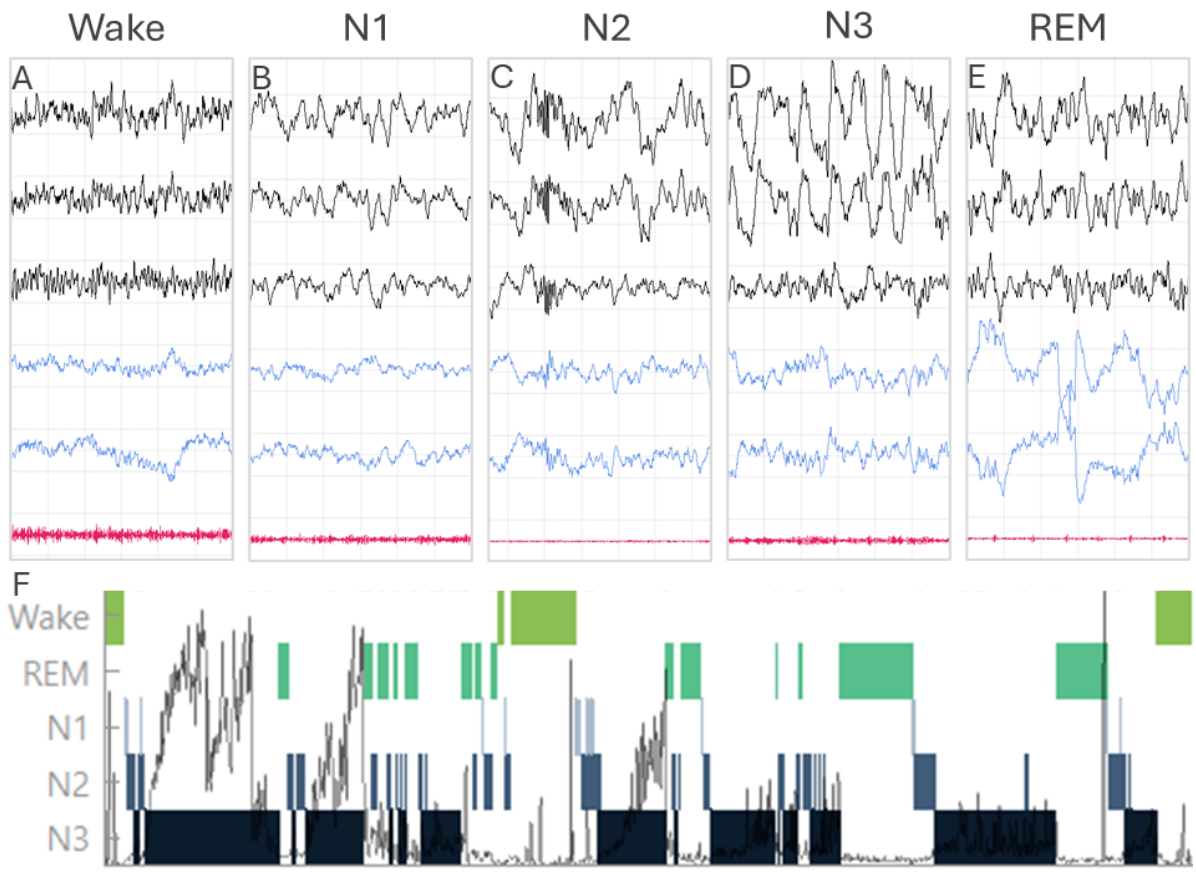

Figure 1.1. Sleep architecture of a child.

For pictures **A**-**E**: EEG, referenced to mastoids is displayed in black (first row: frontal electrode, second row: central electrode, third row: occipital electrode), EOG in blue and EMG in pink. **A:** Resting wake state is characterized by alpha (8-13 Hz) rhythms in the EEG, with high muscle tone and frequent eye movements. **B:** N1 is marked by the emergence of slower waves (4-7 Hz), slow rolling eye movements, and slightly reduced muscle tone. **C:** N2 is dominated by sleep spindles (12-16 Hz) and K-complexes, with slow eye movements and further decreased muscle tone. **D:** N3 is characterized by high-amplitude delta waves (0.5-4 Hz), deepest sleep, and lowest muscle tone with slow eye movements. **E:** REM EEG resembles wakefulness with a mixed wave, rapid eye movements and muscle atonia. **F:** Hypnogram of a child, light green bars indicate wake (Wake), dark green bars indicate REM sleep (REM), light blue bars indicate NREM stage 1 (N1), dark blue bars indicate NREM stage 2 (N2), black bars indicate NREM stage 3 (N3). Sleep stages alternate during night sleep with NREM sleep episodes getting shorter and REM sleep episodes getting longer.

1.2.2 Sleep across development

Sleep undergoes numerous organizational, structural and regulatory changes across development. Newborn sleep is polyphasic and the sleep cycle consists of active (REM-like) and quiet (non-REM-like) sleep. NREM features emerge gradually during the first six months of infancy. Thus, sleep spindles can be detected at 4 weeks of age, SW-like activity at 3 months and KC will arise later. During the first year, active sleep amounts to 50% of the whole sleep time, decreasing to 20–25% in adolescents. The length of the cycles varies from 50 minutes in infancy to 90 – 110 minutes in later childhood (Jenni & Carskadon, 2000).

Slow-wave activity is dramatically greater in early childhood and school-age children, this trend faints in the course of puberty, resulting in a 50% decline of slow-wave sleep length across the night between ages 12 to 14 years and a drastic change in SW amplitude. Such changes in SWA patterns can be explained by age-specific synaptic and neuronal alterations (Feinberg & Floyd, 1979).

School-age children (6 – 12 y.o.) begin to manifest their circadian preferences, which can broadly vary across the population. The length of sleep and its consistency cannot be clearly determined in this age group (Jenni & Carskadon, 2000).

1.3 Sleep-wake cycle regulation

The transition from wakefulness to sleep and backwards has been the topic of research for decades. Constantin von Economo first suggested the crucial role of the brainstem and diencephalon in such regulation, as well as the presence of an ascending arousal system (Von Economo, 1930). Moruzzi and Magoun's findings confirmed von Economo's theory, and the crucial role of the ascending reticular activating system (ARAS) in wakefulness promotion became prevalent in the scientific community (Moruzzi G & Magoun HW, 1949). There are two parallel ARAS branches: a thalamic pathway originates in the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) of the pons and is represented mostly by acetylcholine-releasing neural cells; a hypothalamic pathway originates in the upper brainstem and caudal hypothalamus, its monoaminergic nuclei release a broad list of

neurotransmitters, including histamine, serotonin, and norepinephrine (Saper et al., 2005). Cholinergic neurons mainly input in high-frequency oscillation processes and therefore are active during wakefulness and REM sleep corresponding to cortical activation. During NREM sleep these neurons are less active and consequently less acetylcholine-productive. Monoaminergic nuclei also show the fastest firing in wakefulness, and decreased activity in NREM sleep, but cease in REM sleep. The ARAS system is counterbalanced by the sleep-promoting ventrolateral preoptic nucleus (VLPO) and its γ-aminobutyric acid (GABA) neurons (Pace-Schott & Hobson, 2002). Neurotransmitters' concentration modulates the mutual inhibition of the ARAS (wake) and the VLPO (sleep) systems. This mechanism is called the flip/flop model, analogically to an electrical flip/flop switch (Saper et al., 2005).

1.4 Circadian regulation of sleep

Circadian regulation is a fundamental aspect of human physiology and functioning, which integrates internal and environmental cues. The suprachiasmatic nucleus (SCN) of the hypothalamus is the main driver of circadian processes. It was shown in rodents that the lesion of the SCN disrupts normal circadian rhythms of sleep and wakefulness by altering the secretion of adrenal corticosterone (levels remained constant throughout the day) (Moore & Eichler, 1972). In 2004 Lowrey and Takahashi described a transcriptional-translational negative feedback loop work pattern of the core clock genes (CLOCK, BMAL1, PER1, PER2, PER3, CRY1, and CRY2), as well as the influence of light exposure on the expression of PER genes in the SCN. The same study highlighted the role of the SCN not only in the orchestration of sleep onset but also in the distribution of sleep stages across the night (Lowrey & Takahashi, 2004).

Beyond the SCN, several autonomous circadian oscillators, so-called peripheral clocks, are present in almost all tissues, including the liver, lungs, and heart. Peripheral clocks' regulation processes, as well as specific genes under circadian control, significantly vary among different tissues, ensuring autonomous circadian cycles, based on cell-specific signals. Meanwhile, systemic (central) cues such as the secretion of glucocorticoids also play a

crucial role in synchronizing peripheral clocks, conditioning the complex interplay between circadian and metabolic systems (Mohawk et al., 2012).

1.5 The synaptic homeostasis hypothesis

The synaptic homeostasis hypothesis (SHY) suggests the critical role of sleep in neural plasticity and maintenance of synaptic homeostasis by downscaling synaptic strength that has been built up during wake experience-driven plasticity. The SHY proposes that slow-wave sleep (SWS) is primarily involved in global synaptic weakening (Tononi & Cirelli, 2003). Various studies showed changes in synaptic protein and receptor density throughout the sleep-wake cycle and SWA is considered to promote such adjustments through reduced neurotransmitter release and altered ion channel activity (Cirelli & Tononi, 2020). This hypothesis can be considered as a framework for understanding sleep-dependent memory consolidation. The SHY promotes the dual role of sleep on memory in refining neural circuits by selective preservation and enhancement of relevant synaptic connections and elimination of redundant ones (Tononi & Cirelli, 2014).

1.6 Active System Consolidation Hypothesis

In 2013 Rasch and Born proposed the active system consolidation hypothesis (ASCH), which suggests that sleep, particularly slow-wave activity (SWA), participates in the selective potentiation of synapses that were tagged during wakefulness as relevant to memorisation. According to the ASCH, the reactivation of important memory traces during SWS promotes the redistribution of memory from the hippocampus to permanent storage in the neocortex (Rasch & Born, 2013). Cross-frequency coupling between neocortical slow oscillations, thalamocortical sleep spindles, and hippocampal sharp-wave ripples is considered to ensure a memory replay. The interplay of these oscillatory activities showed increased integration of memory engrams (Van Dongen et al., 2012). The study by Eschenko et al. has shown the synchronisation between phasic brainstem activity and the firing of norepinephrine neurons in the locus coeruleus (Eschenko et al., 2012).

There is evidence for both hypotheses (SHY and ASCH), the key processes of which can be complementary during NREM sleep. The possible explanation, the sleep-dependent performance gain is modulated by large amplitude global slow waves, meanwhile δ waves (smaller amplitude local events) are responsible for the suppression of memory consolidation (Kim et al., 2019).

1.7 Two process model

In 1982 Borbély introduced the two-process model of sleep regulation, which includes the interaction of two independent processes: a circadian process (C) and a homeostatic process (S) (Borbély A. A., 1982). In this model, process S represents the build-up of sleep pressure during wakefulness and its dissipation during sleep. Process S is based on the accumulation of sleep-promoting neurochemical substances during wake hours and its dissociation during SWS. In the case of sleep deprivation, the greater the sleep debt is, the stronger the sleep drive becomes. The recovery after sleep deprivation includes a bigger proportion of SWS and an increase in SWA to repay the sleep debt (Daan et al., 1984). The importance of slow waves in the homeostatic regulation of sleep was shown in preceding studies. Slow oscillations predominate and arousal thresholds are higher in earlier sleep and decrease towards the end of the night (Blake & Gerard, 2001). In a later study, Achermann and Borbély performed several experiments with sleep manipulations. In the sleep deprivation condition, SWA was significantly increased at the beginning of the recovery sleep. This way the level of SWA during the initial NREM sleep episode is a function of prior waking time. The opposite effect was observed during a daytime napping condition. In the night following the day naps, SWA was attenuated (Fig. 1.2. A-B from Achermann & Borbély, 2003).

Process C represents the circadian rhythm that regulates the timing of sleep and wakefulness over a 24-hour cycle. This topic was reviewed in chapter 1.4. According to the two-process model, circadian regulation occurs independently of previous sleep or wakefulness and is primarily influenced by the light-dark cycle, which determines periods of

increased and decreased sleep propensity. Thus, the interaction between process S and process C can be expressed as a dynamic pattern of sleep opportunities throughout a 24-hour cycle. The highest likelihood of sleep episodes appears in the time window where the high homeostatic drive intersects with corresponding signals from the circadian system (Fig. 1.2. C from Silver and M. Rainbow) (Silver & Rainbow, 2013).

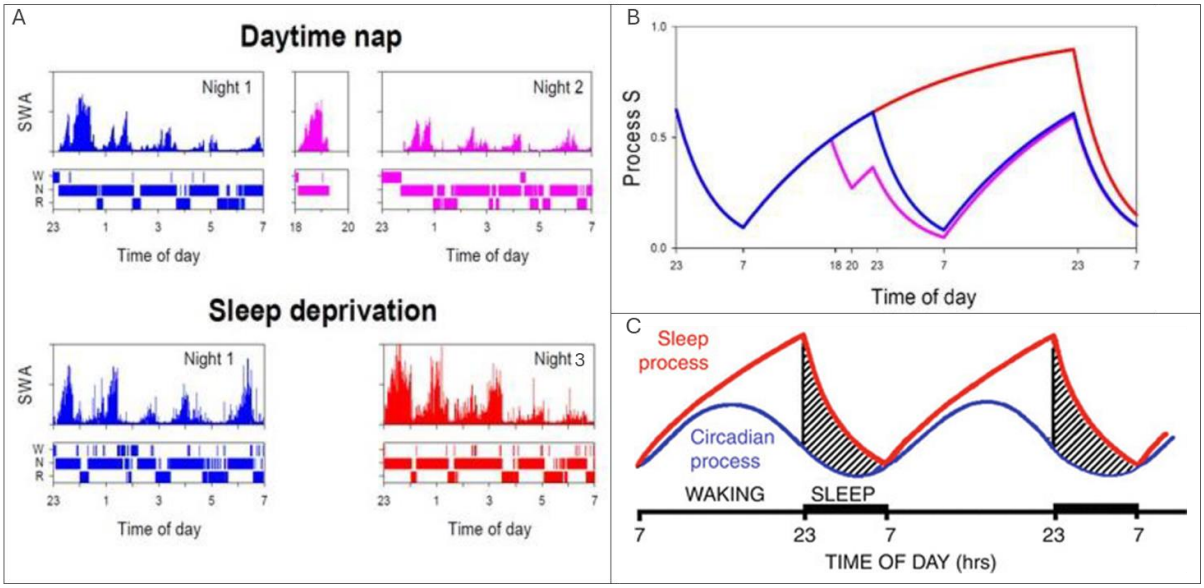

Figure 1.2. Two-process model.

A: Two-process model of Achermann & Borbély, 2003. Time course of SWA and sleep profiles (W: waking and movement time; N: NREM sleep; R: REM sleep) in two baseline nights (Night 1), after 40 h of sleep deprivation (Night 3) and after a daytime nap (Night 2). **B:** Simulations of the homeostatic Process S. Blue: baseline with an 8-h sleep episode; red: sleep deprivation and recovery sleep; pink: 2-h nap at 18:00 h. **C:** Borbély sleep model from Silver & Rainbow, 2013. The circadian system gates the sleep-wake cycle. Sleep is regulated by two processes: homeostatic (red line) and circadian (blue line).

1.8 Slow waves

Slow waves are a particular electrophysiological pattern during NREM sleep (N2 and N3 stages), originating from synchronised neuronal firing. The wave pattern consists of two phases: the down-phase represents a negative half-wave resulting from suppressed spiking activity (off-state) of a large population of neurons in the cortex and hyperpolarization on the

cell level; the up-phase (the positive half-wave) is characterized by a synchronous increase in neuronal firing (on-state) and depolarization on the cell level. Thus, the wave pattern reflects the near-synchronous transitions from up to down states on the level of large neuronal populations (Steriade et al., 2001).

These waves are characterized by high amplitudes and low frequencies from 0.5 to 4 Hz. Maximal SWA is typically detected in frontal areas, the insula, the anterior and posterior cingulate gyri, and the precuneus. Electrophysiological findings showed that slow waves originate from the cingulate gyrus and the insula and that they propagate along the anteroposterior axis (Murphy et al., 2009).

1.8.1 Homeostatic pressure and Slow-wave activity

As was mentioned in chapters 1.5 and 1.7, the homeostatic process S of the two-process model is correlated with SWA levels. Slow waves seem to take part in the sleep-dependent systematic restorative process, reflecting a gradual decrease in sleep pressure across NREM sleep (Borbély & Achermann, 1999). SWA during the initial cycle of sleep can be decreased after day-time naps or oppositely increased after sleep restriction during the previous night, thus these findings support the role of slow waves in sleep pressure dissipation (Achermann & Borbély, 2003; Werth et al., 1996). Later, it was revealed that SWA and other oscillation processes can prevail locally as a result of prior activation during the wake period (Huber et al., 2004; Murphy et al., 2011; Pugin et al., 2015). It seems that specific types of activation during wake evoke oscillation activity in the corresponding brain regions. For example, source modelling allowed to show the increase of SWA in the right premotor and sensorimotor cortices, induced by the implicit learning of a visuomotor adaptation task. An opposite effect was found during arm immobilisation, which resulted in a decrease of SWA in the sensorimotor cortex (Murphy et al., 2011).

1.8.2 Slow oscillation

Slow waves can be distinguished into two populations based on their temporal and spatial characteristics: the slow oscillation (SO; <1 Hz) and delta waves (δ-waves, 1-4 Hz)

(Steriade, Amzica, et al., 1993; Steriade, Contreras, et al., 1993). SOs are originating in frontal areas, characterized by global neural activation, represented in high-amplitude events. Meanwhile, δ-waves are more "local", engaging smaller neural groups. Delta waves can be as well subdivided into thalamic clock-like δ-waves and cortical δ-waves, based on the source of generation. Thereby the surface EEG-recorded SWs result from mixed nature activity patterns (Amzica & Steriade, 2000). Interestingly, an optogenetic modulation study showed that SOs and spindles are involved in sleep-related memory gain, while δ-waves were responsible for overnight memory weakening. This finding supports the dual nature of slow waves in both memory consolidation and forgetting of experiences (Kim et al., 2019).

1.8.3 K-complex

The K-complex (KC) is a high-amplitude sharp-shaped wave, present in N2 and N3 stages and is often associated with spindles. KCs can be spontaneous (not associated with any kind of stimuli) or evoked by external stimuli of different modalities or by internal stimuli, for example, respiratory interruptions Colrain 2005.

The physiological role of KCs is still unclear, but they seem to take part in several processes. One of the hypotheses suggests its function in arousal promotion, as KCs are often accompanied by an increase in muscle tone and heart rate (Wauquier et al., 1995). Other findings show a close relationship between KCs and sleep maintenance and suppression of arousal towards "safe" stimuli (Halász, 2005). Finally, there is evidence for the role of KCs together with slow waves and spindles in subcortico-cortical interaction during memory replay and facilitation of memory consolidation (Cash et al., 2009).

Evoked KCs are associated with several components. Each component is named according to its valency (negative (N) or positive (P) peak) and its latency. Typical sleep auditory evoked potentials (AEPs) are P200, N350, N550, and P900. The origin of the neural generation of KCs is understudied, but the source localisation method can provide information on the generation of the different components. It has been shown that P200 is a modality-specific excitation response, which is supposed to promote the initiation of the off-state, also known as neuronal silence, and trigger the following slow-wave-shape KC

bistability (Laurino et al., 2014). The negative components (N350 and N550) are generally characterised as sensory-modality-independent. While N350 originates exclusively in the modality non-specific brain structures (medial frontal, superior frontal, cingulate gyri, and precuneus), the N550 sources include primary and secondary modality-specific areas as well (Laurino et al., 2019). The source localisation of P900 is not clear yet, but its sensory-modality unspecific nature also suggests its origin from the associative cortical areas (Riedner et al., 2011).

1.9 Closed-loop auditory stimulation

The growing number of findings suggesting the crucial role of SWA in the neurocognitive performance of different levels, including learning, memory and brain recovery abilities, has spurred the development of non-invasive methods of SWA manipulation. These methods aim to establish possible treatments for pathological states and to further investigate the nature and functions of slow waves (Friedrich et al., 2015; Walker & Stickgold, 2006; Wilckens et al., 2014).

There are many approaches to influencing sleep architecture, including such during wakefulness. High physical activity, cognitive exercises and even meditation may result in SWA increase (Huber et al., 2004b; Kredlow et al., 2015; Pugin et al., 2015b).

Besides, several stimulation techniques were developed. One of the broadly used methods of SWA boosting during sleep is transcranial electric stimulation (TES). TES allows to increase 1 Hz neocortical oscillations by applying trains of weak electric pulses to specific brain regions (Marshall et al., 2004).

Currently, the most widely applied solution to non-invasively enhance SWA during NREM sleep is auditory stimulation. There are also several techniques of auditory stimulation. One applies acoustic stimulation to modify sleep architecture by shortening the duration of NREM sleep, causing a decrease in SWA (Landsness et al., 2009).

Real-time closed-loop slow-wave detection and acoustic stimulation systems have been developed to enhance SWA by increasing slow-wave amplitude or extending trains of slow

oscillations without altering the sleep architecture (Ngo et al., 2013; Santostasi et al., 2016). Auditory stimuli with soft sounds of low volume (1/f pink noise bursts with 50 ms duration) are shown to efficiently elicit slow waves (Zhou et al., 2012). Moreover, the precise topological detection of slow waves allows to alter of local SWA rather than global brain activity (Ngo et al., 2013).

The differential neuronal firing patterns during the up- and down-phases of cortical slow waves indicate that stimuli applied at different oscillation phases can influence SWA in varied ways (Steriade et al., 2001). Functional MRI (fMRI) analyses of responses to stimuli presented 300 ms before or after the down-phase of slow waves revealed no difference in the activation of the thalamus or primary sensory areas. However, a significantly higher response was observed in the superior temporal gyrus (STG), a higher-order associative cortex, during the rising slope of slow waves (Schabus et al., 2012).

Given the distinct physiological characteristics of the up- and down-phases, most auditory stimulation studies have employed a phase-targeted approach. Stimuli are applied near either the positive (Ngo et al., 2013; Papalambros et al., 2017) or negative peaks of slow waves (Fattinger et al., 2017; Landsness et al., 2009), depending on the desired outcome. Systematic investigations into the optimal timing for auditory stimuli presentation revealed that the maximum enhancement occurs when stimuli are applied at the positive peak of slow waves, though the specific timing window varies between younger and older participants (Navarrete et al., 2020).

Various algorithms have been developed to optimize the timing of auditory stimuli. Fattinger and colleagues used threshold-based continuous stimulation to disturb SWA by synchronizing stimulation to the down-phase of slow waves. Their study targeted SWA in the primary motor cortex and found that while the capacity for neuroplastic changes was reduced by wakefulness, it was restored during unperturbed sleep. However, this restorative process was markedly attenuated when slow waves were selectively disturbed (Fattinger et al., 2017).

In the study of Ngo and colleagues, the threshold-based two-pulse stimulation with fixed inter-stimulus intervals (ISI) applied to the up-phase of fronto-central slow waves

enhanced memory consolidation and boosted phase-locked spindle activity, likely due to improved synchronization of spindles with slow oscillations (Ngo et al., 2013).

Interestingly, out-of-phase stimulation disrupted slow oscillation activity but did not affect memory consolidation. Ong and colleagues applied a similar approach during an afternoon nap, finding that acoustic stimulation increased slow wave amplitude, theta, and fast spindle activity while reducing the forgetting of word pairs (Ong et al., 2016). Another study extended these findings to older adults, showing that phase-locked overnight auditory closed-loop stimulation enhanced memory consolidation and sustained SWA and spindle activity (Papalambros et al., 2017).

Despite these varied approaches, phase-targeted auditory stimulation (PTAS) consistently demonstrates a positive effect on declarative memory consolidation (Ngo et al., 2013; Ong et al., 2016; Papalambros et al., 2017).

1.10 Sleep and development

During postnatal development, the brain undergoes numerous changes in its structure and connectivity. Naturally, sleep plays a critical role in developmental processes, impacting physical growth, brain maturation, cognitive functioning, and emotional regulation. Historically, several studies have laid the foundation for understanding these relationships. One of the earliest and most influential works is by Kleitman and Aserinsky, who discovered rapid eye movement (REM) sleep and its prevalence in infants, suggesting its importance in early brain development (Aserinsky & Kleitman, 1953). Subsequent research by Anders and Carskadon further highlighted the significant changes in sleep patterns during adolescence, underscoring the importance of sleep in cognitive and emotional development during these formative years (Anders et al., 1980).

More recent studies, such as those by Dahl (1996) and Walker (2009), have expanded on these foundations, exploring the intricate connections between sleep and neurodevelopmental processes. These works collectively emphasize that sleep is not merely a

passive state but an active period crucial for developmental processes (Dahl, 1996; Walker, 2009).

1.10.1 Changes in Sleep Across Development

Infants (0-2 years) spend a substantial amount of their time sleeping, with newborns sleeping up to 16-18 hours a day. Sleep in infancy is characterized by shorter sleep cycles and a higher proportion of REM sleep, which accounts for about 50% of total sleep. This high proportion of REM sleep is believed to be critical for brain development, as it is associated with neural plasticity and the consolidation of new learning and memory. As infants grow, the total sleep duration gradually decreases. By the age of one year, infants typically sleep around 14-15 hours per day, with more consolidated nocturnal sleep and fewer daytime naps. The transition from polyphasic to more monophasic sleep patterns begins during this period (Graven & Browne, 2008).

During early childhood (3-5 years), children usually sleep about 11-13 hours per night. The proportion of REM sleep decreases to around 20-25%, while NREM sleep stages, particularly SWS, become more prominent. SWS is crucial for physical restoration and growth, facilitated by the release of growth hormones during this stage. Sleep patterns also become more consistent, with most children adopting a monophasic sleep pattern. However, naps may still be common, especially in younger children (Rosen, 2010).

In middle childhood (6-12 years), total sleep duration continues to decrease, with children typically requiring about 9-11 hours of sleep per night. Sleep architecture stabilizes, with distinct NREM and REM cycles. Stage N3 of SWS remains important, although its proportion relative to other sleep stages starts to decline gradually (Bathory & Tomopoulos, 2017). During this period, cognitive and academic demands increase, making adequate sleep crucial for learning, memory consolidation, and emotional regulation. Studies by Touchette et al. have shown that children with consistent, adequate sleep patterns perform better academically and exhibit fewer behavioural problems (Touchette et al., 2007).

Adolescence (13-18 years) is marked by significant changes in sleep patterns and architecture. Teenagers typically require about 8-10 hours of sleep per night, but various

factors, including hormonal changes and social pressures, often lead to insufficient sleep. The circadian rhythm shifts during adolescence, causing a natural tendency for later sleep onset and wake times, known as "phase delay" (Carskadon et al., 1993). This shift can conflict with early school start times, leading to chronic sleep deprivation, which is associated with numerous negative outcomes, including impaired cognitive performance, mood disorders, and increased risk-taking behaviours. Efforts to delay school start times have shown positive effects on sleep duration and overall well-being among adolescents (Carskadon et al., 1993).

In adulthood (19-60 years), sleep needs vary but generally stabilize around 7-9 hours per night. Sleep architecture continues to evolve, with a further reduction in the proportion of REM sleep and N3. The quality of sleep can be affected by lifestyle factors, stress, and health conditions.

1.10.2 Changes in SWA during development

In 2009 Campbell and Feinberg conducted a longitudinal study to examine the developmental trajectories of delta and theta EEG activity during NREM sleep as indicators of brain maturation in adolescents. The study tracked participants over several years, from early adolescence through late adolescence, to observe changes in EEG patterns. The study found a significant decline in delta and theta activity during adolescence, corresponding to the period of intensive synaptic pruning and cortical reorganization. The results supported the hypothesis that reductions in delta and theta waves during NREM sleep are indicative of adolescent brain maturation (Campbell & Feinberg, 2009).

Further studies have found that SWA undergoes significant topographical changes during development. In infants, SWA is diffuse and robust across the cortex, supporting the extensive synaptic plasticity needed for early brain growth. The cross-sectional study of Kurth and colleagues showed the SWA being most concentrated over posterior regions during early childhood and then gradually shifted to more central and frontal derivations by adolescence, suggesting back-to-front SWA migration throughout childhood (Kurth, Ringli, et al., 2010). By adolescence, SWA shows a marked reduction in amplitude and becomes more region-specific, indicating ongoing synaptic pruning and maturation of neural circuits.

These findings suggest that SWA can serve as a reliable marker for cortical development and neural maturation, with implications for understanding developmental neurophysiology and identifying potential developmental disorders (Buchmann et al., 2011; Kurth et al., 2010; Ringli & Huber, 2011).

1.11 Event-related potentials (ERPs)

1.11.1 Definition of ERPs

Event-related potentials (ERPs) are electrophysiological signals generated by peripheral, subcortical or cortical structures in response to specific stimuli, such as visual, auditory, or somatosensory inputs. These signals can be recorded from the scalp using electroencephalography (EEG) and are characterized by their time-locked nature to the presentation of the stimulus. When recorded from the scalp surface, their registered amplitude ranges from 0.5 to 15 μV, which is much lower than that of the spontaneous EEG signal of 50-100 μV. Therefore, ERPs remain unseen on the background EEG, however, they “appear” in the EEG signal after simple manipulation of electronic summation or averaging of repeated responses. This technique allows to distinguish the stimuli-dependent time-locked neural activation from the background activities (spontaneous EEG) independent from stimuli (Blackwood & Muir, 1990). As reviewed by Laureys, the ERPs are used to assess the functional integrity of sensory pathways and are valuable in both clinical diagnostics and research settings. The resultant evoked potential is a plot of voltage versus time with an artefact of stimulation at time zero (coincident with the stimulation) and then a series of peaks and valleys at later times, representing different stages of neural processing. ERP responses are usually subdivided into two categories. The early components, which peak approximately within the first 100 milliseconds following a stimulus, are known as 'sensory' or 'exogenous' because they primarily rely on the physical characteristics of the stimulus. Conversely, the components that occur later are indicative of how the subject interprets the

stimulus and are referred to as 'cognitive' or 'endogenous' ERPs, involved in information processing (Laureys et al., 2009).

1.11.2 ERPs in sleep research

Although sensory and cognitive evoked potentials (EPs) can be elicited by various sensory stimuli (such as visual, auditory, somatosensory, olfactory, or nociceptive), auditory tones and somatosensory stimulations have been most employed in sleep studies due to technical considerations. All cortical components of ERPs undergo changes during sleep. NREM sleep ERPs consist of several large amplitude components that seem to be generated independently of the physical characteristics of the stimuli. These include a P2 component around 200 ms, an N350 component between 250 and 400 ms, an N550 component between 500 and 800 ms, and a P900 component between 800 and 1300 ms (Colrain & Campbell, 2007). During wakefulness, long latency auditory evoked potentials (AEPs) or somatosensory evoked potentials (SEPs) manifest as a diphasic, negative-positive complex peaking at 100-150 milliseconds after the stimulus, named N1-P2 “vertex”, afterwards, the response typically flattens out. Sleep significantly alters these late responses. Most researchers agree that the onset of sleep leads to an immediate prolongation in latency and a reduction in the amplitude of the N1 component, along with an increase of the P2 component’s amplitude (Bastuji, 1999).

The most notable impact of sleep on late components is the increased complexity of the response, characterized by the emergence of a sequence of waves typically absent during wakefulness. Loomis et al. and Davis et al. first proposed that the complex auditory late response (components N550 and P900) observed in humans during sleep stages N2 and N3 was due to the summation of K-complexes evoked by sensory stimulation (Davis et al., 1939; Loomis et al., 1938). This was also later verified in the visual and somatosensory modalities (Goff et al., 1966; Roth et al., 1956). The N350 component is prominent in averaged K-complexes but also emerges early in the sleep onset period when the K-complex cannot be elicited. The N350 component seems to indicate activation of the generator responsible for

vertex sharp waves. The N350 and N550 components are the largest responses that can be recorded from the scalp surface, significantly surpassing any ERP recorded during wakefulness. The magnitude of these components suggests a synchronized firing of numerous cortical cells, thereby their presence, latency, and especially amplitude serve as functional indicators of CNS integrity (Colrain & Campbell, 2007). It is hypothesized that the N350 and N550 components are related to sleep protection processes, with the N350 component reflecting an active inhibitory process that facilitates sleep onset and the N550 component representing the evoked K-complex, a precursor to the delta waves observed in SWS (De Gennaro et al., 2000). Bastien and Campbell were the pioneers in separately sorting and averaging responses to stimuli that either elicited a K-complex (KC+) or did not (KC−). They argued that the N550 component observed in the KC+ averages provides an accurate representation of the properties of the "pure" K-complex (C. Bastien & Campbell, 1992). Unlike the N350, the subsequent N550 component is either absent or significantly reduced in KC− averages of responses to both auditory and respiratory stimuli (Goff et al., 1966).

1.12 Individual differences in sleep characteristics

Research on individual differences in sleep and wakefulness revealed significant variability influenced by genetic, physiological, and environmental factors. Van Dongen and colleagues emphasize how genetic predisposition and behavioural traits affect sleep duration and responses to sleep deprivation. These differences are notably larger between individuals than between different nights within the same individual (H. P. A. Van Dongen et al., 2005). Finelli showed that normalized power maps of NREM sleep from a baseline night and a recovery night after sleep deprivation remained remarkably consistent for each subject and could reflect individual traits of functional anatomy, which is largely influenced by genetic factors (Finelli, 2001). Similarly, De Gennaro's team conducted a study over six consecutive nights with substantial experimentally induced changes in sleep architecture and found high intraindividual consistency in the distribution of the electroencephalogram (EEG) power along the anteroposterior cortical axis (De Gennaro et al., 2005). The intraindividual

differences were also shown in studies that investigated human AEPs, showing high variability in AEPs' values depending on the participant's chronotype (McArthur & Bishop, 2002). Further research proved the link between variability in individual sleep recovery and performance impairment due to sleep loss (H. P. A. van Dongen & Belenky, 2009). When performing sleep studies, researchers must consider the high variability of sleep parameters, as studies often focus on population averages. This is especially important to keep in mind when studying a developing population, as some characteristics may reflect the individual rather than a maturational change (Dittrichová et al., 1976; Friedman et al., 2009; Fuligni et al., 2019).

1.13 Aims of the Thesis

The primary objective of this thesis was to examine the impact of closed-loop down-PTAS during NREM sleep in a pediatric population (Leach et al., 2023).

In different PTAS protocols, the stimulation period (ON window) is typically followed by a break of several seconds without any stimuli presented (OFF window). The response to the first stimuli of the ON window is called the 'initial response', and is shown to be larger compared to the responses to the following stimuli of the ON window (Krugliakova et al., 2022). Compared to up-PTAS, in which an increase in SWA follows an initial KC-like response, down-PTAS is a promising approach to elicit isolated KCs without further increase in delta power, as the auditory stimuli are specifically presented at the descending slope of the slow wave, corresponding to the onset of a cortical off-period (Vyazovskiy et al., 2009). To determine if our stimulation protocol effectively evokes the KC-like response in a developing population, we calculated AEPs, focusing on the amplitude and latency characteristics of components P200, N550, and P900 from the sleep EEG of six children (Cote et al., 1999).

The second goal was to compare the characteristics of AEP components (amplitude and latency) between children and adults to identify age-related differences. The majority of studies have investigated responses to auditory stimuli presented to healthy young adults,

meanwhile, the corresponding data for children and elderly people remain relatively sparse and underexplored (Bastien et al., 2002). By examining the parameters of the AEP component, we aimed to identify developmental differences in auditory processing during sleep. This comparison may offer insights into how age influences the neural response to auditory stimulation at the down phase of slow waves, highlighting any significant differences between the two age groups.

Our next objective was to investigate whether the characteristics of early AEP peaks can predict the characteristics of subsequent peaks and to compare these correlations between children and adults. This aim focuses on exploring the potential predictive relationships between early and later AEP component characteristics. By analysing the correlations between the amplitude and latency of early auditory processing components (such as P200) and later KC-like component N550, as well as following P900, we aim to elucidate the sequential processing of auditory stimuli during sleep. Furthermore, comparing these correlations between children and adults will help determine if developmental differences influence these predictive relationships, thus providing deeper insights into the temporal dynamics of auditory processing during sleep.

Our last aim was to explore the potential link between the physiological responses to auditory stimulation and behavioural performance outcomes. As was mentioned in previous chapters (1.8.3, 1.12.2), N550 is a measure of evoked KC response. Experiments employing the up-PTAS protocols consistently showed significant enhancements in verbal declarative memory consolidation (Ngo et al., 2013; Papalambros et al., 2017), these findings were recently reproduced also for down-PTAS in adults (Leach et al., 2023). Unfortunately, due to several reasons, we couldn't collect enough quality data to assess overnight memory consolidation in the children group. Therefore, we decided to concentrate on analysing the overnight changes in reaction time as assessed by the alertness subtest of the test battery for attentional performance (TAP). There are many factors that have been shown to modulate reaction time (RT) in both patients and healthy populations, such as stimulus type, its intensity and complexity, as well as the amount and quality of sleep prior to the test (Cote et al., 2009a, 2009b; Klapp, 2010). In a recent study, Krugliakova and colleagues showed that

PTAS accelerates SWA decline across the night which is associated with an overnight improvement in attentional performance (Krugliakova et al., 2022). Previous findings suggest a possible connection between increased physiological response during PTAS and behavioural outcomes. Our protocol allows us to assess the physiological effects of stimulation on overnight changes in attentional performance. By comparing these effects between children and adults, we aim to understand how the stimulation's impact on behaviour varies with age, thereby contributing to our understanding of developmental differences in sleep-dependent cognitive processes.

2. Method

2.1 Participants

2.1.1 Adult participants

To investigate the differences in down-PTAS effects between adult and pediatric populations, we reanalysed the existing dataset of hd-EEG recordings from a subset of 14 out of 17 participants (4 male,10 female) aged between 18.38 and 26.69 years (mean ± sd = 23.25±2.53 years). Three participants were excluded due to failure to comply with the sleep schedule (n =1), poor sleep efficiency (n =1) or technical problems (n =1).

All participants, except for one, were native Swiss-German speakers. Before the study commenced, participants completed an anonymous online screening in which they self-reported not using any medications, low intake of caffeine and alcohol, and no use of nicotine or other drugs. They also indicated that neither they nor their family members had a history of neurological or psychiatric disorders, including sleep disorders. Participants identified themselves as good sleepers with an average chronotype. Individuals who had engaged in shift work, taken daytime naps, or travelled across more than two time zones in the past 30 days were excluded from the study.

All adult participants underwent the same exact protocol, which was used for pediatric participants. The study design and laboratory environment were kept the same for both age groups to ensure the comparability of collected data.

2.1.2 Pediatric participants

A total of 8 healthy participants (2 females, 6 males) aged between 7 and 12 years old (mean ± sd = 9.03 ± 1.67 years) were recruited. 7 participants were right-handed and 1 ambidexter. Three participants were native Swiss-German speakers, three participants were native Russian speakers, and two participants were native French speakers with German as a second language. Two participants dropped out after first measurement night due to personal reasons. Participants' parents identified their children as good sleepers with average

chronotypes. All parents reported their children's medication-free status, no consumption of caffeine, alcohol, nicotine or drugs, and no personal or family history of psychiatric or neurological disorders (including sleep disorders). Participants were excluded from the study if they were usually waking up at night more than 1-2 times a week, had day naps, or travelled across more than two time zones within the previous 30 days. Prior to the study, all participants and their parents were informed about the procedure and then the consent form was signed by participants' parents.

A	Pediatric group	
Age	Sex	Handedness
8.05	female	right
8.47	male	right
9.38	male	right
7.36	male	right
8.40	male	ambidexter
12.53	female	right
8.38	male	right
11.71	male	right
Mean ± SD = **9.03** ± 1.67 years		

B	Adult group	
Age	Sex	Handedness
25.88	male	right
21.26	female	right
24.82	female	right
18.38	female	right
25.06	female	ambidexter
24.25	male	right
24.12	female	right
24.24	male	right
26.33	female	right
26.69	male	right
20.89	female	right
21.44	female	right
21.35	female	right
20.74	female	right
Mean ± SD = **23.25** ± 2.53 years		

Table 2.1. Participants' characteristics.

A: Characteristics of the pediatric group. **B:** Characteristic of the adult group.

2.2 Recruitment

Participants were recruited through several sources:

- Posts in social media accounts of the University Children's Hospital Zurich
- Ads in public groups on different social media platforms (Meta, Telegram)
- Personal connections of members of the sleep research group

From the initial recruitment source participants were forwarded to the recruitment website, where they familiarised themselves with information about the study and filled in a short entrance questionnaire. Then we contacted all applicants with an answer on whether they corresponded to the including/excluding criteria. Afterwards, respective participants were contacted via email or phone call to clarify further steps. Full study information together with a consent form was sent via post. After signing the consent form, parents together with their children answered a detailed questionnaire about the health conditions and sleep habits of the participants.

2.3 Ethics

The study was conducted following the law of Switzerland. In addition, we followed all internationally recognized guidelines for good clinical practice. The Cantonal Ethics Commission of the Canton of Zurich has examined and approved the study (Kantonale Ethikkommission Zürich, KEK-ZH, BASEC 2019-02134) and was conducted in adherence to the principles outlined in the Declaration of Helsinki. Data collection took place between January 2024 and July 2024.

2.4 Study Design

A randomized, double-blinded, crossover study design was followed. Each participant underwent two experimental conditions one week apart: a stimulation night (STIM) and a sham night (SHAM).

One week before both recording nights in the sleep laboratory of the University Children's Hospital Zurich, participants were asked to follow a strict sleep schedule based on

their habitual bed- and rise times. Bedtimes were monitored using wearable actigraphy devices (Actiwatch Type AWL, CamNtech, Cambridge, UK) and self-report sleep diaries.

Participants were asked to restrict the amount of caffeine to a maximum of one standard portion a day up to 4 days before the recording nights and then to exclude any consumption of caffeine to the night of the experiment. All participants were required to report their caffeine/alcohol and medication use during the study time (16 days). On the day of the recording night, participants had to refrain from unusually high-activity sports and sauna use, as it is known to affect sleep.

On the day of the experimental night, participants arrived at the laboratory at 6:30 p.m. Throughout the experiment, children were always accompanied by a parent. Upon arrival at the laboratory parents together with their children filled out several self-assessment questionnaires (see 2.5 Questionnaires). Following the first behavioural word-pair memory task (WPMT) between 6:45 and 7:30 p.m.. Task motivation was assessed prior to each task. Next, EEG (electroencephalogram), EMG (electromyogram), and ECG (electrocardiogram) placement were done between 7:00 and 8:30 p.m. A 6-minute resting state wake EEG (3-minute eyes open, 3-minute eyes closed) was recorded after the electrode placement between 8:00 to 9:30 p.m. Wake EEG was also recorded during the second behavioural alertness task (Test of Attentional Performance, TAP).

Sleep EEG recordings were started according to each participant's usual bedtime, which varied from 9:00 to 10:15 p.m. Auditory stimulation started after the detection of 10 minutes of stable NREM (non-rapid eye movement) sleep and continued over the N2-N3 stages (progressively deeper stages of NREM sleep) through approximately 9 hours of sleep (mean = 9.07 hours, SD = 0.29). Wake-up times varied individually for each participant between 6:30 and 8:00 a.m. Immediately after awakening, participants filled out a sleep report. After approximately 30 minutes, the resting-state EEG and both behavioural tasks were repeated.

Two weeks after each experimental night, the retrieval of the WPMT task was performed via video or phone call with participants being at their homes. This later retrieval session can provide data about long-term memory consolidation.

A detailed experimental plan is visualised in Fig. 2.1.

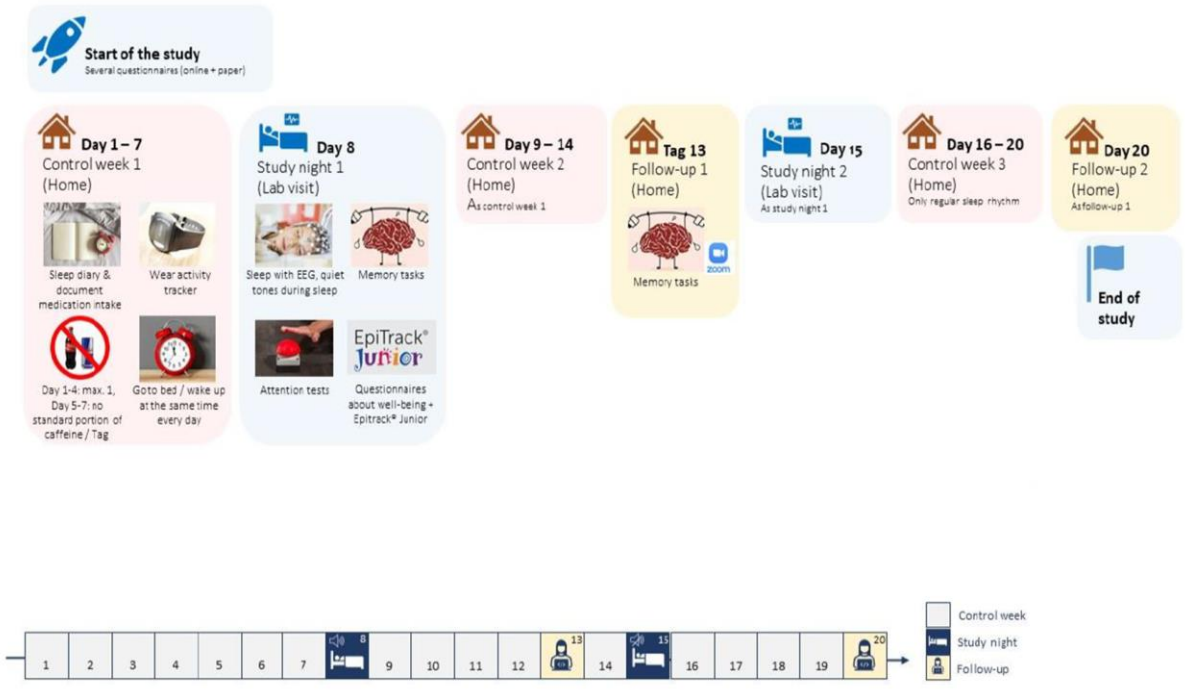

Figure 2.1. Study plan.

2.5 Questionnaires

All questionnaires were self-reports adapted for children, including, adult and pediatric Daytime Sleepiness Scale (Drake et al., 2003, Supplementary Fig. 1) to examine the possible relationship between daytime sleepiness and night sleep; the Edinburgh Handedness Inventory (Oldfield, 1971, Supplementary Fig. 2) to define dominant hand; the Munich Chronotype Questionnaire (MCTQ, Roenneberg et al., 2003, Supplementary Fig. 3) and a socioeconomic status form (SES, Largo, 1989, Supplementary Fig. 4).

2.6 Behavioural Tests

In order to assess a possible connection between overnight acoustic stimulation and particular cognitive functions, two behavioural tasks were carried out: a declarative verbal memory task

(WPMT, (Hager & Hasselhorn, 1994)) and an alertness task (TAP, (Zimmermann & Fimm, 2002)).

Associated word-pair memory task

The task was performed using the PsychoPy (version 2023.2.2) program. Words were auditorily presented through the loudspeakers of the laboratory laptop (ThinkPad E15 Gen 4, Lenovo Inc., Switzerland). All audio files had been pre-recorded by the same person using a microphone (BY-M1, BOYA Audio Equipment CO., LTD).

The word-pair task took place both during evening and morning assessments. In the evening the task started with a training session, followed by an encoding session, during which participants were asked to learn a list of 22 to 32 word pairs, depending on the age of the child, or a list of 40 word pairs for adults. Next, the immediate retrieval session was initiated. Participants listened only to the first word of each newly learned word pair and were required to name the second word from the associated word pair. They were not limited by the time to answer. In case of a wrong answer, the full word pair was presented again. Otherwise, the participant was presented with the word from the following word-pair. The accuracy of the answer was determined by the examiner. To successfully complete the retrieval session participants had to correctly recall at least 60% of the word-pairs. If the recall rate was less than 60% the retrieval session was repeated (Fig. 2.2).

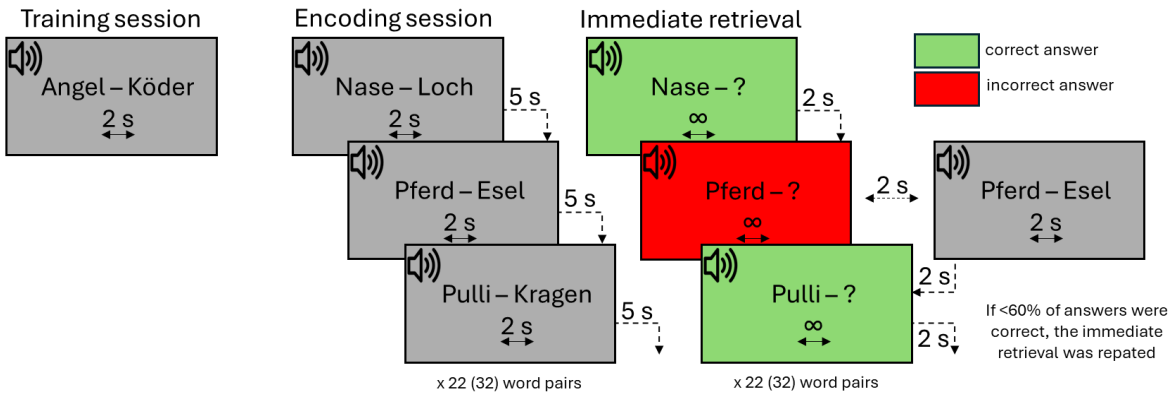

Figure 2.2. Procedure of the associated word-pair memory task.

All words were presented through computer speakers and the screen was not visible for the participant. After a training word pair (left), an encoding phase followed during which participants learned 22(32) associated word pairs (middle). Thereafter, participants immediately retrieved the newly encoded word pairs (right). After a correct answer, the next word pair followed. After a wrong answer, the whole word pair was presented once more. The procedure was repeated until ≥60% of word pairs were recalled correctly.

The morning session consisted of two parts. First, the delayed retrieval. Participants were presented with the first word of each word pair they had learnt the day before and were asked to recall the associated word of the same word pair. In contrast to the immediate retrieval, in the delayed retrieval, no feedback was provided in case of an incorrect answer.

During the second part of the morning task, participants were required to learn (encoding session) and recall (immediate retrieval session) a new list of word pairs. Immediate retrieval of newly learnt words took place only once and no feedback for incorrect answers was provided.

To assess long-term memory consolidation, two weeks after each experimental night, a delayed retrieval session was carried out. The word pairs from the initial evening encoding session were assessed. Participants were examined via video or audio call from their homes and no feedback was given during the retrieval session.

All word pairs were organized into four different lists (Supplementary Fig. 5,6). The words of each pair were meaningfully associated with each other and were characterized by

high tangibility and low emotional intensity. The lists of word pairs were consistently coupled as list 1 (during the evening assessment) plus list 3 (during the morning assessment) or list 2 (during the evening assessment) plus list 4 (during the morning assessment). The respective list couples (1+3 or 2+4) were randomly assigned across conditions (STIM/SHAM). The word pairs within each list were presented in random order during both encoding and retrieval sessions.

A total of 160 word-pairs was used to create four word-pair lists. Word pairs were selected from previous studies (32 from Marshall et al., 2006, 48 from Hager & Hasselhorn, 1994 and 80 from Wilhelm et al., 2008).

The inter-stimuli-intervals (ISI) between words within each word pair and between consecutive word pairs were programmed to 2 and 5 seconds respectively. The ISI is measured from the voice play onset of a word to the onset of the following word.

Alertness task

To assess attention performance, the alertness subtest of the Test of Attentional Performance (TAP) software (version 2.3.1) was employed (Zimmermann & Fimm, 2002). This test evaluates reaction times under two distinct conditions (Fig. 2.3). In the first condition, a white cross appears on the black screen at random intervals, and in response, the participant has to press a key as quickly as possible. This condition assesses the participant's tonic alertness. In the second condition, a warning sound cue is presented first, followed by the appearance of the white cross with random lag time. Participants are instructed to press the key only when the cross appears. A key press in response to the sound is considered a fault. This scenario evaluates phasic arousal, also known as the temporal orientation of attentional focus. The task consisted of a short training session during which the comprehension of the task was assessed, and the main session, which contained four blocks (tonic/phasic alertness) of 20 trials each. Blocks were always presented in the same order: tonic – phasic – phasic – tonic. The reaction time (RT) variability is one of the attentional performance measures sensitive to sleep pressure (Dinges et al., 2009; Doran et al., 2001).

For the correlation with sleep variables, we computed the mean of reaction time across all trials, and normalized values according to the formula below (TAP Δ RT):

$$\mathrm{TAP}\,\Delta\,\mathrm{RT} = \frac{M_{\mathrm{stim}}}{E_{\mathrm{stim}}} - \frac{M_{\mathrm{sham}}}{E_{\mathrm{sham}}}$$

Where:

M_{stim} - morning RT in STIM condition,

E_{stim} - evening RT in STIM condition,

M_{sham} - morning RT in SHAM condition,

E_{sham} - evening RT in SHAM condition

Formula 2.1. Difference in overnight RT change between conditions.

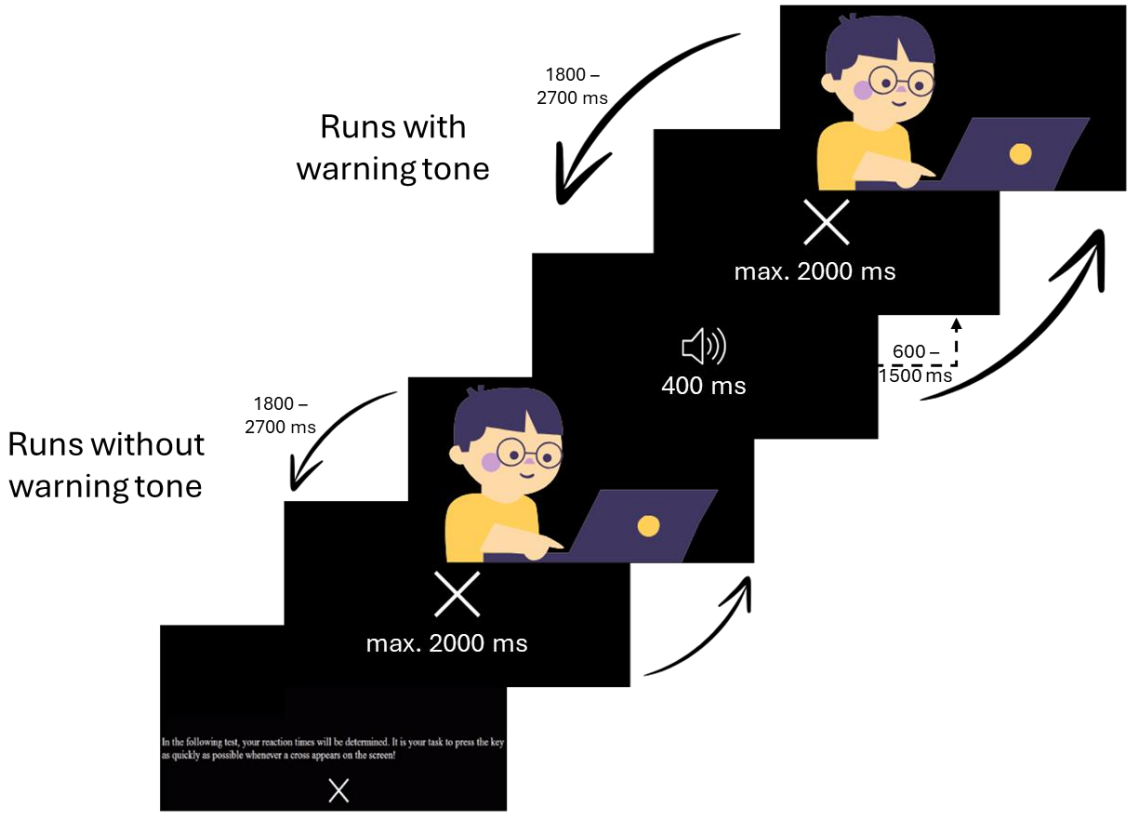

Figure 2.3. Procedure of the alertness test of the test battery of attentional performance (TAP).

The test consists of 4 blocks. In the first and fourth blocks, the tonic RT is assessed (stimuli are presented without a warning tone). In the second and third blocks the phasic RT is assessed (stimuli are presented with a warning tone). Stimulus presentation time: till reaction, max. 2000 ms. Interval reaction-stimulus: 1800 - 2700 ms. Presentation time of warning cue: 400 ms. ISI warning cue – target stimulus SOA: 600 - 1500 ms.

2.7 High-density sleep EEG

Both wake and sleep EEG across two experimental sessions were recorded using 128-channel high-density EEG (HD EEG) Electrical Geodesics Sensor nets (Net Amps 400 series, Electrical Geodesics Inc., EGI, Eugene, OR) and seven additional golden electrodes (gold, Grass Technologies, West Warwick, RI).

Two golden electrodes were attached to the chin muscles to record the electromyogram (EMG) for further manual sleep-scoring use. The other four golden electrodes were attached

to the earlobes and mastoids. Earlobes' electrodes served as alternative references and mastoids' electrodes were used as the reference and ground of the acoustic stimulation device. Electrooculogram (EOG) was measured by channels 1, 32, 125 and 128 within the hd-EEG net.

Electrocardiogram (ECG) was measured with oval solid gel electrodes (Skintact, Innsbruck, AT), the superior electrode was placed two fingers below the right clavicle bone and the inferior electrode was placed two fingers below the lowest left rib. EEG and EMG signals were acquired using EGI Net Station version 5.4.

The net was adjusted to the vertex (Cz) determined by the intersection of midpoints between the two mastoids and between nasion and inion. The skin underneath each net electrode was scrubbed with skin prep gel (Nuprep, Weaver and Company) and an electrolyte gel (ECI Electro-Gel, Electro-Cap International, Inc., Eaton, OH) was injected into each electrode cup to ensure conductance and consistent signal quality. For the golden electrode placement, the skin was preliminarily scrubbed and disinfected (farblos, Octeniderm). Afterwards, electrodes were attached to the skin with EEG paste (SAC2 Electrode Cream, Spes Medica USA) and fixated with medical plaster (Leukoplast, Hypafix).

The impedances of the net and golden electrodes were measured prior to each recording and kept below 50 kΩ and 5 kΩ respectively. EEG was referenced to Cz and sampled at 500 Hz. For ECG the bipolar reference configuration was applied.

EEG data were exported using EGI Net Station software (version 5.4) with a 0.1 Hz high pass filter to eliminate slow drafts and power surges.

2.8 Closed-loop stimulation

A configurable mobile system (Axo, Tosoo AG, Ferster et al., 2019) was utilized for real-time slow-wave detection and phase-locked auditory stimulation. Stimulation triggers were sent in real time to the EGI amplifier from the Axo device via a trigger cable. This way triggers from the auditory stimulation were directly integrated into the high-density EEG recording file.

The Axo device was connected to three golden electrodes (Grass Technologies, West Warwick, RI) that are used for sleep and slow wave phase detection. The active electrode was placed next to C3, positioned in between net electrodes 29, 30 and 36. Two electrodes attached to the mastoids were used as the ground (ipsilateral to the active electrode) and the reference (contralateral to the active electrode).

Auditory stimulation

Auditory stimuli were delivered through comfortable on-ear headphones (sleepPhones, AcousticSheep LLC, Peninsula Drive, Pennsylvania), attached to participants' ears with medical tape. The Loudness of auditory stimuli was measured with a sound level meter (UNI-T UT352 Type 2, Uni-Trend Technology EU GmbH, Augsburg, Germany), which was placed directly on the headphones. The volume for initial stimuli was set to 50 dB. According to a volume control algorithm, the volume usually increased to approx. 58 dB during the night.

To perform real-time slow wave manipulation, the EEG signal was detected online and bandpass filtered (Butterworth 0.5 - 2 Hz, stop-band < 0.1 and > 10 Hz, stop-band attenuation 20 dB, pass-band attenuation 0.1 dB). The Axo device slow-wave detection algorithm consists of three parallel binary classifiers, which allow precise detection of sleep and its architecture. First, the sleep detection classifier assesses the past 80 seconds of the EEG signal to detect N2/N3 sleep stages. Then, the SWA classifier measures slow-wave activity in the past 4 seconds of the EEG signal. Lastly, the phase detection classifier defined the phase (UP/DOWN) of the slow wave. A detailed description of the Axo device algorithm can be found in Dr. Ferster's article (Ferster et al., 2019).

After ten minutes of stable NREM sleep, auditory stimulation (50ms tones, pink $1/f$ noise of 50-58 dB) was initiated and stimuli were presented shortly before the negative peak of the slow wave (DOWN, down-phase stimulation, Fig. 2.4). Auditory stimulation thresholds were previously verified for the specific age group (8 to 12 years old) by running a computer simulation based on previously collected data.

The stimulation protocol included 16 seconds ON windows, given the opportunity for multiple stimulations, followed by 8 seconds OFF windows, during which no stimuli were presented. To perform a valid comparison of two conditions, such temporal structure was inscribed in both SHAM and STIM conditions, but no actual auditory stimuli were delivered during the SHAM condition.

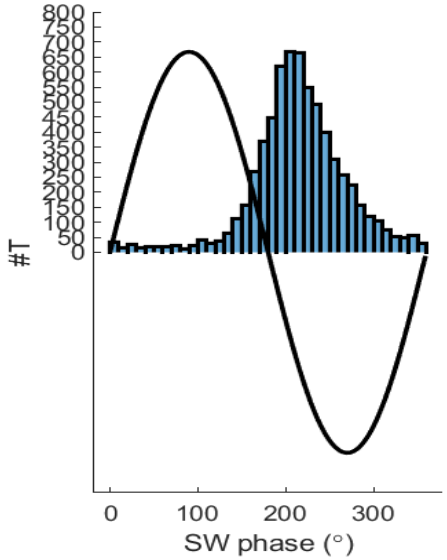

Figure 2.4. Down-phase stimulation.

Example of stimuli distribution during down-phase stimulation in a child participant. On the Y-axis (#T) number of stimuli is shown. The X-axis illustrate the SW phase.

2.9 Data Preprocessing

EEG preprocessing was conducted in MATLAB (version 9.13.0 (R2022b), The MathWorks Inc., Natick, Massachusetts) and EEGLAB (version 2023.0, Delorme & Makeig, 2004) software.

The EEG data were band-pass filtered between 0.5 and 30 Hz and down-sampled to 125 Hz.

Sleep was manually scored in 30-second epochs according to standard criteria (Berry et al., 2017) using single frontal, central and occipital EEG channels, as well as EOG and EMG signals. EEG data for sleep scoring were also down-sampled to 125 Hz and referenced to

contralateral mastoids. The scorer was blinded to the experimental condition but was aware to which participant the data belonged. The five following stages were scored: wake; N1, N2, N3 (NREM sleep); and R (REM sleep). Additionally, visible artefacts were removed (epoch excluded).

Artefact rejection was conducted using the semi-automatic artefact removal program HD-SleepCleaner (Leach et al., 2023). Epochs were excluded if two or more neighbouring channels were artefactual. In the remaining epochs, channel interpolation was performed.

Clean EEG data was re-referenced to the mean value of 110 channels above the ears (average reference).

2.10 Analysis & Statistics

Sleep EEG analysis was performed on artifact-free non-REM (N2 and N3) epochs. First, ERPs were computed for each group for several channels and clusters.

Auditory-evoked potential (AEP)

The EEG data was initially time-locked to the stimuli within the ON windows, then segmented into 4.4-second time windows. The ERPs were computed by averaging the EEG signal of the chosen electrode for a given sample across all ON windows during the night. Averaged ERPs over all trials in N2 and N3 were computed separately for each participant and for both conditions. Then sleep AEP were defined as the difference in the ERP waveform between STIM and SHAM conditions. For positive AEP components, the peak amplitude of the difference curve was quantified as the average amplitude at the local maximum, and the peak amplitude of the difference curve for the negative component was quantified as the average amplitude at the local minimum, occurring at a latency within the respective intervals: 150-250 ms for P200; 500 - 800 ms for N550, and 800 - 1300 ms for P900 (Colrain & Campbell, 2007). Amplitudes were measured from 0 time-point (stimulus presentation) to peak and from peak to peak. The peak latencies of AEP components were calculated as the time from 0 time-point to the components' peak.

Analysis of alertness task

To evaluate alertness performance, the mean overnight change of all trials with (phasic) or without (tonic) warning tome was calculated for STIM and SHAM conditions. Then the TAP Δ RT were calculated using the formula from chapter 2.6. The TAP Δ RT was then correlated to values of AEP components.

Statistics

All statistical analyses were conducted with MATLAB (version 9.13.0 (R2022b)) or R (version 4.2.2) and RStudio (version 2022.12.0).

Given a sample size of 6 participants in a pediatric group, traditional parametric tests like Peason's correlation and multivariate normality tests may not be appropriate due to the small sample size. Instead, the non-parametric method, which assumes no normal distribution is more suitable. For this reason, Spearman's rank correlation coefficient (ρ or rho) was chosen to measure the strength and direction of association between variables.

To perform a comparison between two groups, the two-sample t-test was used. This test compares the means of two independent groups to see if they are significantly defer from each other.

3. Results

3.1. Effects of down-PTAS on EEG in children

The primary objective of this study was to evaluate the efficacy of a closed-loop down-PTAS protocol in eliciting KC-like response and related AEP components during NREM sleep in children. Six participants underwent two experimental nights: one with the down-PTAS protocol (STIM) and another without stimulation (SHAM), with the order of conditions randomized and counterbalanced.

During the STIM night, auditory stimuli were delivered in an ON|OFF window design: 16-second ON windows allowed for stimulus presentation, followed by 8-second OFF windows where no stimuli were presented. In the SHAM night, stimulation flags were recorded without actual auditory stimuli being presented.

To assess the effects of the down-PTAS protocol, we computed ERPs time-locked to the stimuli for both experimental conditions. Given the challenge of background SWA in sleep EEG, which makes the prestimulus baseline correction unreliable, AEPs were computed as the difference in ERP waveforms between conditions, by subtracting the ERPs recorded during the SHAM night from those recorded during the STIM night.

Previous studies suggested the largest AEP response over the frontal region, matching the anticipated topography of the N550 component of KCs (Cote et al., 1999). In our data, the averaged EEG from the Fz channel (Fig. 3.1. C), referenced to linked mastoids and time-locked to the auditory stimuli, demonstrated a prominent AEP compared to SHAM condition ($p < 0.02$; Fig. 3.1. A). The AEP waveform exhibited all classical sleep AEP components, including P200, N350, N550, and P900, confirming the presence of a KC-like response.

While measuring single-channel AEP could provide a clear visualisation of the stimulation effect, as there is no averaging across several channels, representing different underlying sources, it also can be affected by artefacts. To ensure the reliability of the single Fz channel measurements, AEPs were also computed from a cluster of frontal electrodes referenced to linked mastoids (Fig. 3.1. B). A statistical comparison of AEPs between the Fz

channel and the frontal cluster showed no significant differences in amplitude, indicating that the single-channel Fz provides robust data and can be used for further analysis.

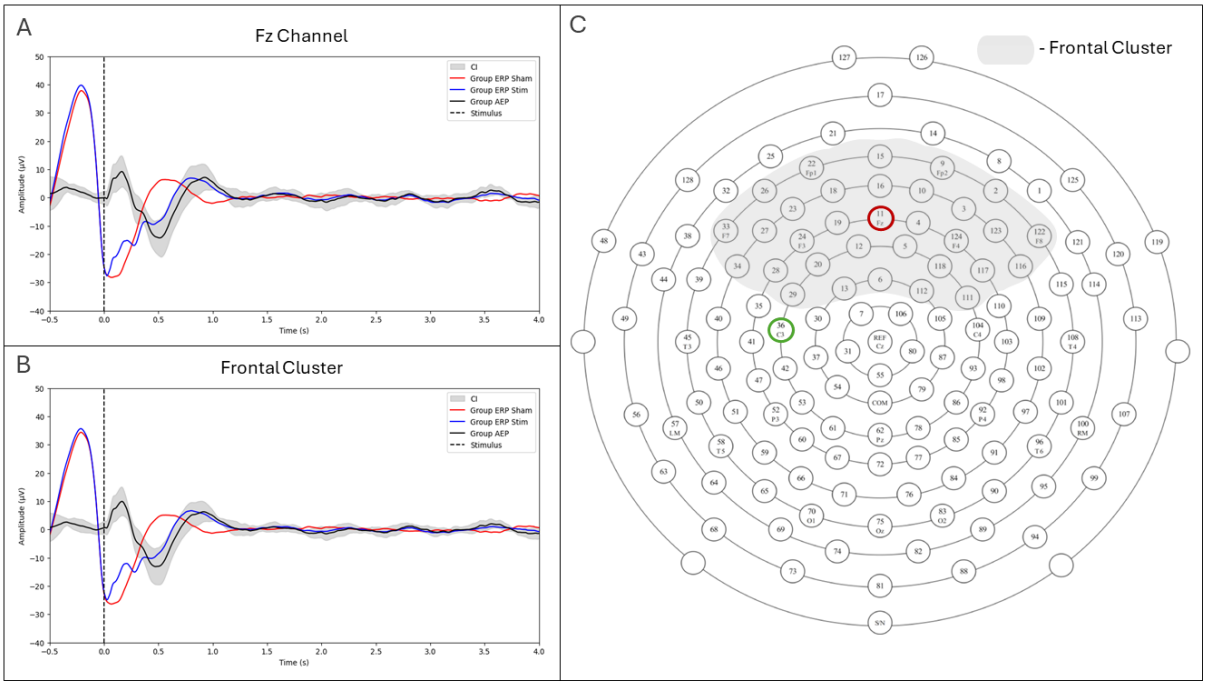

Figure 3.1. AEPs and channel map.

A-B: Group AEP of Fz channel and Frontal cluster. Group ERP in the SHAM condition is in red, group ERP in the STIM condition is in blue, group AEP is in black, and the CI (confidence interval) is in light grey. Dotted vertical lines mark the stimulus onset. **C:** 128 channel-map of hd-EEG electrical geodesics sensor net for long-term monitoring. The position of the stimulation (registering) electrode is marked with a green circle. The Fz electrode is marked with a red circle. The light grey colouring shows the frontal cluster.

3.2. Comparison of sleep AEP characteristics between children and adults

The second aim of this study was to explore age-related differences in the characteristics of auditory evoked potentials (AEPs) during NREM sleep by comparing the amplitude and latency of key AEP components between children and adults. For this analysis, previously collected EEG data from 14 young adults (Fig. 3.2. A) were compared with the EEG recordings from 6 pediatric participants (Fig. 3.2. B).

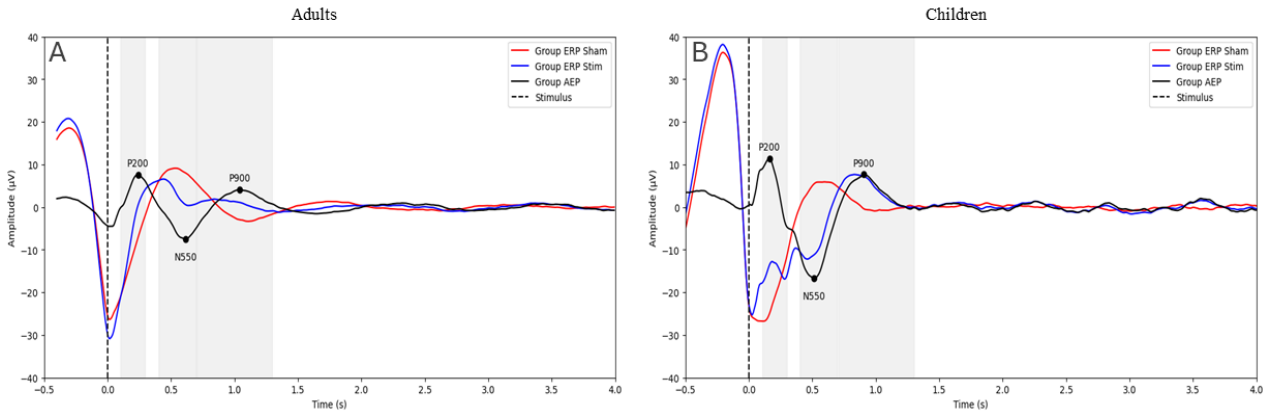

Figure 3.2. AEP components.

A: Children group AEP of Fz channel. **B:** Adults group AEP of Fz channel.

Group ERP in SHAM condition is in red, group ERP in STIM condition is in blue, and group AEP is in black. The interval around each peak (range calculated as +- ¼ of peak value) is in light grey. Dotted vertical lines mark the stimulus onset.

The primary focus was on the three most pronounced components of sleep AEPs: P200, N550, and P900. For each group, we calculated the amplitudes and latencies of previously defined components. Then obtained values were compared between adult and pediatric groups.

P200 Component

The P200 component is a prominent positive peak that typically occurs around 200 ms after stimulus presentation. Our analysis revealed a significant difference in the latency of the P200 component between the pediatric and the adult group ($p < 0.04$). The mean latency of P200 was shorter in children (mean = 170 ms) compared to adults (mean = 240 ms). The P200 amplitude was not significantly different between the two groups ($p > 0.7$).

N550 Component

The N550, also referred to as the KC-like response, is a negative peak emerging between 500 and 800 ms after stimulus onset. The latency of the N550 component was longer

in adults (mean = 600 ms) than in children (mean = 490 ms) on a trend level ($p < 0.09$). Furthermore, there was a possible trend towards a greater amplitude of the N550 component in children (mean = 15.65 mV) compared to adults (mean = 10.1 mV), with a p-value of 0.06. However, as apparent from Figure 3.3, children have much bigger variability in N550 amplitude values than adults.

P900 Component

The P900 component is another positive wave, peaking between 800 and 1300 ms after stimulus onset. Both amplitude and latency comparisons did not reveal significant differences between the two groups.

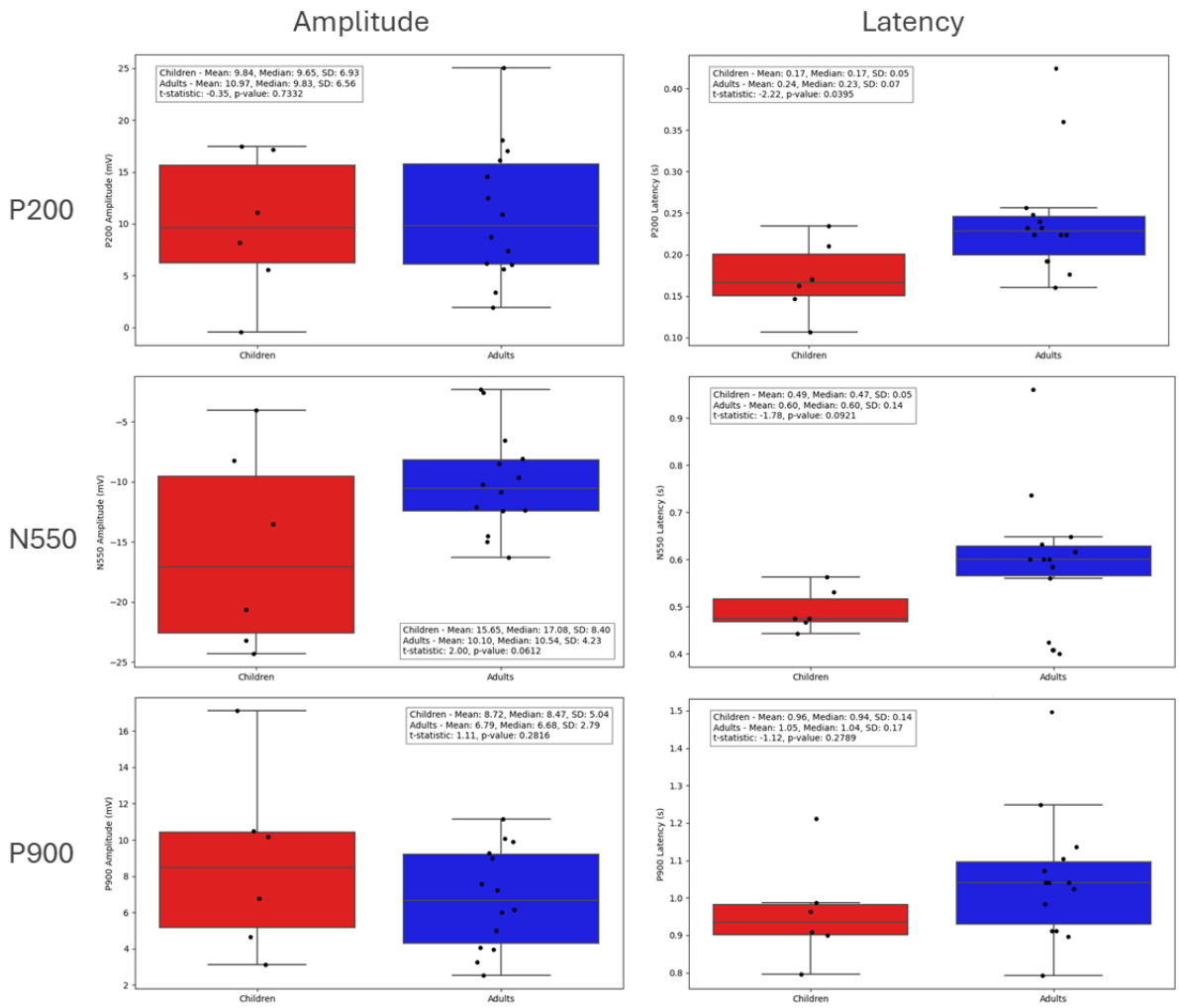

Figure 3.3. Comparison of AEP components characteristics.

On the left side of the figure, the comparison of amplitude values of components P200, N550 and P900 is presented. On the right side, the comparison of latencies of corresponding components is presented. Red box plots represent the children's group. Blue box plots represent the adult group. Individual data points are shown with black dots.

To further investigate a potential trend in the components' amplitude differences, we compared the peak-to-peak indexes between the two groups. This method calculates the amplitude difference between the positive and negative peaks, excluding potential measurement errors due to baseline variations. Some studies suggest that the peak-to-peak method is approximately 20% more sensitive than the base-to-peak method for detecting

amplitude variations. However, the t-test comparison of peak-to-peak indices between children and adults did not yield significant results (Fig. 3.4).

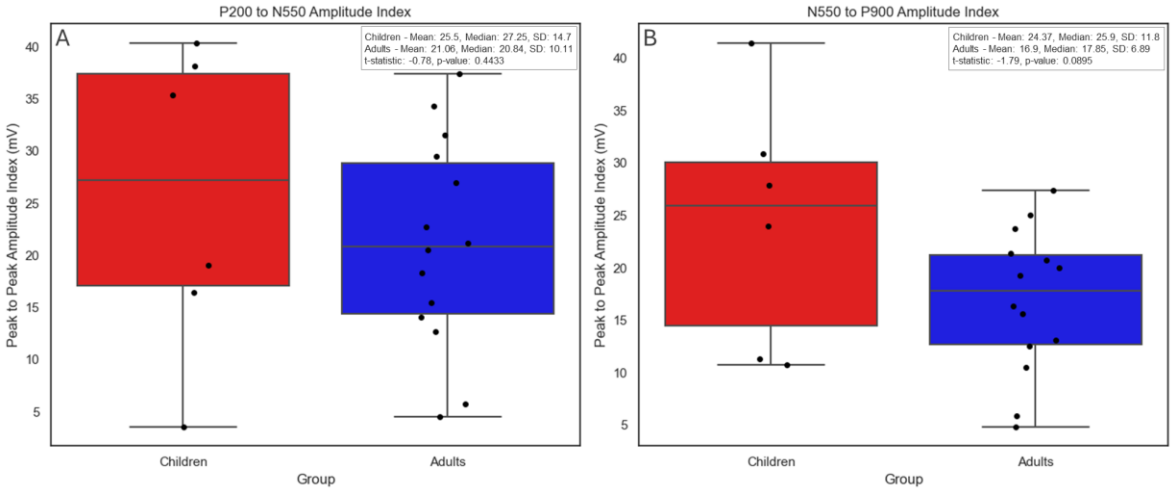

Figure 3.4. Peak-to-peak amplitude index.

The Y-axis shows the range between two peaks in mV. **A:** P200 to N550 amplitude range. **B:** P200 to N500 latencies. Red box plots represent the children's group. Blue box plots represent the adult group. Individual data points are shown with black dots.

3.3. Correlation of age and latency

Based on the results of significant differences in peak latencies of AEP components between children and adults, we further explored the potential relationship between age and the latency of these components. Specifically, we aimed to determine whether age-related changes within the pediatric group could explain the observed differences in AEP characteristics.

To investigate this relationship, we performed a Spearman's rank correlation analysis, which is appropriate for examining non-linear associations between variables. This analysis was conducted separately for the adult and child groups to assess the correlation between age and the latency of the P200, N550, and P900 AEP components.

In the adult group, Spearman's rank correlation analysis did not reveal any significant correlations between age and the latencies of the AEP components.

In contrast, the analysis within the pediatric group uncovered a statistically significant correlation between age and the latency of the N550 component (Spearman's rho = 0.9, $p < 0.02$). This finding indicates that younger children tend to exhibit shorter N550 latencies, while older children have longer N550 latencies (Fig. 3.5, data of P200 and P900 not shown).

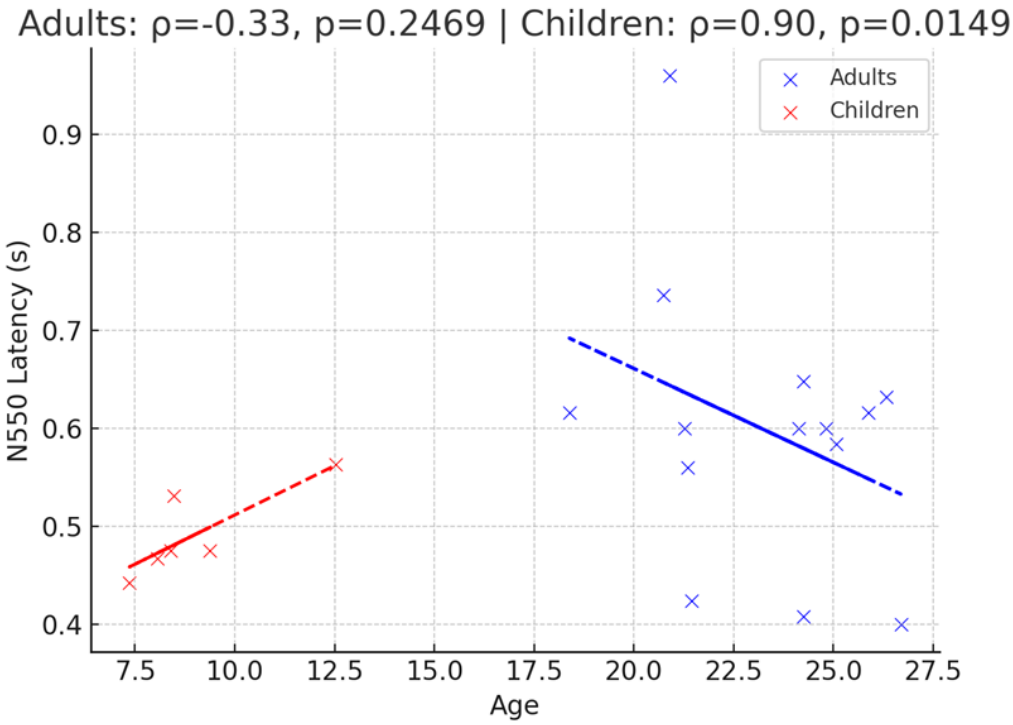

Figure 3.5. Correlation of age and N550 latency.

The red regression line represents the correlation for the children group. Red crosses show the individual data points of the children group. The blue regression line represents the correlation for the adult group. Blue crosses show the individual data points of the adult group. On the top of the plot, statistics are presented: ρ stands for Spearman's correlation coefficient; p stands for p-value.

3.4. Correlation between early and late AEP components.

The next objective of this study was to investigate whether the characteristics of early AEP components, such as P200, can predict the characteristics of subsequent peaks, including the N550 and P900 components.

To assess these relationships, we conducted a Spearman's rank correlation analysis within the amplitudes or latencies of the early and late AEP components. In the adult group, the analysis revealed significant correlations between the characteristics of early and later AEP components. Specifically, the amplitude and latency of the early P200 component were found to predict correspondingly the amplitude ($p < 0.01$) and latency ($p < 0.01$) of the subsequent N550 KC-like response. Additionally, the amplitude of the P200 component correlated significantly with the amplitude of the following positive component P900 ($p < 0.01$), as did the amplitude of the N550 with P900 ($p < 0.01$). Moreover, a significant correlation was also observed between the latencies of the N550 and the P900 components ($p < 0.01$, Fig. 3.6).

Interestingly, in contrast to the findings in adults, the correlation analysis in the pediatric group did not reveal any significant predictive relationships between the early and later AEP components. Neither the amplitude nor the latency of the early P200 component was significantly correlated with the characteristics of the N550 or P900 components in children (Fig. 3.6).

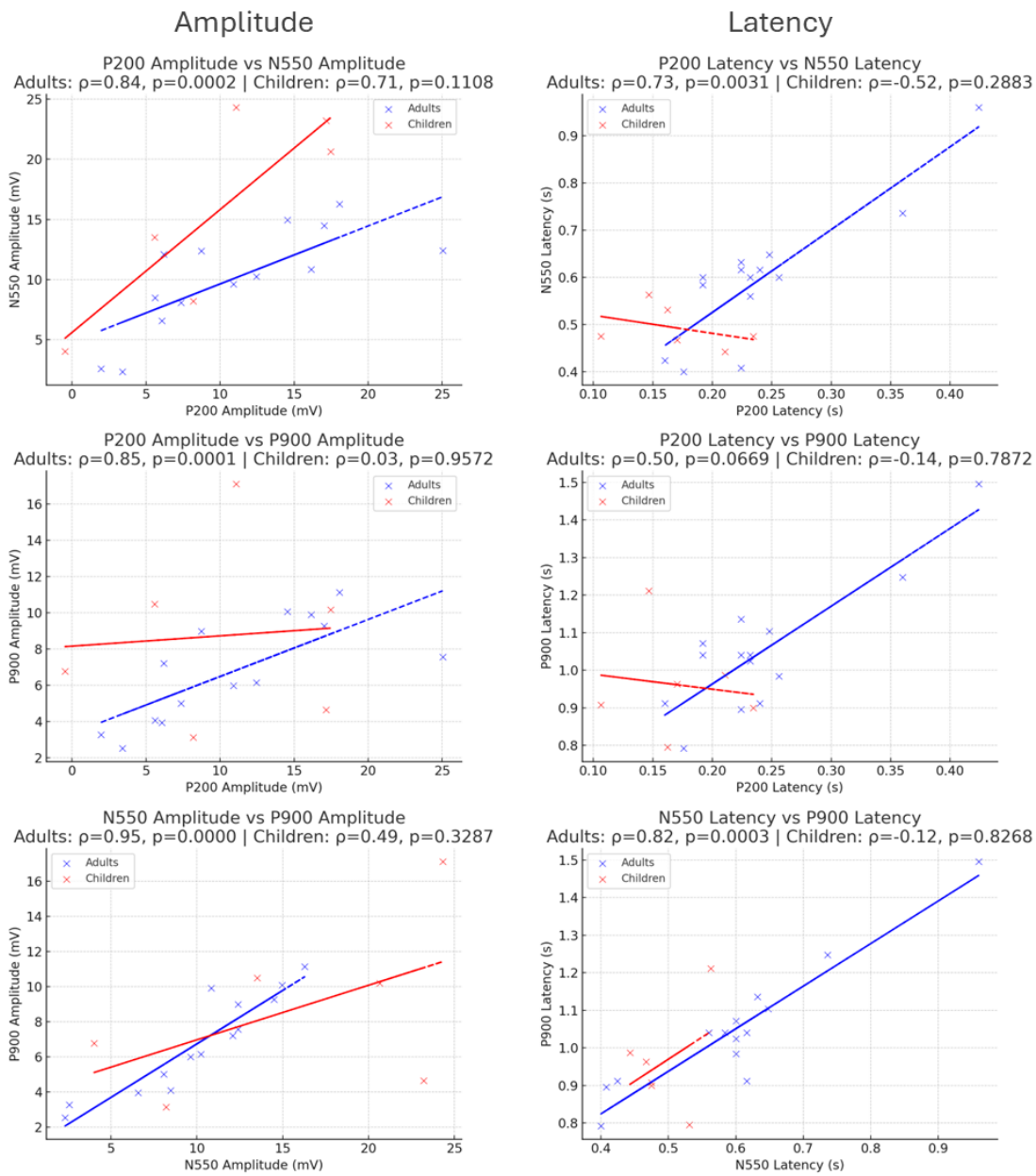

Figure 3.6. Correlations between early and late AEP components in children and adults.

On the left side of the figure, the correlations of amplitude values of components P200, N550 and P900 are presented. On the right side, the correlations of latencies of corresponding components are presented. Red regression lines represent correlations in the children group. Red crosses show the individual data points of the children group. Blue regression lines represent correlations in the adult group. Blue crosses show the individual data points of the adult group. On the top of the plot, statistics are presented: ρ stands for Spearman's correlation coefficient; p stands for p-value.

3.5. Changes in attentional performance and physiological effects of stimulation

The final objective of this study was to explore the potential relationship between physiological responses to auditory stimulation, as measured by AEP components, and behavioural performance outcomes, specifically focusing on attentional performance as assessed by changes in reaction time (RT). This analysis aimed to determine whether the characteristics of the N550 component, indicative of KC responses, could predict overnight changes in RT, and how these relationships might differ between children and adults.

The primary outcome variable was the difference in overnight RT change between conditions (TAP Δ RT), calculated according to Formula 1 in chapter 2.6.

We conducted Spearman's rank correlation analysis to examine the relationship between TAP Δ RT and the characteristics of AEP components, focusing on both amplitude and latency. In the adult group (N=14), the analysis did not reveal any significant correlations between attentional performance (TAP Δ RT) and the characteristics of AEP components, including N550 amplitude and latency.

In contrast, the analysis in the pediatric group (N=5) revealed a significant correlation between TAP Δ RT and N550 amplitude (Fig. 3.7). Specifically, children with a greater N550 amplitude tended to experience a greater slowing of their reaction times from evening to morning after the stimulation night compared to the control night.

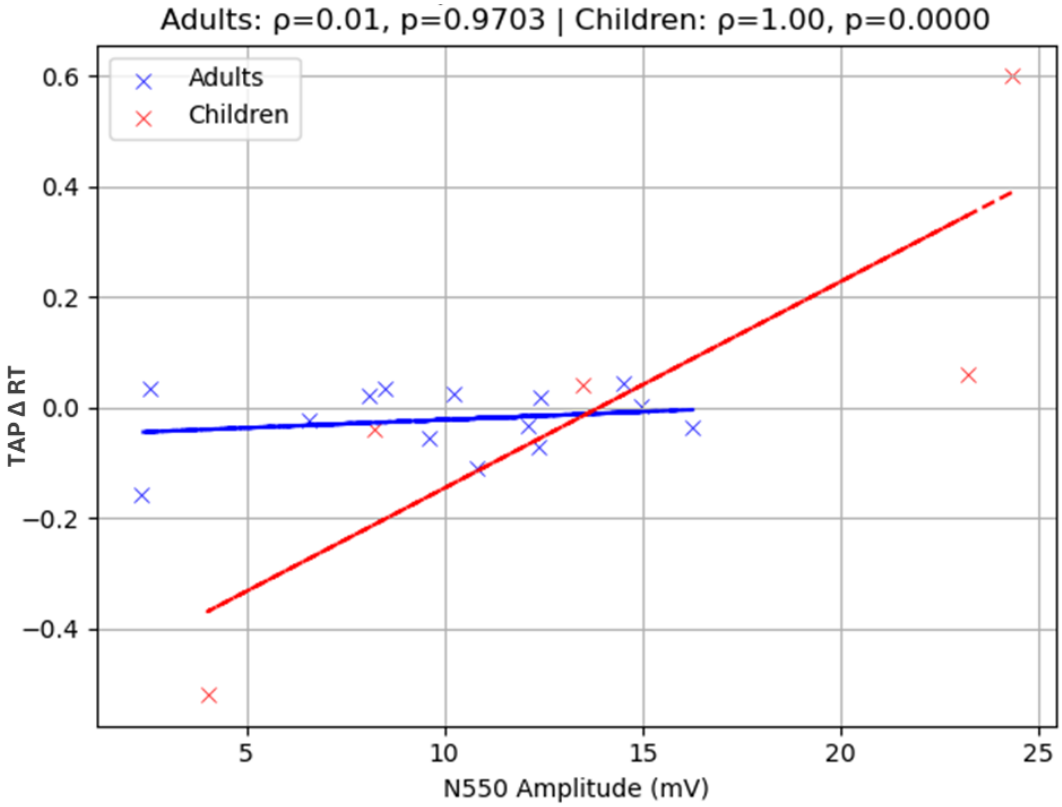

Figure 3.7. Correlation of overnight RT change and N550 amplitude.

Values above 0 (y-axis) show overnight worsening of RT in STIM condition. Values below 0 (y-axis) show overnight improvement of RT in STIM condition. The red regression line represents the correlation in the children group. Red crosses show the individual data points of the children group. The blue regression line represents the correlation in the adult group. Blue crosses show the individual data points of the adult group. On the top of the plot, statistics are presented: ρ stands for Spearman's correlation coefficient; p stands for p-value.

4. Discussion

4.1. Efficacy of down-PTAS in a pediatric population

The primary aim of this study was to assess the efficacy of a closed-loop down-PTAS protocol in eliciting KC-like responses during NREM sleep in children. Our results indicate that the down-PTAS protocol successfully evoked prominent AEP components P200, N350, N550, and P900 in children, confirming the presence of a KC-like response. This finding is significant as it extends the utility of down-PTAS, previously demonstrated in adult populations, to a developing pediatric cohort.

The results of this study are consistent with the findings of Leach et al. (2023), who demonstrated the effectiveness of down-PTAS in adults, showing that this protocol can successfully evoke K-complexes without enhancing SWA. Our findings extend these results to a pediatric population, indicating that the same mechanisms may be at play across different age groups.

Our study, however, did not directly assess changes in SWA during or after stimulation. Therefore, we cannot conclude whether the down-PTAS protocol influenced SWA in our participants. Previous studies have shown that different PTAS protocols, particularly up-PTAS, can enhance SWA following stimulation (Ngo et al., 2013; Papalambros et al., 2017). However, the specific effects of down-PTAS on SWA remain to be explored in future research, particularly in pediatric populations.

4.2. Differences in AEP characteristics between the adult and pediatric groups

The second aim of this study was to explore developmental differences in the characteristics of AEPs during NREM sleep, specifically focusing on the amplitude and latency of the key components P200, N550, and P900. This analysis sought to uncover developmental variations in auditory processing during sleep, which could shed light on how neural responses to auditory stimulation evolve with age.

Age-related differences in the P200 component during NREM sleep

The maturation of the P200 component is known to occur rapidly in early childhood, reflecting the development of specific neural pathways connecting the auditory brainstem to higher brain regions. Ponton et al. (2000) argued that the early maturation of P200 latency is closely linked to the rapid development of pathways between the auditory brainstem and the reticular activating system, which is one of the proposed generators of the P200 peak (Rif et al., 1991).

A mathematical model proposed by Eggermont describes the hyperbolic decrease in latencies of early auditory components, driven by myelogenesis and synaptogenesis, from the age of 1 year, reaching adult-like values by the age of 10 years (Eggermont, 1988).

The effects of ageing on P200 characteristics have not been consistent across different studies. Several studies have not found age-related differences in P200 latencies (Barrett et al., 1987), while others showed a P200 latency increase with age (Amenedo and Diaz, 1999; Picton et al., 1984). Wake studies also showed a non-linear trend in the latency/age slope with a strong decrease in P200 latencies from 5 to 15 years of age, while observing a slow gradual increase in P200 latencies from 16 to 77 years (Enoki et al., 1993).

In our study, we observed that P200 latencies were longer in adults compared to children, a finding that contrasts with those from wakefulness studies but is consistent with observations reported in some sleep studies, as discussed earlier. This outcome might reflect age-related changes in neural processing speed, supporting findings from studies that indicate an increase in latency in adults after puberty. This result may suggest that children of our age cohort have reached their near-peak auditory processing speed, while the adult group started to exhibit a slower neural reaction time.

However, it's important to note that other studies have not found opposite effects in P200 latency, suggesting that the developmental effects of o this component may vary depending on factors such as electrode positioning and individual differences in brain development. The slower response in adults is also possible to be connected to their faster habituation towards the presented stimuli. To further understand these findings it is necessary

to compute AEPs with different inter-stimulus intervals (ISI), which will allow for the evaluation of the effect of habituation on AEP characteristics.

The stability of P200 amplitude across different ages, as observed in our study, is consistent with some findings from the literature on sleep and wakefulness research. Several studies have reported no change in P200 amplitude with age (Barrett et al., 1987; Iragui et al., 1993; Crowley & Colrain, 2004), while others have noted either increases (Amenedo & Diaz, 1998) or decreases (Czigler et al., 1992) in amplitude, often depending on scalp topography.

The lack of significant differences in P200 amplitude between children and adults in our study suggests that the magnitude of the P200 response might be relatively stable once the auditory pathways have matured. This stability may indicate that while the timing of the response (latency) changes with age, the overall strength or magnitude of the response (amplitude) remains consistent.

Age-related differences in the N550 component during NREM sleep

The N550 component, which is closely associated with the KC, represents an endogenous brain response that plays a crucial role in sleep protection by reacting to external stimuli without causing full arousal. The development of KCs and their role in sleep protection has been extensively studied. It was argued that the KC reaches a relative developmental plateau at the age of 12 (Metcalf et al., 1971). Colrain and De Gennaro both highlight that the KC serves as a protective mechanism, particularly in younger individuals, to maintain sleep stability despite external disturbances (Colrain, 2005; De Gennaro et al., 2000). Studies by Crowley and colleagues have also shown a significant reduction in the amplitude and probability of KCs with age (Crowley et al., 2004; Crowley et al., 2002). This reduction could reflect the diminishing need for such pronounced protective mechanisms as the brain matures and sleep architecture stabilizes. Our results, showing a trend towards a larger N550 amplitude in children, support this idea, indicating that the developing brain may rely more heavily on these protective responses to preserve sleep integrity.

Our further analysis revealed a statistically significant positive correlation between age and N550 latency in children, suggesting that as children grow older, the latency of their N550 component tends to increase.

This trend suggests a developmental trajectory in which the neural processes underlying the generation of KC-like responses during sleep may mature as children age, leading to longer response times.

These findings contradict previous developmental studies showing a gradual decrease in N500 latency during childhood, reaching adult-like values during adolescence (Metcalf et al., 1971). This discrepancy may be due to several factors, including our relatively small sample size, which limits the statistical power of our study and the generalizability of our results. Moreover, gender differences could also play a role in the observed results. Previous studies have shown that there may be gender-related differences in information processing in children (Nanova et al., 2008). Another critical consideration is the age distribution within our pediatric sample. The children in our study were not evenly distributed across the age range, with potential clustering around certain ages. This uneven distribution could skew the results, making it difficult to draw definitive conclusions about the relationship between age and N550 latency.

In contrast, no significant correlations were found between age and the latencies of AEP components in the adult group. The absence of a similar correlation in adults supports the notion that this latency shift occurs predominantly during childhood and stabilizes by early adulthood.

Age-related differences in the P900 component during NREM sleep

The P900 component, another late positive wave that follows the N550, did not show significant differences in either amplitude or latency between children and adults. This stability suggests that the P900 component may reflect processes that are less sensitive to age-related changes during the stages of development covered by our study.

4.3. Sequential auditory processing: Analysis of early and late AEP components

The analysis of the correlations between early and late AEP components revealed significant distinctions between adults and children. In adults, significant correlations were observed between the characteristics of early (P200) and later (N550, P900) AEP components. This suggests that in mature auditory systems, early neural responses to auditory stimuli during sleep are predictive of subsequent processing stages. These findings are consistent with previous research indicating that the neural mechanisms underlying sensory gating and sequential auditory processing become more efficient and interconnected with maturation (Kogure et al., 1992).

However, several studies showed a misalignment in the characteristics of early and late AEP components when AEPs were collected in response to frequent and deviant stimuli (Nielsen-Bohlman et al., 1991). In analyses focusing on different ISIs, the characteristics of N550 were dependent on the ISI, while the characteristics of P900 remained unchanged (Bastien & Campbell, 1994).

Contrarily, in children, no significant predictive relationships between the early and later AEP components were observed. This suggests that the developmental stage of the brain plays a crucial role in how auditory information is processed during sleep. The absence of strong correlations in children might reflect ongoing maturation and a less stable or less efficient integration of auditory pathways during sleep.

Other potential explanations may lie within the characteristics of sleep architecture. Several studies showed significant differences in AEP characteristics across sleep stages (Atienzy et al., 2001; Kogure et al., 1992; Nielsen-Bohlman et al., 1991). These differences can be much more distinguishable in pediatric populations, as their sleep is still developing. We analysed AEPs from N2 and N3 stages together, which might have caused the observed discrepancy among children's AEP components.

The much smaller sample of the pediatric group compared to the adult group might also cause the lack of correlation between components in the pediatric group because of less

statistical power. Further research with a bigger pediatric sample and separate analysis for N2 and N3 stages may help to shed light on these differences.

4.4. Relationship between physiological responses to auditory stimulation and attentional performance

The final aim of this study was to investigate whether the physiological responses to auditory stimulation, as measured by AEP components (particularly the N550 indicative of K-complexes), are linked to behavioural performance outcomes, with a focus on attentional performance as assessed by changes in reaction time (RT). The results point toward a developmental difference, as correlations between these variables were observed in children but not in adults.

In the adult group, the lack of significant correlations between AEP components and overnight changes in RT suggests that the physiological effects of down-PTAS, as captured by AEPs, may not directly influence attentional performance. This is consistent with findings from studies of Krugliakova et al. and Leach et al., who employed similar protocols, which demonstrated that while auditory stimulation can enhance certain aspects of sleep architecture, the reaction time performance may not be directly linked to the immediate physiological responses (Krugliakova et al., 2022; Leach et al., 2023).

In contrast, the pediatric group showed a significant correlation between the N550 amplitude and overnight changes in RT. Specifically, a greater N550 amplitude was associated with a more pronounced overnight slowing of RT after the stimulation night compared to the control night. This finding suggests that in children, a higher N550 amplitude may be associated with reduced alertness or cognitive performance the next morning. However, these results require reproducibility in future studies to be able to conclude their meanings.

4.5. Limitations

Sample size and heterogeneity

Despite the promising findings, several limitations should be acknowledged. Some of them were already mentioned in the previous discussion, here they are summarized along with additional considerations regarding the experimental and stimulation protocols. The small and heterogeneous sample size of six participants in the pediatric group limits the statistical power and generalizability of the results. While the within-subjects design and the counterbalancing of conditions mitigate some of the variability, larger studies are needed to confirm these findings and ensure they are representative of the broader population. This limitation is particularly relevant in pediatric research, where variability in developmental stages can introduce confounding factors.

Stimulation protocol

The use of all consecutive auditory stimuli for analysis, without separating them based on the ISI, might have affected the outcomes. Previous studies have demonstrated that KCs are most likely to be elicited by the first tone in the ON window, compared to subsequent tones within the same window (Leach et al., 2023). A similar pattern is observed when trains of stimuli are presented, where the largest KCs are triggered by the initial stimulus of the train rather than by those that follow (Ngo et al., 2015). The lack of ISI-specific analyses could have obscured potential nuances in how different intervals impact AEP characteristics.

The determination of auditory stimuli' loudness is another challenging part of sleep auditory stimulation protocols. The stimulus should be loud enough to evoke the AEPs, but cannot be too loud to not wake or disturb participants (Bellesi et al., 2014). We chose the same loudness for all participants of the pediatric group based on the participants' age of 8 to 12 years. Meanwhile, the individual adaptation of loudness thresholds may have resulted in more optimal stimulation for some participants. While the stimuli successfully evoked brain responses without disrupting sleep, individualised loudness settings could have enhanced the efficacy of the down-PTAS protocol, possibly leading to more significant neurophysiological

and behavioural effects (Henin et al., 2019; Ngo & Born, 2019; Ngo et al., 2013, 2015; Prehn-Kristensen et al., 2020).

Habituation night

The absence of a habituation night, during which participants could have acclimated to the sleeping environment, the high-density EEG net, and the earphones, may have introduced variability due to the first-night-effect. This phenomenon, where individuals experience poorer sleep during the first night in a new environment, could have influenced the results despite counterbalancing the order of stim and sham nights (Henin et al., 2019; Leminen et al., 2017; Ngo & Born, 2019; Ngo et al., 2013).

Unassessed SWA changes

While this study focused on AEP components, it did not directly assess changes in SWA during or after stimulation. Given that previous studies have shown that PTAS protocols can influence SWA, future research should include SWA measurements to fully understand the impact of down-PTAS, particularly in pediatric populations (Ngo & Born, 2019; Ngo et al., 2013, 2015; Leach et al., 2023; Krugliakova et al., 2022).

4.6. Conclusion

The primary objective of this study was to investigate the efficacy of a down-PTAS protocol in eliciting KC-like responses during NREM sleep in children and to explore the developmental differences in AEP characteristics between children and adults. Our findings demonstrate that the down-PTAS protocol is effective in evoking prominent AEP components such as P200, N550, and P900 in children, confirming the presence of an evoked KC-like response. This extends the utility of down-PTAS, previously validated in adults, to a pediatric population, highlighting its potential for a broader application in sleep studies across different age groups.

Furthermore, our analysis revealed significant age-related differences in the characteristics of AEP components during NREM sleep. Specifically, adults exhibited longer P200 latencies compared to children, suggesting age-related changes in neural processing speed. This result contradicts previous findings, which require further research on this topic. The N550 component showed a trend towards larger amplitudes in children but didn't reach significance. This finding could be further confirmed or refuted with a more detailed analysis of a bigger sample.

The study also explored the potential link between physiological responses to auditory stimulation and behavioural performance outcomes, focusing on changes in RT. While no significant correlations were found between AEP components and RT changes in adults, the pediatric group displayed a significant relationship between N550 amplitude and overnight changes in RT. This may suggest that in children, a higher N550 amplitude may be associated with reduced behavioural performance, although further studies are needed to confirm these findings.

4.7. Perspectives for Future Research

Building on the findings and limitations of this study, future research could explore several avenues to further elucidate the role of auditory stimulation during sleep in cognitive and neurophysiological processes:

Future studies should aim to include larger and more homogeneous samples, particularly within specific age groups, to increase statistical power and generalizability. Stratifying participants by developmental stages could help identify more nuanced age-related differences in AEP characteristics.

Given the potential differences in AEP characteristics across sleep stages, future research should conduct separate analyses for the N2 and N3 stages. This approach could provide deeper insights into how different stages of NREM sleep interact with auditory processing and contribute to cognitive outcomes.

To fully understand the impact of down-PTAS, future studies should include measurements of SWA, particularly its modulation across the night following stimulation. Investigating the relationship between SWA changes and behavioural outcomes such as memory consolidation and attentional performance would offer a more comprehensive view of how auditory stimulation influences sleep-dependent cognitive processes.

Longitudinal research tracking AEP characteristics and cognitive outcomes over time could shed light on the developmental trajectories of auditory processing during sleep. Such studies would help clarify how neural responses evolve from childhood to adulthood and how these changes relate to cognitive development.

5. Acknowledgement

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- My family and friends for their unlimited patience and mental support.

6. Statement of Authorship

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

30.09.2024, Zurich

Date and Place | Signature

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8. Supplementary materials

Supplementary Figure 1. Pediatric Daytime Sleepiness Scale.

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PN #	Date

Daytime Sleepiness Questionnaire

(Pediatric Daytime Sleepiness Scale, Drake 2003)

Who fills out that questionnaire?

☐ Mother ☐ Father ☐ Other

The following questions refer your normal daily life in the recent past. Please try to imagine how the situations below would affect your child, even if your child has never experienced them before. How likely you believe is it that your child gets drowsy or falls asleep (not just feeling tired)?

How often does your child fall asleep or get drowsy during class periods?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often does your child get sleepy or drowsy while doing your homework?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

Is your child usually alert most of the day?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often is your child ever tired and grumpy during the day?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often does your child have trouble getting out of bed in the morning?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often does your child fall back to sleep after being awakened in the morning?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often does your child need someone to awaken you in the morning?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

How often does your child think that you need more sleep?

never ☐ seldom ☐ sometimes ☐ frequently ☐ always ☐

Supplementary Figure 2. Edinburgh Handedness Inventory.

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PN #	Date

Edinburgh Handedness Inventory

Please indicate your child's preferences in the use of hands in the following activities or objects:

	Always left	Usually left	Both equally	Usually right	Always right
Writing	☐	☐	☐	☐	☐
Drawing	☐	☐	☐	☐	☐
Throwing	☐	☐	☐	☐	☐
Scissors	☐	☐	☐	☐	☐
Toothbrush	☐	☐	☐	☐	☐
Knife (without fork)	☐	☐	☐	☐	☐
Spoon	☐	☐	☐	☐	☐
Broom (upper hand)	☐	☐	☐	☐	☐
Striking Match (match)	☐	☐	☐	☐	☐
Opening box (lid)	☐	☐	☐	☐	☐
Which foot do you prefer to kick with?	☐	☐	☐	☐	☐
Which eye do you use when using only one?	☐	☐	☐	☐	☐

Supplementary Figure 3. Munich Chronotype Questionnaire.

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PN #	Date

Instruction

In this questionnaire, you report on your typical sleep behaviour over the past 4 weeks. We ask about work days and work-free days separately. Please respond to the questions according to your perception of a standard week that includes your usual work days and work-free days.

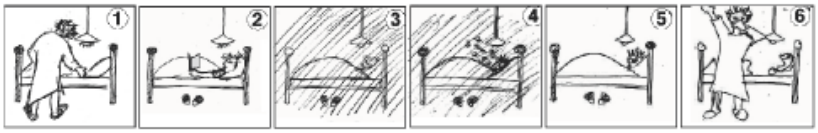
MCTQ

I go to school on a regular basis

Yes ☐ I go to school on 1☐ 2☐ 3☐ 4☐ 5☐ 6☐ 7☐ day(s) per week.

No ☐

Is your answer "*Yes, on 7 days*" or "*No*", please consider if your sleep times may nonetheless differ between regular 'school days' and 'weekend days' and fill out the MCTQ in this respect.



Please use 24-hour time scale (e.g. 23:00 instead of 11:00 pm)!

School Days

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

I use an alarm clock on school days or my parents wake me up: Yes ☐ No ☐

If "Yes": I regularly wake up BEFORE the alarm rings: Yes ☐ No ☐

Free Days

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

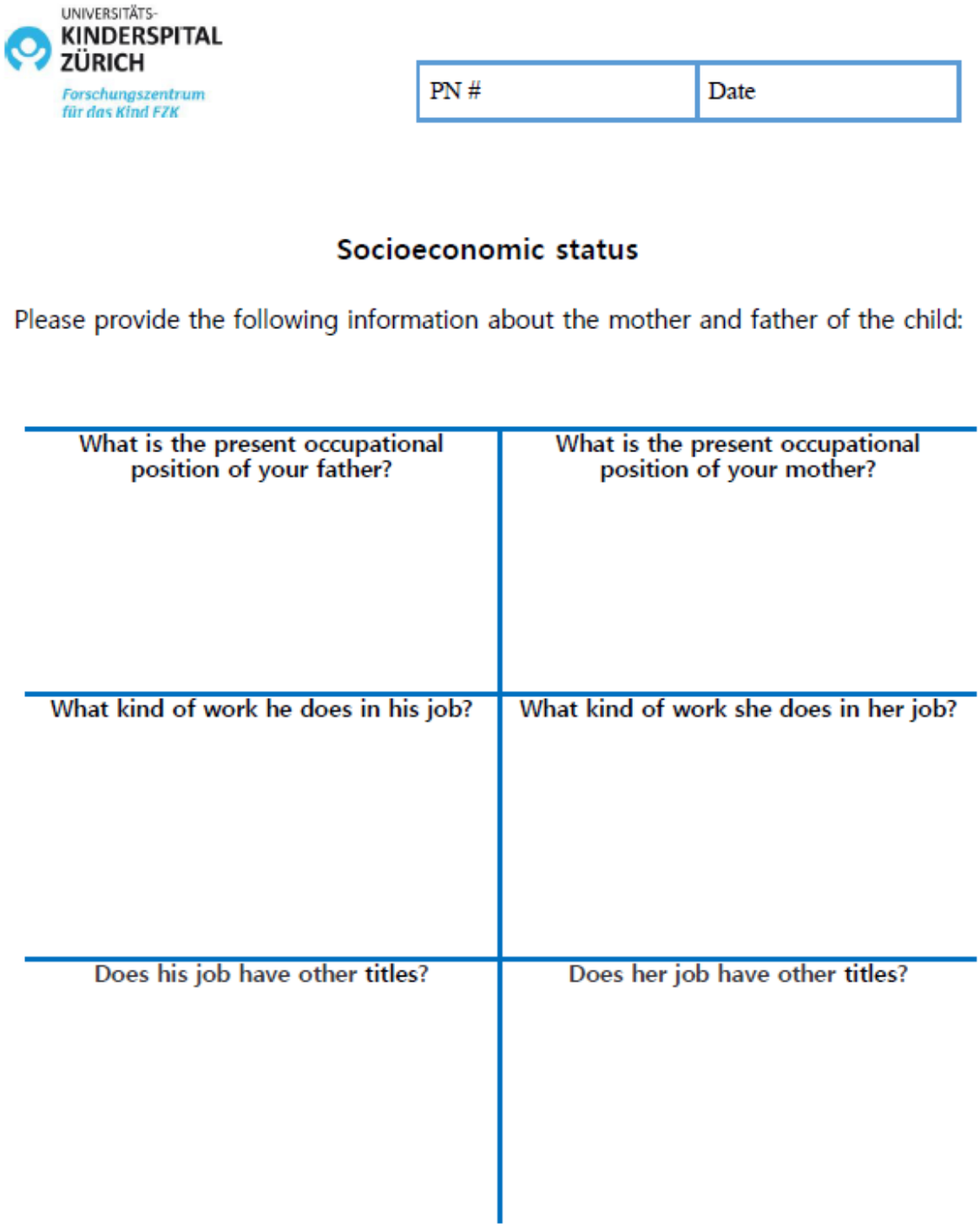
My wake-up time (Image 5) is due to the use of an alarm clock or my parents waking me up:
Yes ☐ No ☐

There are particular reasons why I cannot freely choose my sleep times on free days:

Yes ☐ If "Yes": Familymembers/pet(s) ☐ Hobbies ☐ Others ☐, for example:_____

No ☐

Supplementary Figure 4. Socioeconomic status form.



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PN #	Date

Socioeconomic status

Please provide the following information about the mother and father of the child:

What is the present occupational position of your father?	What is the present occupational position of your mother?
What kind of work he does in his job?	What kind of work she does in her job?
Does his job have other titles?	Does her job have other titles?

Supplementary Figure 5. Word-pair lists (German)

List 1

Nase	Loch	Sumpf	Schlamm
Pferd	Esel	Brust	Schulter
Pulli	Kragen	Stirn	Muetze
Schaukelstuhl	Oma	Strumpf	Stoff
Bikini	Strand	Fass	Schiff
Delphin	Robbe	Geschoss	Kugel
Eule	Adler	Auftrag	Arbeit
Frosch	Fliege	Huette	Tal
Gummistiefel	Jacke	Halle	Palast
Mauer	Stein	Ereignis	Fest
Mantel	Handschuh	Raupe	Kohl
Sessel	Decke	Herrscher	Befehl
Stier	Arena	Flocken	Bergung
Kueche	Salat	Glaube	Verzicht
Tuer	Keller	Seehund	Schwanz
Uhr	Diamant	Angabe	Zeuge
Erde	Kartoffel	Aufstand	Schild
Gras	Schlange	Brechstange	Schloss
Wald	Fuchs	Krebs	Augen
Bluete	Schmetterling	Allee	Dickicht

List 3

Truhe	Gold	Augenbraue	Pupille
Vogel	Rabe	Fischer	See
Waschbecken	Zahnbuerste	Pflanze	Getreide
Zeigefinger	Nagel	Weste	Knopf
Garage	Haus	Kind	Puppe
Tasse	Loeffel	Lager	Raeuber
Telefon	Freund	Gletscher	Felsblock
Erdbeere	Kuchen	Zucker	Apfel
Schule	Tafel	Koerper	Gelenk
Urlaub	Sonne	Schatten	Scheinwerfer
Geld	Muenze	Feuer	Stern
Lied	Text	Sport	Zeit
Koffer	Reise	Riese	Schritt
Zahn	Mund	Klippe	Lawine
Schnee	Berg	Museum	Fund
Maus	Kaese	Ufer	Damm
Papier	Brief	Anstand	Hoeflichkeit
Zeitung	Bleistift	Aquarell	Galerie
Einbrecher	Polizist	Beruf	Fleischer
Zehen	Knie	Bibliothek	Signatur

List 2

Loewe	Zoo	Butter	Kuehlschrank
Ofen	Brot	Becher	Pudding
Ohr	Backe	Kaffee	Dampf
Ampel	Auto	Gewitter	Wasser
Saege	Axt	Guertel	Leder
Schal	Kaelte	Hammer	Zange
Schaufel	Garten	Schrank	Griff
Schere	Blatt	Zitrone	Pfirsich
Arm	Blut	Harfe	Klavier
Badewanne	Ente	Kueste	Meer
Bart	Haut	Tinte	Plakat
Bauch	Herz	Gitter	Gefaengnis
Bein	Knochen	Reptil	Insekt
Bett	Schlaf	Stuhl	Polster
Daumen	Ringfinger	Zigarre	Pfeife
Hund	Dackel	Infektion	Schmerzen
Post	Fahrrad	Instrument	Oboe
Kirche	Glocken	Paechter	Vertrag
Heft	Note	Schauspiel	Ausdruck
Zimmer	Hotel	Stift	Kappe

List 4

Buch	Brille	Lampe	Schirm
Strasse	Laterne	Giesskanne	Rost
Heizung	Winter	Maschine	Eisenbahn
Gitarre	Konzert	Streichholz	Fabrik
Foto	Rahmen	Verkaeufer	Moebel
Radio	Stimme	Eisen	Schmied
Baum	Natur	Naesse	Fluss
Fenster	Wetter	Panne	Anruf
Regal	Brett	Bakterien	Mikroskop
Gemuese	Kochtopf	Kleidung	Buegeleisen
Kerze	Nacht	Staub	Dachboden
Teppich	Flug	Schueler	Buecherei
Parkplatz	Einkauf	Laden	Reklame
Katze	Fell	Landschaft	Moor
Kino	Leinwand	Macht	Kampf
Himmel	Gebet	Labor	Pipette
Getraenk	Schaum	Schlinge	Seil
Doktor	Krankenhaus	Maedchen	Verlobung
Messer	Metall	Naht	Kreuzstich
Flasche	Korken	Orkan	Luft

Supplementary Figure 6. Word-pair lists (Russian)

List 1

Нос	Дырка	Лес	Лиса
Лошадь	Осел	Снаряд	Пуля
Свитер	Воротник	Цветок	Бабочка
Гусеница	Капуста	Болото	Грязь
Купальник	Пляж	Кухня	Салат
Грудь	Плечо	Зал	Дворец
Дельфин	Морж	Мероприятие	Фестиваль
Сова	Орел	Правитель	Приказ
Лягушка	Муха	Дорога	Кусты
Сапоги	Куртка	Заявление	Свидетель
Стена	Камень	Бунт	Щит
Пальто	Перчатка	Задание	Работа
Кресло	Одеяло	Станция	Киоск
Бык	Арена	Мост	Ручей
Лоб	Шапка	Союз	Договор
Носок	Ткань	Флот	Палуба
Бочка	Корабль	Мысль	Высказывание
Дверь	Подвал	Лицо	Маска
Почва	Картофель	Группа	Человек
Трава	Змея	Армия	Пехота

List 2

Лев	Зоопарк	Шторм	Вода
Печь	Хлеб	Решетка	Тюрьма
Ухо	Щека	Масло	Холодильник
Сигнал	Машина	Кофе	Пар
Пила	Топор	Рептилия	Насекомое
Шарф	Холод	Лимон	Персик
Лопата	Сад	Сигара	Трубка
Ножницы	Лист	Тетрадь	Оценка
Рука	Кровь	Инфекция	Боль
Ванна	Утка	Палец	Ладонь
Борода	Лицо	Молоток	Плоскогубцы
Живот	Сердце	Арфа	Фортепиано
Нога	Кость	Чернила	Плакат
Кровать	Сон	Чашка	Пудинг
Пояс	Кожа	Церковь	Колокола
Собака	Поводок	Арендатор	Договор
Шкаф	Полка	Почта	Велосипед
Стул	Подушка	Игра	Выражение
Берег	Море	Ручка	Колпачок
Комната	Гостиница	Теория	Практика

List 3

Сокровище	Золото	Растение	Зерно
Птица	Ворон	Рыбак	Озеро
Раковина	Щетка	Газета	Карандаш
Жилет	Пуговица	Бандит	Полиция
Ступня	Колено	Ребенок	Кукла
Палец	Ноготь	Склад	Грабитель
Гараж	Дом	Сахар	Яблоко
Бровь	Зрачок	Тело	Сустав
Кружка	Ложка	Тень	Прожектор
Телефон	Друг	Огонь	Звезда
Клубника	Торт	Скала	Лавина
Школа	Доска	Гигант	Шаг
Отпуск	Солнце	Спорт	Время
Деньги	Монета	Музей	Открытие
Песня	Текст	Берег	Плотина
Чемодан	Путешествие	Манеры	Вежливость
Зуб	Рот	Акварель	Галерея
Снег	Гора	Работа	Мясник
Мышь	Сыр	Библиотека	Подпись
Лист	Письмо	Энергия	Бензин

List 4

Книга	Очки	Студент	Библиотека
Улица	Фонарь	Врач	Больница
Отопление	Зима	Спичка	Завод
Гитара	Концерт	Цепь	Ржавчина
Фото	Рамка	Происшествие	Вызов
Нож	Металл	Напиток	Пена
Бутылка	Пробка	Продавец	Мебель
Кино	Экран	Железо	Кузнец
Дерево	Природа	Петля	Веревка
Лампа	Абажур	Поток	Река
Кот	Шерсть	Полка	Доска
Свеча	Ночь	Овощь	Горшок
Ковер	Полет	Лаборатория	Пипетка
Окно	Погода	Магазин	Повышение
Парковка	Шоппинг	Пейзаж	Болото
Рай	Молитва	Власть	Борьба
Машина	Поезд	Девушка	Обручение
Бактерия	Микроскоп	Шов	Игла
Одежда	Утюг	Ураган	Воздух
Пыль	Чердак	Радио	Голос