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IMPACT OF SPATIAL NAVIGATION TRAINING DURING 14-DAY BED REST ON MAINTAINING MOTOR FUNCTIONS AND ON BRAIN ACTIVITY IN OLDER ADULTS

PhD Thesis

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sem avtor doktorske disertacije z naslovom:

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S svojim podpisom zagotavljam, da je predložena doktorska disertacija izključno rezultat mojega lastnega dela. Prav tako se zavedam, da je predstavljanje tujih del kot mojih lastnih kaznivo po zakonu.

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Key words: bed rest, physical inactivity, older adults, computerized cognitive training, spatial navigation, gait variability, dual-task effect, electroencephalography, event-related potential

Abstract: INTRODUCTION: Prolonged physical inactivity or bed rest due to illness, long-term postoperative immobilization and sedentary lifestyle can result in significant declines in cardiovascular, sensory-motor, musculoskeletal, cognition or even brain functioning. Computerized cognitive training (CCT) with virtual spatial navigation task was proposed as a technique to mitigate sensorimotor-related declines following prolonged bed rest. METHODS: Sixteen healthy older men (59.6±3.6 years) completed a 14-day bed rest. Eight were randomly chosen for the CCT program involving 12 sessions of spatial navigation training during the bed rest period, while eight others served as an active controls. Before and after the bed rest, virtual maze navigation, gait performance and the brain electrocortical activity was measured. RESULTS: Results showed that participants in the Intervention group, as compared to the Control group, improved in virtual maze navigation at the end of bed rest ($p=.006$) and the effect of CCT persisted up to 400 days after the study ($p=.021$). Moreover, participants in the Intervention group significantly reduced negative dual-task effects after the bed rest in both the normal ($p<.001$) and fast paced walking conditions ($p=.030$). Swing time variability in fast pace walking condition with dual-task was increased only in Control group at the end of bed rest ($p=.006$). Furthermore, behavior data of finger and foot tapping were not affected by bed rest ($p>.05$) while stimulus-related event related potentials, in particular P1 amplitude increased only in the Control group at the end of bed rest ($p=.009$). P1 latency decreased in both group ($p<.029$), while P2 latency only in the Intervention group at the end of bed rest ($p=.031$). CONCLUSION: Our findings show that bed rest has a detrimental effect in terms of functional outcomes, such as the volume of the quadriceps femoris muscle, knee extension maximal voluntary contraction and maximal aerobic capacity. Furthermore, CCT has a significant effect on the cognitive and motor performance, as well as the brain

electrocortical activity during the 14-day bed rest in older adult men. CCT had an effect on especially those domains related to a higher task complexity, which can be most likely explained by the engagement of brain structures known to be involved in mobility or improving cognitive domains associated with control of mobility. Overall, this study provides empirical evidence that CCT, during the absence of physical activity, can be effective and transferable, and open a new perspective on basic research on developing new methods regarding mitigation of detrimental effects of prolonged inactivity.

Ime in PRIIMEK: Uroš MARUŠIČ
Naslov doktorske disertacije: Vpliv treninga prostorske navigacije med 14-dnevnim horizontalnim ležanjem na ohranjanje gibalnih funkcij in možgansko aktivnost pri starejših odraslih.
Kraj: Koper Leto: 2015
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Somentor: Doc. dr. Voyko Kavcic
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Ključne besede: Horizontalno ležanje, fizična neaktivnost, starejši odrasli, informacijsko podprt kognitivni trening, prostorska navigacija, variabilnost hoje, učinek dvojne naloge, elektroencefalografija, z dogodkom povezan potencial
Povzetek: UVOD: Dlje trajajoča gibalna neaktivnost ali dolgotrajno ležanje zaradi bolezni, po-operativna imobilizacija ali značilen sedentarni načina življenja lahko povzroči izrazit upad na srčno žilnem, senzorično-gibalnem, mišičnem in skeletnem sistemu ter celo vpliva na kognicijo in delovanje možganov človeka. Informacijsko podprt kognitivni trening (KT) z nalogo virtualne prostorske navigacije je bil uveden kot možna rešitev za ublažitev senzorično-motoričnih sprememb, ki nastopijo med dolgotrajnim horizontalnim ležanjem (HL). METODE: Šestnajst zdravih starejših moških (59,6±3,6 let) je sodelovalo v študiji 14-dnevnega HL. Osem jih je bilo naključno izbranih za KT, ki je vključeval 12 treningov prostorske navigacije med HL, med tem ko je drugih osem služilo kot aktivna kontrolna skupina. Pred in po HL je bilo izmerjeno stanje virtualne navigacije, učinkovitosti hoje in raven možganske električne aktivnosti. REZULTATI: Rezultati so pokazali, da so udeleženci intervencijske skupine, v primerjavi s kontrolno skupino, izboljšali sposobnosti prostorske navigacije po HL (p=,006). Učinek treninga je deloval do 400 dni po študiji (p=,021). Poleg tega so udeleženci v intervencijski skupini pomembno zmanjšali negativne učinke dvojne naloge po HL, tako v samo-izbrani (p<,001) kot tudi hitri hoji (p=,030). Variabilnost zamašne faze pri hitri hoji z dvojno nalogo se je povečala le pri kontrolni skupini na koncu HL (p=,006). Nadalje, pri vedenjskih podatkih prstnega in nožnega tapkanja ni bilo zaznati pomembnih vplivov HL (p>,05) medtem ko se je pri z dogodkom povezanih potencialih vidnega procesiranja P1 amplituda povišala le pri kontrolni skupini na koncu HL (p=,009). P1 latenca se je skrajšala pri obeh skupinah (p<,029), medtem ko se je P2 latenca pomembno skrajšala le pri intervencijski skupini (p=,031). ZAKLJUČEK: Ugotovitve naše študije kažejo, da ima HL negativen vpliv na funkcionalne sposobnosti, kot so upad obsega štiriglave stegenske mišice, največje izometrične hotene sile kontrakcije in največje aerobne zmožnosti. Po drugi strani KT pomembno vpliva na kognitivne in motorične

zmožnosti ter tudi na raven možganske električne aktivnosti med HL pri starejših odraslih moških. KT je vplival zlasti na tista področja povezana z bolj zahtevnimi nalogami kar je najverjetneje mogoče pojasniti z aktivacijo tistih možganskih struktur za katere je znano, da so vpletene v gibanje telesa ali v izboljšanje kognitivnih domen povezanih z nadzorom gibanja. V splošnem omenjena študija empirično dokazuje, da je lahko KT v odsotnosti telesne aktivnosti učinkovita in na druge pod-domene prenosljiva intervencija in tako odpira nove poglede na temeljne raziskave za razvoj novih metod v zvezi z blaženjem škodljivih vplivov dolgotrajne neaktivnosti.

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1 INTRODUCTION

Functional as well as cognitive decline and/or impairment are among the most feared and costly aspects of aging (Deary et al., 2009; Morrison & Newell, 2012). The aging process is commonly defined as an accumulation of diverse, adverse changes that increase the risk of death, and changes that are attributed to development, genetic defects, the environment, disease, and the in-born aging process (Harman, 1998). Since 1987, Rowe and Kahn posed some basic concerns to the contemporary understanding of the aging process which was published in *Science,* still extensively cited today (Rowe & Kahn, 1987). Authors highlighted the importance of identifying successful mediations that would enhance the proportion of older adults who will age successfully (Rowe & Kahn, 1997). Their model of "successful aging" contains three interconnected elements: i) avoiding disease and disability, as well as an absence of risk factors for disease, ii) engagement with life, which covers interpersonal activity such as social engagement in productive activities, also in the advanced older age, and iii) maintaining high cognitive and physical function. Unfortunately, according to the World Health Organization (WHO), long-life physical inactivity alone is responsible for causing up to 3.2 million deaths per year and it is the fourth leading risk factor, after high blood pressure, use of tobacco and high blood glucose (WHO, 2010).

The proportion of elderly [defined as ≥65 years (WHO, 2012)] is increasing worldwide, and it is projected to double by the year 2030 with an expansion of life span for another 10 years by the year 2050 (Centers for Disease & Prevention, 2003). According to the EU Report on Ageing, the number of people in Europe over the age of 65 years is also projected to increase from 85 million in 2008 to 151 million in 2060. Moreover, the number of super-olds (≥80 years) is expected to triple, from 22 to 61 million from year 2008 to 2060, respectively (Commission, 2009). With the accompanying trend of population aging, higher demands on public health and aging services are also foreseen. Together, the above-mentioned statistical predictions demand new research on healthy aging and the subsequent development of new interventions to enhance both physical and cognitive performance in older adults. Indeed, Hayflick (2000) highlighted the importance of aging research in *Nature*: "the goal of research on ageing is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence". Disability can be an outcome of frailty, which is a common clinical syndrome that increases the vulnerability of older adults to functional decline, falls, hospitalization, and mortality (Fried et al., 2001; Xue, 2011). Frailty can be further defined

using five indices, including: slow gait velocity, low physical activity, unintentional weight loss, exhaustion, and muscle weakness (Gill, McGloin, Gahbauer, Shepard, & Bianco, 2001). Rothman, Leo-Summers, and Gill (2008) later added cognitive impairment as a key indicator of frailty and argued for inclusion of the value of self-reported exhaustion and muscle weakness. However, for the prediction of physical frailty, gait velocity was reported to be the most sensitive measure (Gill et al., 2001; Rothman et al., 2008).

There is ample evidence to conclude that regular physical activity is associated with better quality of life and health outcomes, and is therefore beneficial for healthy aging (Netz, Wu, Becker, & Tenenbaum, 2005; Penedo & Dahn, 2005; Pišot, 2012). Improvements in cognitive function during aerobic exercise suggest a close relationship between both physical and neurocognitive performance (Smith et al., 2010). In the later life, physically active individuals, as compared to the sedentary controls, are less likely to experience cognitive decline or even clinical dementia (Buchman et al., 2012; Sofi et al., 2011; van Gelder et al., 2004; Weuve et al., 2004). Dinse (2006) reported that in adult persons cortical reorganization can occur in two cases: i) first, during the aging process, and ii) second, during treatment of age-related changes (Dinse, 2006). Most studies that evaluated the modifications of cognitive performance in the older adults have reported significant improvements in cognitive functions directly associated to the specifically targeted cognitive areas (Ball et al., 2002; Ball, Edwards, & Ross, 2007; Klusmann et al., 2010; Willis et al., 2006). To our knowledge, only one study has demonstrated an improvement of mobility in sedentary seniors after their engagement in computerized cognitive training (CCT) (Verghese, Mahoney, Ambrose, Wang, & Holtzer, 2010). This study suggests that CCT (targeting attention and executive functions) could translate cognitive practice into more efficient walking patterns, giving the strong association between these cognitive processes and control of walking. In addition, Lovden and colleagues (2012) have reported that sustained spatial navigation training can modify hippocampal volumes in humans, demonstrating a linkage between CCT and cerebral changes.

These findings lend support to using CCT as a possible strategy for mitigating the possible negative effects of prolonged physical inactivity (or immobilization) in the elderly, especially when it involves virtual navigation. Therefore, the aim of the present study was to evaluate whether CCT could be an effective tool for reducing and/or preventing the negative physical and cognitive declines observed after 14 days of bed rest. We hypothesized that CCT with spatial navigation will attenuate motor decline after bed rest evidenced by reductions in brain electrocortical activity compared to active controls.

The PhD thesis contains three related topics discussing the impacts of CCT on specially trained cognitive domain, and its generalization to a distal untrained domain such as motor output and the brain electrocortical activity. The results of this PhD thesis are published (or in the peer-review process) in the following articles:

- Marusic, U., Meeusen, R., Pisot, R., and Kavcic, V. (2014). The brain in micro- and hypergravity: The effects of changing gravity on the brain electrocortical activity. Eur J Sport Sci, 1-10. doi: 10.1080/17461391.2014.908959
- Goswami, N., Kavcic, V., Marusic, U., Simunic, B., Rossler, A., Hinghofer-Szalkay, H., & Pisot, R. (2015). Effect of computerized cognitive training with virtual spatial navigation task during bed rest immobilization and recovery on vascular function: a pilot study. Clin Interv Aging, 10, 453-459. doi: 10.2147/CIA.S76028
- Marusic, U., Pišot, R., Moffat, S., Petrič, M., Dolenc, P., and Kavcic, V., Effectiveness of Computerized Cognitive Training in Older Adults during 14-day of Physical Inactivity in the Bed Rest Study, submitted.
- Marusic, U., Kavcic, V., Giordani, B., Gerževič, M., Meeusen, R., and Pišot, R., Computerized spatial navigation training during 14 days of bed rest in healthy older adult men: Effect on gait performance, Accepted February 17, 2015 in Psychology and Aging.

2 LITERATURE REVIEW

2.1 Age-related changes in gait performance and brain electrocortical activity

The common definition of aging phenomena consists of a process that is genetically determined and environmentally modulated (Rogina, Reenan, Nilsen, & Helfand, 2000). Some authors add a progressive or a gradual functional decline leading to decreased fecundity and increased vulnerability to death (López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013). On the other hand, it could refer to the intrinsic, inevitable, and irreversible age-related process of loss of viability and increase in vulnerability (Comfort, 1964) or frailty (Morley, Kim, Haren, Kevorkian, & Banks, 2005; Rockwood et al., 2004). Gait parameters could serve as a sensitive measure for prediction of physical frailty (Gill et al., 2001; Rothman et al., 2008). In the following chapters we discuss the age-related changes in the gait performance as well as in brain electrocortical activity.

Walking is a rhythmic motor task that involves complex motor, sensory and cognitive processes (Holtzer, Verghese, Xue, & Lipton, 2006; Scherder et al., 2007). When a person is walking, there is a need for ongoing integration of visual, proprioceptive, and vestibular sensory information, as well as the positioning of joints to avoid falls, assimilation of feedback from the terrain to allow for routine changes in positioning and stride length, and constant observation of the environment to avoid falls risk-heightening situations (Giordani & Persad, 2005; J. M. Hausdorff, Yogev, Springer, Simon, & Giladi, 2005). However, in the elderly, more attention for motor control is required while walking, indicating the compounded involvement of attentional resources during gait (Olivier Beauchet & Berrut, 2006; Gschwind, Bridenbaugh, & Kressig, 2010; Kressig, 2010). Age-related neuromotor changes such as reduced motor strength or decreased sensory input (vision, hearing, and proprioception) increase the attentional demands needed for walking. This increased demand is met at the cost of a reduction in the central processing capacity for attentional reserve (Gschwind et al., 2010; Kressig, 2010). The effects of divided attention on motor performance and gait control could be assessed by a "dual-task" methodology.

Walking whilst simultaneously performing a secondary attention-demanding task is called a "dual-task paradigm" and has been used to assess the interactions between cognition, gait, and risk of falls (O. Beauchet et al., 2008). The underlying hypothesis is that two simultaneously performed tasks interfere with each other and compete for brain cortical

resources (Yogev-Seligmann, Hausdorff, & Giladi, 2008). Gait modifications also known as dual-task effects (DTEs) are a sensitive relative measures allowing comparison of combined single- and dual-task walking conditions across groups and time (V. E. Kelly, Janke, & Shumway-Cook, 2010; Remaud, Boyas, Lajoie, & Bilodeau, 2013; Siu & Woollacott, 2007). A negative value of DTE represents a decrement (dual-task costs), while a positive value represents an improvement (dual-task benefits) under dual-task condition. Dual-task costs reflect the increased cost of cognitive attentional processes under the dual-task condition (O. Beauchet et al., 2008; Beurskens & Bock, 2012; Lindenberger, Marsiske, & Baltes, 2000), while dual-task benefits may represent higher arousal under more challenging dual-task condition (Huxhold, Li, Schmiedek, & Lindenberger, 2006; V. E. Kelly et al., 2010). This might also give insight on possible mechanisms involved in risk of falls in older people in clinical practice (O. Beauchet et al., 2009) as well as the prediction of future mobility loss or even progression to dementia (Montero-Odasso, Verghese, Beauchet, & Hausdorff, 2012).

Studies reporting age-related changes in gait performance have shown that healthy elderly have a more complex gait pattern compared to their younger counterparts, which is reflected in reduced comfortable gait speed, shorter stride and step length, increased double support phase and increased step-to-step variability (Menz, Lord, & Fitzpatrick, 2003; Winter, Patla, Frank, & Walt, 1990). These changes represent either a degeneration of the balance control system or compensatory adaptations providing safer gait (Kressig, 2010). Similar to age-related changes in gait have been found after a period of bed rest: Dupui, Montoya, Costessalon, Severac, and Guell (1992) concluded that bed rest induces certain sensorimotor changes involved in the decrease of gait and balance performance without a clarification of central mechanism. Due to the fact that aging process is accompanied with both, functional and cognitive decline, a certain structural and functional age-related alterations occur within the brain as well (Raz, 2000).

Functional age-related changes are reflected with decreased gray and white matter volume as well as with specific brain regions such as caudate, cerebellum, hippocampus and association cortices (Raz et al., 2005). The same authors reported that the shrinkage in the hippocampus and the cerebellum is accelerated with age (Raz et al., 2005). However, age-related changes within the brain are complex mix of mechanisms that allow the brain to adapt and compensate. This suggests that brain plasticity is operational into old age (Dinse, 2006). Furthermore, behavior age-related slowing relates to perceptual, cognitive and motor processes and it is reflected in slower reaction times to the onset of motion (Fozard, Vercryssen, Reynolds, Hancock, & Quilter, 1994) as well as age-related changes

in attention, orientation, working memory, executive functioning, slower reaction times (Park, 2012; Peltz, Gratton, & Fabiani, 2011). Slower response times can have dramatic outcomes in terms of everyday life of an elderly individual, for instance, older adult driving a car (Boot, Stothart, & Charness, 2014). Given the importance of understanding age-related declines in motor and cognitive processes and understanding the mechanisms of such as phenomena, researchers raised the question of whether these processes are followed by perceptual or motor deficit *per se*, or a combination of both. The following paragraphs will discuss a method that was used for recording electrical activity of the brain along the scalp to be able to distinguish between stimulus- and motor-related processes and analyze how they alter within 14-day of bed rest in older individuals.

Scalp electroencephalography (EEG) is a routine research and clinical tool, even though its relationship to the underlying neural activity is not completely understood. Despite the small magnitude of measured scalp electrical potentials, surface EEG signals reflect the summation of synchronized afferent or efferent excitatory or inhibitory neural activity. The most common analysis of EEG is spectral analysis using Fast Fourier transform (FFT). Spectral content is usually divided in four frequency bands: delta (0.5–3.5 Hz), theta (4–7.5 Hz), alpha 1 (8–10.5 Hz), alpha 2 (11–12.5 Hz), beta 1 (13–19.5 Hz), and beta 2 (20–29.5 Hz). For over half a century, thousands of research publications have reported the relationships between spectral changes of EEG and the various global brain states reflecting physiological processes (e.g., brain hemodynamics, cortical arousal, baseline eyes-closed, eyes-open), mental states (e.g., anxiety, emotional valence), or cognitive processes (e.g., attention, memory) (for review see Regan, 1989). Indeed, all frequencies are usually present whenever EEG is measured. Specific frequency bands, however, may reflect important considerations. For example, the predominant "slow wave" activity (delta and theta) is indicative of lower cortical activity and can be indicative of neuropathology (e.g., Alzheimer's disease) (for review see Vecchio et al. (2013)), while increased "fast activity" (alpha and beta) is indicative of increased arousal and cognitive engagement. It is well understood that spectral changes in EEG are context sensitive. An additional approach to investigate electrocortical activity is via direct slow cortical potentials (SCPs). Event-related direct current shifts of the EEG reflect the excitability of the cerebral cortex (Elbert, 1993) either related to cortical generators such as epileptic seizures (Elbert, 1993), vigilance (Marshall, Mölle, Fehm, & Born, 1998), or extra-cortical generators such as a change in CO2 levels (Voipio, Tallgren, Heinonen, Vanhatalo, & Kaila, 2003), and changes in cerebral blood flow (Vanhatalo et al., 2003).

During reaction time tasks, as well as synchronization tasks, event-related potentials (ERPs) reflect fast neuroelectric events that are related with specific aspects of information processing (Blackwood & Muir, 1990; Regan, 1988). ERPs are defined as time-locked EEG activity to sensory, motor or cognitive events. This technique allows one to record and analyze specific processes in the brain with a precision of milliseconds (Gaudreault et al., 2013). The ERPs are typically divided into two categories, the early and later waves, according to their latency and amplitude. The early waves are termed as "sensory" or "exogenous" and appear approximately 50-200 milliseconds after the stimulus, resulting as a peak activity (Kuperberg, 2004). On the other hand, the later ERP waves are termed as "cognitive" or "endogenous" as they reflect the manner in which subject evaluates the stimulus- examination of information processing or the meaning of the stimulus (Kuperberg, 2004). ERPs are not affected by cultural and educational influences, and therefore represent a non-invasive and objective means for cognitive process investigation (Lai, Lin, Liou, & Liu, 2010). ERP components are defined by their positive or negative polarity, timing, scalp distribution, and sensitivity to task manipulations (Woodman, 2010). The evaluation of processing stages can be divided into three categories:

- Stimulus processing,
- sensorimotor integration/ response selection/ decision making, and
- motor processing/ response execution.

Stimulus processing can be further divided in perception and task-stimulus classification. In the perception phase, the stimulus is being processed and therefore its recognition occurs. This stage is reflected by P1 and N1 components of stimulus-locked ERP (s-ERP). For the visual modality, P1 and N1 components are the most positive deflection within 40-140 milliseconds and the most negative deflection within 120-200 milliseconds after the stimulus onset, respectively. Both components are extracted from the occipital locations at the scalp. The extraction of latencies and amplitudes of P1 and N1 components allow to examine the speed and intensity of early perceptual mechanisms (E. Amenedo & Díaz, 1998). Furthermore, the P2 component is a positive deflection around 200 milliseconds after the stimulus onset and reflects working memory (Finnigan, O'Connell, Cummins, Broughton, & Robertson, 2011; Lefebvre, Marchand, Eskes, & Connolly, 2005). Aging effects on s-ERP are mixed; while some of the studies reported enhanced and delayed stimulus onset of P1 and N1 component in the older age (De Sanctis et al., 2008; Falkenstein, Yordanova, & Kolev, 2006; Yordanova, Kolev, Hohnsbein, & Falkenstein, 2004), some studies found an unchanged age-related stimulus processing (Stothart, Tales, & Kazanina, 2013). In addition Czigler and Balázs (2005) found a decrease in P1 and N1 amplitude while comparing younger and older participants. Moreover, the later stages of

stimulus processing are reflected with endogenous P3 component which is defined as the most positive peak within 260-370 milliseconds after the stimulus occurs in the mid-line electrodes, such as CPz. The P3 amplitude decreases with advancing age in adults, while its latency increases (Polich, 1997b, 2007).

Sensorimotor integration is a process when a response from an external stimulus is requested. In this process motor cortical areas are activated immediately or in parallel with stimulus identification. Sensorimotor integration is composed from the following phases (Krakauer & Mazzoni, 2011; Magill, 2011): i) sensory input – from stimulus detection with sense organs to its transmission to the central nervous system; ii) sensory integration – organization of new sensory stimulation and integration with the previous stimuli (i.e. memory); iii) motor interpretation – recalibration in terms of internal motor decision-making (i.e. recalibration based on current stimulus and previously memorized information); iv) motor activation – implementation of movement; v) feedback – evaluation of movement in the basis of many sensory details which enables the assessment of the whole process and repetition of the cycle. In terms of analyzing the initiation of motor-related processes from the EEG signal, the lateralized readiness potentials (LRPs) have been most often used (Coles, 1989; Gladwin, t Hart, & de Jong, 2008; Praamstra, 2007; Roggeveen, Prime, & Ward, 2007). The measure of the preparation and execution of motor actions was first observed by Kornhuber and Deecke (1965) while analyzing voluntary and passive movements. Before the hand movement, a negative potential occurs above the motor cortex at the contralateral site of the responding hand (Vaughan, Costa, & Ritter, 1968). Thus, when the participant prepares to move his/her right hand, a negativity is larger over the left side of the scalp. Measured with high density EEG system, this would be reflected as a negativity obtained at the electrode C3 (contralateral site). Such as negativity could not be obtained at ipsilateral site/motor cortex and therefore the start of the difference between contra- and ipsi-lateral s-ERP represents the time when sensorimotor integration or response selection is completed and the effective movement is started (Yordanova et al., 2004). When a stimulus requires both hands, the LRPs are being calculated such as a sum of subtraction of left and right hand and therefore dividing by two the electrodes C4 and C3 for both hands (for details see Coles (1989)), while for responses being processes with one hand, the LRP is calculated as a C3-C4 difference (for a right) and C4-C3 (for a left) hand, respectively (Yordanova et al., 2004). Finally, the LRP analysis cannot distinguish decisional from perceptual processes but it provides more direct approach to differentiate motor preparation and execution (Roggeveen et al., 2007). When using LRP analysis in an aging context, LRP analysis reveals slower behavior data/response

times in older adults arose from slower programming or execution of a response, rather than perceptual processing (Roggeveen et al., 2007).

Motor-related potentials (MRP) is the EEG method that is used for analysis of motor processing. MRPs are extracted at contralateral motor cortical areas as response-locked potentials (r-ERPs). According to the scheme of the human homunculus (Nakamura et al., 1998; Stancák & Wackermann, 1998), MRPs are usually extracted from the electrode C3 and C4 for finger and foot movements, respectively. Age-related changes in MRP seem to be reflected in higher activation levels in older as compared to younger individuals which prolongs reaction times with age (Falkenstein et al., 2006). Yordanova et al. (2004) showed that functional dysregulation of the contralateral motor cortex causes behavioral slowing in older adults and that this deficit becomes more evident with higher task complexity.

2.2 The role of physical activity and inactivity on healthy aging and well-being

When participating in regular physical activity, there are numerous physiological and cognitive benefits for the human body (see the following paragraph), in contrast, an inactive or sedentary lifestyle increases the risk for cardiovascular disease (Warren et al., 2010), diabetes (Hu, 2003; Katzmarzyk et al., 2003; Mayer-Davis & Costacou, 2001), metabolic syndrome (Bertrais et al., 2005; Ford, Kohl, Mokdad, & Ajani, 2005), obesity (Mayer-Davis & Costacou, 2001), cancer (Lynch, 2010) and even depression (Teychenne, Ball, & Salmon, 2010). According to the WHO, physical inactivity is one of the main features of modern lifestyle linked to a number of health hazards, and leads to ~6% of deaths worldwide (WHO, 2010). Older persons are more often subjected to periods of physical inactivity (e.g. reduced mobility or hospitalization), which is often reflected in substantial decline in functional status (Fortinsky, Covinsky, Palmer, & Landefeld, 1999; Kortebein et al., 2008). Frequently such a decline in function results in nursing home placement (Fortinsky et al., 1999) which causes loss of independency and elevated costs for the national health care systems. Therefore, regular exercise, including walking should be of a higher priority to the frail older individuals not to become physically disabled (Pahor et al., 2014).

Next to functional decline, an additional hotly debated topic is the role of physical activity in relation to the cognitive functioning. Ample evidence has demonstrated that physical activity may serve as an effective cognitive intervention (for meta-analysis see: Smith et

al. (2010)). However, recent systematic review and meta-analysis showed a limited impact (3 in favor vs. 26 with no effect) of physical exercise on the cognitive functioning in healthy older adults (M. E. Kelly et al., 2014). Positive correlations between cognitive function and physical activity/aerobic training have been reported for the following: simple reaction times, attention, memory and executive functions (Ratey & Loehr, 2011; Ruscheweyh et al., 2011; Weuve et al., 2004). Furthermore, accumulating evidence from several studies suggests that regular exercise positively affects medial temporal structure, the region that plays an integral role in memory and special cognition by promoting synaptic plasticity and neurogenesis (Chapman et al., 2013; Erickson et al., 2011; Griffin et al., 2011). For instance, aerobic exercise resulted in increased hippocampal blood volume in healthy older adults (Colcombe et al., 2004) and participants who were exposed to one year of aerobic exercise increased the hippocampal volume for 2%, compared to the controls who were involved in the stretching training and decreased a hippocampal volume for 1.4% (Erickson et al., 2011). Positive effects of aerobic activity on medial temporal brain structures have been also registered in patients with Alzheimer's disease (Erickson et al., 2011; Yuede et al., 2009) and schizophrenia patients (Pajonk et al., 2010).

However, much less is known about the effects of physical inactivity on cognition. Horizontal bed rest is one of the most widely used methods to study the consequences of prolonged physical inactivity (Convertino, Bloomfield, & Greenleaf, 1997). The bed rest model was first introduced in the 1960s to simulate the acute adaptations to a microgravity environment (Adams, Caiozzo, & Baldwin, 2003). In this experimental approach, participants must spend a specified number of days in an uninterrupted horizontal position, with strict restriction of their movement, requiring all their daily needs to be performed in a horizontal way (i.e. eating, daily hygiene). However, during bed rest the force of gravity is still present where head-to-foot loading of the body and the input from cutaneous pressure receptors of the foot sole are reduced to the minimum (Reschke et al., 2009). The effects of bed rest on the human organism appear similar to those that take place after physical inactivity, sedentary lifestyle, immobilization, and spaceflight or in microgravity environment. Moreover, bed rest can represent an accelerated form/model of the ageing process (Timiras, 1994; Vernikos & Schneider, 2009). Summarized, the impact of longer periods of bed rest is most pronounced in the cardiovascular (Blomqvist et al., 1994; Perhonen et al., 2001; Traon et al., 1998), skeletal (Leblanc, Schneider, Evans, Engelbretson, & Krebs, 1990; Rittweger et al., 2009) and muscular (Grogorieva & Kozlovskaia, 1987; Pisot et al., 2008) systems of human body with some evidence of potential deficits on brain functioning (Marusic, Meeusen, Pisot, & Kavcic, 2014) and cognition (Ioseliani, Narinskaia, & Khisambeev Sh, 1985; Lipnicki & Gunga, 2009). Long

periods of inactivity and concomitant sensory decline leading to delayed recovery, can substantially impair mobility functions (Dupui et al., 1992), contribute to poor quality of life (Newman et al., 2003) and significantly increase health care costs, especially in older adults (Deary et al., 2009). Hospitalizations of over 8 days are associated with significant changes in body composition, aerobic capacity and lower extremity strength and power in older persons (Alley et al., 2010; Kortebein et al., 2008). The development of new and effective approaches for mitigating or preventing the detrimental effects of prolonged immobilization is thus critical to both containment of healthcare costs and improvement the quality of life (Graf, 2006; Kortebein et al., 2008).

Whilst assessing the effectiveness of physical activity interventions, or CCTs on cognitive function, researchers have often very limited or minimal control over participant's daily activity beyond what is occurring during the training session (see the next chapter). However, the bed rest model provides an opportunity to plan and control the majority of daily activities of all participants 24/7, such as physical activity, and nutrition. As reviewed by Lipnicki and Gunga (2009), eight of 17 bed rest studies reported significant detrimental effects on cognitive performance. Six studies reported unchanged cognitive functioning after bed rest, whereas three studies surprisingly showed improvements in cognitive performance. In the latter case, task exposure and practice effects could mask the underlying detrimental effect of bed rest on cognitive functioning (Lipnicki & Gunga, 2009). However, to our knowledge, no bed rest study has yet been conducted with additional computerized cognitive training (CCT) intervention.

2.3 The role of cognitive interventions in healthy older adults

The rapid increase in longevity, and increase in the proportion of elderly in the general population, not to mention age- and disease-related cognitive declines, have important and impactful socio-economic implications for society at-large (Walter et al., 2012; Wimo, Winblad, Aguero-Torres, & von Strauss, 2003). Thus, development of reasonable, economically viable, and culturally acceptable interventions to maintain cognitive function in later life could promote both social and economic benefits. In this regard, different interventions comprising of modifiable lifestyle factors, such as cognitive, social and physical activity, have each been gaining interest (Coley et al., 2008). Of these strategies, cognitive training is aimed at optimizing the cognitive functioning and/or slowing brain aging which generally involves a guided practice on a standard set of cognitive tasks, such as memory or attention (Martin, Clare, Altgassen, Cameron, & Zehnder, 2011). These training tasks are often designed to present increasing challenge to cognitive abilities and thereby induce learning.

From a non-pharmacological point of view, this cognitive intervention is further divided into various approaches and concepts, such as: cognitive stimulation, cognitive rehabilitation and cognitive training. While cognitive rehabilitation is designed to cover only the population with cognitive impairments itself, the other two concepts can be applied to both healthy and cognitively impaired persons (for review see (Tardif & Simard, 2011)). Although the promising results of cognitive training and stimulation on tasks measuring memory, executive functions, attention, and speed of processing, the generalization and benefit to everyday life are usually not addressed directly (Tardif & Simard, 2011). Furthermore, generalization of cognitive intervention programs to a distal untrained domain, such as person's mobility, has been addressed in healthy older population only once, where the authors suggested that targeting attention and executive functions may translate into more efficient walking patterns in the sedentary seniors (Verghese et al., 2010). Traditional face-to-face training programs can be expensive, labour intensive and time-consuming (Kueider, Parisi, Gross, & Rebok, 2012; Wadley et al., 2006); therefore, computer-based cognitive interventions are a potentially effective alternative, offering a more flexible, yet personalized approach, and still providing real-time performance feedback (Kueider et al., 2012; Rebok, Carlson, & Langbaum, 2007; Wadley et al., 2006).

Over the past decade several reviews have highlighted the beneficial effects of cognitive training in healthy older adults (Kueider et al., 2012; Lampit, Hallock, & Valenzuela, 2014; Martin et al., 2011; Papp, Walsh, & Snyder, 2009; Tardif & Simard, 2011; Teixeira et al.,

2012; Valenzuela & Sachdev, 2009), suggesting that cognitive and neural plasticity is maintained in the old age (Toril, Reales, & Ballesteros, 2014). Most of the reviewed studies reported significant improvements in performance across the specific cognitive functions trained (e.g., (Ball et al., 2002; Mahncke et al., 2006; Willis et al., 2006)). In some studies, the improvements are sustained for several years (Rebok et al., 2014), but in most cases, studies demonstrate only a limited transfer to other cognitive functions and/or activities of daily living (Tardif & Simard, 2011). Some authors claim that the absence of transfer to real-world outcomes is not particularly surprising, since most cognitive intervention trials do not include everyday functioning as outcome measures (Reijnders, van Heugten, & van Boxtel, 2013).

Cognitive training programs are commonly run as a time-limited, daily session for some specified period of intervention (e.g., two hours per day for five days a week for 20 sessions). However, researchers often have only minimal control of participant's daily activity beyond what is occurring during the training session. In other words, other uncontrolled and unassessed factors besides cognitive training may affect measured outcomes. Ideally, training effects should be isolated from the potentially confounding effects of other daily activities. Especially for older individuals, the potential confounding factors affecting the success of cognitive training could involve the extent of cognitive and physical activity, and their level of social contacts (especially in the more isolated elderly). In addition, because participants know that they are enrolled in a cognitive intervention study, they could (knowingly or unknowingly) alter their engagement in cognitive activities outside the lab environment as well.

For the purposes of this study, we conducted a horizontal 14-day bed rest with older adult men in a highly controlled environment that allowed complete control of possible confounding factors affecting cognitive training, such as caloric intake, social interaction, and physical activity. The spatial navigation task used for the present CCT protocol represents a unique aspect of spatial navigation intervention; since it is highly hippocampal integrity dependent (Lovden et al., 2012) it engages medial temporal structures and may therefore be effective in preventing deleterious effects of physical inactivity during bed rest. By definition, spatial navigation is a complex cognitive skill which depends on multiple cognitive domains including spatial skills, explicit memory, working memory and executive processes (S. D. Moffat, 2009). Spatial navigation refers to the process of determining and maintaining a trajectory from point A to B and therefore it is critically important for functioning in the everyday environment. It has been well documented that spatial navigation declines with age (Klencklen, Despres, & Dufour, 2012; S. D. Moffat, 2009;

Monacelli, Cushman, Kavcic, & Duffy, 2003). Furthermore, age-related decline in spatial navigation is linked to decline in spatial integration of visual perception (Kavcic, Vaughn, & Duffy, 2011), and significantly decreased electrophysiological response to motion (Kavcic, Fernandez, Logan, & Duffy, 2006; Kavcic, Martin, & Zalar, 2013), relevant for visually guided daily activities, which can restrict daily activities and consequently significantly impair the quality of life in old age.

There are only a few studies, which used spatial navigation task for the purposes of cognitive training: Lovden and colleagues (2012) reported that spatial navigation training (every other day for 4 months) improved subsequent spatial navigation performance. Moreover, the intervention group maintained stable hippocampal volumes during and after four months of training while control group showed small age-related volume decline. The behavioral training-related effect declined in magnitude measured four months after spatial navigation training intervention (Lovden et al., 2012). Recently, Hotting, Holzschneider, Stenzel, Wolbers, and Roder (2013) conducted 6-month long aerobic endurance training with 40-55 years old sedentary adults. In the last month of physical intervention, participants were also exposed to a cognitive intervention (spatial navigation or perceptual training). Their results showed that only spatial navigation training improved spatial navigation performance. Moreover, spatial navigation training resulted in lower brain activations in the hippocampus and parahippocampal gyrus as well in frontal and temporal brain areas, a network of brain areas associated with spatial navigation. Authors suggested that decreased brain activations associated with spatial navigation reflect more efficient neural processing (Hotting et al., 2013).

We anticipated that CCT training would improve performance on the practiced task (Ball et al., 2002; Willis et al., 2006) and that CCT will also have an effect on the mobility (Verghese et al., 2010) and the brain electrocortical domain (Marusic et al., 2014). In addition, imaging studies have shown that the same premotor cortex and supplementary motor areas that are involved in real walking are also activated during the observation of gait, as well as during mental imagery of movement (Iseki, Hanakawa, Shinozaki, Nankaku, & Fukuyama, 2008; Wagner et al., 2008). Both observation of walking and virtual walking produce similar and even higher levels of cerebral activation compared to real walking (Kranczioch, Mathews, Dean, & Sterr, 2010; la Fougere et al., 2010). Together, these results suggest that a CCT approach emphasizing virtual movement may not only improve gait based on cognitive enhancement, but also improve movement based on activating the same neural systems involved in overall mobility. Such an approach would be ideal as a possible strategy for preventing negative effects of prolonged physical inactivity or bed

rest. The study was conducted with older adult individuals undergoing 14 days of bed rest in a highly controlled environment that allowed complete control of possible confounding factors affecting cognitive training (e.g., caloric intake, cognitive and physical activity).

3 OBJECTIVES AND HYPOTHESES

3.1 Objectives of research study

The purpose of the present study was to investigate the effects of CCT using a virtual spatial navigation task during 14-day bed rest on a virtual maze performance, a motor domain, and brain electrocortical activity. The primary goal of this research was to quantify the efficacy of the CCT intervention on human brain function, particularly whether sensorimotor functions are influenced by the protocol following prolonged immobilization (i.e. bed rest). Specifically, to assess:

- the effectiveness of CCT with spatial navigation task (measured with virtual maze performance) immediately following
    - 14 days of bed rest
    - 28 and 400 days after the intervention period
- gait performance in normal- and fast-paced conditions with and without dual-task immediately after 14 days bed rest. Gait would be measured via:
    - Gait speed parameter
    - Dual-task effects (DTEs)
    - Gait variability
- the brain electrocortical activity in specific regions of the brain, such as motor cortex and occipital regions during synchronization task, also 14 days after the bed rest.
    - Behavioral data of synchronization task while finger and foot tapping,
    - Analysis of stimulus-related processes,
    - Analysis of motor-related processes for finger and foot tapping.

3.2 Hypotheses

The following hypotheses were developed based on the subject matter and the objectives of the research study:

H1: Older adults in the Intervention group will significantly increase their performance in the virtual maze task after 12 session of CCT as compared to the control group. Specifically:

H1.1: Virtual maze performance will differ between groups based on the distance traveled and number of errors after 14-day bed rest.
H1.2: Differences in virtual maze performance will persist following the bed rest intervention both 28 and 400 days post-intervention.

H2: Older adults in the Intervention group as compared to Control group will have lesser decline in gait performance in normal- and fast-paced walking conditions after 14-day bed rest.

H2.1: There will be a significant difference in the gait speed drop in both normal- and fast-paced walking conditions with and without dual-task between participants in the Intervention and Control groups at the end of 14-day bed rest.
H2.2: There will be a significant interaction effect in dual-task effects (DTEs) in both normal- and fast-paced walking conditions between participants in the Intervention and Control group at the end of 14-day bed rest.
H2.3: There will be a significant difference in gait variability between participants in the Intervention and Control groups at the end of 14-day bed rest in all four walking conditions.

H3: Older adults in the Intervention group as compared to Control group will have better performance on behavior data of synchronization task after 14-day bed rest.

H3.1: The mean synchronization rate, its variability and the number of non-responded stimuli while finger-tapping task will significantly differ between participants in the Intervention and Control group at the end of 14-day bed rest.
H3.2: The mean synchronization rate, its variability and the number of non-responded stimuli while foot-tapping task will significantly differ between participants in the Intervention and Control group at the end of 14-day bed rest.

H4: Older adults in the Intervention group as compared to the Control group will have lesser changes in the brain electrocortical activity during synchronization task after 14-day bed rest.

H4.1: The stimulus-locked event related potentials during finger tapping task will significantly differ between participants in the Intervention and Control group at the end of 14-day bed rest.
H4.2: The response-locked event related potentials during finger tapping task will significantly differ between participants in the Intervention and Control group at the end of 14-day bed rest.

H4.3: The response-locked event related potentials during foot tapping task will significantly differ between participants in the Intervention and Control group at the end of 14-day bed rest.

4 METHODS

4.1 Participants

A total of twenty-three males, (16 older adults aged 53 to 65, and 7 younger adults aged 19 to 28) volunteered to participate in the project "*Bed Rest Study – PANGeA, Valdoltra 2012 – The effects of simulated weightlessness on the human organism*". Participants underwent 14 days of horizontal bed rest with a supervised 28-day recovery period. All participants were right-handed, had normal or corrected-to-normal vision, and reported no history of cardiovascular disease, neurological or psychiatric conditions. All procedures were carried out in accordance with the Declaration of Helsinki and were approved by the Republic of Slovenia National Medical Ethics Committee. Written informed consent was obtained from all participants prior to the bed rest experiment.

For the purposes of the present study, a total of 16 healthy, untrained, older male participants (59.6 ± 3.6 years; mean ± SD) were considered, due to the fact that only one group of older adults had an additional cognitive intervention during the bed rest (for details see below). They were recruited through public promotions of the project, newspaper advertisements, and word-of-mouth recommendation from three coastal towns in Slovenia. Details on age and body mass index can be found in Table 1. The level of education of the majority (10/16) of older adults was at a secondary level (gymnasium or vocational secondary school: 12 years of education), three participants had 16 years of education (college or university diploma), and three of them had lower than secondary level degree. The usage of computer: 5 participants reported not use it at all (1 from Intervention and 4 from Control group), while the other 11 participants used it daily or often, approximately 2 hours per day, mainly for the purpose of information search, e-mailing and e-bank (table 1). Furthermore, participants were medically examined prior the study inclusion with an interview, routine blood and urine analysis, and fitness battery test. Exclusion criteria were: regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer < 500 μg·L-1; acute or chronic skeletal, neuromuscular, metabolic and cardiovascular disease condition; pulmonary embolism; a Short Physical Performance Battery score < 9; and a VO_2max < 21 mlO2·kg-1·min-1.

Table 1: Basic characteristics of included participants

Parameter		**Value**	**p value**
BMI ($kg \cdot m^{-2}$)			.844
	Intervention	26.2 ± 5.2	
	Control	26.6 ± 4.0	
Age (years)			.778
	Intervention	59.4 ± 3.6	
	Control	59.9 ± 3.3	
Level of education			.442
	Intervention	4.4 ± 1.8	
	Control	3.8 ± 1.0	
Usage of computer (hours/day)			.648
	Intervention	2.2 ± 2.0	
	Control	1.8 ± 1.8	

Note: Means and standard deviations of age, body mass index (BMI), educational level and usage of computer for Intervention and Control group. Statistical evaluation of differences between the two groups are presented in the right column and represent p value of independent Mann-Whitney U test.

4.2 Study design

This bed rest study was a controlled, longitudinal, interventional study. To achieve the aims of the study (simulate prolonged physical inactivity), the participants had to lay in their bed continuously for 14 days. During the bed rest, participants were allowed only to turn on all sides of the body, or put no more than two pillows under the head, and were not allowed to stand up, sit on the bed, or raise the arms above the level of their head. Hospital staff regularly checked physical condition of the participants and transferred them with their beds to the bathroom for personal hygiene. They received standard hospital meals three times a day at 7.30 a.m., 12 a.m. and 6 p.m. The bedrooms (3-4 persons per room) were air-conditioned and the room temperature was kept comfortably below 25 °C. During the bed rest study participants were allowed to read books and newspapers, use the internet, watch TV and listen to the radio, and freely communicate to each other. After the bed rest, all participants followed a 28-day supervised recovery (physical intervention). Additionally, 400 days after the end of bed rest participants were assessed again in terms of cognitive performance in the virtual maze. The 4th measurement served for assessment of the effectiveness of cognitive training.

Eight participants were randomly selected for the CCT (Intervention group), while the other eight served as active controls (Control group). In separate rooms, the interventional group performed cognitive training for approximately 50 minutes a day, whereas the Control group watched documentaries at the same time, and for the same duration. The bed rest study was performed at Orthopaedic Hospital Valdoltra, University of Primorska, Ankaran, Slovenia.

4.3 Procedures

4.3.1 Assessment of muscle volume, function, and aerobic capacity

Quadriceps muscle volume of the right leg was measured from turbo spin-echo, T1-weighted, magnetic resonance images (MRI) obtained with 1.5 T (Magnetom Avanto; Siemens Medical Solution, Erlangen, Germany). On each MRI slice, contours corresponding to the quadriceps muscles were delineated by an expert of MRI imaging, using the image processing tools OsiriX (Pixmeo Sarl, v.4.1.2). The muscle volume was then derived by summation of a series of evenly spaced truncated cones comprised between each two axial images, a process that included an average of 25 images (range 23-28) and covered the entire length of the quadriceps.

Knee extensors muscle strength of the right leg was estimated from maximal voluntary isometric contraction (MVC) at a 110° knee angle, with hip fixed at 90°. Force was measured by an electrical transducer (TSD121C, BIOPAC Systems, Inc., USA), with 1kHz sampling frequency, implemented on a custom built chair for isometric contractions of knee extensor muscle groups. After a familiarization, participants performed two MVC, with two minutes rest. The highest MVC was taken into account for further analysis.

Maximal aerobic power (VO2max) was determined by a graded exercise test on a mechanically braked cyclo-ergometer (Monark Ergomedic 839E). During the test, ventilatory and gas exchange responses were measured continuously with a metabolic unit (Quark-b2, Cosmed, Italy). After determining rest values, participant warmed-up at 80W and thereafter, 20W increments were imposed every minute until volitional exhaustion. A leveling off of oxygen uptake (defined as an increase of no more than 2 ml•kg-1•min-1) observed during the last one or two steps of the exercise test indicating that VO2max had

been attained. VO2max was calculated as the average oxygen uptake of the last 20s of the test normalized to body weight.

4.3.2 Cognitive assessment

Cognitive assessments were performed with the virtual spatial navigation assessment task. The virtual maze navigation assessment task was administrated on the first day (BR d1), at the end of the bed rest (BR d14), and at the end of the physical recovery intervention (REC d28). Additionally, to assess the long-term maintenance of CCT, a fourth measurement 400 days after the bed rest study (REC d400) was also conducted. All the computerized assessments were performed in supine position to assure the same testing conditions at each measuring point (see Figure 1).

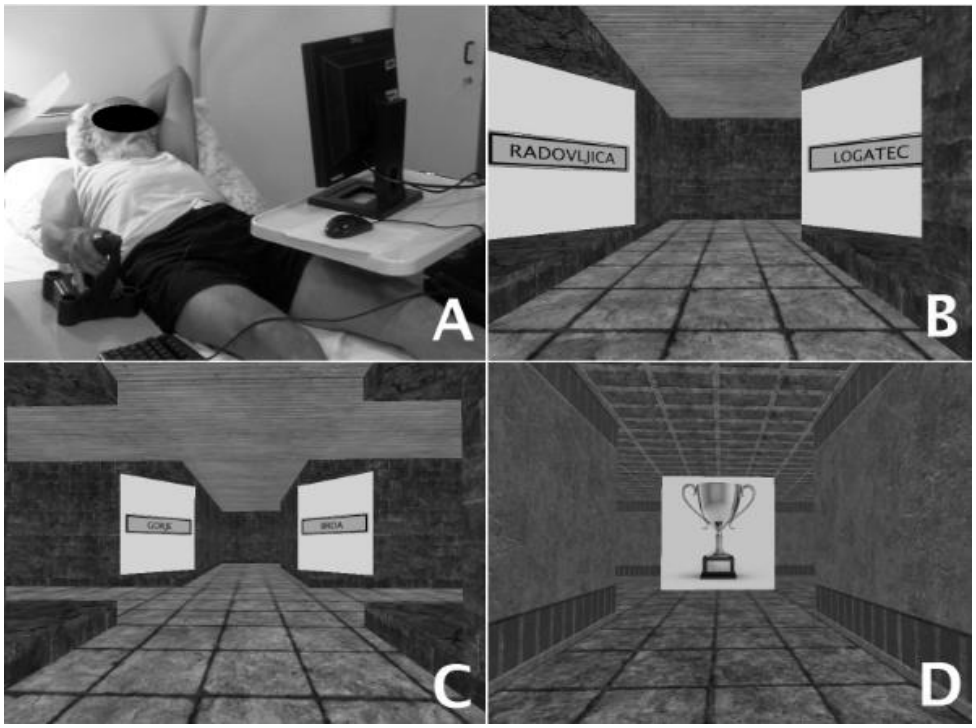
Figure 1: Two participants during the virtual spatial navigation task performance

Figure source: U. Marušič

All virtual environments were designed using modified version of Unreal Tournament 2003 and the Unreal Editor 3.0 (Epic Games, Inc) software package (Nowak, Diamond, Land, & Moffat, 2014; Nowak & Moffat, 2011). During periods of cognitive training, all the participants were lying in bed and controlled their movements within the virtual environments using a Trust Predator Joystick GM-2550 (for task description details see below). The tasks were presented on a 17-inch flat panel LCD monitor situated approximately 60 centimeters in front of participants. While performing virtual environment tasks, the participant's head was not restrained, but comfortably resting on their pillow (see Figure 1).

The virtual maze navigation task was presented from a first person perspective and comprised of a series of interconnected hallways and alleys with three available paths at each intersection or decision point. Some hallways of the maze ultimately led to the goal (designated by a trophy), and others led to dead ends. A pair of verbal or non-verbal cues were displayed in the virtual maze at each decision point (i.e. intersection), placed at either opposite corner of the intersection, and in corridors at various non-decision points. Verbal cues (Figure 2 B and C) consisted of signs with country names, city names, and animal names. Non-verbal cues consisted of country flags, animal pictures, and human faces. Participants were instructed to select the correct path as quickly and efficiently as possible in order to move toward the goal area. Upon reaching the goal area, participants were "transported" back the starting point to complete another learning trial of the same virtual environment.

Figure 2: Participant during CCT and figures of virtual maze



Note: Photograph depicting a random participant during the bed rest study performing virtual maze navigation task with joystick (A). Illustrations of virtual maze navigation task: (B) Hallway in virtual maze task with two non-critical cues, (C) Decision intersection in virtual maze with three available paths together with two critical cues, (D) The end of virtual maze task with a trophy designating the goal. *Figure source: U. Marušič*

Practice period before testing: To ensure that all participants were equally adept at controlling their movement, the participants were given joystick training prior to cognitive training/testing in virtual environment. This involved navigating through an environment consisting of a corridor with several turns and that was equal in width to those seen in the training mazes. All participants completed the joystick-training environment under the required time of 120 seconds.

Virtual maze scoring: For each maze, the coordinate position and heading bearing was sampled every 20 milliseconds and this record was used to compute time to completion, distance travelled and average speed. Due to the slightly different path lengths across multiple mazes, we transformed distance travelled into z scores. In addition, the number of navigation errors was also calculated. An error was classified as any deviation from the correct route into a dead end corridor. However, during the first trial or the virtual environment learning task, participants had no way of knowing which corridors ultimately

led to the goal object, thus the number of errors of trials 2-5 was used as the dependent measure, as suggested in the recent manuscript (Gazova et al., 2013).

4.3.3 Assessment of subjective workload

The National Aeronautics and Space Administration Task Load Index (NASA-TLX) was applied after the conclusion of the bed rest to assess subjective workload (Hart & Staveland, 1988). NASA-TLX is multidimensional questionnaire comprising six subscales using twenty points scale. For mental, physical and temporal demand, frustration, effort and performance participants estimated their experience of these burdens by crossing the line between very low (1) and very high (20): mental demand was defined as amount of mental and perceptual activity required for a task; physical demand, how much physical activity was required; temporal demand, how much time pressure participants felt due to duration of bed rest; performance as participants' satisfaction with their performance, and frustration as level of irritation, stress, and annoyance present during the bed rest. A twenty-step bipolar scale resulting in a score between 0 and 100 is also rated for each of the six subscales and the total raw workload experienced by the participants is represented by an average of these subscales (Hart & Staveland, 1988). Initially, a weighting procedure was applied to the raw test scores of each dimension to estimate the individual sources of workload (Hart & Staveland, 1988). However, over the years many researchers have eliminated the weighting procedure and instead used the raw test scores (RTLX) for improved applicability (Hart, 2006). Six individual raw subscale ratings were analyzed in our study in addition to the total raw workload (i.e., average of the six subscales) (Hart, 2006). An average of the six scales reflects the contribution of each factor to the total workload of a specific activity and can be used as an integrated measure of overall workload (Hart & Staveland, 1988). An example of NASA-TLX is available in the appendix section.

4.3.4 Virtual navigation training

For virtual navigation training we used virtual maze navigation task as described above, albeit with well-matched alternate versions. Participants in the Intervention group were asked to navigate through virtual mazes with the use of a joystick for approximately 50 minutes for each of 12 days of bed rest. Upon successful joystick training, participants were given cognitive training in several virtual mazes of increasing length. Participants

were trained in virtual mazes with three intersections, five intersections, and seven intersections. Three variants of virtual mazes differed by the cues used:

- Cues with the names of Slovenian cities (Figure 2 B and C),
- Cues with the names of world's countries and
- Cues with the pictures of forest animals.

Any given virtual maze had only one of these cue categories throughout. For each virtual maze, participants were instructed to navigate to the goal area until successfully completing two consecutive trials with no errors. At this point, participants had received adequate experience with the virtual maze to learn the correct path and then recall it again, and could then move on to the next virtual maze. Across all training days, participants were to complete six three-intersection mazes, followed by six five-intersection virtual mazes, and twelve seven-intersection virtual mazes. The cognitive training was progressive in terms of increasing difficulty; when a participant has finished one virtual maze twice in a row withouth error he was administred a maze of a higher level of difficulty. Although the number of seven-intersection virtual mazes completed by the end of the training period varied depending on how quickly each participant reached criterion on each virtual maze, all participants who went through cognitive training completed at least four of the seven-intersection mazes. For all levels of virtual maze complexity, alternate forms were used which contained different spatial layouts and used different cues (i.e. city/country names and images) to minimize practice effects across repeated testing. Virtual navigation training was, the same as testing, performed in the supine position (see Figure 1 and Figure 2 A).

4.3.5 Measurements of functional gait parameters

Gait spatio-temporal parameters were measured with the OptoGait system (Microgate, Bolzano, Italy), which gives valid and reliable data (Lienhard, Schneider, & Maffiuletti, 2013). Ten transmitting and ten receiving bars were placed in parallel to each other along a 10 m x 2 m hall and the first bar was placed approximately 30 cm from the starting point. Data were sampled at 1000 Hz and analyzed with OptoGait software, version 1.6.0. The first two and the last two steps were systematically excluded for each individual basis due to accelerations at the beginning and at the end of each 10-meter measuring distance.

Participants were asked to perform in a randomized order, the following four conditions: 1. walking in their preferable self-selected speed, 2. brisk walking to the best of their

capacity, and 3 & 4 both speeds under a dual-task condition. The dual-task condition was composed of walking whilst at the same time using mathematical subtraction from a randomly chosen number between 400 and 500 (by threes). Each participant completed one minute of walking for each testing conditions, starting out always with the right foot.

The outcome variable chosen for the analyses was dual-task effect (DTE), a sensitive relative measure allowing comparison of combined single- and dual-task walking conditions across groups and time (V. E. Kelly et al., 2010; Remaud et al., 2013; Siu & Woollacott, 2007). For each walking condition (normal, fast paced), the measure of dual-task effects (DTEs) was calculated as follows:

$$\mathrm{DTE} = \frac{\text{dual task walking condition} - \text{single task walking condition}}{\text{single task walking condition}} \times 100 \qquad \text{(Equation 1)}$$

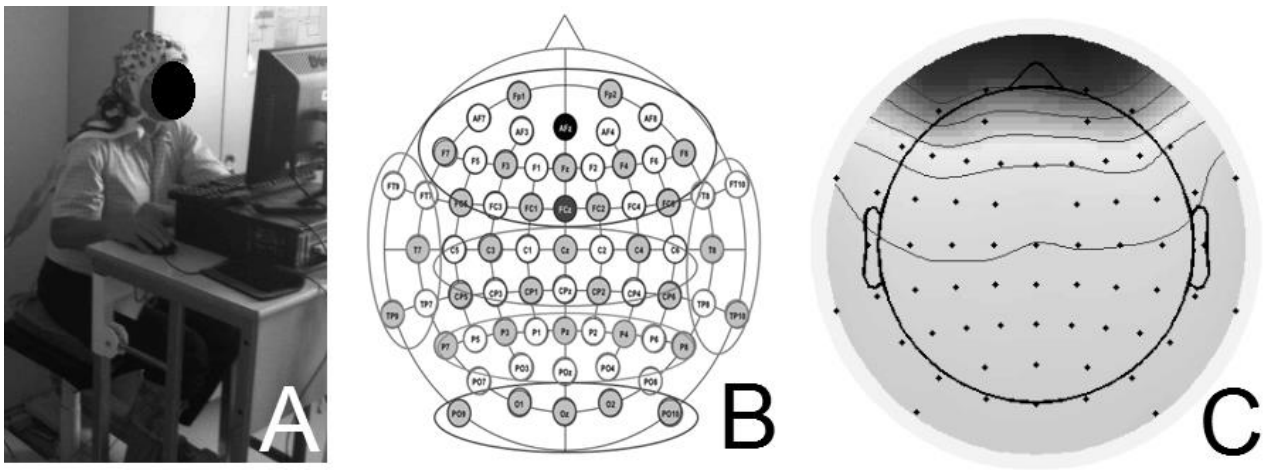
where a negative value of DTE represents a decrement (dual-task costs), while a positive value represents an improvement (dual-task benefits) under dual-task condition. Dual-task costs reflect the increased cost of cognitive attentional processes under the dual-task condition (O. Beauchet et al., 2008; Beurskens & Bock, 2012; Lindenberger et al., 2000), while dual-task benefits may represent higher arousal under more challenging dual-task condition (Huxhold et al., 2006; V. E. Kelly et al., 2010).

Gait variability was assessed with swing time variability and stride-to-stride fluctuations of the gait cycle (Verghese, Holtzer, Lipton, & Wang, 2009).

4.3.6 Electroencephalographic (EEG) measurements

Scalp electroencephalographic (EEG) activity was recorded using Brain Vision (Brain Vision, Inc.) equipment, with high density electrodes Acti Cap (64 electrodes) modified according to the International 10-20 System (see figure 3 A and B). The recording locations included eight midline sites, with the FCz electrode as an on-line reference and a ground at midline location AFz. During EEG measurements, low and high pass filter settings were set at 70 Hz and 0.1 Hz, respectively. The cutoff frequencies for these filters were set at 3 dB down; the roll off was 12 dB per octave at both sides. Impedances were maintained below 10 kΩ for each channel and balanced across all channels within a 5 kΩ range. The sampling rate was 2000 Hz with 32 bit resolution.

Figure 3: EEG measurements and finger tapping task, regions of interest and independed component of eye blink

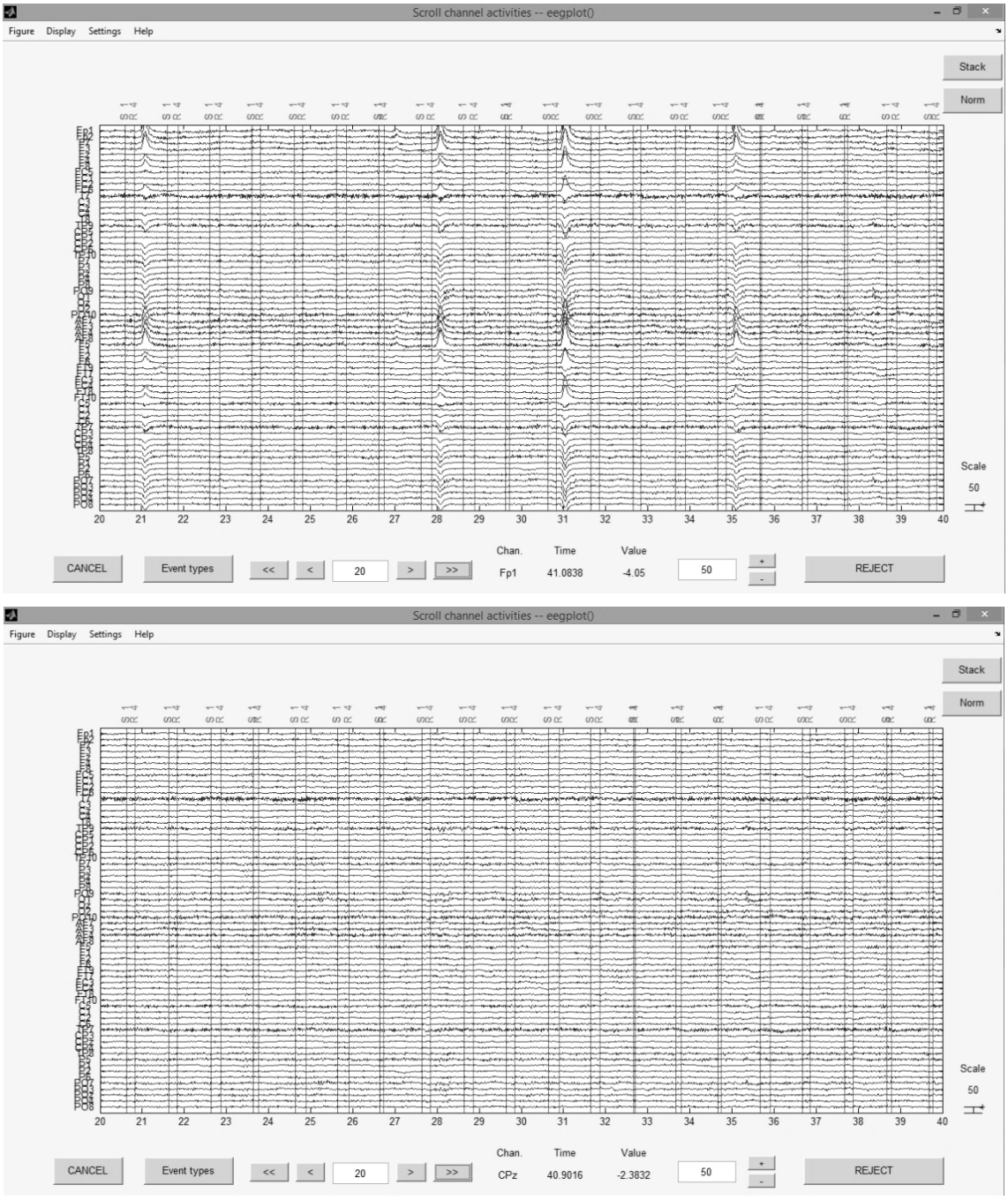


Note: Photograph depicting a participant during the EEG finger tapping task (A). Sixty-four electrodes and Regions of Interest (ROI): frontal, Left and Right temporal, central, parietal, and occipital (B). An example of eye blink component, which was removed after Independent component analysis (ICA) (C). *Figure source: U. Marušič*

After baseline measurements with eyes open and closed, participants were instructed to perform finger and foot tapping motion as accurately as possible, following the visual stimuli presented on a 17-inch flat panel LCD monitor situated approximately 50 centimeters in front of them. Responses were recorded with standard computer mouse for finger tapping and specially made response box for foot tapping. No feedback was provided to participants regarding accuracy of their tapping motion. The familiarization protocol was performed to adapt motion mechanisms prior to the task.

All data was analyzed with EEGLAB Matlab toolbox (Delorme & Makeig, 2004). After importing the EEG data into EEGLAB, the channel location information was updated for all 64 electrodes. The next step was the off-line visual inspection of EEG signal to identify and remove segments contaminated by either excessive noise, saturation or lack of EEG activity. Due to the process optimization the EEG data was resampled from 2000 Hz to 500Hz and applied 50 and 0.5 Hz, low and high pass filter, respectively. Afterwards, an independent components analysis (ICA) was used to remove eye blinks (see Figure 3 C and Figure 4). Segmentation for either stimulus or response was performed as follows.

Figure 4: An example of EEG signal (pre- and post-processing) in the EEGLAB software



Note: Upper figure represents original EEG signal before ICA, while lower figure represents EEG signal after ICA after the eye blink artifact removal. *Figure source: U. Marušič*

The electroencephalographic measurements are represented into two parts: a) behavioral (foot and finger tapping responses) and b) electroencephalographic data with further stimulus- and response-locked evoked related potentials (ERP) analyses.

Behavioral data:

Altogether, there were 200 visual stimuli for finger and foot tapping tasks. Depending on subject's synchronization rate the number of responses varied, however in the optimal conditions were exactly 200. Finger and foot tapping data were reordered in the ".vmrk" text file and stored next to header ".vhdr" and EEG ".eeg" file of Brain Vision EEG system. The visual stimuli were presented in the middle of a monitor (duration 300 ms, intensity 50 cd/m2, visual angles 1° horizontal/1.5° vertical,) placed in front of the subject's eyes.

EEG baseline frequency analysis:

Baseline EEG was decomposed with Fast Fourier Transform (FFT) to different band ranges: theta (4–7.5 Hz), alpha 1 (8–10.5 Hz), alpha 2 (11–12.5 Hz), beta 1 (13–19.5 Hz), and beta 2 (20–29.5 Hz). Prior to FFT, EEG epochs were transformed into the reference-free current source density (CSD) distribution. 64 channels were pooled in 6 regions of interest (ROI): frontal, left and right temporal, central, parietal and occipital (see Figure 3 B).

Stimulus processing:

Stimulus-locked event related potentials (s-ERPs) were segmented from -200 to +800 milliseconds with the baseline set from -200 to 0 milliseconds. For each tapping task that lasted 200 seconds, we were able to extract 200 epochs. After epoch extraction, both, automatic and visual inspection were performed with a cutoff value 100 μV. In all cases, at least 120 stimuli epochs were averaged for each electrode.

Visual modality was assessed over the occipital locations over the scalp with the best electrode taken into further analysis. The following analyses were performed:

- P1 was detected as most positive peak (amplitude and latency) within the range 40-140 milliseconds after the stimuli occurred,
- N1 was the most negative peak (amplitude and latency) within the range of 120-200 milliseconds and
- P2 was the first positive peak (amplitude and latency) after 200 milliseconds.

Motor response processing:

For finger and foot tapping, the response-locked ERPs were segmented from -800 to +200 milliseconds with the baseline set from -800 to -600 milliseconds before the response occurred. For each tapping task that lasted 200 seconds, we were able to extract at least 93 epochs due to the fact that not all participants were able to perform all 200 responses, especially while foot taping after the bed rest. The same as for s-ERP, also for r-ERP after epoch extraction, automatic and visual inspection were performed with a cutoff value 100 μV.

r-ERP, i.e. motor response potentials (MRP) for finger and foot tapping responses were analyzed on the electrode C3 and C4 respectively, which was positioned on the contralateral side of right hand or foot, above motor cortex (Figure 3: electrodes position and ROIs).

The following calculations were extracted from each MRP at contralateral side of right hand and foot (Yordanova et al., 2004):

- the most negative displacement of MRP (latency and amplitude),
- the threshold of 15% of MRP maximum was set for onset latency of the MRP,
- the MRP rise time representing the duration of motor-related activation was calculated as the difference between MRP peak latency and MRP onset latency.

4.4 Statistical procedures

The data were analyzed with IBM SPSS Statistics 20.0 software for Windows (SPSS, Inc., Chicago, Ill, USA). Normality of the distribution of the parameters was tested with the Shapiro-Wilk's test. Results, which did not violate any assumptions for parametric statistics, were further analyzed with analysis of variance (ANOVA), otherwise the appropriate non-parametric equivalent was used. The baseline characteristics was assessed with independent sample t-test. Statistical significance was set at the level of $p < .05$ and at $p < .10$ for interactions.

Cognitive assessment: For virtual maze navigation performance, however, there were violations assumptions for ANOVA, therefore we used non-parametric Mann- Whitney U test for testing the differences between two independent groups, e.g., Intervention and Control group. To maintain family-wise error rate at .05 we used Holm-Bonferroni method (Holm, 1979). According to this statistical approach, the effectiveness of cognitive training will be supported by significant differences between the two groups at BR d14, REC d28 and REC d400.

Assessment of subjective workload: Subjective evaluation of mental, physical and temporal demand, frustration, effort, performance and total workload was compared between Intervention and Control groups with independent sample t-test.

Measurements of gait parameters: Gait speed, DTEs, and gait variability were entered into a 2x2 mixed design repeated measures ANOVA with Group (Intervention and Control group) as the between subject variable and Time (pre and post) as the within subject variable. Post hoc comparisons were carried out with a Bonferroni adjustment.

Electroencephalographic measurements: Amplitudes and latencies of P1, N1 and P2, as well as for MRP, MRP onsets, and rise time were entered into a 2x2 mixed design repeated measures ANOVA with Group (Intervention and Control group) as the between subject variable and Time (pre and post) as the within subject variable. Where independent sample t-test showed significant changes at baseline condition, analysis of covariance (ANCOVA) was used. Post hoc comparisons were carried out with a Bonferroni adjustment.

Correlations between cognitive assessment, gait and EEG parameters: For normally distributed data we used a Pearson's correlation analysis, while where were we detected violation of parametric statistics we used a nonparametric equivalent, such as Spearman's correlation analysis.

5 RESULTS

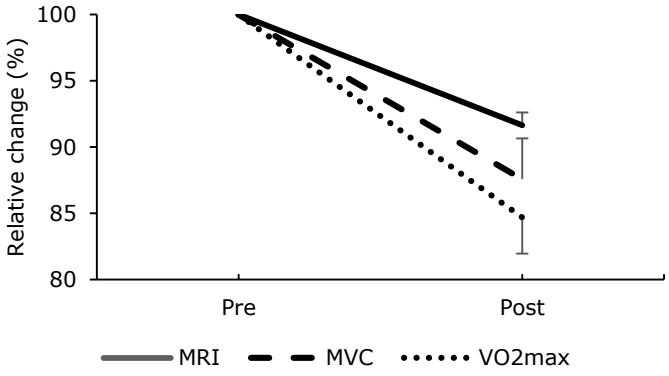
5.1 Muscle volume, function, and aerobic capacity

These results highlight the general decline of skeletal muscles volume, function, and aerobic capacity after 14-day bed rest in older adults *(Results are in submission to the Journal of the American Medical Association)*. The summary of bed rest results on muscle mass, muscle strength and aerobic capacity are presented in the Figure 5. At baseline there was no significant change between Intervention and Control group in the volume of the quadriceps femoris muscle ($p = .540$), knee exstension MVC ($p = .521$) and VO_2max ($p = .551$).

RM ANOVA showed no interaction effect between Intervention and Control group for the volume of the quadriceps femoris muscle ($p = .996$), knee exstension MVC ($p = .166$) and VO_2max ($p = .609$).

However, there was a significant time effect for the volume of the quadriceps femoris muscle [$F(1,13) = 62.88$, $p < .001$, $\eta^2 = .83$], knee exstension MVC [$F(1,14) = 16.37$, $p = .001$, $\eta^2 = .54$], and VO_2max [$F(1,14) = 16.74$, $p = .001$, $\eta^2 = .55$] with post hoc tests showing a significant reduction in all presented parameters at the end of bed rest ($p < .05$).

Figure 5: Relative change of the volume of the quadriceps femoris muscle, knee exstension MVC, and VO_2max



Note: X axis shows mesurements before bed rest (Pre) and post bed rest (Post). Results are in submission to the Journal of the American Medical Association.

5.2 Cognitive training effectiveness

At baseline, both groups performed a virtual maze learning task to assess baseline navigation performance. Independent t-tests demonstrated that the Intervention and Control groups did not differ in the number of errors committed during virtual environment learning (7.88 vs 9.71, p = .613, respectively) (Figure 5), nor on their average distance travelled (z scores) through the environment (-.16 vs .18, p = .541, respectively) (Figure 6). Therefore, both groups were not different in terms of their navigation skill prior to the intervention. The virtual maze task assessments for all four time points are presented in the table 2.

Table 2: Results on virtual maze navigation task performance

	PRE-CCT	POST-CCT	REC d28	REC d400
Sum of errors (N)				
Intervention group	7.88 ± 4.58	1.50 ± 1.60 $	1.25 ± 2.05 *	2.00 ± 2.78 *
Control group	9.71 ± 6.50	7.14 ± 4.49	6.71 ± 6.05	6.14 ± 4.45
Mean travelled distances (z score)				
Intervention group	-.16 ± 0.91	-.72 ± .57 $	-.51 ± .59 *	-.38 ± .80
Control group	.18 ± 1.14	.83 ± .68	.58 ± 1.09	.44 ± 1.08

Note: PRE bed rest indicates day of baseline data collection; POST bed rest indicates day of post-measurements immediately after 14-day bed rest period; REC d28 indicates day of post-measurements after 28-day of recovery period; REC d400 indicates day of post-measurements 400 days after the CCT; * - denotes statistical significant difference from the Control group at P < .05; $ - denotes statistical significant difference from the Control group at P < .01.

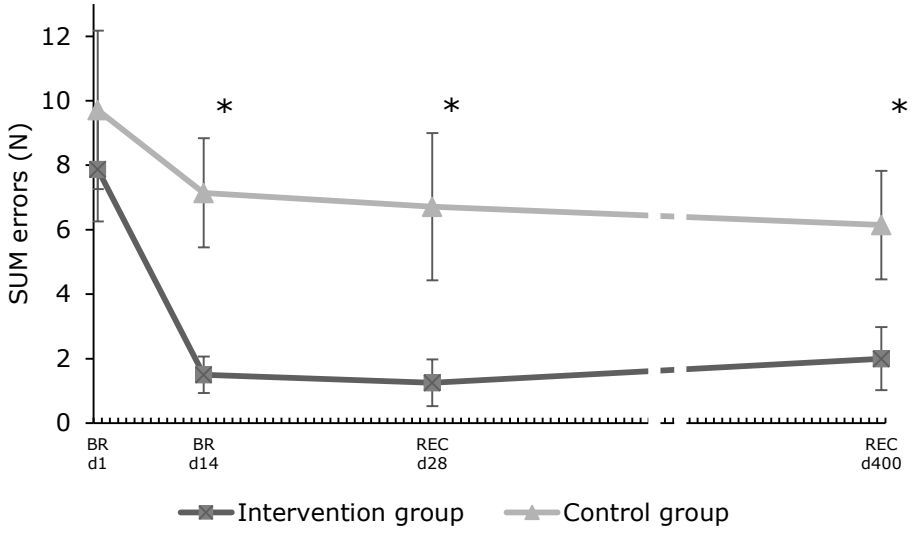
Total number of errors:

The difference in sum of errors at BR d14, REC d28 and REC d400 between the two groups were tested with Mann-Whitney U test which showed that Intervention group had significantly fewer errors at BR d14 (Mann-Whitney; U-test, z = -2.683 p = .006), REC d28 (Mann-Whitney U-test; z = -2.302, p = .021), and REC d400 (Mann-Whitney U-test; z = -2.277, p = .021) (see Figure 6), confirming the effectiveness of CCT up to 400 days after the initial training.

Mean travelled distances:

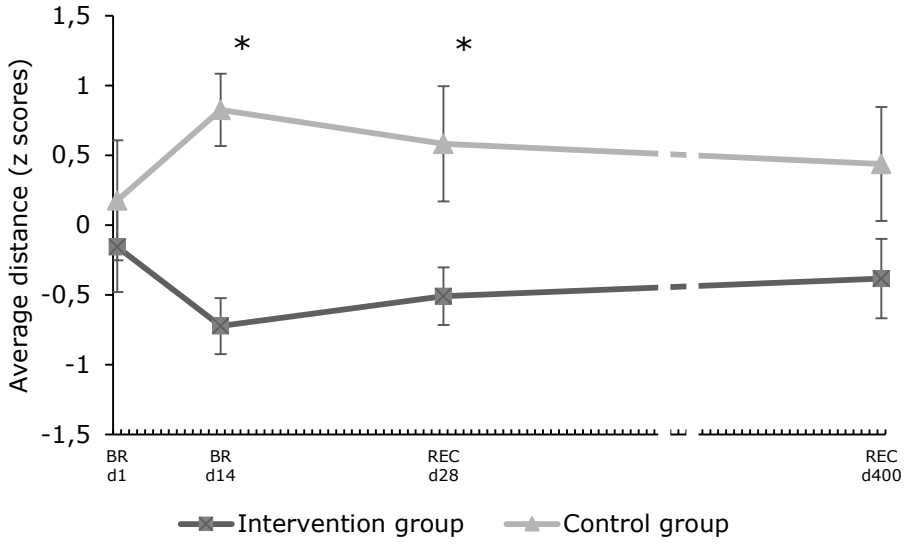
Results for average travelled distances represented in z scores (Figure 6) showed that there was significantly shorter distance traveled in the Intervention compared to Control group at BR d14 (Mann-Whitney U-test; z = -3.009, p = .001), and at REC d28 (Mann-Whitney U-test; z = -2.083, p = .020), but not at REC d400 (Mann-Whitney U-test; z = -1.504, p = .076) (see Figure 7).

Figure 6: Performance on virtual maze navigation task in terms of summed errors



Note: Y axis shows sum of errors made from 2nd – 5th trial in the virtual maze. X axis shows mesurements before CCT (BR d1), at the end of CCT (BR d14), at the end of recovery period (REC d28), and 400 days after the CCT (REC d400) for the Intervention (squares) and Control groups (triangles). * markes a significant difference between both groups.

Figure 7: Performance on virtual maze navigation task in terms of average distance travelled



Note: Y axis shows z scores of average distances travelled from 2nd – 5th trial in the virtual maze. X axis shows mesurements before CCT (BR d1), at the end of CCT (BR d14), at the end of recovery period (REC d28), and 400 days after the CCT (REC d400) for the Intervention (squares) and Control groups (triangles). * markes a significant difference between both groups.

5.3 Assessment of subjective workload

Subjective evaluation of mental, physical and temporal demand, frustration, effort, performance and total workload are presented in table 3. Independent sample t test analyses showed that there were no statistically-significant differences between Intervention and Control group in any of subscales ($p > .35$).

Table 3: The National Aeronautics and Space Administration Task Load Index (NASA-TLX)

Demands	NASA-TLX Values	T test p values
Mental		.982
Intervention	41.88 ± 14.38	
Control	41.67 ± 19.15	
Physical		.719
Intervention	12.50 ± 9.64	
Control	15.00 ± 15.81	
Temporal		.430
Intervention	50.63 ± 14.74	
Control	57.50 ± 16.66	
Performance		.931
Intervention	26.88 ± 18.70	
Control	25.83 ± 25.58	
Effort		.353
Intervention	36.88 ± 16.02	
Control	47.50 ± 25.25	
Frustration		.643
Intervention	21.88 ± 19.63	
Control	26.67 ± 17.22	
Total workload		.472
Intervention	37.77 ± 8.69	
Control	35.69 ± 11.15	

Note: Means and standard deviations of NASA-TLX assessing subjective workload after completion of bed rest study for Intervention and Control group. Statistical evaluation of differences between the two groups are presented in the right column and represent p value of independent sample t-test.

5.4 Measurements of gait parameters

Means and standard deviations of pre- and post-bed rest gait speeds without (Gait speed) and with dual-task (Gait speed plus dual-task), as well as the derived DTE parameters and gait variability (for both groups) during normal and fast pace walking conditions are presented in Table 4.

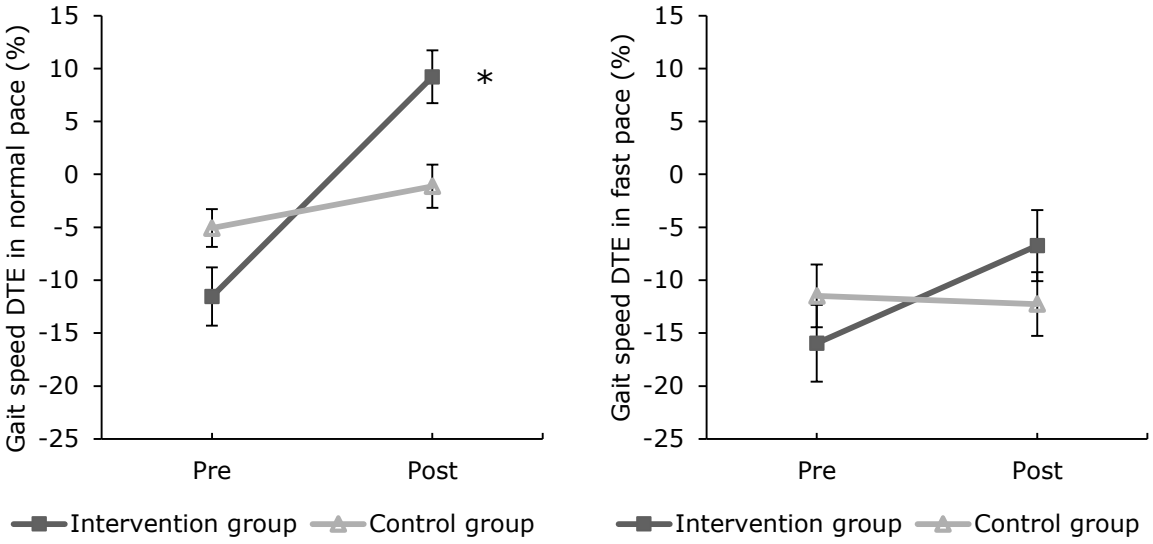
5.4.1 Gait speed

For normal gait speed, fast gait speed and the combined normal gait speed plus dual-task gait performance, the 2x2 ANOVA showed no significant interactions, group, or time effect. There was a significant time effect, however, in gait speed in dual-task condition during fast walking [$F(1,13) = 7.002$, $p = .02$, $\eta^2 = .35$]. Post hoc analysis showed no significant decrease in gait speed in the Intervention group ($p = .37$), whereas the average gait speed during dual-task in control subjects significantly decreased by approximately 17 cm/s, from 1.71 to 1.54 m/s ($p = .005$) (see Table 4).

5.4.2 Dual-task effects (DTEs)

For the normal pace walking condition, DTEs were entered into a 2x2 mixed design ANOVA which showed a significant effect of time [$F(1,13) = 58.12$, $p < .001$, $\eta^2 = .82$] and an interaction between time and group [$F(1,13) = 26.93$, $p < .001$, $\eta^2 = .67$]. Post-hoc comparisons of interest demonstrated that the significant effect of time was driven by Intervention group; whilst there were no significant differences in the DTEs between Pre- and Post-bed rest for the Control group (–5.07 % vs. –1.13 %, $p = .124$), participants in the Intervention group showed a significant reduction of negative DTE (resulting as dual-task benefits) in Post- as compared with Pre-bed rest (–8.66 % vs. 9.22 %, $p < .001$) (Figure 8– left panel).

Figure 8: Dual-task effects (DTEs) for gait speed results



Note: Dual-task effects (DTEs) for gait speed results (presented mean ± SEM). DTE scores refer to the percentage of decrease (dual-task costs) or increase (dual-task benefits) in gait speed in dual-task condition as compared to single-task, for Pre- and Post-bed rest (Pre and Post, respectively). Left panel: DTEs during normal pace walking condition for the Intervention (squares) and Control group (triangles). Right panel: DTEs during fast pace walking condition for the Intervention (squares) and Control group (triangles). * markes a significant increase in the Intervention group at the end of bed rest.

For the fast pace walking condition, there was a significant interaction effect between time and group [$F(1,13) = 5.70$, $p = .03$, $\eta^2 = .31$] during fast pace walking. Post-hoc analyses showed that the significant effect of time was driven by Intervention group: while there was no significant difference in the negative DTE between pre- and post-bed rest for Control group (–11.48 % vs. –12.25 %, $p = .716$), participants in the Intervention group showed in post-bed rest as compared to pre-bed rest suggestive (Figure 8, right panel) but not significantly smaller negative DTE (–15.95 % vs. –6.74 %, $p = .056$).

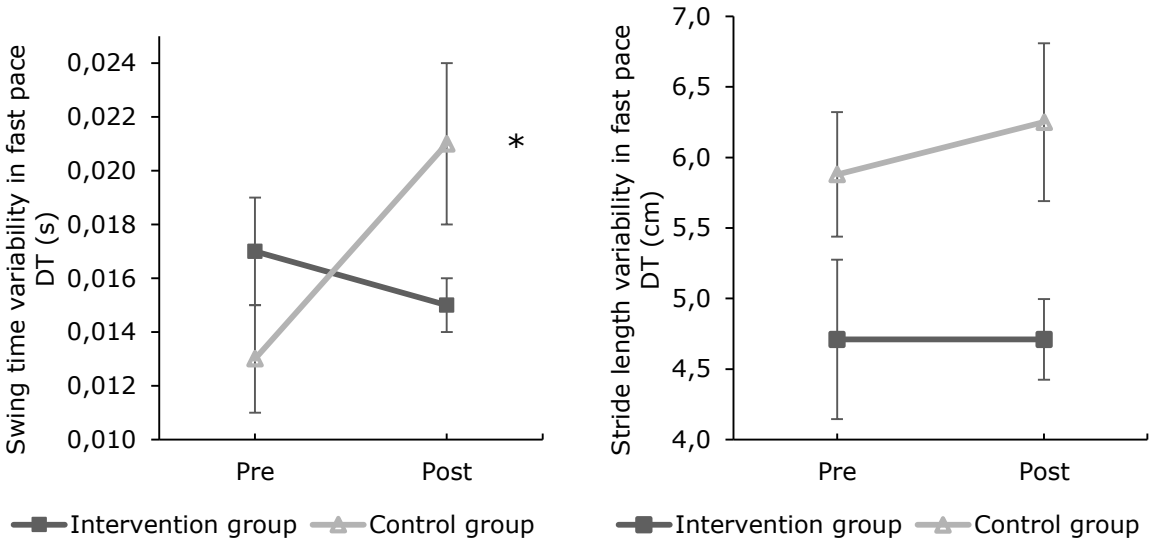
5.4.3 Gait variability

No significant changes between both groups were found at the Pre-bed rest baseline measurement ($p > .114$) at all eight entered parameters (four walking conditions X swing time and stride length variability).

Standard deviations of swing times were entered into 2x2 ANOVA. There were no significant interaction effects ($p > .05$) in all three walking conditions, except for the most

demanding one; for fast walking with dual-task there was a trend of time [F (1,13) = 4.29, p = .059, η^2 = .25], and significant interaction effect between time and group [F (1,13) = 12.81, p = .003, η^2 = .50]. Post-hoc analysis showed that the significant effect of time was driven by Control group: while there was no significant change in the swing time variability between Pre- and Post-bed rest for Intervention group (.017 ± .005 vs .015 ± .003 seconds, p = .302), participants in the Control group showed significantly higher swing time variability in Post- as compared with Pre-bed rest (Pre-bed rest .013 ± .005; Post-bed rest .021 ± .009 seconds, p = .006) (Figure 9, left panel).

Figure 9: Gait variability for swing time and stride length gait parameters



Note: Gait variability for swing time and stride length gait parameters (mean ± SEM). Left panel: Swing time variability (presented in seconds) in fast pace walking condition with dual-task for the Intervention (squares) and Control group (triangles). Right panel: Stride length variability (presented in centimeters) in fast pace walking condition with dual-task for the Intervention (squares) and Control group (triangles). * markes a significant increase in the Control group at the end of bed rest

For stride length variability, no significant interaction or time effects were found. However, the 2x2 ANOVA showed a significant group effect in fast walking with dual task (p = .018, η^2 = .36) (Figure 9, right panel). Post hoc analysis showed that there were no differences at the Pre-bed rest measurement between the two groups (p = .125), while there was a significant difference at Post-bed rest (p = .034). The Control group had higher stride length variability at the end of the bed rest (Pre-bed rest 5.88 ± 1.25; Post-bed rest 6.25 ± 1.58 cm), compared to the Intervention group, for whom stride length variability stayed at the same mean level (Pre bed rest 4.71 ± 1.50; Post bed rest 4.71 ± .76 cm).

Table 4: Gait measurements for normal and fast pace walking conditions

Variables	Normal pace walking			Fast pace walking		
	Pre	Post	$P_{INTERACTION}$ (η^2)	Pre	Post	$P_{INTERACTION}$ (η^2)
Gait speed (m/s)			.490			.466
Intervention group	1.20 ± .22	1.04 ± .15		1.83 ± .35	1.52 ± .21	
Control group	1.36 ± .19	1.27 ± .17		1.94 ± .19	1.76 ± .17	
Gait speed DT (m/s)			.276			.452
Intervention group	1.06 ± .18	1.14 ± .15		1.51 ± .27	1.42 ± .20	
Control group	1.29 ± .23	1.25 ± .15		1.71 ± .22	1.54 ± .19 †	
Gait speed DTE (%)			**< .001 (.674)**			**.033 (.305)**
Intervention group	-11.53 ± 7.28	9.22 ± 6.59 ‡		-16.51 ± 9.63	-6.74 ± 8.87	
Control group	-5.07 ± 5.04	-1.13 ± 5.77		-11.48 ± 8.38	-12.25 ± 8.52	
Swing time variability (s)			.464			.909
Intervention group	.014 ± .004	.019 ± .005		.012 ± .002	.018 ± .010	
Control group	.011 ± .003	.014 ± .003		.011 ± .002	.017 ± .012	
Swing time variability DT (s)			.137			**.003 (.496)**
Intervention group	.026 ± .012	.020 ± .007		.017 ± .005	.015 ± .003	
Control group	.018 ± .011	.021 ± .012		.013 ± .005	.021 ± .009 †	
Stride length variability (cm)			.344			.583
Intervention group	4.86 ± 1.77	5.00 ± 1.00		4.86 ± 1.22	5.71 ± 2.43	
Control group	4.00 ± 1.31	4.25 ± .71		5.25 ± 2.38	5.50 ± 1.07	
Stride length variability DT (cm)			.985			.693
Intervention group	4.71 ± 1.11	4.71 ± 1.11		4.71 ± 1.50	4.71 ± .76	
Control group	5.13 ± 1.89	5.25 ± 1.91		5.88 ± 1.25	6.25 ± 1.58	

Note: All values are means ± SD unless otherwise stated. DT = dual-task walking condition. DTE = calculated dual-task effect (negative value represents dual-task costs while positive values represents dual-task benefits).

† Significantly different from Pre-bed rest at $p < .01$

‡ Significantly different from Pre-bed rest at $p < .001$

5.5 Electroencephalographic (EEG) measurements

Behavioral data are summarized in the tables 5 and 6, representing the mean and standard deviation of the synchronization rate, as well as the number of responses given by participants in the Intervention and Control groups. Furthermore, all results for stimulus processing are presented in the table 7, while for response processing for finger and foot tapping are in tables 8 and 9 respectively, with means and standard deviations for both groups as well as interaction, group and time effects of Repeated Measures ANOVA.

5.5.1 Behavioral data

Finger tapping:
The independent sample t-test showed no significant changes in synchronization rate at baseline testing between Intervention and Control groups for finger tapping ($p > .05$). More specifically, there were no significant changes in average synchronization rate ($p = .346$), standard deviations ($p = .387$) and the number of not responded stimuli ($p = .547$) at the first baseline measurement.

The 2-way RM ANOVA revealed a non-significant interaction, group and time effect ($p > .409$) for all analyzed parameters (see table 5).

Table 5: Behavioral data of finger tapping task

	Pre	Post	P_{TIME} (η^2)	P_{GROUP} (η^2)	$P_{TIMExGROUP}$ (η^2)
Mean synchronization rate (ms)			.617	.605	.409
Intervention group	52.52 ± 28.84	68.16 ± 39.35			
Control group	73.80 ± 54.59	69.90 ± 65.89			
Standard deviation of synchronization rate (ms)			.861	.528	.732
Intervention group	47.64 ± 16.74	50.49 ± 25.84			
Control group	55.38 ± 17.89	54.45 ± 22.45			
Number of not responded stimuli			.875	.676	.805
Intervention group	7.63 ± 6.44	7.87 ± 12.69			
Control group	9.88 ± 8.06	8.75 ± 8.08			

Note: All values are means ± SD unless otherwise stated

Foot tapping:

The independent sample t-test showed no significant changes at baseline testing between Intervention and Control groups for foot tapping, for parameters standard deviation of synchronization rate (p = .741) and the number of not responded stimuli (p = .782). Because of significant changes in average synchronization rate during baseline group measures (p = .025), an ANCOVA was used to eliminate the baseline bias.

The RM ANOVA revealed a non-significant interaction, group and time effect (p > .145) for all analyzed parameters (see table 6). Similarly, the ANCOVA obtained no significant effects (p = .661).

Table 6: Behavioral data of foot tapping task

	Pre	Post	P_{TIME} (η^2)	P_{GROUP} (η^2)	$P_{TIMExGROUP}$ (η^2)
Mean synchronization rate (ms)			*	*	*
Intervention group	32.94 ± 34.87	66.30 ± 36.53			
Control group	74.32 ± 31.18	68.09 ± 58.99			
Standard deviation of synchronization rate (ms)			.282	.293	.407
Intervention group	51.73 ± 29.45	39.90 ± 7.74			
Control group	55.90 ± 18.85	54.32 ± 21.22			
Number of not responded stimuli			.569	.853	.975
Intervention group	17.75 ± 17.65	23.13 ± 29.33			
Control group	15.13 ± 19.53	21.13 48.78			

Note: All values are means ± SD unless otherwise stated; Pre- bed rest indicates day of baseline data collection; Post- bed rest indicates day of post-measurements immediately after 14-day bed rest period *ANCOVA was used due to significant changes between two groups at Pre-measurements.

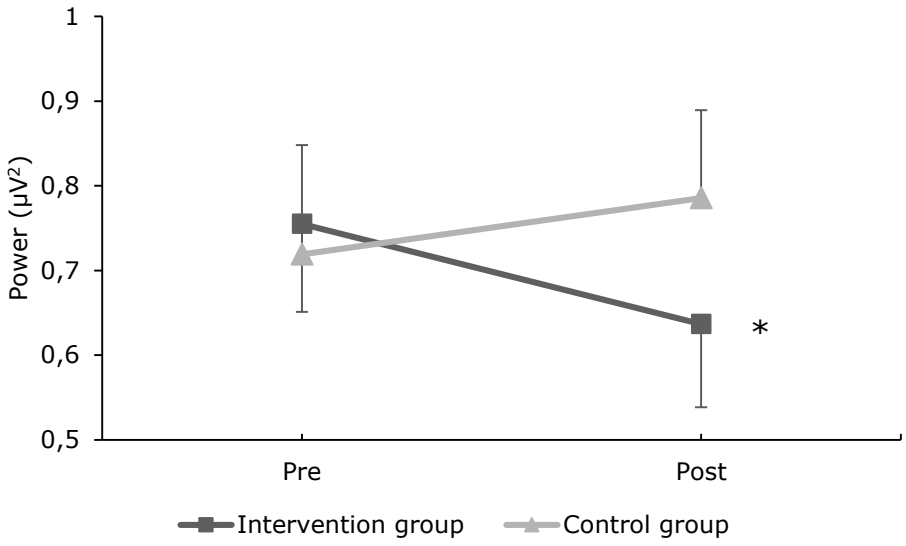
5.5.2 EEG frequency baseline analysis

An EEG frequency baseline analysis was performed in two different conditions: eyes-open and eyes-closed. In the eyes-open condition the power did not change neither in the Intervention nor in the Control group at the end of bed rest. To the contrary, in the eyes-closed condition, there was a significant effect of bed rest and CCT in Theta and Beta 2 frequency ranges. For Theta oscillations there was a significant interaction in central (p =

.043), parietal (p = .033) and occipital (p = .004) ROIs (2x2 group, time ANOVA). Post hoc anaylsis showed that there was a significant decrease of Theta frequency after the bed rest in the central ROI in the Intervention group (p = .018) (Figure 10), and a significant increase of Theta frequency in the occipital ROI (p = .016) in the Control group at the end of bed rest (Figure 11).

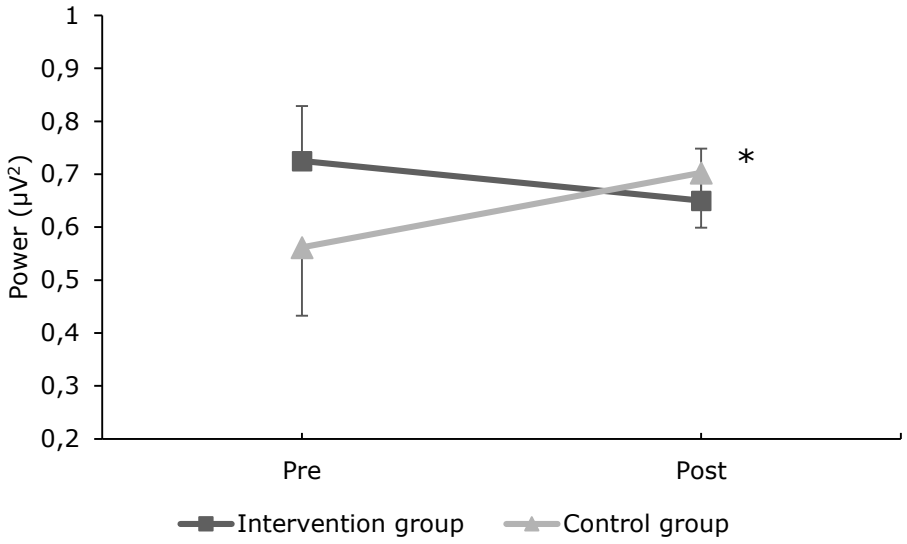
In the Beta 2 range RM ANOVA showed significant interaction in frontal (p = .048), right (p = .029) and left (p = .047) temporal and occipital (p = .023) ROIs. These interactions were driven by a significant increase in Beta 2 power in the Intervention group in the frontal (p = .006) (Figure 12) and right temporal (p = .012) ROIs (Figure 13).

Figure 10: The power of Theta rhythm in the central ROI at baseline EEG with eyes-closed



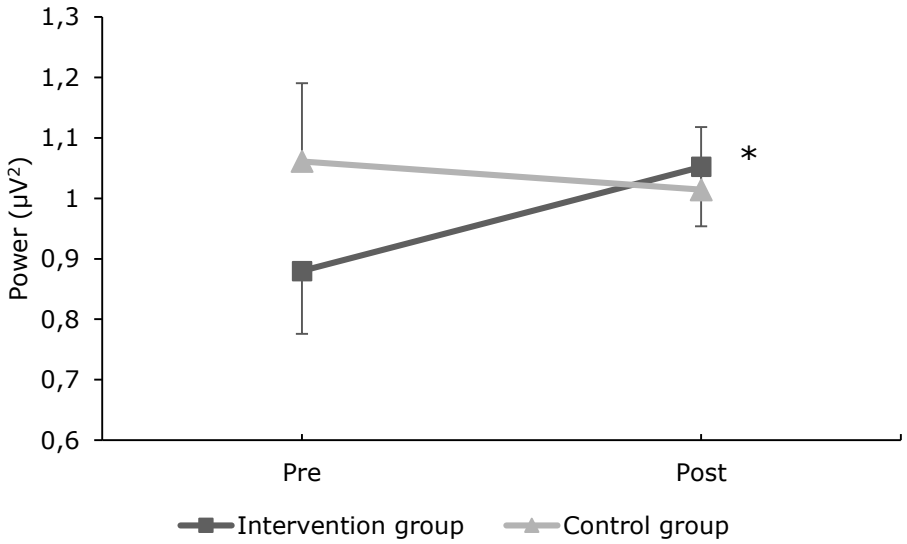
Note: * markes a significant reduction in the Intervention group at the end of bed rest

Figure 11: The power of Theta rhythm in the occipital ROI at baseline EEG with eyes-closed



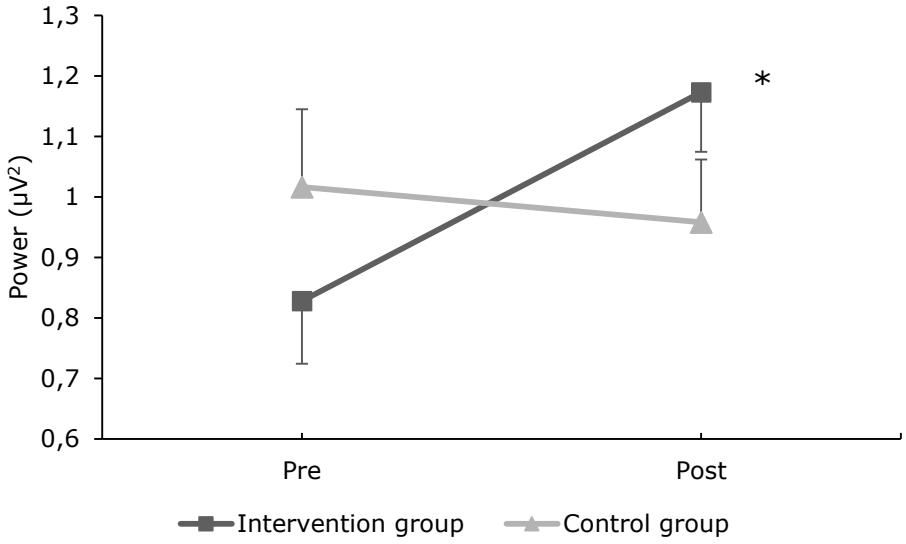
Note: * markes a significant increase in the Control group at the end of bed rest

Figure 12: The power of Beta 2 rhythm in the frontal ROI at baseline EEG with eyes-closed



Note: * markes a significant increase in the Intervention group at the end of bed rest

Figure 13: The power of Beta 2 rhythm in the right temporal ROI at baseline EEG with eyes-closed



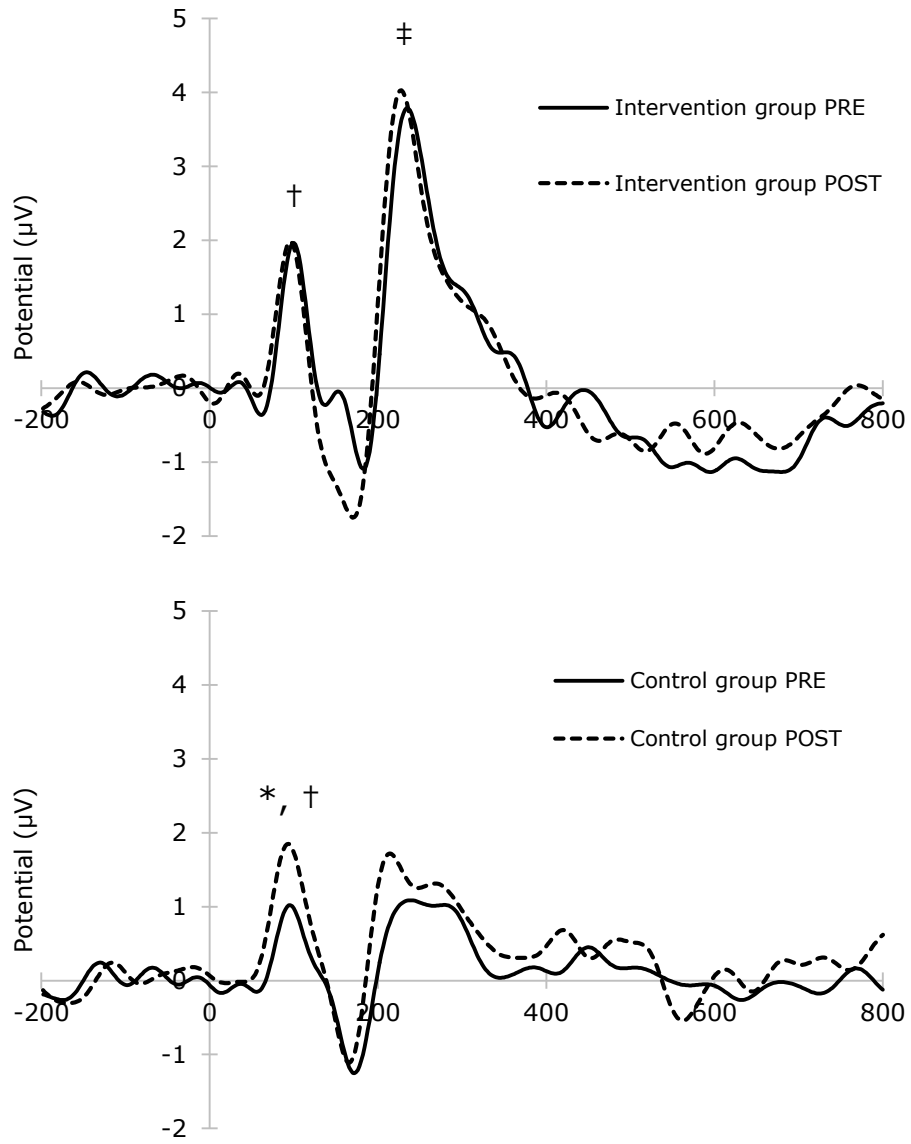
Note: * markes a significant increase in the Intervention group at the end of bed rest

5.5.3 Stimulus-related processes

For baseline characteristics of stimulus-related processes, the independent sample t-test showed no significant changes between groups for peak P1 amplitude ($p = .087$) and latency ($p > .999$), N1 amplitude ($p = .587$) and latency ($p = .829$), and P2 amplitude ($p = .073$) and latency ($p = .756$).

There were no significant group ($p > .183$) and interaction ($p > .145$) effects for all parameters of stimulus-related processes. However, there was a significant time effect in peak P1 amplitude [$F(1,14) = 7.078$, $p = .019$, $\eta^2 = .336$], P1 latency [$F(1,14) = 19.802$, $p = .001$, $\eta^2 = .586$], and P2 latency [$F(1,14) = 6.044$, $p = .028$, $\eta^2 = .302$].

Figure 14: Stimulus-locked event-related potential (ERP)



Note: Stimulus-locked ERP for the Intervention (upper) and Control (lower) group. The full line represents the Pre-bed rest, while the dotted line represents the Post-bed rest measurement. * markes a significant increase of P1 amplitude at the end of bed rest. † markes a significant reduction of P1 latency at the end of bed rest. ‡ markes a significant reduction of P2 latency at the end of bed rest.

Peak P1 amplitude:

Post-hoc analysis showed that the significant effect of time in peak P1 amplitude was driven by the Control group; while there was no significant change in the Intervention group ($p = .513$), participants in the Control group increased the peak P1 amplitude at the end of the bed rest (Pre-bed rest 1.47 ± 1.23; Post- bed rest 2.57 ± 1.35 μV, $p = .009$) (Figure

14). Additionally, the percent of change analysis showed that the peak P1 amplitude was increased for 32.17 ± 62.30 % in the Intervention group, while in the Control group the percent of change was 208.53 ± 268.84 %.

P1 latency:

Post-hoc analysis showed that P1 latency was significantly reduced in both groups. In the Intervention group the reduction was for -2.89 ± 2.36 % (Pre- bed rest 111.50 ± 21.61; Post-bed rest 108.25 ± 20.80 ms, $p = .010$), while in the Control group it was -2.26 ± 2.54 % (Pre-bed rest 111.50 ± 21.51; Post-bed rest 109.00 ± 21.41 ms, $p = .028$) (Figure 14).

P2 latency:

Post-hoc analysis showed that the significant effect of time in the P2 latency was driven by the Intervention group; while there was no significant change in the Control group ($p = .188$), participants in the Intervention group significantly reduced their P2 latency at the end of the bed rest (Pre-bed rest 235.25 ± 9.68; Post-bed rest 230.50 ± 9.12 ms, $p = .031$) (Figure 14). Additionally, the percent change analysis (Post to Pre measurement) showed that the P2 latency was significantly decreased by -1.99 ± 2.18 % in the Intervention group, while in the Control group the percent change was -2.09 ± 4.37 % but, however, non-significant.

Table 7: Results for stimulus-locked event related potentials

	Pre	Post	P_{TIME} (η^2)	P_{GROUP} (η^2)	$P_{TIMExGROUP}$ (η^2)
P1 amplitude (μV)			**.019 (.336)**	.206	.145
Intervention group	2.87 ± 1.77	3.16 ± 1.91	.513		
Control group	1.47 ± 1.23	2.57 ± 1.35 $^{\$}$	**.009 (.646)**		
P1 latency (ms)			**.001 (.586)**	.972	.571
Intervention group	111.50 ± 21.61	108.25 ± 20.80 $^{\$}$	**.010 (.640)**		
Control group	111.50 ± 21.51	109.00 ± 21.41 *	**.028 (.521)**		
N1 amplitude (μV)			.434	.354	.349
Intervention group	-2.57 ± 2.69	-3.33 ± 2.99			
Control group	-1.97 ± 1.48	-1.90 ± 1.63			
N1 latency (ms)			.206	.933	.466
Intervention group	167.25 ± 24.42	162.75 ± 19.33			
Control group	164.75 ± 21.00	163.50 ± 17.66			
P2 amplitude (μV)			.252	.183	.429
Intervention group	4.03 ± 2.71	4.18 ± 4.00			
Control group	1.82 ± 1.75	2.64 ± 2.24			
P2 latency (ms)			**.028 (.302)**	.762	.860
Intervention group	235.25 ± 9.68	230.50 ± 9.12 *	**.031 (.507)**		
Control group	239.25 ± 33.89	233.75 ± 30.70	.188		

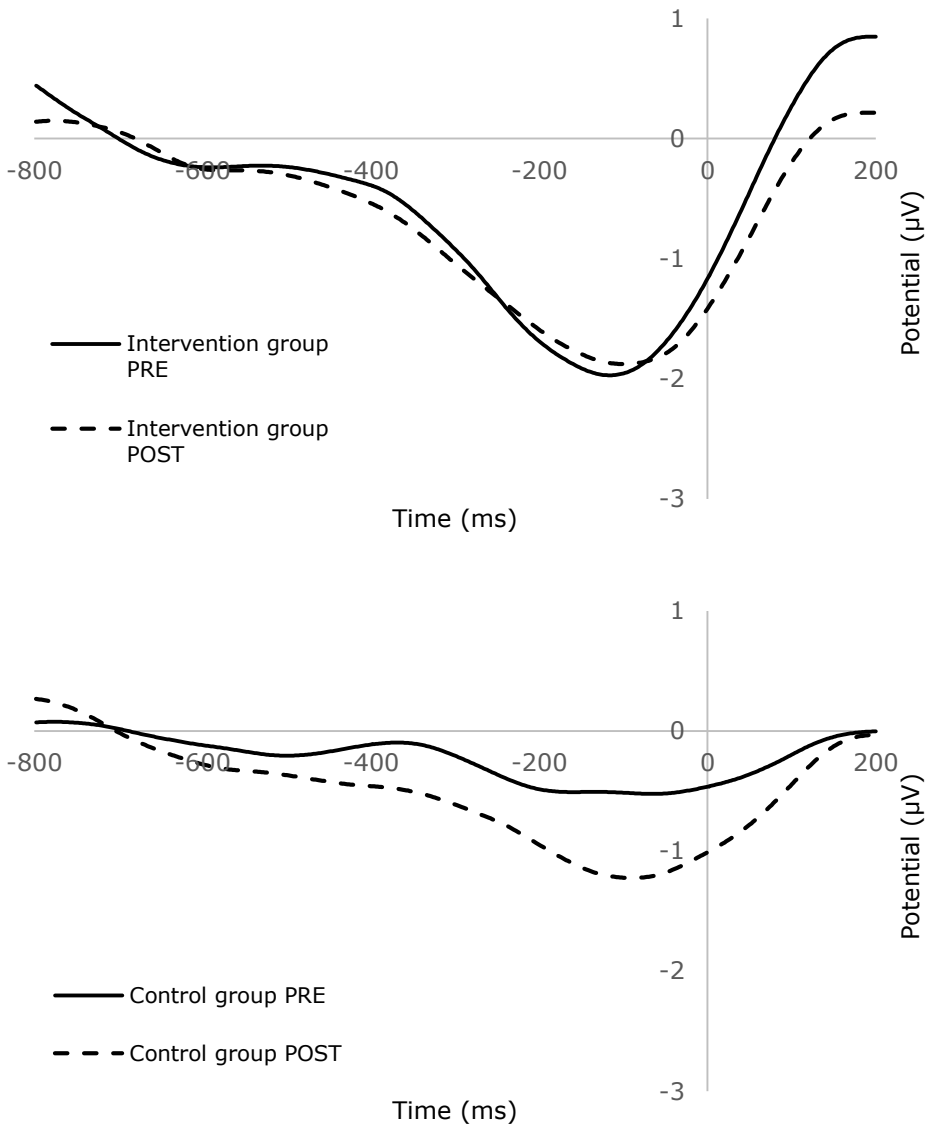
Note: All values are means ± SD unless otherwise stated; Pre-bed rest indicates day of baseline data collection; Post- bed rest indicates day of post-measurements immediately after 14-day bed rest period; μV represents micro volts; ms represents milliseconds; * - denotes statistical significant difference from PRE bed rest at $P < .05$; $^{\$}$ - denotes statistical significant difference from PRE bed rest at $P < .01$; The η^2 represents the effect size and is present only where significance level was reached; μV represents micro volts; ms represents milliseconds.

5.5.4 Motor-related processes (MRP) for finger tapping

The independent sample t-test revealed no significant changes in baseline measurements from motor-related processes (finger tapping) in maximum peak amplitude ($p = .226$) and latency ($p = .216$), onset ($p = .963$) and rise time ($p = .468$) between Intervention and Control subjects.

The 2-way RM ANOVA showed no significant time ($p > .191$), group ($p > .188$), and interaction ($p > .243$) effects for all parameters of motor-related processes or finger tapping.

Figure 15: Response-locked ERP for finger tapping task



Note: Response-locked ERP for finger tapping task for the Intervention (upper) and Control (lower) group. The full line represents the Pre-bed rest, while the dotted line represents the Post-bed rest measurement.

Table 8: Results from motor-related processes for finger tapping task

	Pre	Post	P_{TIME} (η^2)	P_{GROUP} (η^2)	$P_{TIMExGROUP}$ (η^2)
MRP peak amplitude (μV)			.235	.293	.495
Intervention group	-2.07 ± 1.97	-2.19 ± 1.99			
Control group	-1.11 ± 0.79	-1.55 ± 0.88			
MRP latency (ms)			.392	.188	.948
Intervention group	-120.75 ± 35.28	-131.50 ± 68.73			
Control group	-70.75 ± 99.84	-80.00 ± 87.17			
MRP onset (ms)			.191	.728	.339
Intervention group	-428,75 ± 120.10	-438.25 ± 128.67			
Control group	-425.63 ± 141.83	-484.00 ± 128.29			
MRP rise time (ms)			.266	.231	.243
Intervention group	308.00 ± 118.99	306.75 ± 125.94			
Control group	354.88 ± 132.18	404.00 ± 110.88			

Note: All values are means ± SD unless otherwise stated; Pre-bed rest indicates day of baseline data collection; Post- bed rest indicates day of post-measurements immediately after 14-day bed rest period; μV represents micro volts; ms represents milliseconds;

5.5.5 Motor-related processes (MRP) for foot tapping

The independent sample t-test revealed no significant changes in baseline measurements from motor-related processes (foot tapping) in maximum peak amplitude ($p = .215$) and latency ($p = .099$), onset ($p = .979$) and rise time ($p = .447$) between Intervention and Control subjects.

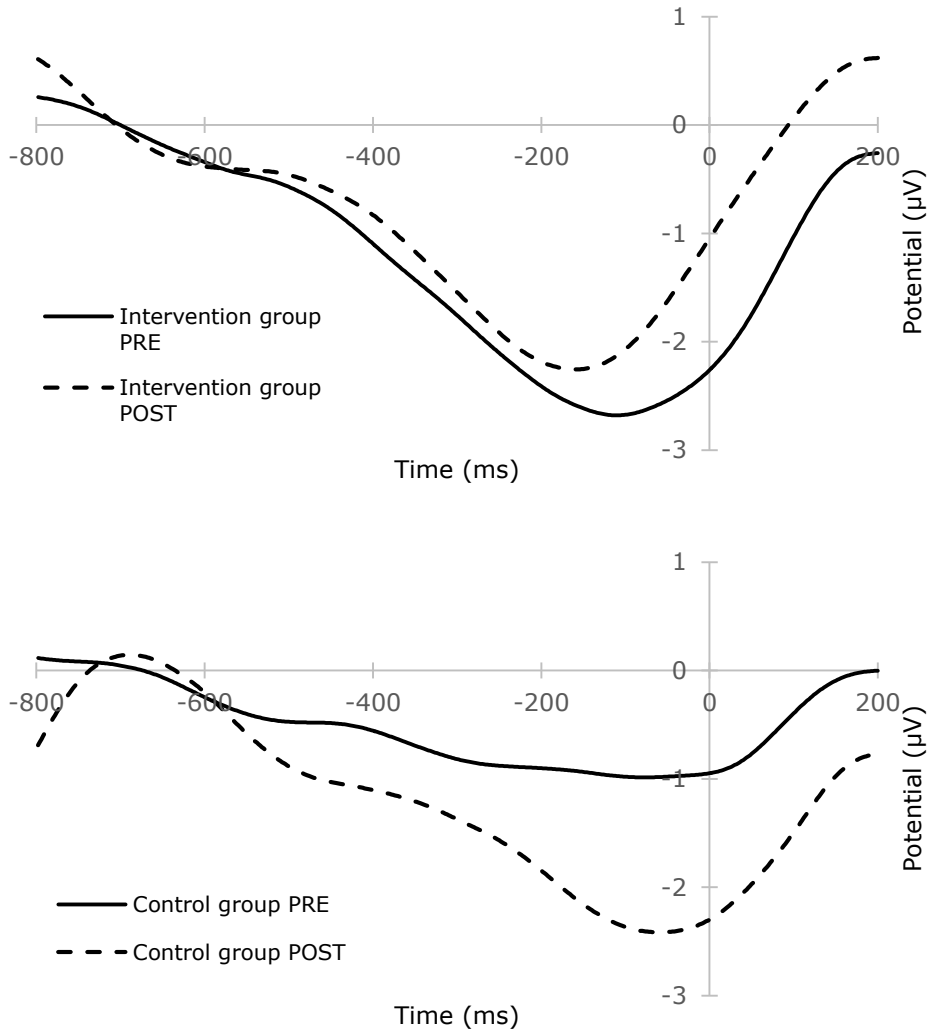
The 2-way RM ANOVA showed no significant time effects ($p > .154$) for all parameters. Furthermore, there was a significant interaction effect for MRP peak amplitude [$F(1,14) = 4.042$, $p = .064$, $\eta^2 = .224$], and significant group effect for MRP latency [$F(1,14) = 9.917$, $p = .007$, $\eta^2 = .415$] (see Table 9).

MRP peak amplitude:
Post-hoc analysis showed that there was a trend of time in the Control group ($p = .115$), while no significant time effect for the Intervention group ($p = .392$). Additionally, the percent of change analysis showed that the MRP peak amplitude was decreased for -10.71 ± 65.66 % in the Intervention group, while in the Control group it was increased for 148.35 ± 190.25 %.

MRP latency:

Post-hoc analysis showed no significant difference between Intervention and Control groups at the Pre-bed rest measurement (p = .099), while there was a significant difference between-groups at the Post-bed rest measurement (p = .005).

Figure 16: Response-locked ERP for foot tapping task



Note: Response-locked ERP for foot tapping task for the Intervention (upper) and Control (lower) group. The full line represents the Pre-bed rest, while the dotted line represents the Post-bed rest measurement.

Table 9: Results from motor-related processes for foot tapping task

	Pre	Post	P_{TIME} (η^2)	P_{GROUP} (η^2)	$P_{TIMExGROUP}$ (η^2)
MRP peak amplitude (μV)			.312	.673	**.064 (.224)**
Intervention group	-3.10 ± 3.07	-2.63 ± 2.97			
Control group	-1.64 ± 0.82	-3.14 ± 2.06			
MRP latency (ms)			.154	**.007 (.415)**	.205
Intervention group	-96.88 ± 61.29	-134.75 ± 56.21			
Control group	-54.50 ± 21.75	-56.88 ± 35.99 ≠			
MRP onset (ms)			.704	.668	.622
Intervention group	-533.38 ± 114.49	-536.38 ± 62.46			
Control group	-532.13 ± 59.55	-509.25 ± 81.93			
MRP rise time (ms)			.360	.213	.882
Intervention group	436.50 ± 132.39	401.63 ± 73.57			
Control group	477.63 ± 64.18	452.38 ± 94.56			

Note: All values are means ± SD unless otherwise stated; Pre-bed rest indicates day of baseline data collection; Post- bed rest indicates day of post-measurements immediately after 14-day bed rest period; μV represents micro volts; ms represents milliseconds;

5.6 Correlations between cognitive assessment, gait and EEG parameters

Statistically significant correlations between EEG, behavioral data of synchronization task, virtual maze and gait performance are reported bellow. All correlations were performed on 8 subjects from the Intervention and 8 from the Control group for all measures, with the exception of gait measurements where one subject was eliminated on a priory criteria (for details please see the methods section). Correlations were conducted with both groups together (N=16) for Pre- and separated for Intervention (N=8) and Control group (N=8) for Post-bed rest testing. Prior to the final reporting, all the correlations were checked visually with scatter plots for a possible outlier effect.

5.6.1 Correlations at PRE-bed rest testing

Correlations between EEG data and synchronization task at Pre-bed rest testing:

- No significant correlations were found.

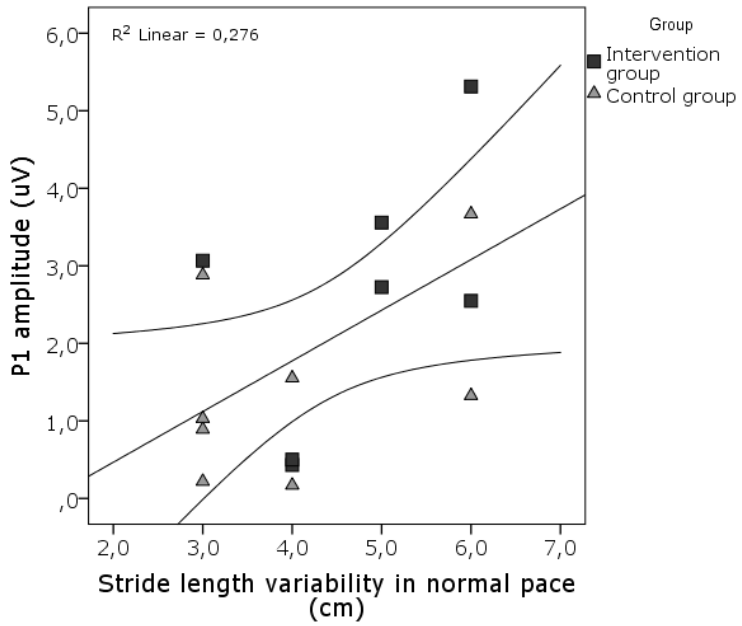
Correlations between EEG data and virtual maze learning task at Pre-bed rest testing:

- No significant correlations were found.

Correlations between EEG data and gait performance at Pre-bed rest testing:

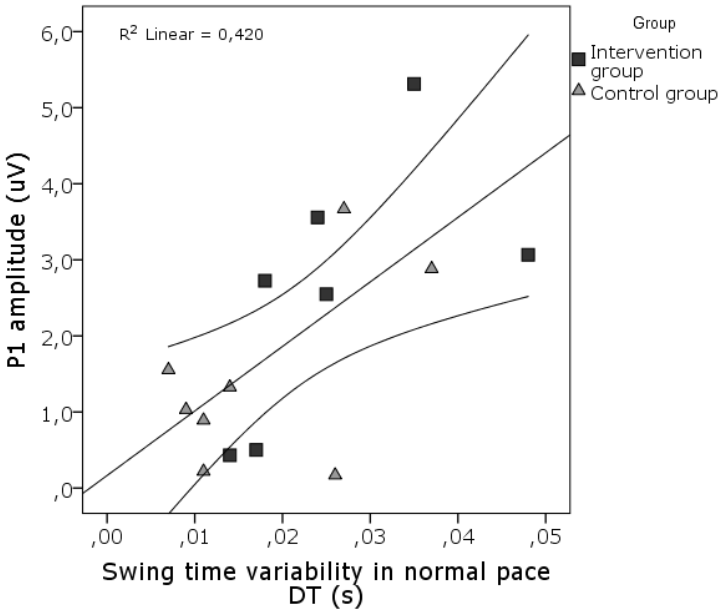
- P1 amplitude is positively correlated with stride length variability in normal-paced walking condition without dual-task (r =.526, p = .044);
- P1 amplitude is positively correlated with swing-time variability in normal-paced walking condition with dual-task (r =.648, p = .009);
- MRP rise time for foot tapping is positively correlated with stride length variability in face-paced walking condition with dual-task (r =.520, p = .047);
- MRP onset time for foot tapping is negatively correlated with swing-time variability in fast-paced walking condition without dual-task (r = -.546, p = .035);

Figure 17: Correlation between P1 amplitude and stride length variability in normal-paced walking condition without dual-task



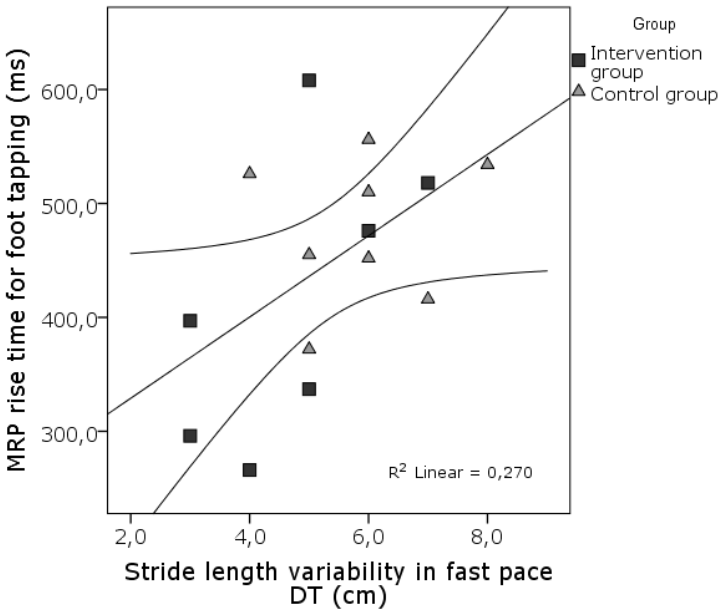
Note: Figure reporesents both groups combined at Pre-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

Figure 18: Correlation between P1 amplitude and swing-time variability in normal-paced walking condition with dual-task



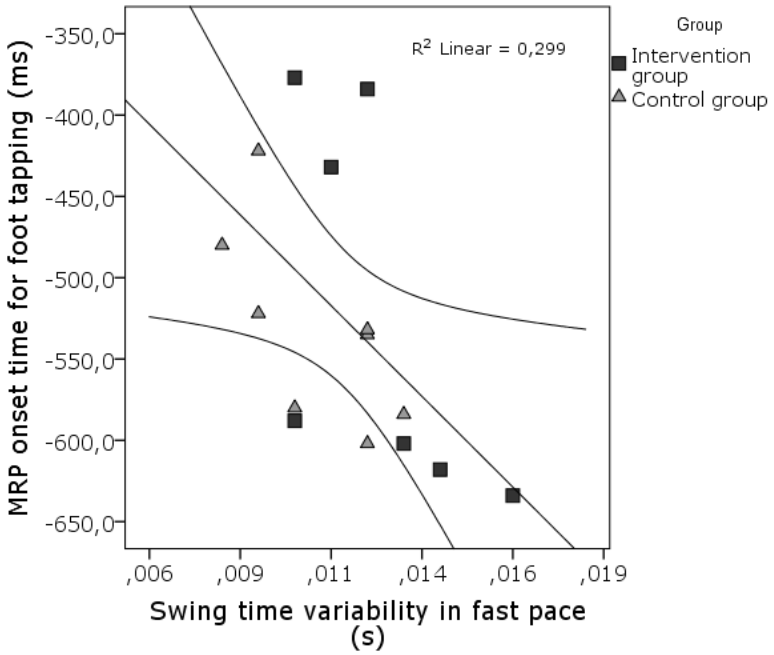
Note: Figure reporesents both groups combined at Pre-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

Figure 19: Correlation between MRP rise time for foot tapping and stride length variability in face-paced walking condition with dual-task



Note: Figure reporesents both groups combined at Pre-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

Figure 20: Correlation between MRP onset time for foot tapping and swing-time variability in fast-paced walking condition without dual-task



Note: Figure reporesents both groups combined at Pre-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

5.6.2 Correlations at POST-bed rest testing

Correlations for Intervention group between EEG data and synchronization task at Post-bed rest testing:

- No significant correlations were found.

Correlations for Control group between EEG data and synchronization task at Post-bed rest testing:

- No significant correlations were found.

Correlations for Intervention group between EEG data and virtual maze learning task at Post-bed rest testing:

- No significant correlations were found.

Correlations for Control group between EEG data and virtual maze learning task at Post-bed rest testing:

- No significant correlations were found.

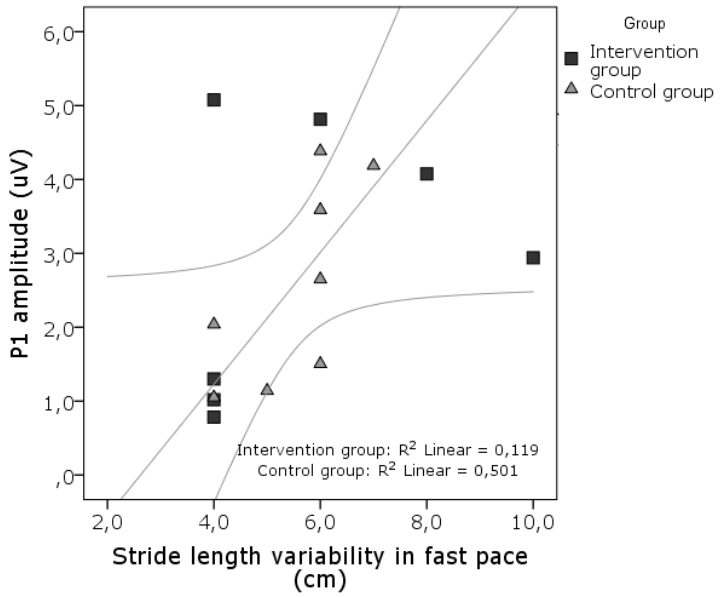
Correlations for Intervention group between EEG data and gait performance at Post-bed rest testing:

- No significant correlations were found.

Correlations for Control group between EEG data and gait performance at Post-bed rest testing:

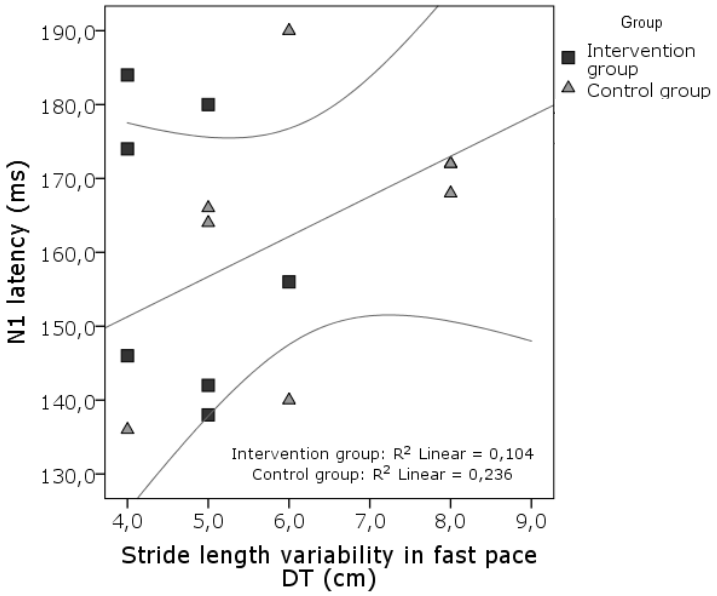
- P1 amplitude is positively correlated with stride length variability in fast-paced walking condition without dual-task (r = .708, p = .050);
- N1 latency is positively correlated with stride-length variability in fast-paced walking condition with dual-task (r =.713, p = .047);
- MRP amplitude for foot tapping is negatively correlated with swing-time variability in fast-paced walking condition with dual-task (r = -.789, p = .020);

Figure 21: Correlation between P1 amplitude and stride length variability in fast-paced walking condition without dual-task in the Control group



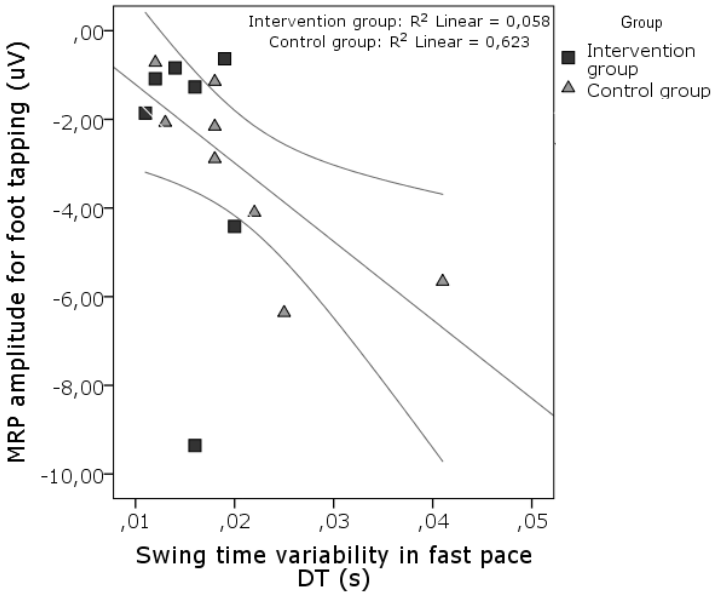
Note: Figure reporesents both groups combined at Post-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

Figure 22: Correlation between N1 latency and stride-length variability in fast-paced walking condition with dual-task in the Control group



Note: Figure reporesents both groups combined at Post-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

Figure 23: Correlation between MRP amplitude for foot tapping and swing-time variability in fast-paced walking condition with dual-task in the Control group



Note: Figure reporesents both groups combined at Post-bed rest measurement. Lines represent a linear fit line and the 95% confidence interval for the mean.

6 DISCUSSION

The structure of current PhD thesis covers three major parts. First, it evaluates the effectiveness of computerized cognitive training (CCT) with spatial navigation task during the 14-day bed rest period, 28-day rehabilitation process and 400-day after the study. Secondly, it deals with a possible transfer of CCT to subsequent motor output/gait performance after the 14-day bed rest; and third, it covers a possible transfer of CCT to the subsequent brain electrocortical activity, containing behavioral foot and finger tapping paradigm, baseline EEG frequency analysis, and stimulus- and response-related evoked electrocortical potentials.

The motivation for undertaking this study was triggered by an observation that after a period of immobilization or bed rest a significant decline was detected on the functional (e.g. Kortebein et al., 2008) as well as cognitive (e.g. Lipnicki & Gunga, 2009) domain. Commensurate with previous research (Akima et al., 2000; Berry, Berry, & Manelfe, 1993; Coker, Hays, Williams, Wolfe, & Evans, 2015; Ferrando, Stuart, Brunder, & Hillman, 1995; Ferretti et al., 1997), our data showed a significant decline in volume of the quadriceps femoris muscle (see Figure 5), knee extension MVC, and VO_2max after 14-day bed rest in older adults *(results are in submission to the Journal of the American Medical Association)*.

Therefore, the aim of the present study was to evaluate if CCT incorporating spatial navigation could be an effective technique for reducing/preventing negative effects of 14 days of bed rest in older men.

6.1 The effectiveness of CCT with spatial navigation task

To the best of our knowledge, this is the first study to apply a cognitive training intervention during prolonged physical inactivity in a highly controlled lab environment. The generally expected worsening in cognitive functioning after the bed rest was not detected in our study (Marušič, Kavcic, Moffat, Petrič, Dolenc & Pišot, *submitted*). Participants in the Control group did not experience cognitive decline but rather a non-significant learning effect on the subsequent times of measurement. Lipnicki & Gunga (2009) reviewed cognitive functioning during bed rests and found 17 studies which reported significant detrimental effects, six with unchanged cognitive functioning and three with significant improvements in cognitive performance at the end of bed rest. Similar to our case, task

exposure and practice effects could mask the underlying detrimental effect of bed rest on cognitive functioning (Lipnicki & Gunga, 2009).

Moreover, CCT intervention significantly improved performance in virtual maze navigation compared to controls where The effectiveness of this virtual navigation training persisted for 400 days after the study. Commensurate with prior research, we found that CCT significantly increased the performance in virtual maze testing, and therefore improved specifically targeted cognitive abilities (Ball et al., 2002; Ball et al., 2007; Klusmann et al., 2010; Kueider et al., 2012; Lampit et al., 2014; Pressler et al., 2011; Willis et al., 2006). Participants in the Intervention group significantly reduced their number of errors and distance travelled when tested at the end of bed rest. The effects the of CCT intervention were also present 28 and 400 days after the intervention in terms of errors made in the virtual maze, which support previous research findings of different other CCT interventions based on standard (non-bed rest) protocols (Ball et al., 2002; Papp et al., 2009; Tardif & Simard, 2011). The transfer of CCT was observed in one subdomain of the computerized CogState tests, measuring attention and processing speed which, however, was not a part of the PhD thesis and was discussed elsewhere (Marušič, Kavcic, Moffat, Petrič, Dolenc & Pišot, *submitted*). An additional important component of this study is that the sustained improvement in spatial navigation can be attributed to CCT training itself, since the potential confounding factors through our physical immobility and dietary control paradigm were controlled as closely as possible.

In the past decade, several reviews have discussed the beneficial effects of cognitive interventions in healthy older adults (Kueider et al., 2012; Lampit et al., 2014; Martin et al., 2011; Papp et al., 2009; Tardif & Simard, 2011; Valenzuela & Sachdev, 2009). Each concluded that cognitive intervention can be effective in improving various aspects of objective cognitive functioning, such as memory performance, executive functioning, processing speed, attention, fluid intelligence, and subjective cognitive performance. These reviews also showed that cognitive interventions varied in terms of sessions (from 3 to 180) while the durations of the intervention sessions lasted from 0.5 to 4 hours. A recent systematic review and meta-analysis reported that sessions either shorter than 30 minutes or more frequent than 3 times per week are ineffective (Lampit et al., 2014). This can be supported by a study which reported that synaptic plasticity occurs after 30 minutes of stimulation (Luscher, Nicoll, Malenka, & Muller, 2000), on the other hand another study has suggested that cognitive fatigue can interfere with training gains (Holtzer, Shuman, Mahoney, Lipton, & Verghese, 2011). Interestingly, even if our intervention was more frequent (12 sessions in 12 days) compared to other research studies, participants in the

Intervention group reported the same level of mental demands during the bed rest as compared to the controls. This could be interpreted as the fact that participants in the Control group were instructed to watch documentaries (in a separate room) at the same time and for the same amount of time and as such served as active controls. In addition, both groups had frequent contact with medical staff, researchers, and participants could freely communicate with each other, read newspaper or use their own computers. Therefore, we believe that during the bed rest study, the two groups did not suffer differences in cognitive fatigue.

The spatial navigation task used for the CCT was selected due to widespread network of brain structures involved in spatial navigation, such as hippocampus, parahippocampal gyrus, posterior cingulate gyrus, parietal lobes and prefrontal cortex (Ekstrom et al., 2003; Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000; Lovden et al., 2012). Spatial navigation was found to include multiple cognitive domains as well, such as spatial skills, explicit memory, working memory and executive processes (S. D. Moffat, 2009). Lovden and colleagues (2012) used spatial navigation for cognitive training purposes. The intervention group showed a significant improvement in spatial navigation task performance and stable hippocampal volumes as compared to the controls that showed expected age-related decline in the hippocampal volume (Lovden et al., 2012). Moreover, training-related gains were observed as long as 4 months after the study. Authors concluded that by engaging in such spatially challenging tasks might preserve their spatial cognition and its neural substrates (Lovden et al., 2012). Another study that targeted spatial abilities, reported improvement in related virtual maze task (Hotting et al., 2013). Same authors reported that participants in the spatial navigation training showed lower brain activations in the hippocampus and parahippocampal gyrus as well in frontal and temporal brain areas after the training (Hotting et al., 2013). Similar assumptions of more efficient neural processing after the spatial engagement might be done for our Intervention group that showed training-related gains up to 400 days after the intervention.

The bed rest paradigm presents unique opportunities for the investigation of cognitive training since it allows for complete control of physical activity, diet, social interactions and other factors during the study. However, it is not yet well understood how the prolonged bed rest itself affects cognition per se. As reviewed in the Introduction section, there are mixed reports regarding the effects of the bed rest on cognitive function. Admittedly, most of the bed rest studies were performed with younger participants, so it can be expected that the effects of bed rest on cognition on these individuals differs from the effects on older adults. Next to it, in such a short period of time task exposure and practice effects

could mask the underlying detrimental effect of bed rest on cognitive functioning as well (Lipnicki & Gunga, 2009). In our study we did not find a decline in performance on virtual maze navigation task in the Control group which, besides 45 minutes of passively watching documentaries on the television, did not have any other physical or cognitive obligations. The lack of cognitive decline in the Control group could be partially explained by rich activities during bed rest: participants had at least two medical staff visits per day, unlimited verbal interactions with other participants, a possibility to watch a TV or use internet on their own computers, and daily contacts with researchers and the possibility of visits by family members. Regardless of involvement in the CCT intervention, participants from both groups reported the same level of mental, physical, temporal and total workload, as well as same performance, effort and frustration level during the bed rest study (see Table 3).

6.2 Generalization of CCT to the motor output domain

When investigating the effects of CCT on motor output domain/gait performance, participants in the Intervention group who underwent 12 sessions of virtual spatial navigation training showed no significant reduction in gait speed after bed rest when compared to controls. Moreover, in contrast with our expectations, the Intervention group showed a non-significant increase in the normal-paced walking condition with dual-task gait at the end of 14-day bed rest as compared to pre-mean values. Together, these results support the hypothesis that CCT was a protective intervention to the detrimental effects in mobility generally observed following bed rest (Dupui et al., 1992). Whereas most of the studies applying cognitive training have evaluated effectiveness in the same cognitive domain in which the training occurred (Ball et al., 2007; Karbach & Kray, 2009; Pressler et al., 2011; Schmiedek, Lovden, & Lindenberger, 2010; Willis et al., 2006), results for CCT in our study demonstrated a generalization to the mobility domain. Our findings are in general agreement with those of Verghese et al. (2010), who demonstrated that CCT improved mobility (in a non bed rest setting) in sedentary seniors in both normal and dual-task walking conditions. This study suggested that CCT could be one of non-pharmacological interventions for modifying gait performance, especially during most demanding dual-task walking conditions (Verghese et al., 2010). Other studies that used non-physical types of training and were performed on non-healthy older individuals, such as demented patients (Schwenk, Zieschang, Oster, & Hauer, 2010) and patients with Parkinson's disease (Mirelman et al., 2010), reported a significant improvement in walking abilities as well.

The relationship between walking alone and walking while performing a secondary task was often reported with dual-task effects (Bock & Beurskens, 2011; V. E. Kelly et al., 2010; Krampe, Schaefer, Lindenberger, & Baltes, 2011; Lindenberger et al., 2000; Sparrow, Begg, & Parker, 2006). In the case of our study, a significant improvement in DTEs (a switch from dual-task costs to benefits) in gait speed with normal pace walking during post bed rest was found. In the fast pace walking condition, the post-intervention reduction of negative DTEs was marginally non-significant. Participants in the Control group, however, showed approximately the same level of negative DTEs in Pre- as compared with Post-bed rest, in normal as well as in fast pace walking condition. According to the Yerkes-Dodson law (Yerkes & Dodson, 1908) and extensive later research (Deviterne, Gauchard, Jamet, Vancon, & Perrin, 2005; V. E. Kelly et al., 2010; Riley, Baker, & Schmit, 2003; Vuillerme, Nougier, & Camicioli, 2002), the relationship between postural/mobility control and cognitive demand in dual-task situations could be explained as an inverted U-shaped curve. Positive DTEs which were found only at the end of bed rest in our Intervention group could therefore represent a low demand of secondary task, higher arousal level under more challenging dual-task condition or that cognitive task directed attention away from walking, which was showed not anymore as dual-task costs, but as a dual-task benefits.

Furthermore, inspection of basic gait data shows that the only statistically significant reduction of gait speed was found in the Control group, in the more attention-demanding fast pace dual-task walking condition. This is the condition in which the ameliorating effect was most evident for the Intervention group. Studies examining gait performance after bed rest are scarce. Younger adults undergoing 30 days of head-down-tilt bed rest have shown impaired gait performance, which resulted in reduced gait speed, stride length and balance stability (Dupui et al., 1992). Together, they suggested that bed rest-induced sensorimotor changes are involved in the decrease of gait and balance performance and have shown a link between cardiovascular and sensorimotor deconditioning (Dupui et al., 1992). However, the aforementioned study is not directly comparable due to the nature of bed rest (head-down-tilt which is more extreme position comparing to the horizontal or supine position), including the taget participants (younger vs. older) and the duration of bed rest (30- vs. 14-day bed rest).

During gait performance, balance control, and consistency of the stepping pattern are reflected in gait variability parameters which are frequently reported as within-subject standard deviation, or coefficient of variation (Moe-Nilssen, Aaslund, Hodt-Billington, & Helbostad, 2010; Verghese et al., 2009). These fluctuations are low in healthy adults and increased in patients with syndromes such as falling, frailty, and neuro-degenerative

diseases, such as Parkinson's (Blin, Ferrandez, & Serratrice, 1990) and Alzheimer's (Sheridan, Solomont, Kowall, & Hausdorff, 2003). Our results showed that gait performance, in terms of variability, was shown to be affected only in the Control group after the bed rest. More specifically, participants in the Control group showed a significantly higher swing time variability at the end of bed rest in the most demanding walking condition (fast pace walking condition with dual-task) as compared to the Intervention group. For instance, gait variability was increased only in elderly fallers, but not in elderly non-fallers and young adults (Springer et al., 2006). Commensurate with previous results from a non-bed rest study where elderly non-fallers and young adults maintained a stable gait under different dual-task conditions (Springer et al., 2006), which after the bed rest our participants in the Control group did not, raises a question of increased risk for falls after prolonged bed rest without countermeasure in older adults.

6.3 Generalization of CCT to the brain electrocortical activity

We reviewed the existing literature regarding brain electrocortical changes that occur during extreme circumstances such as space and parabolic flights, as well as ground based model of microgravity such as bed rest (Marusic et al., 2014). In summary, results showed that bed rest reduced EEG (increase in theta power) while this was reversed in the intervention group (increased power in Beta 2 range). The reduction in EEG caused by bed rest may reflect diminished cognitive functioning while the increase is indicative of greater alertness and attention in intervention group. Moreover, an increase in Theta power is characteristic in subjects with variety neurological disorders and for the late part of the life span such as aging process (Klimesch, 1999). Different effects were observed for those individuals involved in CCT: in the intervention group we observed a decrease in Theta power and an increase in Beta 2 power. These findings are indicative of increased attentional capacity (MacLean, Arnell, & Cote, 2012). Due to the scarce literature in the field of EEG frequency analysis and bed rest, a direct comparison is not possible. However, two abstracts from Chinese authors reported findings of a six days head down tilt (HDT) bed rest on human EEG spectral changes in younger adult men. They reported that Alpha power increased during the bed rest, while peak frequency of EEG gradually slowed down. Beta 1 and Theta rhythms increased in the frontal regions and reached maximum power on the 3rd day. The authors concluded that "there exists a potential influence of bed rest on brain functioning, headache can be easily induced and that brain adaptation function decrease" (Han et al., 2001; Han et al., 2002). The following paragraphs focus on

behavioral, stimulus- and response-related EEG changes that occurred at the end of the 14-day bed rest in older adult men.

6.3.1 Behavioral data

When evaluating the generalization of CCT on other motor-related processes, there were, however, no significant interaction effects on either the finger or foot tapping task. More specifically, neither bed rest, nor CCT, had an effect on the measures of synchronization tasks. Stable synchronization rate obtained in all analyzed parameters could be due to a not-enough-demanding task. Namely, sensorimotor synchronization is commonly studied by synchronization tapping paradigm (Drewing, Aschersleben, & Li, 2006; Repp, 2005; Repp & Su, 2013). In this paradigm the complexity of sensory and motor processes is reduced to a minimum, enabling participant to focus properly on the external stimuli (Repp & Su, 2013). If the intervals are constant and from 399 to 1500 milliseconds long, this represents the simplest case for sensorimotor synchronization task (Drewing et al., 2006). In our case, the inter-stimulus intervals were constant and exactly 1000 milliseconds long therefore not-enough challenging task to be affected in any of the two groups at the end of the bed rest.

6.3.2 Stimulus-related processes

To the opposite of behavioral data while finger and foot tapping, there were a few significant time, group and interaction effects between Intervention and Control group while analyzing stimulus- and motor-related electrocortical processes. For stimulus-locked event-related potentials (s-ERP), there was a significant increase in P1 peak amplitude after the bed rest in the Control group, while this was not the case in the Intervention group, which remained stable peak values of P1 component. Increased P1 peak amplitude can reflect a higher activation level needed after the bed rest to compensate for the same speed and intensity of early perceptual mechanisms (E. Amenedo & Díaz, 1998). This finding is comparable with aging studies, which examined age-related change in the stimulus processing and reported enhanced P1 peak component in older vs. younger participants (De Sanctis et al., 2008; Falkenstein et al., 2006; Yordanova et al., 2004). Interestingly, P1 latency was significantly reduced in both groups with a higher effect size observed in the Intervention group. Shorter P1 latencies in both groups at the end of the bed rest probably reflects higher attentional levels to the forthcoming stimulus, which can

modulate the processing of visual information as reflected with shorter P1 latencies (Schuller & Rossion, 2001). Next, N1 amplitude, latency, and P2 amplitude did not change after the bed rest in both groups, while P2 latency was significantly reduced just in the Intervention group. This finding might reflect the generalization of CCT on other previously mentioned cognitive functions, in particular enhanced working memory (Finnigan et al., 2011; Lefebvre et al., 2005). Results on aging studies and P2 latency parameter are mixed; some studies have shown increased P2 latencies (Goodin, Squires, Henderson, & Starr, 1978; Iragui, Kutas, Mitchiner, & Hillyard, 1993; A. Pfefferbaum, Ford, Roth, Hopkins, & Kopell, 1979; A. Pfefferbaum, Ford, Roth, & Kopell, 1980; Adolf Pfefferbaum, Ford, Roth, F. Hopkins Iii, & Kopell, 1979), while others have reported no significant age-related changes (E. Amenedo & Díaz, 1998; Elena Amenedo & Díaz, 1999). Studies analyzing age-related changes in P3, which presumably reflects speed of task-related stimulus classification (Polich, 1998), have found a decreased amplitude and increased latency of this component (Polich, 1997a, 1997b, 2007). However, in our case we were not able to properly extract a P3 component. This could be interpreted that our participants needed to synchronize with visual stimuli instead of react, therefore P3 (which reflects later stages of stimulus processing), was less evident or undetectable. Other ERP studies that were conducted (in terms of changing body position or short-term head-down-tilt bed rest) were focusing on pain-related somatosensory processing (Fardo, Spironelli, & Angrilli, 2013; Spironelli & Angrilli, 2011) or emotional cortical processes (Messerotti Benvenuti, Bianchin, & Angrilli, 2013) that are not directly comparable with our results. From the other point of view of ERP perceptual components and effects of physical activity, studies have exanimated mostly P3 component (Berchicci, Lucci, Perri, Spinelli, & Di Russo, 2014; Hillman, Weiss, Hagberg, & Hatfield, 2002; Kamijo & Takeda, 2009; Winneke, Godde, Reuter, Vieluf, & Voelcker-Rehage, 2012). Results for P3 showed enhanced amplitude and delayed latency in sedentary young and older adults while this was reversed in both groups who reported higher levels of physical activity (Hillman et al., 2002). Again, direct comparison to our results is not possible due to the lack of existing literature on early perceptual ERPs and absent P3 component in our data.

6.3.3 Motor-related processes

Furthermore, when analyzing response-locked event-related potentials (r-ERP), there were no significant changes for finger tapping. To the contrary, r-ERP for foot taping revealed a significant interaction effect between two groups. The increased peak amplitude in foot tapping in the Control group is in agreement with age-related changes between

younger and older participants found in a previous study (Falkenstein et al., 2006). The increased r-ERP amplitude in the control group could be interpreted as reflecting compensatory mechanisms enabling participants in Control group to maintain motor performance at the comparable level at post- as compared to pre-bed rest. The significant effects obtained for foot but not finger r-ERP appears to be logical since it is well established that the bed rest affects to greater degree lower as compared to upper extremities. This finding additionally supports that bed rest does not affect only peripheral muscular processes but most likely also motor control processes.

Correlations between EEG data and gait performance:

When evaluating correlations among EEG and gait data at pre bed rest testing, most of the significant correlations were found between EEG data (more specifically, P1 peak amplitude of visual modality and MRP peak response of foot tapping) and gait variability parameters, whereas no basic gait parameter was correlated with EEG data. The correlations are reasonable due to the fact that impaired mobility is reflected with higher variability in walking performance (J. M. Hausdorff, 2005; Jeffrey M. Hausdorff, Rios, & Edelberg, 2001), and enhanced P1 peak amplitude can reflect age-related changes in early stages of visual processing (Falkenstein et al., 2006; Yordanova et al., 2004). Interestingly, visual modality was correlated with normal-paced walking condition, while MRP rise and onset time for foot tapping were correlated as well with gait variability parameters but only with fast-paced walking condition. The latter might be explained with fast pace walking variability being more sensitive to cortical neuroelectric activity. In other words, for fast motor activity participants were instructed to walk to the best of their current capabilities, which was then correlated to MRP parameters of foot tapping. It might be that in the normal pace walking condition, there is intrusion of some other processes that somewhat interfere with MPR, therefore there was no significant correlation.

Throughout post bed rest testing there were no significant correlations for the Intervention group between EEG data and gait performance. On the other hand, in the Control group there were significant correlations between N1 latency and P1 amplitude, and stride length variability in fast-paced walking condition with and without dual-task, respectively. Participants in the Control group who were more negatively effected by bed rest, as showen by longer N1 latency and higher P1 amplitude, had higher variability in fast pace walking performance. Increased N1 latency and enhanced P1 peak amplitude are characteristic for age-related changes (De Sanctis et al., 2008; Falkenstein et al., 2006;

Yordanova et al., 2004). Thus, the results show that bed rest "aged" the brain processes in the Control group moreso than the Intervention group, and it can thus be concluded that the Control group effects were similar to processes that take place during the normal aging process.

Overall, the EEG data acquired during synchronization tasks suggest that bed rest negatively affects brain functioning and accelerates correlates of brain aging as indicated by increased P1 peak amplitude and increased MRP peak amplitude while foot tapping in the Control group. Bed rest presumably engages/stimulates compensatory central mechanisms to maintain motor performance after the bed rest at the same level shown as enhanced MRP peak amplitude in the Control group. To the contrary, it appears that CCT is a beneficial intervention, as indicated by reduced P2 latency in the Intervention group after bed rest (table 7 in the result section). Such changes could be indicative of an improvement in working memory (Finnigan et al., 2011; Lefebvre et al., 2005).

6.4 Interpretation of underlying mechanisms

The effectiveness of CCT with the virtual spatial navigation to mitigate bed rest effects may relate in whole or part to the following potential mechanisms: CCT improved cognitive functions specifically involved with facilitating dual-task performance (e.g., executive functioning, speed of processing) and/or spatial navigation training engaged brain areas involved in mobility and hence "rehearsing" walking while the patient was immobilized/in bed rest. It has been well established that success in spatial navigation is associated with superior spatial memory, speed of processing, as well as executive functions (S. D. Moffat, Kennedy, Rodrigue, & Raz, 2007), and that during normal walking and especially in dual-task walking condition, similar cognitive processes are engaged (Giordani & Persad, 2005). Thus, CCT may have been most successful during the dual-task walking condition because efficient dual-task walking requires attention, appropriate speed of processing, and executive functions that are trained by the CCT (Verghese et al., 2010). This is also due to the fact that frontal subcortical circuits overlapping the circuits of executive control and attention functions are those that are responsible for gait control (Parihar, Mahoney, & Verghese, 2013). Furthermore, virtual spatial navigation has also been shown to activate neural structures involved in mobility (Lovden et al., 2012; S. D. Moffat et al., 2007). As already described in details above, Lovden and colleagues reported that sustained spatial navigation training mitigates age-related declines of hippocampal volume (Lovden et al., 2012), a critical brain structure for long and short term memory and one closely involved

in spatial navigation. The fact that the control group actually slowed in the dual-task condition, which the intervention group did not, supports the finding that CCT was effective in mitigating the effect of bed rest. The fact that this pattern was not evident in the normal pace walking most likely suggests that this condition did not represent a sufficient enough challenge to bring out the differential effects of training (Lindenberger et al., 2000).

Another possible mechanism is that cognitive training could have activated brain structures involved in mobility and walking, which, in turn, could affect peripheral blood flow. For instance, imaging studies have shown that the same premotor cortex and supplementary motor areas that are involved in real walking are activated during mental imagery of movement suggesting a close neurophysiologic link between locomotion and its mental imagery (Iseki et al., 2008; Wagner et al., 2008). Moreover, motor imagery and active observation of movement together were shown to promote motor learning and are highly advised to be implemented during periods of inactivity or immobilization (Taube, Lorch, Zeiter, & Keller, 2014). Indeed, both observations of walking and virtual walking produce similar, and even higher, levels of cerebral activation compared to real walking (Yinlai et al., 2012). These findings suggest that our CCT emphasizing virtual movement may activate the same neural systems involved in mobility. In turn, activation of cerebral structures involved in the walking may to same degree also generate subliminal or even supraliminal muscle contractions/activation, which in turn may affect blood flow to the muscles. In the study of Yinlai et al. (2012) authors discussed the importance of mental representation of locomotion for faster rehabilitation process and highlighted the importance of diversity of stimuli for a higher oxygenation of the brain (Yinlai et al., 2012). In the other words, the task must be challenging enough to not decrease the activation of the brain due to adaptation to the presented stimuli. The continuous increasing difficulty of our CCT within the bed rest period may represent a continuous higher activation of the brain circuits and its oxygenation compared to our controls who passively observed the documentaries on the television for the same amount of time.

6.5 Limitations and future research

While contributing to the study of the cognitive training and spatial navigation during bed rest our study has methodological limitations.

The bed rest paradigm imposed also disadvantages in our cognitive training study. A limiting factor is that bed rest imposes very specific demands on cognitive training: i)

training has to be accomplished within the timeframe of the bed rest protocol which is, in most cases, of relatively short duration and thus requires massed rather than distributed practice ii) the training itself has to be conducted in supine position and iii) bed rest may result in digestive upset, boredom or higher frustration levels in comparison to the traditional CCTs. In addition, it is necessary to keep in mind that bed rest studies are very costly, labor intensive, and limited by hospital/institutional capacity. Consequently, our study was based on relative small sample size – 16 men and has thus limited generalizability.

Additional shortcomings of our study are the utilization of exclusively male participants, since sex differences in navigation have been observed (Gron et al., 2000; Scott D. Moffat, Hampson, & Hatzipantelis, 1998; Sandstrom, Kaufman, & Huettel, 1998). Admittedly, the majority of bed rest studies have been conducted on male participants although more recent research is investigating females participants as well (Demiot et al., 2007; Edgell et al., 2007; Guinet et al., 2009; Kortebein et al., 2008).

The reason why we did not include participants older than 65 years is the requirement from the National Medical Ethics Committee, which allowed us to perform this research only with mid-older adult men aged younger than 65 years due to concerns that elderly men could be more susceptible to the increased detrimental effects of prolonged bed rest; however, none of our participants developed any serious medical conditions after 14-day of bed rest, suggesting that older participants could be recruited safely for the future studies.

While planning future investigations, the following suggestions might be considered as well: i) in the present study, we did not follow the number of subtracted numbers and errors made by participants during the dual-task walking conditions. The instructions about focus on either walking or cognitive task may partially explain the positive dual-task effects obtained at the end of the bed rest in our Intervention group (V. E. Kelly et al., 2010). Additionally, before measuring walking with additional dual-task is it recommended to measure the secondary task in seated position (Springer et al., 2006); ii) The 2D Optogait system, which was not available during the period of our bed rest campaign, would be of additional help to analyze gait parameters such as gait width and gait width variability to investigate the stability of gait performance before and after the bed rest; iii) while analyzing behavior data of tapping task we detected some of participants reacting to the task of synchronization with the response to the visual stimuli rather than trying to synchronize their tapping to 1 Hz rhythm. Instructions must be administrated with a high

focus, following the subject performing subsequent task and explaining task rules again if needed, especially when working with older adults.

7 CONCLUSIONS

Improving patients' mobility after prolonged physical inactivity or immobilization can affect multiple areas of quality of life, including a faster return to premorbid daily functioning. Patient-based evaluations of CCT will also be important, as it has been reported that older hospitalized patients, despite an ability to walk independently, spent most of their time lying in bed, and therefore may represent a group particularly at risk for bed rest effects (Brown, Redden, Flood, & Allman, 2009). In addition, CCT effects may be specifically beneficial in terms of decreasing the risk of falls immediately after bed rest or immobility by leading to a faster return to pre-immobilization activity (E. L. Bouldin et al., 2012). Falls among patients in hospital and long-term care facilities are common, in part related to prolonged immobilization and poorer executive functioning (Herman, Mirelman, Giladi, Schweiger, & Hausdorff, 2010), leading to increased cost for health services (E. L. D. Bouldin et al., 2013). Prevention of falls in these situations may represent an important facet of patient safety and public health. Kortebein et al. (2008) have recommended that bed rest days in hospitals and/or nursing homes must be limited as much as possible in older adults. In addition to the cognitive training, other interventions such as resistance exercise and nutritional or pharmacological intervention could be pursued and applied, as suggested also by same authors (Kortebein et al., 2008).

In conclusion, to our knowledge, this is the first bed rest study that directly evaluated the effectiveness of virtual spatial navigation-based CCT in older adult men. Our findings provide new information regarding mitigation of detrimental effects of bed rest or prolonged immobilization/inactivity, especially those related to a higher task complexity. Overall, this study provides empirical evidence that cognitive intervention during the absence of physical activity can be effective and may be transferable. In contrast with other CCT studies, in our study, physical activity/inactivity and food intake across the whole duration of cognitive intervention was controlled. Thus, significant duration and generalizability effects could not be associated with aerobic activity or foot intake. Despite the small sample size, the current study provides important evidence that CCT may be effective in mitigating the detrimental effects of bed rest and emphasizes the generalization of CCT to a distal untrained domain, such as gait performance, and stimulus- and response-related electrocortical processes. Future studies should replicate our findings and evaluate the effects of different approaches to CCTs on mobility in older females and males, as well as with patients in hospital settings. Outcomes of this study provide empirical validation for the effectiveness of cognitive intervention during physical inactivity and thus, may provide support the development of similar intervention programs, as a part of

rehabilitation protocols applied during long-term inactivity caused by aging, illness, sedentary jobs and lifestyle, or even space flights.

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POVZETEK V SLOVENSKEM JEZIKU

1 UVOD

Po podatkih Svetovne zdravstvene organizacije (World Health Organisation, WHO) je gibalna neaktivnost vzrok za do 3,2 milijona smrti letno in s tem četrti najpogostejši dejavnik tveganja, za visokim krvnim tlakom, uporabo tobaka in visoke ravni glukoze v krvi (WHO, 2010). Delež starejših ljudi se na svetovni ravni povečuje in napovedi kažejo, da se bo do leta 2030 podvojil, pri čemer se bo do leta 2050 življenjska doba podaljšala za 10 let (Centri za bolezni in preventivo, 2003). Skladno s Poročilom o staranju Evropske unije bo število ljudi, ki živijo v Evropi in so starejši od 65 let povečalo s 85 milijonov leta 2008 na 151 milijonov v letu 2060. Poleg tega se bo število zelo starih oseb (≥80 let) potrojilo, z 22 na 61 milijonov od leta 2008 do leta 2060 (Evropska komisija, 2009). Ob trendu staranja in nezdravega načina življenja prebivalstva lahko pričakujemo tudi povečanje povpraševanja po storitvah v javnem zdravju (Deary idr., 2009). Omenjene statistične napovedi kličejo po novih raziskavah o zdravem staranju in, temelječ na teh,razvoju novih intervencij za krepitev tako funkcionalnih kot tudi kognitivnih sposobnosti starejših odraslih.

Trenutno obstajajo dokazi, na podlagi katerih lahko zaključimo, da je redna gibalna/športna aktivnost povezana z boljšo kakovostjo življenja in zdravjem ter ima zato tudi pozitivne učinke na zdravo staranje (Netz idr., 2005; Penedo in Dahn, 2005). Izboljšanja na področju kognitivnih sposobnosti med aerobno vadbo kažejo na tesno zvezo med funkcionalnimi in nevro-kognitivnimi sposobnostmi (Smith idr., 2010). Kasneje v življenju so gibalno aktivni posamezniki v primerjavi s sedentarnimi vrstniki manj podvrženi kognitivnemu upadu in celo demenci (Buchman idr., 2012; Sofi idr., 2011; van Gelder idr., 2004; Weuve idr., 2004). Dinse (2006) je poročal, da se lahko reorganizacija možganov zgodi v dveh primerih: i) prvič, med procesom staranja in ii) drugič, med zdravljenjem sprememb, povezanih s starostjo (Dinse, 2006). Večina študij, ki so preučevale spremembe kognitivnih sposobnosti starejših odraslih, so poročale o pomembnih izboljšanjih tistih kognitivnih funkcij, ki so jih neposredno trenirali (Ball, Berch, Helmers, Jobe, Leveck, Marsiske, Morris, Rebok, Smith, Tennstedt, Unverzagt, Willis in Grp, 2002; Ball idr., 2007; Klusmann idr., 2010; Willis idr., 2006). Le ena študija je pokazala izboljšanje mobilnosti sedentarnih starejših oseb po tem, ko so bili vključeni v informacijsko podprt kognitivni trening (KT) (Verghese idr., 2010). Ta študija kaže, da lahko KT (usmerjen na izvršne funkcije ter pozornost) vpliva na bolj učinkovite vzorce hoje

ter oblikuje močno povezavo med kognitivnimi procesi in nadzorom nad hojo. Poleg tega so Lovden in sodelavci (2012) poročali, da lahko dolgotrajni trening prostorske navigacije tudi spremeni volumen hipokampusa pri posameznikih vključenih v vadbo, kar kaže na povezavo med KT in možganskimi spremembami.

Te ugotovitve podpirajo uporabo KT z virtualno navigacijo kot možne strategije za blažitev negativnih vplivov dolgotrajne fizične neaktivnosti (ali imobilizacije) starejših ljudi. Zato je bil namen naše študije oceniti, ali bi lahko KT uporabili kot primerno orodje za zmanjšanje in/ali preprečevanje negativnih funkcionalnih in kognitivnih upadov, zaznanih po 14-dnevni študiji horizontalnega ležanja (HL). Predvideli smo, da bo KT, skupaj s prostorsko navigacijo omilil upad motoričnih funkcij po študiji 14-dnevnega HL, kar se bo odražalo tudi v manjših spremembah na ravni možganske električne aktivnosti.

Doktorska študija vsebuje tri povezane vsebine, ki razpravljajo o vplivih KT na trenirano kognitivno razsežnost prostorske navigacije in posplošitev vpliva KT na netrenirano gibalno razsežnost ter na raven možganske električne aktivnosti. Rezultati te doktorske študije so objavljeni (ali v procesu objavljanja) v naslednjih znanstvenih publikacijah:

- Marusic, U., Meeusen, R., Pisot, R., and Kavcic, V. (2014). The brain in micro- and hypergravity: The effects of changing gravity on the brain electrocortical activity. Eur J Sport Sci, 1-10. doi: 10.1080/17461391.2014.908959
- Goswami, N., Kavcic, V., Marusic, U., Simunic, B., Rossler, A., Hinghofer-Szalkay, H., & Pisot, R. (2015). Effect of computerized cognitive training with virtual spatial navigation task during bed rest immobilization and recovery on vascular function: a pilot study. Clin Interv Aging, 10, 453-459. doi: 10.2147/CIA.S76028
- Marusic, U., Pišot, R., Moffat, S., Petrič, M., Dolenc, P., and Kavcic, V., Effectiveness of Computerized Cognitive Training in Older Adults during 14-day of Physical Inactivity in the Bed Rest Study, *v postopku recenzije*.
- Marusic, U., Kavcic, V., Giordani, B., Gerževič, M., Meeusen, R., and Pišot, R., Computerized spatial navigation training during 14 days of bed rest in healthy older adult men: Effect on gait performance, *Sprejet v objavo dne 17. februarja 2015 v Psychology and Aging*.

2 CILJI IN HIPOTEZE

2.1 Cilji raziskave

Namen študije je bil raziskati učinke informacijsko podprtega kognitivnega treninga (KT) z nalogo virtualne prostorske navigacije na sposobnost virtualne navigacije in funkcionalno učinkovitost hoje ter na raven možganske električne aktivnosti med 14-dnevnim horizontalnim ležanjem (HL).

Natančneje smo želeli preveriti:

- Učinkovitost KT (merjeno s testom virtualne prostorske navigacije) po:
    - 14 dneh HL
    - 28 in 400 dni po HL
- Učinkovitost hoje pri normalni in hitri hoji brez in z dodatno nalogo po 14 dneh HL. Učinkovitost hoje smo preverili s:
    - Parametrom hitrosti hoje
    - Učinkom dvojne naloge
    - Variabilnostjo izbranih parametrov (zamašna faza, dolžina koraka) hoje
- Raven možganske električne aktivnosti 14 dni po HL v specifičnih možganskih predelih, kot so motorični korteks in okcipitalne regije, med nalogo sinhronizacije:
    - Vedenjski podatki prstnega in nožnega tapkanja,
    - Analiza z dogodkom povezanih potencialov vidnega procesiranja,
    - Analiza z dogodkom povezanih potencialov motoričnega procesiranja pri prstnem in nožnem tapkanju.

2.2 Hipoteze

Na osnovi predmeta in ciljev raziskovalne naloge smo razvili naslednje hipoteze:

H1: Starejši odrasli v intervencijski skupini bodo v primerjavi s kontrolno skupino po dvanajstih KT znatno izboljšali svojo uspešnost pri testu virtualne prostorske navigacije.

H1.1: Uspešnost virtualne navigacije po 14 dneh HL se bo med intervencijsko in kontrolno skupino pomembno razlikovala.

H1.2: Razlike med skupinama v uspešnosti virtualne navigacije se bodo ohranile tudi 28 in 400 dni po HL.

H2: Starejši odrasli v intervencijski skupini bodo imeli v primerjavi s kontrolno skupino manjši upad spremljanih parametrov hoje pri normalni in hitri hoji po 14 dneh HL.

H2.1: Po 14 dneh HL bo med skupinama pomembna razlika v upadu učinkovitosti normalne in hitre hoje z in brez dodatne kognitivne naloge.

H2.2: Med skupinama bo prišlo do pomembnega interakcijskega učinka pri učinkih dvojne naloge pri normalni in hitri hoji.

H2.3: Po 14 dneh HL bo med skupinama pomembna razlika v variabilnosti hoje v vseh štirih pogojih.

H3: Starejši odrasli v intervencijski skupini bodo imeli v primerjavi s kontrolno skupino boljše rezultate pri vedenjskih podatkih sinhronizacijske naloge po 14 dnevnem HL.

H3.1: Povprečna stopnja sinhronizacije in njena variabilnost ter število neodgovorjenih dražljajev pri prstnem tapkanju se bodo pomembno razlikovali med skupinama po koncu 14 dnevnega HL.

H3.2: Povprečna stopnja sinhronizacije in njena variabilnost ter število neodgovorjenih dražljajev pri nožnem tapkanju se bodo pomembno razlikovali med skupinama po koncu 14 dnevnega HL

H4: Starejši odrasli v intervencijski skupini bodo imeli v primerjavi s kontrolno skupino manjši upad možganske električne aktivnosti med sinhronizacijsko nalogo po 14 dneh HL.

H4.1: Z dogodkom povezani potenciali vidnega procesiranja se bodo značilno razlikovali med udeleženci intervencijske in kontrolne skupine po 14 dneh HL.

H4.2: Z dogodkom povezani potenciali motoričnega procesiranja med prstnim tapkanjem se bodo značilno razlikovali med udeleženci intervencijske in kontrolne skupine po 14 dneh HL.

H4.3: Z dogodkom povezani potenciali motoričnega procesiranja med nožnim tapkanjem se bodo značilno razlikovali med udeleženci intervencijske in kontrolne skupine po 14 dneh HL.

3 METODE DELA

3.1 Preiskovanci

V študiji 14-dnevnega HL je sodelovalo 16 starejših moških (59.6 ± 3.6 let; povprečje ± standardna deviacija). Vsi udeleženci so bili desničarji, brez predhodnih obolenj srca in ožilja, nevroloških ali psihičnih motenj in so pred študijo opravili zdravniški pregled. Vsi postopki so bili izvedeni v skladu s Helsinško deklaracijo in so bili odobreni s strani Komisije Republike Slovenije za medicinsko etiko. Študija je potekala v prostorih Ortopedske bolnišnice Valdoltra. Udeleženci so pred začetkom študije 14-dnevnega HL podali pisno soglasje.

3.2 Zasnova študije

Da bi pri študiji HL dosegli cilj študije (simulirati podaljšano fizično neaktivnost), so morali preiskovanci študije ležati v postelji 14 dni brez prestanka. Tekom študije HL so se preiskovanci lahko le obračali na vse strani ali pod glavo podložili največ dve blazini, niso smeli vstati, sedeti na postelji ali dvigniti rok nad glavo. Preiskovancem je bilo omogočeno tri krat tedensko pasivno razgibavanje. Bolnišnično osebje in raziskovalni asistenti so redno preverjali fizično stanje udeležencev in jim omogočali dostop do posebnih postelj za namene osebne higiene. Udeleženci so prejemali tri standardne bolnišnične obroke dnevno ob 7.30, 12.00 in 18.00. Sobe (3-4 osebe na sobo) so bile klimatizirane in temperatura je bila vzdrževana udobno pod 25°C. Med študijo HL so udeleženci lahko prebirali knjige in časopise, uporabljali internet, gledali televizijo in poslušali radio, ter prosto komunicirali med seboj. Po koncu študije HL so bili vsi udeleženci vključeni v 28-dnevni program nadzorovanega okrevanja (fizična intervencija).

Že pred pričetkom študije je bilo osem preiskovancev naključno izbranih za KT (intervencijska skupina), med tem, ko je preostalih osem služilo kot aktivna kontrolna skupina (kontrolna skupina). V ločeni sobi je intervencijska skupina izvajala kognitivne treninge približno 50 minut na dan, med tem, ko je kontrolna skupina v istem časovnem intervalu gledala dokumentarne filme.

3.3 Postopki

3.3.1 Ocena mišičnega volumna, funkcije ter aerobne zmožnosti

Volumen štiriglave stegenske mišice (m. quadriceps femoris) desne noge je bila merjena z uporabo magnetne resonance (MRI) 1,5 T (Magnetom Avanto; Siemens Medical Solution, Erlangen, Nemčija). Kontinuirane MRI slike so bile obdelane s pomočjo programa za obdelavo slik IOsiriX (Pixmeo Sarl, v.4.1.2). Mišični volumen je bil izračunan z uporabo metode prisekanega stožca (Jones & Pearson, 1969), z uporabo naslednje formule: V = 1/3 h (a + (√ab) + b), kjer sta a in b področji prečnih prerezov (CSA) dveh vzporednih MRI rezin v aksialni ravnini pri čemer je h celotna razdalja med njima (vključno z debelino ene MRI rezine). Skupni ocenjeni volumen smo dobili iz vsote posameznih prisekanih stožcev za posameznega preiskovanca.

Največja mišična sila iztegovalk kolena je bila ocenjena s pomočjo največje hotene izometrične kontrakcije (NHK) pri kotu 110° v kolenu in 90° v kolku. Sila je bila izmerjena z električnim dinamometrom (TSD121C, BIOPAC Systems, Inc., ZDA) s frekvenco vzorčenja 1kHz. Preiskovanci so po uvajalnem delu izvedli dva NHK z 2-minutnim počitkom. Najvišja NHK je bila uporabljena za nadaljnjo obdelavo.

Največja aerobna moč (VO2max) je bila dosežena s pomočjo večstopenjskega obremenitvenega testa na ciklo-ergometru z mehansko zavoro (Monark Ergomedic 839E, Monark, Varberg, Sweden). S pomočjo mobilne naprave (Quark-b2, Cosmed, Italija) so bili med testom spremljani tako ventilacijski kot plinski parametri. Po oceni stanja v mirovanju je preiskovanec pričel z ogrevanjem na obremenitvi 80 W. Po zaključku ogrevanja se je obremenitev progresivno povečevala (20 W/minuto) do subjektivne izčrpanosti preiskovanca. Pojavnost platoja največje porabe kisika (t.j. povečanje porabe kisika za največ 2 ml•kg-1•min-1 pri predzadnji ali zadnji stopnji testa) potrjuje dosego vrednosti VO2max. Največja poraba kisika je definirana kot povprečna poraba kisika v zadnjih 20 sekundah testa, normalizirana na telesno maso.

3.3.2 Ocena uspešnosti pri testu virtualne prostorske navigacije

Kognitivni preizkus smo opravili z nalogo preizkusa virtualne prostorske navigacije. Preizkus navigacije v virtualnem labirintu je bil izveden na pravi dan (HL d1), ob koncu študije HL (HL d14) in ob koncu intervencije fizičnega okrevanja (FO d28). Poleg tega je

bila izvedena četrta meritev 400 dni po koncu študije HL (FO d400) z namenom ocene dolgoročnega učinka KT. Vsi računalniški preizkusi so bili izvedeni v ležečem položaju, da bi zagotovili enake pogoje testiranja za vsako meritev.

3.3.3 Potek KT

Trening virtualne navigacije je vključeval nalogo virtualnega labirinta. Udeležence intervencijske skupne smo prosili, da se »virtualno gibljejo« skozi virtualne labirinte s pomočjo računalniške igralne palice približno 50 minut vsakega od 12 dni tekom študije HL. Uporabili smo tri različice virtualnih labirintov glede na smerokaze:

- smerokazi z imeni slovenskih mest,
- smerokazi z imeni držav sveta in
- smerokazi s slikami gozdnih živali.

Udeležence smo trenirali v virtualnih labirintih s tremi, petimi in sedmimi križišči. Vsak virtualni labirint je vseboval le eno od navedenih kategorij. Preiskovancem smo naročili, da po labirintu potujejo, dokler ga ne uspejo dvakrat zaporedoma zaključiti brez napak. Do takrat so udeleženci že pridobili dovolj izkušenj, da so se naučili pravilne poti in se je ponovno spomnili. Šele nato so lahko napredovali v naslednji virtualni labirint. V celotnem obdobju KT so preiskovanci zaključiti šest labirintov s tremi križišči, šest labirintov s petimi križišči in dvanajst labirintov s sedmimi križišči. Čeprav je končno število zaključenih labirintov s sedmimi križišči med preiskovanci intervencijske skupine nihalo glede na to, kako hitri so bili posamezni udeleženci pri izpolnjevanju pogojev v posameznem virtualnem labirintu, so vsi udeleženci, ki so sodelovali v KT, zaključili vsaj štiri labirinte s sedmimi križišči. Za vsako raven kompleksnosti virtualnega labirinta smo uporabili različne prostorske postavitve in različne smerokaze (imena mest/držav in slike živali), da bi minimizirali učinke predhodne vaje na ponovno testiranje. Trening virtualne navigacije je bil, prav tako kot testiranje, opravljen v ležečem položaju.

3.3.4 Meritve parametrov hoje

Meritve hoje so bile izvedene z opremo OptoGait system (Microgate, Bolzano, Italy) pod štirimi različnimi pogoji (običajna hoja, hitra hoja, običajna hoja z dodatno nalogo in hitra hoja z dodatno nalogo) v naključnem vrstnem redu. Dodatna kognitivna naloga je bila sestavljena iz hoje in hkratnega odštevanja po tri od naključno izbrane številke med 400 in 500.

Učinek dodatne naloge (UDN) je relativna mera, ki primerja običajno hojo s hojo z dodatno nalogo (Kelly idr., 2010; Remaud idr., 2013; Siu in Woollacott, 2007). Za običajno in hitro hojo je bil UDN izračunan tako:

$$\text{UDN} = \frac{\text{hoja z dodatno nalogo} - \text{običajna hoja}}{\text{običajna hoja}} \times 100$$

Variabilnost hoje je bila ovrednotena z variabilnostjo zamašne faze ter variabilnosti dolžine koraka (Verghese, Holtzer, Lipton in Wang, 2009).

3.3.5 Elektroencefalografske meritve (EEG)

Meritve EEG so bile posnete z opremo Brain Vision (Brain Vision, Inc.) in 64 elektrodami (Acti Cap). Obdelava zajetih podatkov je bila izvedena s programsko opremo EEGLAB (Delorme & Makeig, 2004). Preiskovanci so opravili nalogo sinhronizacije prstnega in nožnega tapkanja z vidnim dražljajem, ki se je prikazoval na 17-inčnem LCD ekranu.

Vedenjski podatki:
Preiskovanci so morali svoje prstne in nožne odgovore čim bolj sinhronizirati z dvestotimi vidnimi dražljaji. V idealnih pogojih (ko se je preiskovanec ustrezno odzval na vse vidne dražljaje) je bilo skupno zapisanih 200 dražljajev in pripadajočih motoričnih odzivov.

Procesiranje vidnega dražljaja:
Z dogodkom povezani potenciali vidnega procesiranja so bili segmentirani od -200 do +800 milisekund s postavljenim izhodiščem med -200 in 0 milisekundami. Modalnost vidnega procesiranja je bila ocenjena nad okcipitalnem delu pri čemer je bila elektroda z največjim odzivom nadaljnje analizirana:

- Vrh komponente P1 je predstavljal najbolj pozitivno odstopanje med 40 in 140 milisekundami za vidnim dražljajem,
- komponenta N1 je bila zaznana med 120 in 200 milisekundami za vidnim dražljajem in
- komponenta P2 je bila zaznana kot prvi pozitivni vrh signala po 200 milisekundah.

Procesiranje motoričnega odgovora:
Z dogodkom povezani potenciali motoričnega procesiranja so bili segmentirani od -800 do +200 milisekund s postavljenim izhodiščem med -800 in -600 milisekundami pred motoričnim odgovorom. Modalnost motoričnega procesiranja je bila ocenjena nad motoričnim korteksom (C3 za prstno in C4 za nožno tapkanje), pri čemer so bili analizirani naslednji parametri (Yordanova idr., 2004):

- Najbolj negativno odstopanje (amplituda in latenca),
- prag 15% najbolj negativnega odstopanja je bil določen kot mera začetne latence in
- čas vzpona je bil določen kot trajanje aktivacije od začetne latence do najbolj negativnega odstopanja.

3.3.6 Statistična analiza

Statistična analiza je bila opravljena s programsko opremo SPSS 20.0 (SPSS, Inc., Chicago, Ill, USA). V primeru zadostitve pogojem parametričnih testov (Shapiro-Wilk test), smo izvedli analizo variance za ponovljene meritve (RM ANOVA). Statistična značilnost je bila določena na ravni $p < .05$ ter na ravni $p < .10$ za interakcijski efekt.

4 REZULTATI

4.1 Učinkovitost KT

Mann-Whitney U test je pokazal, da je naredila intervencijska skupina pomembno manj napak v virtualnem labirintu pri merjenju HL d14 (Mann-Whitney; U-test, z = -2.683 p = .006), FO d28 (Mann-Whitney U-test; z = -2.302, p = .021) in FO d400 (Mann-Whitney U-test; z = -2.277, p = .021) kar potrjuje učinkovitost KT do 400 dni po začetku izvajanja KT.

4.2 Meritve parametrov hoje

RM ANOVA ni pokazala pomembnega interakcijskega, skupinskega ali časovnega učinka v treh pogojih hoje (p>.05). Le pri hitri hoji z dodatno kognitivno nalogo je prišlo do časovnega učinka [F (1,13) = 7.002, p = .02, η^2 = .35]. Nadaljnji testi so pokazali, da je le kontrolna skupina pomembno znižala hitrost hoje po HL (p = .005), pri čemer je intervencijska skupina ostala nespremenjena (p = .37).

Pri meri UDN pri običajni hoji je prišlo do pomembnega časovnega [F (1,13) = 58.12, p < .001, η^2 =.82] in interakcijskega učinka [F (1,13) = 26.93, p < .001, η^2 =.67]. Nadaljnje analize so pokazale, da je prišlo pri intervencijski skupini do obrata, iz negativnih v pozitivne vplive učinka dodatne naloge (p < .001), pri čemer je kontrolna skupina ostala nespremenjena (p = .124).

Pri meri UDN pri hitri hoji je prišlo do pomembnega interakcijskega učinka [F (1,13) = 5.70, p = .03, η^2 = .31]. Nadaljnje analize so pokazale, da je prišlo pri intervencijski skupini do trenda zmanjševanja negativnih kazalcev mere UDN (p = .056), medtem ko je kontrolna skupina ostala nespremenjena (p = .716).

Pri variabilnosti hitre hoje z dodatno kognitivno nalogo in sicer pri parametru zamašne faze pri hoji, je prišlo do pomembnega interakcijskega učinka F (1,13) = 12.81, p = .003, η^2 = .50]. Nadaljnje analize so pokazale, da je prišlo pri kontrolni skupini do pomembnega povečanja variabilnosti hoje po HL (p = .006), pri čemer je intervencijska skupina ostala nespremenjena (p = .302).

4.3 Elektroencefalografske meritve (EEG)

4.3.1 Vedenjski podatki

Pri vedenjskih podatkih prstnega in nožnega tapkanja ni RM ANOVA pokazala nobenega pomembnega interakcijskega, skupinskega ali časovnega učinka ($p > .05$).

4.3.2 Z dogodkom povezani potenciali vidnega procesiranja

Pri z dogodkom povezanih potencialih vidnega procesiranja ni prišlo do pomembnega skupinskega ($p > .183$) in interakcijskega učinka ($p > .145$). Pomemben časovni učinek smo dobili pri parametru P1 amplitude [$F (1,14) = 7.078$, $p = .019$, $\eta^2 = .336$], P1 latence [$F (1,14) = 19.802$, $p = .001$, $\eta^2 = .586$] in P2 latence [$F (1,14) = 6.044$, $p = .028$, $\eta^2 = .302$].

P1 amplituda se je po HL pomembno povišala le pri kontrolni skupini ($p = .009$), P1 latenca se je pomembno skrajšala pri obeh skupinah ($p < .029$) in P2 latenca se je pomembno skrajšala le pri intervencijski skupini ($p = .031$).

4.3.3 Z dogodkom povezani potenciali motoričnega procesiranja

RM ANOVA ni pokazala pomembnega časovnega ($p > .191$), skupinskega ($p > .188$) in interakcijskega učinka ($p > .243$) za vse parametre povezane s motoričnim procesiranjem pri prstnem tapkanju.

Pri motoričnem procesiranju nožnega tapkanja ni prišlo do pomembnega časovnega učinka pri nobenem izmed parametrov ($p > .154$). Prišlo je do pomembnega interakcijskega učinka pri parametru amplitude maksimalnega odmika [$F (1,14) = 4.042$, $p = .064$, $\eta^2 = .224$] ter parametru latence maksimalnega odmika [$F (1,14) = 9.917$, $p = .007$, $\eta^2 = .415$].

RAZPRAVA

Študija predstavlja prvo tovrstno študijo HL s starejšimi odraslimi osebami z dodatno kognitivno intervencijo, ki je potekala med 14-dnevnim HL v kontroliranih laboratorijskih pogojih. KT je bil učinkovit, saj so preiskovanci v intervencijski skupini v primerjavi s kontrolno skupino po 12 KT izboljšali svoje sposobnosti pri testu virtualne prostorske navigacije. Še več, učinek KT je bil viden do 400 dni po študiji HL. Skladno s predhodnimi raziskavami, smo tudi v naši raziskavi ugotovili, da je KT znatno izboljšal sposobnost virtualne prostorske navigacije in s tem izboljšal specifično trenirane kognitivne sposobnosti (Ball idr., 2002; Ball idr., 2007; Klusmann idr., 2010; Kueider idr., 2012; Lampit idr., 2014; Pressler idr., 2011; Willis idr., 2006). Prenos vplivov KT je bil pri naši študiji ugotovljen tudi na pod-domeni informacijsko podprtega CogState testa, ki meri stopnjo pozornosti ter hitrosti procesiranja, vendar ta del študije ne predstavlja dela tega doktorata in je predstavljen drugje (Marušič, Kavcic, Moffat, Petrič, Dolenc, & Pišot, *v postopku recenzije*).

Prostorska navigacija je bila izbrana kot naloga KT prav zaradi razloga, ker vključuje aktivacijo širokega spektra možganskih struktur, ki so potrebne za uspešno realizacijo prostorske navigacije. Med te sodi hipokampus, parahipokampalni girus, posteriorni cingulatni girus, parietalni reženj in prefrontalni korteks (Ekstrom idr., 2003; Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000; Lovden idr., 2012). V sorodni študiji, kjer so preiskovanci trenirali veščine prostorske navigacije v virtualnem okolju, so ugotovili, da so bili učinki treninga prisotni še 4 mesece po intervenciji ter, da je intervencijska skupina zadržala volumen hipokampusa nespremenjen. Pri tem pa je bila kontrolna skupina podvržena učinkom staranja pri katerih je bil razviden upad volumna te pomembne možganske strukture (Lovden idr., 2012). Ugotovljene učinke KT prostorske navigacije lahko tako pripišemo učinkovitejšemu nevronskemu procesiranju po KT, saj so Hotting idr. (2013) po treningu prostorske navigacije ugotovili manjše možganske aktivacije v hipokampusu, parahipokampalnem girusu in frontalnem ter temporalnem predelu možganov.

Pri preučevanju učinkov KT na učinkovitost hoje smo ugotovili pomembno znižanje hitrosti hoje po 14-dnevnem HL le pri kontrolni skupini. Pomemben upad hitrosti hoje je bil prisoten le pri najzahtevnejši hoji (hitra hoja z dvojno nalogo). Negativne učinke HL na hojo lahko povežemo z nekaterimi že ugotovljenimi dejstvi raziskave, ki je ugotovila pomemben upad pri parametrih hoje med HL (Dupui idr., 1992). Prenos vpliva KT na gibalno učinkovitost pri zdravih posameznikih pa je po našem vedenju preiskovala le ena študija. Verghese idr.

(2010) so poskušali s pomočjo KT, ki je specifično vplival na kognitivne domene pozornosti in izvršnih funkcij, izboljšati učinkovitost hoje pri sedentarnih starejših osebah. Ugotovili so pozitiven prenos KT na vzorec hoje in s tem nakazali tesno povezavo med treniranimi kognitivnimi procesi in motorično kontrolo lokomocije (Verghese idr., 2010). V naši raziskavi smo med drugim ugotovili pomembno povečanje variabilnosti hoje po 14-dnevnem HL samo pri kontrolni skupini. Ena predhodnih študij je pokazala, da je variabilnost hoje povečana samo pri odraslih osebah, ki so dovzetne za padce (Springer idr., 2006). Omenjeno nas napeljuje k spoznanju, da obstaja večja možnost pojavnosti padcev pri preiskovancih v kontrolni skupini po 14-dnevnem HL.

Pri analizi EEG podatkov smo ugotovili, da pri vedenjskih podatkih ni bilo razlik med skupinama po 14-dnevnem HL. Uporabljena naloga prstnega in nožnega tapkanja na konstanten vidni dražljaj (naloga sinhronizacije s konstantnim vidnim dražljajem) očitno ni bila dovolj zahtevna, da bi na eni strani ugotovili učinke HL in na drugi učinke KT (Drewing idr., 2006). Pri z dogodkom povezanih potencialih vidnega procesiranja smo ugotovili pomembno povečanje amplitude P1 samo pri kontrolni skupini po HL. Omenjeno ugotovitev lahko povežemo s študijami staranja, ki so že ugotovile, da je amplituda P1 povečana pri starejših osebah v primerjavi z mlajšimi (De Sanctis idr., 2008; Falkenstein idr., 2006; Yordanova idr., 2004). Latenca P2 je bila skrajšana le pri intervencijski skupini na koncu 14-dnevnega HL kar lahko interpretiramo in povežemo s predhodnimi študijami, ki so ugotovile povezavo med skrajšano latenco P2 in izboljšanim delovnim spominom (Finnigan idr., 2011; Lefebvre idr., 2005). Pri preučevanju z dogodkom povezanih potencialov motoričnega procesiranja smo ugotovili pomemben vpliv KT. Povečana amplituda pri nožnem tapkanju samo pri kontrolni skupini nakazuje na kompenzatorne mehanizme centralnega živčnega sistema po 14-dnevnem HL, ki jih lahko povežemo tudi z ugotovitvami študij vpliva staranja na z dogodki povezanimi potenciali motoričnega procesiranja (Falkestein idr, 2006). Pomemben vpliv, povezan z nožnim, in ne ročnim, tapkanjem lahko logično povežemo z dejstvom, da ima HL večje negativne vplive na periferne mišične procese in najverjetneje na oteženo motorično kontrolo.

Izboljšanje pacientove mobilnosti z ustreznimi intervencijami po daljšem obdobju fizične neaktivnosti ali imobilizacije lahko vpliva na veliko področij kakovosti življenja, vključno s hitrejšo vrnitvijo v stanje pred poškodbo. Padci so med pacienti v bolnišnicah in institucijah za dolgotrajno nego pogosti in se jih delno pripisuje slabšim izvršilnim funkcijam (Herman idr., 2010). Pozitivni učinki KT kažejo smer zmanjšanja nevarnosti padcev takoj po koncu HL ali imobilizacije in s tem prispevajo k hitrejši vrnitvi v stanje pred imobilizacijo (Bouldin idr., 2012). Preprečevanje padcev lahko v teh primerih predstavlja pomemben element za

varnost pacienta in splošen prispevek javnemu zdravju. Kortebein idr. (2008) so priporočali, da naj bodo dnevi HL v bolnicah in/ali domovih za starejše kolikor se le da kratki. Druge intervencije, kot so vaje za moč in prehranske ali farmakološke intervencije, bi morale biti razvite med dolgotrajnim ležanjem kot preventiva negativnim vplivom HL, predlagajo nekateri avtorji (Kortebein et al., 2008).

Zaključimo lahko, da je naša študija prva tovrstna študija HL, ki je neposredno ocenila učinkovitost KT na podlagi virtualne prostorske navigacije pri starejših odraslih moških. Naše ugotovitve podajo nova znanja o možnostih ublažitve negativnih učinkov HL, predvsem tistih, ki so povezani s kompleksnejšimi nalogami. Na splošno je naša študija podala empirične dokaze, ki dokazujejo, da je lahko kognitivna intervencija učinkovita, njeni učinki pa se kažejo tudi na drugih ne-ciljno-treniranih domenah in dlje časa. V nasprotju z ostalimi študijami KT smo v naši raziskavi nadzorovali gibalno aktivnost/neaktivnost ter vnos hrane tekom celotnega trajanja kognitivne intervencije. Predstavljena študija ponuja pomembne ugotovitve, ki nakazujejo, da bi lahko bil KT učinkovit pri ublažitvi negativnih učinkov HL in poudarja generalizacijo KT na ostale merjene parametre kot so učinkovitost hoje in raven možganske električne aktivnosti. Bodoče študije bi lahko replicirale naše rezultate in evalvirale učinke različnih pristopov h KT na mobilnost starejših žensk in moških ter tudi pacientov v bolnišnicah. Rezultati naše študije predstavljajo empirično potrditev učinkovitosti kognitivne intervencije med fizično neaktivnostjo in lahko služijo kot podpora razvoju podobnih programov intervencij kot dela rehabilitacijskih protokolov, uporabljenih med dolgotrajno neaktivnostjo, ki nastopi kot posledica staranja, bolezni, sedentarnih poklicev ali vzorcev gibalno neaktivnega življenja, vključno z vesoljskimi poleti.

APPENDIX

Appendix 1: Study approval by the Republic of Slovenia National Medical Ethics Committee

KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

Prof. dr. Rado Pišot, predstojnik,
prof. dr. Darko Darovec, direktor UP ZRS,
in prof. dr. Dragan Marušič, rektor UP po pooblastilu
Znanstveno-raziskovalno središče Koper
Inštitut za kineziološke raziskave
Univerza na Primorskem, Garibaldijeva 1, 6000 Koper

Štev.: 103/04/12
Datum: 28. 4. 2012

Spoštovani gospodje profesorji,

Komisiji za medicinsko etiko (KME) ste 6. 4. 2012 poslali v oceno predlog raziskave z naslovom:

"Factors of Healthy Aging – BED REST." Šifra: Ageing BED REST.

KME je na seji 17. aprila 2012 ocenila, da je raziskava etično še sprejemljiva, in Vam s tem izdaja svoje soglasje. Zavezuje pa vas, da skrbno opazujete osebe v raziskavi in nemudoma reagirate na sum škodljivih učinkov. KME želi dobiti ustrezno vmesno in končno poročilo, kakor tudi obvestila o zapletih.

Lep pozdrav,

prof. dr. Jože Trontelj
predsednik Komisije RS za medicinsko etiko

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