Frequency-specific insight into short-term memory capacity

Matteo Feurra,1,2 Giulia Galli,1,3 Enea Francesco Pavone,1,4 Alessandro Rossi,1 and Simone Rossi1

1Department of Medicine, Surgery and Neuroscience, Unit of Neurology and Clinical Neurophysiology, Brain Investigation and Neuromodulation Laboratory (SI-BIN Lab), Azienda Ospedaliera Universitaria di Siena, Policlinico Le Scotte, Siena, Italy; 2School of Psychology, Centre for Cognition and Decision Making, National Research University Higher School of Economics, Russian Federation, Moscow, Russia; 3Department of Psychology, Faculty of Arts and Social Sciences, Kingston University, Kingston Upon Thames, United Kingdom; and 4Department of Psychology, Sapienza University of Rome, Rome, Italy

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Feurra M, Galli G, Pavone EF, Rossi A, Rossi S. Frequency-specific insight into short-term memory capacity. J Neurophysiol 116: 153–158, 2016. First published April 27, 2016; doi:10.1152/jn.01080.2015.—The digit span is one of the most widely used memory tests in clinical and experimental neuropsychology for reliably measuring short-term memory capacity. In the forward version, sequences of digits of increasing length have to be reproduced in the order in which they are presented, whereas in the backward version items must be reproduced in the reversed order. Here, we assessed whether transcranial alternating current stimulation (tACS) increases the memory span for digits of young and midlife adults. Imperceptibly weak electrical currents in the alpha (10 Hz), beta (20 Hz), theta (5 Hz), and gamma (40 Hz) range, as well as a sham stimulation, were delivered over the left posterior parietal cortex, a cortical region thought to sustain maintenance processes in short-term memory through oscillatory brain activity in the beta range. We showed a frequency-specific effect of beta-tACS that robustly increased the forward memory span of young, but not middle-aged, healthy individuals. The effect correlated with age: the younger the subjects, the greater the benefit arising from parietal beta stimulation. Our results provide evidence of a short-term memory capacity improvement in young adults by online frequency-specific tACS application.

digit span; short-term memory; posterior parietal lobe; transcranial alternating current stimulation; beta frequency

NEW & NOTEWORTHY

We provided novel evidence of a frequency-specific effect by transcranial alternating current stimulation (tACS) of the left posterior parietal cortex on short-term memory, during a digit span task. The effect was prominent with stimulation at beta frequency for young and not for middle-aged adults and correlated with age. Our findings highlighted a short-term memory capacity improvement by tACS application.

MEMORY SPAN CAPACITY REPRESENTS a measure of temporary short-term memory. In everyday life, the concept of short-term memory span may be illustrated by a “smart-pad” that allows the temporary storage and manipulation of information such as a new telephone number, a PIN code, a password, or the amount of items in our basket when we proceed to checkout at the supermarket. All these processes are essential in our routine activities. Here, we want to make a distinction between what we refer as working memory and short-term memory capacity. The former better fit with tasks such as the operation span (OSPAN), reading span (RSPAN), and counting span (CSPAN), thus requiring more attentional resources. The latter, relies to tasks such as forward span-dissimilar (FSPAN), forward span-similar (FSPANS), and backward span (BSPAN), that require less attentional resources (Engle et al. 1999).

One way to assess short-term memory capacity is the digit span (DS) test, which is a subtest of the Wechsler Adult Intelligence Scale (WAIS). DS is widely used in neuropsychological assessment, given that the ability to maintain items in memory for a short period of time decreases in physiological aging, mild cognitive impairment (MCI), and neurodegenerative diseases, even at an initial stage (Orsini et al. 1987; Reynolds 1997). In the forward version [digit forward (DF)], subjects repeat sequences of digits of increasing length in the order in which they are presented, whereas in the backward version [digit backward (DB)] items must be produced in the reversed order. DF is thought to reflect verbal short-term memory capacity, while DB primarily taxes executive functioning resources (Sair et al. 2006). Neuroimaging studies have shown that a wide prefrontal-parietal network underlies DS performance (Bor et al. 2004) and that a dissociation exists between the neural correlates of DF and DB. DF engages the mid-ventrolateral frontal cortex and the left parietal cortex (Koenigs et al. 2009; Paulesu et al. 1993), whereas the DB involves a wide-spread prefrontal network including ventral and dorsal aspects (Owen 2000). The difference in neural correlates may be explained, at least in part, by the different cognitive processes underlying performance in the DS. DB has a stronger visuospatial component than DF. Moreover, performance at the DB correlates with general intelligence abilities and requires mental transformation, an element missing from rote, forward recall (Reynolds 1997).

Memory for digits (as assessed with both DF and DB) is a relatively stable individual trait in young adults, but linearly decreases as individuals age. Classical memory studies most often compare extreme age groups of young vs. older adults (e.g., above 65 yr of age) to investigate age-related cognitive decline. However, cognitive decline may occur at earlier stages of aging (Rossor et al. 2010). According to recent studies on the aging human brain, memory, reasoning, and comprehension skills start to decline during...
the middle-age and not after 60 yr as previous studies suggested (Lu et al. 2004; Yankner et al. 2008). There is evidence that short-term verbal memory starts to decline between 40 and 50 yr of age (Singh-Manoux et al. 2012). In addition, middle-aged individuals differ from younger adults also in the neural correlates of working memory (WM). Recent studies have demonstrated that successful WM maintenance is correlated with greater activation of the fronto-parietal network in midlife adults (Klaassen et al. 2014). In addition, WM maintenance is related to specific oscillatory patterns in the brain in this age range, which differ from both younger and older adults (Barr et al. 2014). This suggests that the comparison between extreme age groups may not entirely capture age-related changes in cognition, which in most cases follow a linear trajectory rather than reflecting an on-off phenomenon. It is therefore essential to examine the effectiveness of interventions aimed at reducing age-related cognitive decline not only in groups of older adults, but also in middle-aged individuals, and to assess the age window at which potential interventions are likely to be most beneficial. One intervention that has recently proven to be a potential neuro-rehabilitation tool is transcranial alternating current stimulation (tACS) (Brittain et al. 2013).

In healthy young adults it has been recently demonstrated that tACS boosts cortical excitability and behavioral performance on cognitive motor and working memory and intelligence tasks and induces state-dependent effects (Battleday et al. 2014; Feurra et al. 2013; Polania et al. 2012, 2015; Santar necchi et al. 2013, 2016; Vosskuhl et al. 2015). Moreover, it seems that tACS may entrain endogenous oscillatory activity of a specific target region and/or network, thereby inducing a frequency-specific increase of EEG activity (Helfrich et al. 2014a,b).

In the current study, we decided to combine the DS test with tACS to modulate verbal short-term memory performance by stimulating at different frequencies in a theta-to-gamma range. We examined the effects of tACS in a group of young and in a group of middle-aged individuals, in line with the hypothesis of a verbal short-term and working memory decline at an earlier stage of aging (Orsini et al. 1987; Singh-Manoux et al. 2012; Yankner et al. 2008). We administered a computerized version of DS in which sequences of numbers of increasing length (from 2 to 9 digits) were spelled out by a synthesized voice. At the end of each sequence, subjects had to repeat the numbers in the exact or reversed order until they got to their maximum span. During each sequence, we stimulated the left posterior parietal cortex (PPC), which is known to be involved in verbal short-term memory, especially in tasks that engage the articulatory loop (Baddeley et al. 1998, 2000; Henson et al. 2000; Koenigs et al. 2009; Paulesu et al. 1993; Romero et al. 2006; Vallar and Papagno 2002), and is associated with an increase of parietal beta and parieto-frontal theta oscillatory activity during maintenance processes (Engel and Fries 2010; Roberts et al. 2013; Sauseng et al. 2010; Vosskuhl et al. 2015). Since tACS has been shown to induce reliable effects when delivered online (Battleday et al. 2014), theta-, alpha-, beta-, and gamma-band and placebo (sham) stimulation frequencies (5, 10, 20, and 40 Hz and sham, respectively) were delivered during the task.

### MATERIALS AND METHODS

**Participants.** Twenty-eight participants took part in the experiment. Participants aged 18 to 35 yr old were allocated to the group of young adults (6 males and 8 females; mean age: 27.6 yr; SD: 4.3 yr; age range: 21–35 yr), whereas participants aged 36 to 60 yr old were included in the group of middle-aged adults (7 males and 7 females; mean age: 45.4 yr; SD: 7.8 yr; age range: 37–59 yr). There is no agreement in the literature as to what should be considered a middle-aged range, and the cut-offs to differentiate young from middle adulthood in memory studies vary from 30 (Barr et al. 2014; Shapiro and Levy-Gigi 2016), to 40 (Kwon et al. 2015; Park et al. 2013), and to 50 yr of age (Cansino et al. 2012; Klaassen et al. 2014). In the current study we used 35 yr of age as cut-off for two reasons. First, it has been shown that brain oscillatory patterns during working memory tasks start to change as early as 30 yr of age (Barr et al. 2014). Second, the most evident age demarcation in DS performance seems to occur at 35 yr of age (Orsini et al. 1987). All subjects had at least 13 yr of scholastic education and had no history of neurological or psychiatric problems or substance abuse. Subjects signed an informed consent before starting the experiment. The study was performed according to the Declaration of Helsinki and approved by the local Ethics Committee of University of Siena.

**Apparatus and materials.** Acoustic digits were numbers from 1 to 9, presented centrally by two stereo audio speakers (Logitech Digital USB Speaker System). The stimuli were presented using Eprime (PST Software). Subjects sat down on a comfortable reclining chair at a distance of ~60 cm from the audio speakers. Acoustic digits were computed by a freeware (DSpeech) Test to Speech program, which reads aloud a given written text and saves the acoustic stimuli in a wave stereo file format at 16 KHz and 16 bit. The purpose of using this method was to make sure any human accent or inflection was absent on the audio track. The volume of the stimuli was individually adjusted.

**tACS.** Stimulation was delivered by a battery-driven current stimulator (BrainStim, EMS) through surface saline-soaked sponge electrodes (size, 5 × 7 cm). Stimulating electrodes were positioned according to the International 10–20 EEG System. The target electrode was centered on the left posterior parietal cortex in correspondence of P3. The “return” electrode was placed over the ipsilateral shoulder (Santar necchi et al. 2014; Vandermeeren et al. 2010). tACS was delivered at an intensity of 1,000 mA (500 mA peak-to-peak). The maximum current density at the stimulation electrode was ~14.2 μA/cm². The waveform of the stimulation was sinusoidal, and there was no direct current offset. The low intensity of stimulation was used to avoid a perception of flickering lights (Paulus 2010), usually reported with higher stimulation intensities (Kanai et al. 2008). To minimize skin sensations, the electrodes were placed inside sponges and constantly saturated with a saline solution. Impedances were kept below 10 kΩ throughout stimulation sessions.

**Task.** Each subject performed the DF and DB in two different sessions spaced 4–7 days apart. Half participants started with the DF, the other half with the DB. For each session (DB or DF), two runs of five DS blocks were delivered. A span block corresponded to a complete DS set (from a 2- to a 9-digit sequence for a possible maximum span). In each block, one of the five stimulation frequencies were delivered (5, 10, 20, and 40 Hz and sham), so that for each run the whole set of stimulation frequencies was administered. After subjects completed a DS block (lasting about 3–4 min depending on the maximum span reached by the subject), a new one was subsequently run, with a different stimulation frequency. The stimulation started at the beginning of one digit span block (e.g., at the 2-digit sequence) and ceased when the subject reached his/her maximum span, corresponding to two consecutive mistakes (Fig. 1). Within each run, the order of the stimulation frequency was randomized. Individual blocks were interspersed by a 120-s interval. The maximum score
reached by each subject was collected as accuracy rate. Accuracy rate was then averaged between the two runs for each frequency condition.

Data analysis. Two mixed model ANOVAs, with five levels of the within-subject factor Stimulation (5, 10, 20, and 40 and sham) and two levels of the between-subject factor Group (young and middle-aged) were performed, separately for forward and backward digit span scores. Greenhouse-Geisser correction was applied when necessary to compensate for the violation of the assumption of sphericity. In the presence of significant interactions, pairwise comparisons contrasting each pair of stimulation frequency, separately in young and middle-aged adults, were performed using the Bonferroni correction. The level of significance was set at $P < 0.05$.

RESULTS

The mixed model ANOVA on DF scores showed no main effect of Stimulation [$F(4,104) = 1.985$, mean square error (MSE) = 0.604, $P = 0.102$, partial Eta$^2 = 0.071$] or Group [$F(1,26) = 1.125$, MSE = 4.464, $P = 0.299$, partial Eta$^2 = 0.041$]. A significant interaction between Stimulation and Group [$F = 4.031$, MSE = 1.228, $P = 0.004$, partial Eta$^2 = 0.134$] emerged; Post hoc comparisons revealed that in young adults tACS at 20 Hz significantly improved DF performance compared with the sham stimulation ($P = 0.002$; Fig. 2A). No other pairwise comparison was significant (all $P$s > 0.143). In middle-aged participants, the performance during 20-Hz stimulation did not significantly differ from sham ($P = 0.286$), neither were other comparisons significant (all $P$s > 0.197).

The selective effect of beta stimulation was further supported by a direct contrast between the DF performance of young and middle-aged adults during 20-Hz stimulation, ($P = 0.021$). To further characterize the age-selective effect of 20-Hz stimulation, we conducted a Pearson correlation between age and normalized DF scores (indicating the percentage change with...
respect to the sham condition). The correlation was significant \((r = -0.535, P = 0.003)\), suggesting that the effects of 20-Hz stimulation decrease linearly with age (Fig. 2B).

The ANOVA on DB scores showed no main effect of Stimulation \((P = 0.392)\), Group \((P = 0.073)\), or interaction between Stimulation Condition and Group \((P = 0.132)\).

To rule out the possibility that our results could be affected by practice effects between the first and the second DS run, we conducted an additional ANOVA with within-subject factors Run (1st, 2nd) and Stimulation (5, 10, 20, and 40 Hz and sham), and the between subject factor Group (young, middle-aged). No main effect of Run or interaction involving this factor emerged (all \(P_s > 0.087\)), suggesting that practice effects were negligible.

DISCUSSION

Our results showed a considerable improvement of DF span (near to 0.7 points) induced by 20 Hz tACS of the posterior parietal cortex in young adults, by taking into account that standard DS corrections applied for normalizing the effects of age and education in the normal population range from 0.25 to 0.6 points (Orsini et al. 1987). This represents the first step towards the causal evidence that 1) a frequency-dependent electrical stimulation boosts memory retention and 2) memory capacity across the life span is associated with age-related changes in parietal oscillatory activity.

Among the full set of applied oscillations at a physiological range, only 20-Hz tACS in the beta range increased DF performance. Since the WM system is a fluid system that comprises different subcomponents such as the executive functions, the episodic buffer, the visual spatial sketchpad, and the phonological short-term memory (Baddeley 2000), the effect of parietal beta-tACS on the DF may be related to sequential maintenance, top-down, and memory load processes (Engel and Fries 2010). Moreover, such activity might have not induced reliable effects due to an initial stage of development in middle-aged networks (Polania et al. 2012; Vosskuhl et al. 2015). The study of Vosskuhl and collaborators (2015) targeted FCz and Pz using individual theta-to-gamma frequencies. However, our study is substantially different: here we targeted the left PPC, a cortical region involved in the verbal short-term phonological loop of the WM system (Henson et al. 2000; Paulesu et al. 1993). Moreover, our subjects were submitted to a vocal response task and stimuli were acoustically delivered to simulate a standard neuropsychological assessment like the WAIS-R Digit Span subtest (Wechsler 2008). The current results are instead consistent with a recent study that found no effect of theta stimulation on WM abilities (Santarnecchi et al. 2016). One hypothesis that deserves further investigation is that theta stimulation may influence WM only when phase-coupled in fronto-parietal networks (Polania et al. 2015; Vosskuhl et al. 2015). Although a few studies tried to show online tACS/EEG effects (Helfrich et al. 2014a,b; Voss et al. 2014), more technical advances are needed to understand which are the exact action mechanisms that allow sinusoidal transcranial electrical stimulation to modulate endogenous oscillatory cortical activity, and in particular whether tACS in living humans may induce an oscillatory “entrainment” of the endogenous human brain activity. However, tACS has been showed to induce robust increases not only at a physiological (Feurra et al. 2011) but also at a behavioral level as shown in declarative and working memory and in fluid intelligence (Marshall et al. 2006; Polania et al. 2012; Santarnecchi et al. 2013).

In this study only young adults had a benefit of a 20-Hz stimulation while the middle-aged group did not benefit from any of the applied stimulation frequencies. One possible reason could be that aging brain is more susceptible to regional activation shift towards bilateral parietal and/or anterior prefrontal cortices as an index of compensation for age-related neural degradation that would otherwise adversely affect cognitive performance (Miller et al. 2008); Older adults recruit parietal cortex bilaterally whereas young adults show unilateral recruitment; this compensatory index is commonly associated with better task performance in normal aging (Huang et al. 2012). Thus it is likely to infer that here tACS on middle-aged might have not induced reliable effects due to an initial stage of a regional neuronal activity shift (Landolt and Borbely 2001; Li et al. 2013) and consequent lack of local entrainment of oscillatory task-related activity. Alternatively, a recent study (Barr et al. 2014) has shown atypical prefrontal gamma oscillatory activity in middle-aged individuals during a WM task. A similar age-related change in oscillatory patterns may also occur in parietal beta activity. This notion is supported by evidence indicating abnormal event related synchronization (ERS) and desynchronization (ERD) of oscillatory activity in elderly and even more in Alzheimer’s disease patients during...
recognition memory processes (Karrasch et al. 2004; Kurimoto et al. 2012). One may speculate that a similar oscillatory pattern may emerge even before the typical age that is considered for inclusion in aging studies. This might be in line with the idea that cognitive decline and associated neural changes occur well before 65 yr of age (Klaassen et al. 2014; Lu et al. 2004; Singh-Manoux et al. 2012). In summary, by providing the first evidence of enhanced memory span by online brain alternating frequency stimulation in young adults, this study supports the view of tACS as a neurotechnique that induces neuro-enhancement effects. In addition, we showed that this modulation of short-term memory capacity may be state dependent or more specifically age dependent at an initial stage of aging. Stimulation parameters inside this study ruled out the possibility that our results might be affected by subjective discrimination of different frequencies: the amplitude of stimulation is the main factor determining intensity of neurosensory side-effects including tingling and itching and phosphenes perception. In our study the size of electrode was rather large (7 × 5 cm); thus current density distribution was quite low (~14.2 μA/cm²). This is in line with a recent study that shows tACS to be more unlikely to induce neurosensory effects when delivered at lower intensities and far from retina, meaning far from prefrontal sites (Raco et al. 2014), as in the current study. At the end of experiment our subjects were asked if they were perceived any tingling or itching sensation on their skin. All of them did not report any of these side-effects. Despite that a growing number of studies has shown that tACS exerts its influence on behavior when delivered online (Helfrich et al. 2014a,b; Feurra et al. 2013; Polania et al. 2012, 2015; Santarnecchi et al. 2013, 2016; Voss et al. 2014), we cannot rule out carry-over effects of tACS in the present investigation. It seems unlikely, however, that any such effect would result in a frequency-specific modulation of performance, given the randomized order of stimulation frequencies.

The absence of any effect in the middle aged group might discourage the belief that tACS may be a promising neurorehabilitation tool. However, there is interesting evidence about facilitatory effects of a different noninvasive brain stimulation technique such as tACS on WM performance accordingly to the education factor in older adults (Berryhill and Jones 2012). This may be a source of inspiration for a further investigation on aging population. So far, just a few studies showed tACS induces beneficial effects on patients with Parkinson’s disease and with visual impairment (Brittain et al. 2013; Fedorov et al. 2011; Krause et al. 2013). However, additional mechanisms of action of tACS beyond “resonance” with oscillatory activity still need to be fully elucidated. There may also be other mechanisms related to the coupling of neural activity and subsequent change in cerebral blood flow, the so called “neurovascular coupling” (Dutta 2015). Thus further advanced functional magnetic resonance (fMRI) and EEG studies combined with online tACS may help to selectively localize a specific temporal-spatial domain hot spot to apply stimulation in aging individuals and in patients.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


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