

Effects of Aging on Cerebral Oxygenation during Working-Memory Performance: A Functional Near-Infrared Spectroscopy Study

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Abstract

Working memory is sensitive to aging-related decline. Evidence exists that aging is accompanied by a reorganization of the working-memory circuitry, but the underlying neurocognitive mechanisms are unclear. In this study, we examined aging-related changes in prefrontal activation during working-memory performance using functional Near-Infrared Spectroscopy (fNIRS), a noninvasive neuroimaging technique. Seventeen healthy young (21–32 years) and 17 healthy older adults (64–81 years) performed a verbal working-memory task (n-back). Oxygenated and deoxygenated hemoglobin concentration changes were registered by two fNIRS channels located over the left and right prefrontal cortex. Increased working-memory load resulted in worse performance compared to the control condition in older adults, but not in young participants. In both young and older adults, prefrontal activation increased with rising working-memory load. Young adults showed slight right-hemispheric dominance at low levels of working-memory load, while no hemispheric differences were apparent in older adults. Analysis of the time-activation curve during the high working-memory load condition revealed a continuous increase of the hemodynamic response in the young. In contrast to that, a quadratic pattern of activation was found in the older participants. Based on these results it could be hypothesized that young adults were better able to keep the prefrontal cortex recruited over a prolonged period of time. To conclude, already at low levels of working-memory load do older adults recruit both hemispheres, possibly in an attempt to compensate for the observed aging-related decline in performance. Also, our study shows that aging effects on the time course of the hemodynamic response must be taken into account in the interpretation of the results of neuroimaging studies that rely on blood oxygen levels, such as fMRI.

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Introduction

Working memory is one of the cognitive functions that is particularly sensitive to aging-related decline [1–3]. Aging is associated with both decrements in working-memory capacity and alterations in working-memory processing networks [4]. In contrast to young adults, older people have been reported to demonstrate more bilaterally organized prefrontal components of the working-memory circuitry [5]. Further, reduced hippocampal activity [6] and additional activation of prefrontal regions during working-memory performance has been reported [7].

The precise underlying neurocognitive mechanisms of altered prefrontal activation in older adults are unclear. Two main hypotheses regarding the alterations have been proposed: the dedifferentiation and compensation hypotheses. The dedifferentiation view holds that due to decreased neural responsivity and increased neural noise, the cortical representations become less distinctive in the aging brain. This leads to the recruitment of similar brain systems by different neurocognitive functions,

regardless of whether it is beneficial for behavioral performance or not [8]. According to the compensatory reorganization hypothesis, additional recruitment of brain regions may represent compensatory mechanisms recruited to counteract aging-related neurocognitive decline, in order to achieve or attempt to achieve the same performance levels as younger adults [9,10].

The recruitment of additional neural circuitry is not unique to the aging brain. The Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) proposes that, irrespective of age, neural engagement varies with the level of task demand [9]. According to CRUNCH, declined neural efficiency in older adults leads to recruitment of more neural resources than young adults at low levels of task demand. However, as task demands increase, older adults reach a limit of neural resource availability, resulting in underactivation relative to young adults at higher loads. Consistent with this notion, previous functional Magnetic Resonance Imaging (fMRI) and electroencephalography (EEG) re-

search showed that working-memory processing is modulated by working-memory load and age [11–15].

The current study utilizes functional Near-Infrared Spectroscopy (fNIRS), a noninvasive neuroimaging technique, to gain more insight into aging-related changes in functional prefrontal activation patterns during working-memory performance. The principles of fNIRS have been extensively described (for review see [16–18]). fNIRS is particularly sensitive to the microvasculature [19,20] and enables monitoring of concentration changes in cortical oxygenated ($[O_2Hb]$) and deoxygenated hemoglobin ($[HHb]$) with high temporal resolution. Based on the tight coupling of neural activity and oxygen delivery [21], both increases in $[O_2Hb]$ and decreases in $[HHb]$ are taken as indicator of cortical activation [22]. fNIRS has been used to gain more insight into the physiological mechanisms of the BOLD response during fMRI [22] and may, for example, be a useful technique for brain-computer interfaces [23]. In comparison to fMRI, fNIRS has low running costs, a high portability, it is relatively insensitive to movement artifacts, and it enables measurements in a natural setting. These advantages of fNIRS make the technique perfectly suitable to study functional brain activation in a broad range of participants including children, psychiatric patients, and elderly.

Few studies have applied fNIRS in the field of cognitive aging. These fNIRS studies showed an aging-related decline in prefrontal activity during performance of an arithmetic task [24], a verbal fluency task [25], a Stroop task [26], three subtests of the Wechsler Adult Intelligence Scale [27], or a walking-while-talking task [28]. Herrmann et al. [29] found aging-related decline in prefrontal activity during a verbal-fluency task. Young adults showed left-hemispheric lateralization, but older adults did not show a lateralization effect. Tsujii et al. [30] reported that the right inferior frontal cortex was more activated than the left inferior frontal cortex in young adults during performance of a deductive reasoning task. Older adults additionally recruited the left inferior frontal cortex in order to compensate for age-related decline. To our knowledge, no fNIRS studies on the relationship between prefrontal activation and working-memory load in older adults have been published to date.

In fMRI research, the n-back paradigm has reliably and validly been employed in establishing cerebral activity patterns in the prefrontal cortex in relation to increasing working-memory load [31,32]. Performance of the n-back task requires on-line monitoring, updating, and manipulation of remembered information. Therefore it is assumed that large demands are placed on several key processes within working memory. The executive processing components of working-memory are assumed to be more affected by aging than the storage components [33]. In contrast to, for example, delayed-matching-to-sample tasks, the n-back task requires not only passive maintenance, but also executive processing operations, especially at higher task loads. Therefore, the n-back task is highly suitable for cognitive aging research.

fMRI studies reported increased prefrontal activity in older adults in comparison with young adults during performance of a verbal 1-back task. Higher working-memory loads (2- and 3-back) resulted in reduced prefrontal activity [11,12]. Nagel et al. [13] reported that in young adults, activation increased linearly from 1-back up to the highest level of working-memory load (3-back) in several prefrontal areas except the right ventrolateral cortex. By contrast, for older adults no linear increase was reported, except for the left frontopolar cortex, suggesting compensatory activation at low load. The EEG study of Missonnier et al. [14] showed that in older adults the amplitude of the frontal early positive-negative working memory (PNwm) component, reflecting neural activity,

reached maximal values during the less demanding 0-back task. In young adults, maximal values were reached during performance of the 2- and 3-back task.

The aim of the current study was to investigate how prefrontal brain activity is changing as a function of parametric manipulations of working-memory load in both young and older adults. Based on theories of prefrontal compensatory mechanisms in aging [9,34] we hypothesized that, compared to young, older people show increased prefrontal recruitment during working-memory performance. To test this hypothesis we measured prefrontal activation during performance of a verbal n-back task using fNIRS.

Methods

Participants

Thirty-four healthy Dutch speaking volunteers participated in the study (17 young adults, 10 female, mean age = 25.9 ± 3.0 years, range 21–32; 17 older adults, 11 female, mean age = 70.7 ± 5.2 years, range 64–81). The young adults were recruited from the social network of the authors and were just as naive about the study as the older adults. The older adults were recruited from local bridge, chess, and senior clubs. They did not experience memory problems (self report), were living independently at home, and had unimpaired cognitive function as assessed with the Mini Mental State Examination [35] (mean score = 29.2 ± 0.9 , range 27–30). The educational level of the participants was assessed based on the Dutch educational system [36], using seven categories (1 = less than primary school, 7 = university degree). The educational level slightly differed between the young ($M = 6.7 \pm 0.7$, range 5–7) and older adults ($M = 5.5 \pm 0.9$, range 4–7) (Mann Whitney $U = 49.50$, $p < .001$), but all participants completed secondary school or higher. Also, in older adults, educational level often underestimates actual intelligence, because many had limited access to advanced schooling. All participants had an IQ > 85 as estimated by the Dutch version of the National Adult Reading Test [37]. All were right-handed and had normal or corrected-to-normal vision. None of the participants had a history of neurological/psychiatric disease or used psychopharmacological drugs. Six older adults used antihypertensive medication. All participants refrained from alcohol, caffeine, and nicotine from at least 3 hours before the experimental session.

Ethics statement

The research proposal of the present study was submitted to the regional medical-ethics committee (CMO Arnhem-Nijmegen, no. 2009/198), but was deemed exempt from formal medical ethical evaluation, because the study does not fall within the remit of the Medical Research Involving Human Subjects Act (WMO). All participants gave written informed consent. All data were anonymized before analysis. The study was performed according to the Helsinki Declaration.

Experimental paradigm

Participants performed three versions of a verbal n-back task (see Figure 1): 0-back (control condition), 1-back (low working-memory load condition), and 2-back (high working-memory load condition). Prior to all conditions, participants practiced the task for one minute and received feedback about their performance. The conditions were preceded by a baseline period of one minute, during which a black fixation cross was displayed at the center of the 15 inch screen. Stimuli were presented in black on a grey background using E-prime 2.0 software (Psychology Software Tools, PA, USA), which also registered the behavioral perfor-

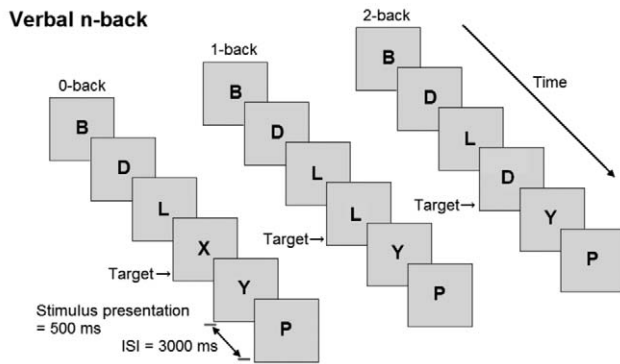


Figure 1. Schematic overview of the verbal n-back task. Stimulus presentation was 500 ms, interstimulus interval (ISI) 3000 ms. During the ISI, a fixation cross was displayed. Participants were allowed to respond until the next stimulus appeared. doi:10.1371/journal.pone.0046210.g001

mance. Stimulus presentation time was 500 ms. Interstimulus interval was set to 3000 ms to optimize the behavioral response of the older participants [33,38]. All conditions consisted of 60 trials of which 17 were target trials. The distribution of targets over the task period was random. In each trial participants indicated whether the stimulus was a target by pressing the button under the right index finger, or a non-target by pressing the button under the right middle finger (PST Serial Response Box, Psychology Software Tools Inc., PA, USA). On each trial, a letter that was randomly selected from a set of 20 consonants was displayed at the center of the screen. In the 0-back condition, the letter “X” was defined as target. In the 1-back and 2-back condition, the target was any letter that was identical to the letter presented *n* trials before, while the letter “X” was no longer shown. The whole experimental procedure lasted around 20 minutes per participant. To minimize effects of fatigue, participants were able to rest a couple of minutes between conditions.

Instrumentation

[O₂Hb] and [HHb] (μmol/L) were measured by a continuous-wave NIRS device (Oxyon, Artinis Medical SystemsTM, The Netherlands) using light of three wavelengths (775, 845, 904 nm). Near-infrared light penetrates biological tissue such as the skull and brain rather easily. In the cerebral cortex, the near-infrared light is absorbed by the chromophores O₂Hb and HHb, which have different absorption spectra. Assuming constant scattering [39] and by using the modified Lambert-Beer Law, it is possible to calculate the concentration of these chromophores in the penetrated brain tissue based on changes in the detected light intensity. Both increases in [O₂Hb] and decreases in [HHb] are indicators of cortical activation. Concentration changes in total hemoglobin ([tHb]), defined as the sum of changes in [O₂Hb] and [HHb], are used as an indicator of alterations in total blood volume. Since absolute concentration of the chromophores O₂Hb and HHb cannot be determined by a continuous-wave NIRS device, all measurements are expressed as absolute concentration changes from an arbitrary zero at the start of the measurement period. Data were sampled at 125 Hz.

Two pairs of optodes were bilaterally attached to the forehead and were tightly fixed in a customized headband (Spencer technologiesTM, Seattle, Wa). The detection optodes were placed 25–30 mm above the midpoint of the eyebrow, at approximately FP1 and FP2 according to the international 10–20 electrode system (see Figure 2). The emission optodes were laterally placed

at approximately F7 and F8. The emitter-detector spacing was 50 mm to minimize contamination from the extra-cerebral circulation and maximize signal intensity [40,41]. Based on Monte Carlo simulation, the average photon path from emitter to detector is estimated to be ellipsoid or banana-shaped with a penetration depth of approximately 2 to 3 cm [42,43]. Hence, the cerebral areas under investigation were the left and right superior and middle frontal gyrus (Brodmann’s area 10/46) [44]. The differential pathlength factor (DPF), which accounts for the increased distance travelled by light due to scattering, is age-dependent [45]. For the young adults, DPF was calculated by the formula $4.99+0.067 \times \text{Age}^{0.814}$. At present however, no data are available on the actual variation of DPF in adults aged above 50 years. Therefore, in the older adults, the DPF was set to 6.61, corresponding to age 50 [45]. Although this results in inaccurate estimation of true DPF in the elderly, it will have no effect on the accuracy of our assessment of relative changes in [O₂Hb] and [HHb] within and between conditions [46].

Cerebral hemodynamic changes during the n-back task may potentially be influenced by systemic changes. To account for this, blood pressure was measured simultaneously using a photoplethysmography cuff on the index or middle finger of the left hand of the participant (Finometer, Finapres Medical SystemsTM, the Netherlands). Blood pressure data were available from 15 young and 14 older adults.

Data processing

fNIRS data were analyzed using commercially available software (Oxysoft, Artinis Medical SystemsTM, The Netherlands). A moving average window of 1 s was applied to the [O₂Hb] and [HHb] signals to filter out the noise of the heart beat frequency. The first three trials (all non-targets) of all conditions were excluded from behavioral and fNIRS data analyses. For the fNIRS signals, the value at the start of the fourth trial was taken as zero. Changes of [O₂Hb], [HHb], and [tHb] were recalculated for 180 s from this point. Subsequently, each task period was divided into six time segments of 30 s. Mean values of [O₂Hb], [HHb], and [tHb] were calculated for each time segment. To quantify asymmetric prefrontal activation, the laterality index (LI) was calculated for the time segments, and for each task the average LI was determined. LI was defined as: $100 \times (|\Delta Q_{\text{left}}| - |\Delta Q_{\text{right}}|) / (|\Delta Q_{\text{left}}| + |\Delta Q_{\text{right}}|)$, where Q_{left} and Q_{right} represent the fNIRS



Figure 2. Positioning of the two pairs of fNIRS optodes. The subject of the photograph has given written informed consent (as outlined in the PLoS consent form) to publication of her image. doi:10.1371/journal.pone.0046210.g002

parameters [O₂Hb], [HHb], and [tHb], measured by the left and right fNIRS channel respectively. The value of the LI ranges from -100, indicating pure right-hemispheric dominance, to +100, indicating pure left-hemispheric dominance. A value near zero indicates no dominant hemisphere.

Statistical analysis

Statistical analysis was performed using PASW Statistics software version 18.0 (SPSS Inc., Chicago, IL, USA). Behavioral performance on the verbal n-back tasks was assessed by number of hits, misses, correct rejections, and false alarms. The non-parametric discrimination index (i.e., sensitivity) A' was calculated by the formula: $0.5 + ((\text{hit rate} - \text{false alarm rate}) \times (1 + \text{hit rate} - \text{false alarm rate})) / (4 \times \text{hit rate} \times (1 - \text{false alarm rate}))$. A' is a performance variable derived from signal detection theory [47] and ranges from 0.5 (chance level) to 1 (perfect discrimination between targets and non-targets). Shapiro-Wilk tests and Q-Q plots indicated that the assumptions for performing an ANOVA were not met. Accuracy (i.e. A') of the young and older adults was compared by Mann-Whitney U tests. In both groups, the effects of working-memory load were established by performing Wilcoxon signed-rank tests. For reaction time on targets and non-targets a 2 (group: young, old) × 3 (load: 0-,1-,2-back) repeated measures ANOVA was performed.

The assumption of normality was met by the fNIRS data acquired during the verbal n-back task, as was established by Shapiro-Wilk tests. For each of the hemodynamic measures, [O₂Hb], [HHb], and [tHb], a 2 (location: left, right hemisphere) × 2 (group: young, old) × 3 (load: 0-,1-,2-back) × 6 (time: time segments 1, 2, 3, 4, 5, 6) repeated measures ANOVA was performed. Due to violations of the sphericity assumption, Greenhouse-Geisser corrections were applied. Significant main and interaction effects were further analyzed by means of planned contrasts. With respect to the aim of the current study, primarily significant effects involving the factors load and age are reported, as these are the factors of interest. Because we expected that activation in the left and right hemisphere is differentially affected by the factors load and age, data from the left and right channel were analyzed separately. With respect to the factor time, a trend analysis was performed to characterize the time course over the whole task period.

Results

Behavioral performance

Table 1 shows the behavioral results of the young and older adults during the n-back tasks. Compared to the control condition, both low and high working-memory load led to a declined accuracy in older adults (0- vs. 1-back $z = -3.045$, $p < .001$; 0- vs. 2-back $z = -3.295$, $p < .001$; 1- vs. 2-back $z = -2.480$, $p = .011$), but not in young adults. However, with greater load reaction times on targets and non-targets increased significantly in both groups ($p < .001$). No group × load interaction was found for reaction time.

fNIRS results

Figure 3 shows the courses over time of the raw fNIRS signals that were measured during the 20 s pre-task baseline period and during the 180 s verbal n-back tasks. Figures 4 and 5 display the mean changes of [O₂Hb], [HHb], and [tHb] for each of the six 30 s time segments in which we divided the 180 s n-back tasks. The four-way interaction of location × time × group × load was not significant ([O₂Hb]: $F_{(5.23, 167.50)} = 1.70$, $p = .134$; [HHb]: $F_{(5.53, 177.68)} = 0.44$, $p = .839$; [tHb]: $F_{(4.40, 140.92)} = 2.30$, trend $p = .055$). A significant location × time × group interaction indicated lateralization effects, which were not consistent across time and groups ([O₂Hb]: $F_{(2.64, 84.46)} = 5.00$, $p = .004$; [HHb]: $F_{(2.49, 79.52)} = 0.96$, $p = .404$; [tHb]: $F_{(2.47, 78.98)} = 4.51$, $p = .009$). Further testing at group level revealed however no significant lateralization effects in the older adults (all LIs $< \pm 12$), indicating bilateral activation during 0-back, 1-back and 2-back performance. In the young adults, however, a trend was found for the location × time × load interaction, but only for changes of [O₂Hb] ($F_{(10, 160)} = 1.80$, $p = .065$). The location × time interaction was significant ([O₂Hb]: $F_{(2.82, 45.15)} = 5.15$, $p = .004$; [HHb]: $F_{(2.17, 34.75)} = 0.96$, $p = .295$; [tHb]: $F_{(2.68, 42.89)} = 4.84$, $p = .007$). LIs suggested slight right-hemispheric dominance during 0-back ([O₂Hb]: LI = -21.7; [HHb]: LI = -4.8; [tHb]: LI = -10.1) and 1-back ([O₂Hb]: LI = -14.9; [HHb]: LI = -14.5; [tHb]: LI = -19.8), but not during 2-back performance ([O₂Hb]: LI = -4.6; [HHb]: LI = -0.28; [tHb]: LI = -15.8). Therefore, to establish effects of working-memory load and age, data from the left and right fNIRS channel were analyzed separately.

Working-memory load. In the young adults, left prefrontal activation was, overall, larger with increasing working-memory load ([O₂Hb]: $F_{(2, 32)} = 3.98$, $p = .029$; [HHb]: $F_{(2, 32)} = 5.99$, $p = .006$; [tHb]: $F_{(2, 32)} = 5.48$, $p = .009$). The 2-back vs. 1-back

Table 1. Accuracy and reaction times (Mean ± SD) for the verbal n-back tasks.

		Young adults			Older adults			U- or F-statistic
A' (accuracy)	0-back	0.99	±	0.01	0.99	±	0.01	139.00
	1-back	0.98	±	0.02	0.97	±	0.03	94.00
	2-back	0.99	±	0.01	0.94	±	0.05	51.50**
RT target (ms)	0-back	496.60	±	79.92	660.95	±	125.05	20.85**
	1-back	550.56	±	77.49	689.89	±	99.91	20.64**
	2-back	643.03	±	134.01	870.59	±	154.52	21.04**
RT non-target (ms)	0-back	498.38	±	84.32	610.38	±	87.41	14.46**
	1-back	573.76	±	101.29	667.98	±	97.97	7.60*
	2-back	728.73	±	155.20	832.19	±	159.75	3.67

For group comparisons (young vs. older adults) of accuracy Mann-Whitney-U test values are given. For comparisons of reaction time F values are given.

** $p \leq .001$,

* $p < .01$.

doi:10.1371/journal.pone.0046210.t001

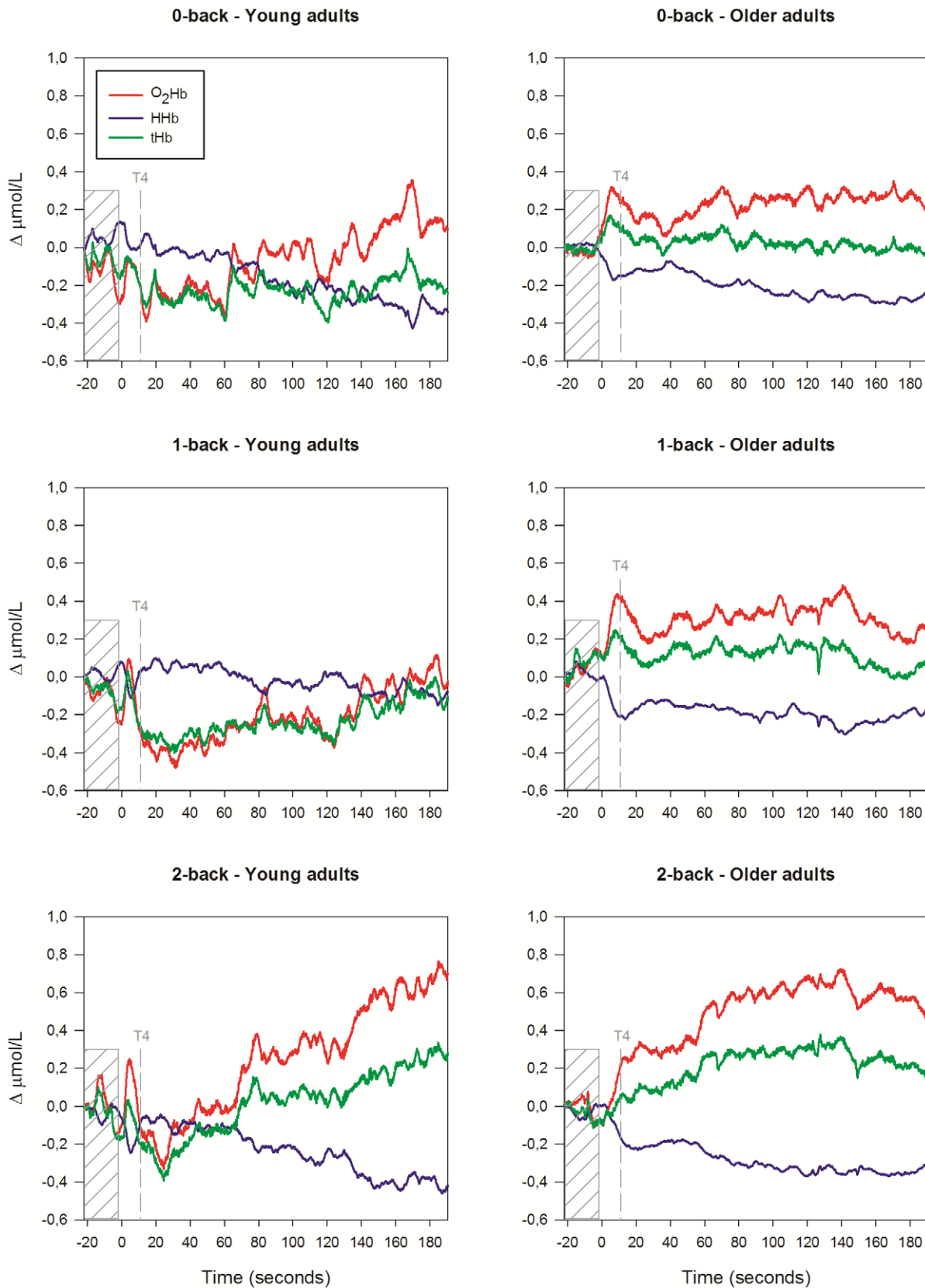
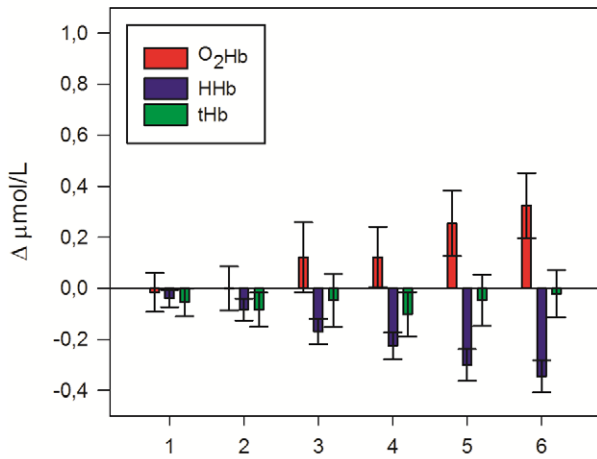
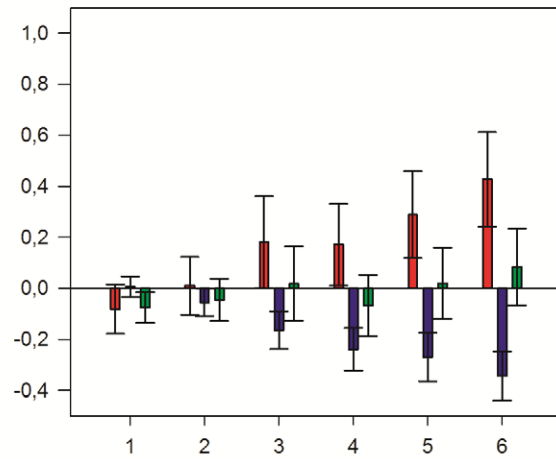


Figure 3. Grand average waveforms of [O₂Hb], [HHb] and [tHb] changes in the prefrontal cortex. The Figure shows raw fNIRS signals of young and older adults during performance of the verbal 0-back task (*upper panels*), 1-back task (*middle panels*) and 2-back task (*lower panels*). The last 20 s of the pre-task baseline period are marked in the Figure. The n-back task starts at 0 s. The first three trials were excluded from further data analysis. The start of the fourth trial has been marked by the dashed line (T4). For illustrative purposes, in this Figure the signals were nulled at -22 s and were averaged over the left and right optode pair.
doi:10.1371/journal.pone.0046210.g003

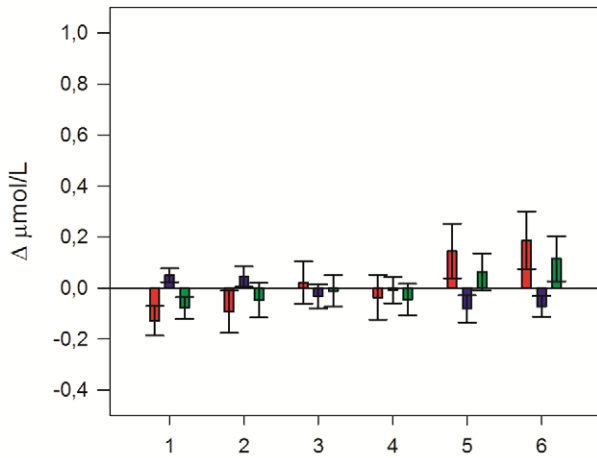
0-back - Young adults - Left channel



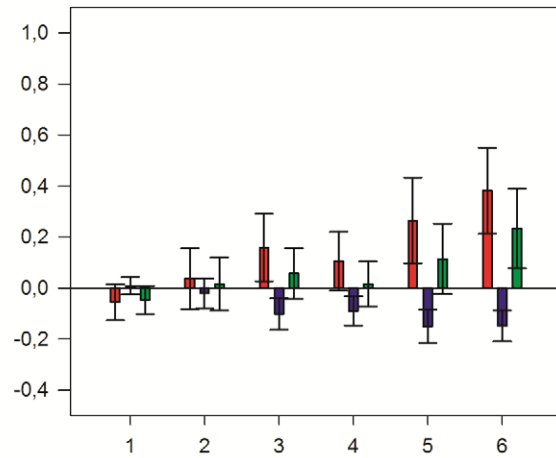
0-back - Young adults - Right channel



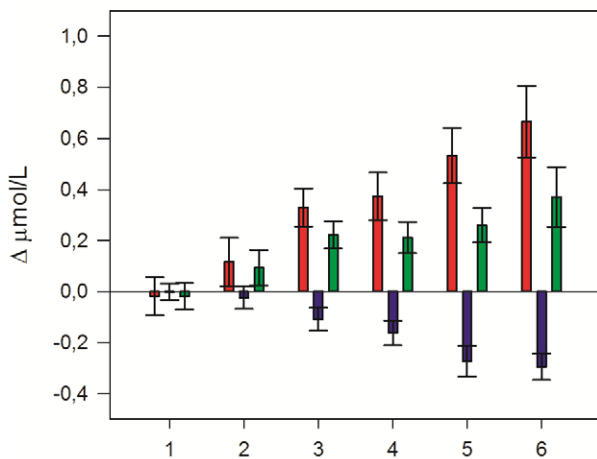
1-back - Young adults - Left channel



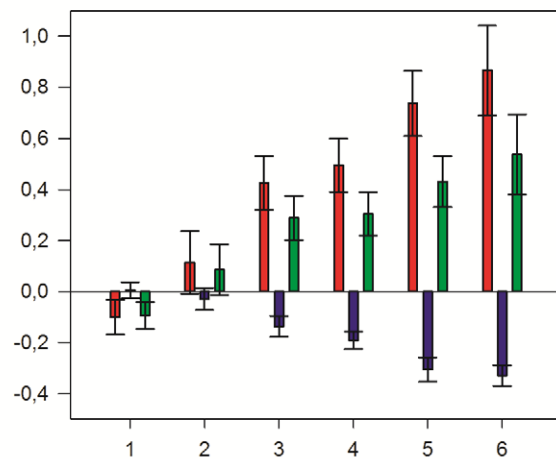
1-back - Young adults - Right channel



2-back - Young adults - Left channel



2-back - Young adults - Right channel



Time Segment

Time Segment

Figure 4. Hemodynamic concentration changes in young adults. Mean (\pm SEM) changes of [O₂Hb], [HHb] and [tHb] in the left and right hemisphere during the six 30-s time segments of the verbal 0-back task (*upper panels*), 1-back task (*middle panels*) and 2-back task (*lower panels*). doi:10.1371/journal.pone.0046210.g004

comparison showed increased [O₂Hb] ($p = .007$), decreased [HHb] ($p = .015$), and increased [tHb] ($p = .020$). The 2-back vs. 0-back comparison revealed increased [O₂Hb] (trend; $p = .058$) and increased [tHb] ($p = .003$). The 1-back vs. 0-back comparison showed decreased [HHb] ($p = .005$). For the right prefrontal cortex, there were no clear effects of working-memory load in the young adults. Only the 2-back vs. 0-back comparison showed increased [O₂Hb] (trend; $p = .057$) and increased [tHb] ($p = .016$).

In the older adults, both left and right prefrontal activation increased with rising load (left: [O₂Hb]: $F_{(2, 32)} = 10.94$, $p < .001$; [HHb]: $F_{(2, 32)} = 2.41$, $p = .106$; [tHb]: $F_{(2, 32)} = 11.94$, $p < .001$; right: [O₂Hb]: $F_{(2, 32)} = 10.96$, $p < .001$; [HHb]: $F_{(2, 32)} = 7.17$, $p = .003$; [tHb]: $F_{(2, 32)} = 6.89$, $p = .003$). The 2-back vs. 1-back comparison revealed increased [O₂Hb] (left and right: $p < .001$), decreased [HHb] (left: $p = .013$; right: $p < .001$), and increased [tHb] (left: $p < .001$; right: $p = .006$) in both hemispheres. The 2-back vs. 0-back comparison revealed increased [O₂Hb] (left: $p = .008$; right: $p = .003$) and increased [tHb] (left and right: $p = .001$) in both hemispheres. No significant changes of the fNIRS parameters were found for the 1-back vs. 0-back comparison.

Group differences. For the left fNIRS channel, significant time \times group interactions were found for the 1-back and 2-back task, but not for the 0-back task, suggesting differences in left prefrontal activation over time between young and old. The 1-back condition elicited a significantly different response between groups. Young adults showed a small initial drop of [O₂Hb] and [tHb], and subsequently an increase of [O₂Hb] and [tHb]. Contrary to that, older adults showed a decrease of [O₂Hb] and [tHb] ([O₂Hb]: $F_{(3.35, 107.14)} = 2.97$, $p = .030$; [HHb]: $F_{(3.00, 95.90)} = 0.42$, $p = .741$; [tHb]: $F_{(2.70, 86.26)} = 3.26$, $p = .030$). During 2-back performance, both groups showed an increase of [O₂Hb] and a decrease of [HHb], although the time courses significantly differed ([O₂Hb]: $F_{(2.43, 77.84)} = 4.00$, $p = .016$; [HHb]: $F_{(2.45, 78.35)} = 4.34$, $p = .011$; [tHb]: $F_{(2.24, 71.65)} = 1.83$, $p = .163$).

For the right fNIRS channel, we found significant time \times group interaction effects that were similar to the left fNIRS channel for the 1-back and 2-back task (1-back: [O₂Hb]: $F_{(2.87, 91.79)} = 2.93$, $p = .040$; [HHb]: $F_{(2.68, 85.72)} = 0.99$, $p = .393$; [tHb]: $F_{(2.44, 78.15)} = 2.60$, $p = .069$; 2-back: ([O₂Hb]: $F_{(2.46, 78.63)} = 9.34$, $p < .001$; [HHb]: $F_{(2.75, 88.09)} = 4.70$, $p = .005$; [tHb]: $F_{(2.26, 72.35)} = 7.59$, $p = .001$).

In summary, no group difference in temporal pattern of prefrontal activation was found for the 0-back task. Older adults showed a decrease of the hemodynamic response during 1-back performance, while young adults showed an increased response. During 2-back performance, young adults showed in comparison to older adults stronger activation of the prefrontal cortex over time.

Time course. The time courses of the hemodynamic changes differed between young and older adults. In young adults the changes of [O₂Hb] and [HHb] during performance of all n-back tasks and the changes of [tHb] during performance of the 2-back task followed a linear trend ($p < .05$). In the older adults the changes of [O₂Hb] (left: $p = .055$; right: $p = .048$) and [tHb] (left: $p = .031$; right: $p = .078$) tended to follow a quadratic course during the 1-back task. For the 2-back task, clear quadratic trends were found ([O₂Hb]: left and right: $p = .002$; [HHb]: left: $p = .180$; right: $p = .099$; [tHb]: left and right: $p = .001$).

In addition to this difference in linear vs. quadratic shape of the time-activation curve, we further analyzed group differences in

prefrontal activation during the six time segments of the 2-back task. Young adults showed a further increase of [O₂Hb] and [tHb] and a decrease of [HHb] after the fourth time segment ($p \leq .01$, with the exception of [tHb] left: $p = .128$). Contrary to that, in older adults the changes of the fNIRS parameters reached a maximum and plateau level during the third time segment. [tHb] significantly declined after the fifth time segment ($p < .05$).

Blood pressure

Mean blood pressure slightly increased during performance of the 0-back (young: 0.9 ± 2.0 mmHg; old: 3.2 ± 3.9 mmHg), 1-back task (young: 1.3 ± 2.3 mmHg; old: 4.2 ± 5.8 mmHg), and 2-back task (young: 3.9 ± 4.5 mmHg; old: 6.1 ± 4.9 mmHg) in comparison to baseline measurements. These increases in mean blood pressure did however not significantly differ between groups (0-back: $F_{(1, 27)} = 3.85$, $p = .060$; 1-back: $F_{(1, 27)} = 3.33$, $p = .079$; 2-back: $F_{(1, 27)} = 1.59$, $p = .218$). Therefore, we conclude that the cerebral hemodynamic differences between the groups cannot be explained by systemic changes, but are truly the result of aging effects.

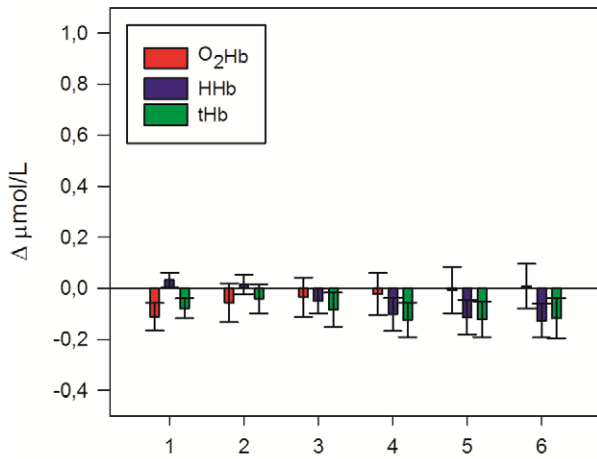
Discussion

The present study investigated the effects of aging on activation of the prefrontal cortex, by measuring changes of [O₂Hb], [HHb], and [tHb] during verbal working-memory performance. Based on our results it can be hypothesized that young adults were better able to keep the prefrontal cortex recruited over time during high working-memory load performance. Older adults appear to recruit both hemispheres already at low levels of working-memory load, possibly in an attempt to compensate for the observed aging-related decline in performance.

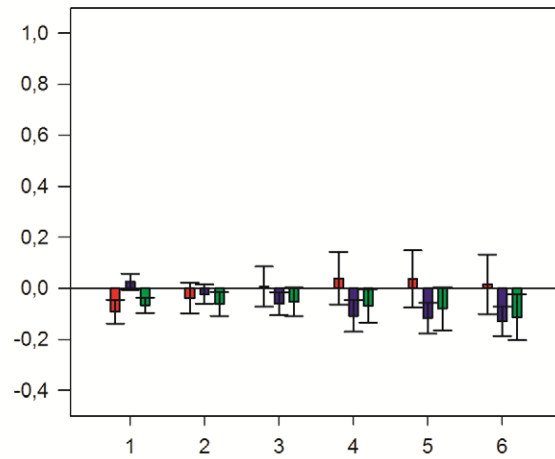
The fNIRS analyses revealed that in both young and older adults prefrontal activation increased with rising working-memory load. The results of the young adults are in agreement with other fNIRS studies on verbal n-back performance. Hoshi et al. [48], Herrmann et al. [49], and Honma et al. [50] reported that the degree of increase of [O₂Hb] and decrease of [HHb] showed a positive correlation with working-memory load in young adults. Previous fNIRS studies on cognitive aging suggest aging-related decline in prefrontal activity during cognitive performance. However, to our knowledge, no fNIRS studies using a verbal n-back task in older adults have been published to date. Some fNIRS studies indicate reduced prefrontal lateralization in older adults [29,30]. Unfortunately, not all previous fNIRS studies performed measurements on both hemispheres or performed analyses on lateralization effects or reported on these. The results of fMRI and EEG studies on the relationship between working-memory load and prefrontal activation are mixed. However, a common finding is that older adults show maximum prefrontal activation at low working-memory loads and that young adults show increasing prefrontal activation up to high working-memory loads [11–14]. In our study, the largest prefrontal activity was found during 2-back performance for both the young and the older adults.

Our behavioral results showed that low and high working-memory load resulted in a declined accuracy in comparison to the control condition in older adults, while young adults demonstrated the same level of accuracy in all conditions. We consider it unlikely that the aging-related differences in prefrontal activation are related to task difficulty in general. Barch et al. [38] found that the dorsolateral prefrontal cortex is specifically involved in working-

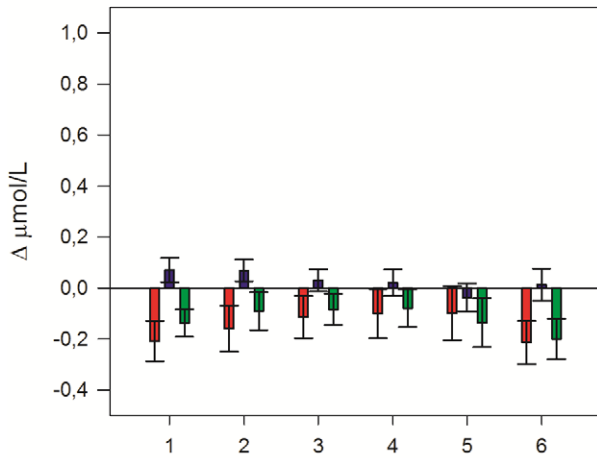
0-back - Older adults - Left channel



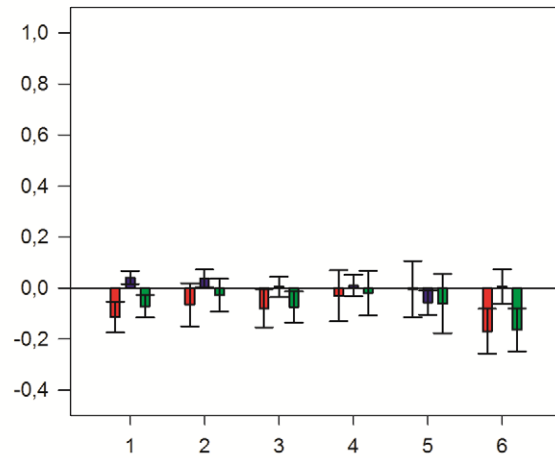
0-back - Older adults - Right channel



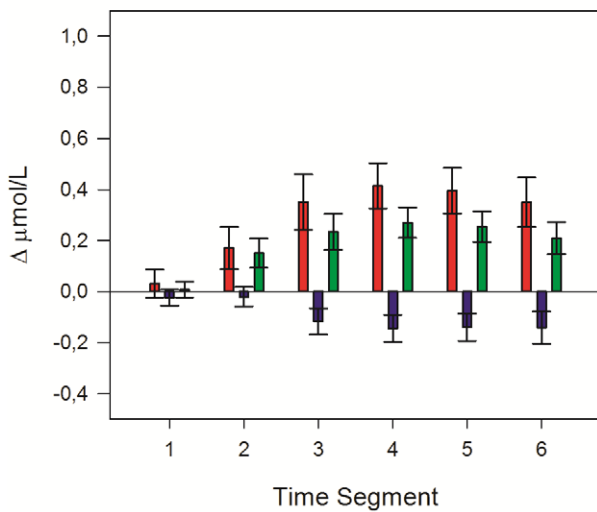
1-back - Older adults - Left channel



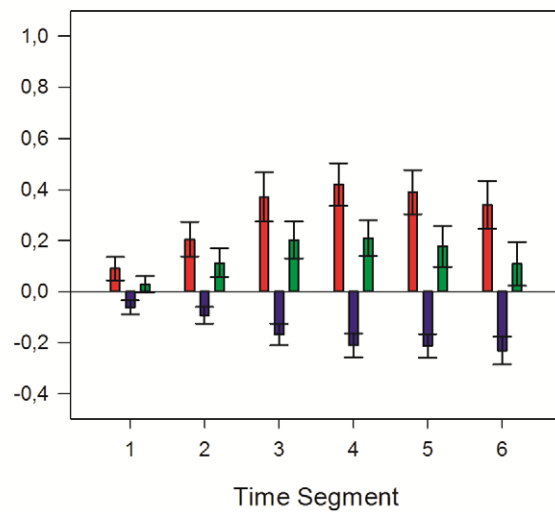
1-back - Older adults - Right channel



2-back - Older adults - Left channel



2-back - Older adults - Right channel



Time Segment

Time Segment

Figure 5. Hemodynamic concentration changes in older adults. Mean (\pm SEM) changes of [O₂Hb], [HHb] and [tHb] in the left and right hemisphere over the six 30-s time segments of the verbal 0-back task (*upper panels*), 1-back task (*middle panels*) and 2-back task (*lower panels*). doi:10.1371/journal.pone.0046210.g005

memory function, and that this area is not responsive to task difficulty. Moreover, in the present study, accuracy during 2-back performance was on average very high in both groups. This suggests that working-memory capacity was not exceeded in either of the groups. Furthermore, we found a consistent difference in reaction time between the young adults and elderly, which is in line with the notion that many aspects of information processing become less efficient with increasing age [33]. In both groups reaction times slowed with increasing load. Taken together, our results provide a reliable estimate of the relationship between working-memory load and activation of the prefrontal cortex.

The pattern of prefrontal activation differed between the age groups. Older adults showed bilateral activity during all conditions, whereas young adults showed slight right-hemispheric dominance during 0-back and 1-back performance. These results are in agreement with the HAROLD model ([34], p. 85) which states that “under similar circumstances, prefrontal activity during cognitive performance tends to be less lateralized in older adults than in younger adults”. The function of age-related reductions in asymmetry is unclear, but different interpretations have been introduced such as the dedifferentiation [8] and compensation hypotheses [9]. Our results are in line with CRUNCH [9]. According to CRUNCH, activity in cortical regions is upregulated as task load increases, independent of age. Older adults however, may need to recruit more cortical regions at lower levels of task demand in order to compensate for reduced neural efficiency. In the current study we found that older adults showed bilateral activity already at lower loads. Young adults showed slight right-hemispheric dominance at lower loads. At high task load, young adults no longer showed a lateralization effect, indicating additional recruitment of the left hemisphere.

Furthermore, the time-activation curve during high working-memory load performance differed between groups; it tended to follow a linear course in young adults and a quadratic course in older adults. Maximum and plateau level of activation were reached earlier in older adults than in young adults. Possibly, older adults reached the limit of available neural resources before the young adults did. Alternatively, performance may have become more automatic in older adults. Evidence exists that prefrontal activity increases during initial learning and decreases as a task becomes more practiced [51]. However, we consider this explanation less plausible, because an automatic response would also have occurred in young adults and especially in high performers.

The time course of hemoglobin changes during prolonged cognitive activation has been assessed by means of fNIRS in children [52], in young adults [53–55], in middle aged adults [24], and in Alzheimer patients versus older adults [56]. However, we are not aware of any other fNIRS study that directly compared young and older adults during prolonged cognitive activation using the same task.

Butti et al. [57] provided a detailed description of the time course of the fNIRS parameters in the prefrontal cortex of young healthy adults, while they performed a continuous performance task for ten minutes. The task elicited a significant change of the fNIRS parameters which did not remain constant during the task period. The authors concluded that this fluctuation of fNIRS parameters, which was not correlated with behavioral performance, is the result of a complex mechanism of coupling and uncoupling between cerebral blood flow and cerebral metabolic

rate of oxygen. In addition, our study indicates that aging has an effect on the time course of [O₂Hb] and [HHb] changes during sustained activation which is possibly the result of changes in neurovascular coupling that accompany aging, although we could not establish the relationship between fNIRS parameter change and behavioral performance. Neurovascular coupling refers to the relationship between local neural activity and subsequent changes in hemodynamic properties of the surrounding vasculature, including cerebral blood flow, cerebral blood volume, and cerebral metabolic rate of oxygen. The mechanisms that underlie neurovascular coupling are not fully understood. However, evidence exists that these mechanisms alter with aging [58]. Aging is accompanied by a degeneration of the vascular system, probably beginning as early as the fourth decade of life [59]. Regional cerebral blood flow, for example, decreases with age and vessel stiffness is enhanced [60]. These phenomena are accompanied by a reduction in the cerebral metabolic rate of oxygen [61]. Accordingly, fNIRS may be a valuable complementary method to fMRI in unraveling the effects of aging on the time course of the hemodynamic response.

For data analysis it is important to keep the findings of León-Carrión et al. [62,63] and Butti et al. [57] in mind. Their fNIRS studies showed that exposure to stimuli can cause prefrontal activation lasting long after stimuli cessation and may even induce more robust prefrontal activation after than during the task period. In several fNIRS studies, the mean of a pre- and post-task baseline was calculated to exclude slow drift during the task period by means of a linear fitting procedure. An overshoot of [O₂Hb] during the post-task period might lead to an overestimation of drift, resulting in an underestimation of the genuine effects. In our study, we also found a substantial overshoot of [O₂Hb] in both young and older adults after task cessation (data not reported). Therefore, we argue for an analysis procedure that does not incorporate post-task baseline activation.

Finally, we recognize some limitations of our study. Since we only monitored activation of the prefrontal cortex, we cannot rule out the possibility that other brain regions were under- or overactivated in older adults in comparison to young adults. We cannot determine whether bilateral brain activity leads to improved performance or whether an underlying mechanism causes both functional brain reorganization and the decrement in performance. Further, in our study, we found that effects of [O₂Hb] were not always accompanied by effects of [HHb]. This is in line with the notion that in fNIRS research [O₂Hb] may be a more robust indicator for changes in regional cerebral blood flow, due to larger changes in amplitude. Since [HHb] changes are less pronounced, this may have led to slightly weaker statistical results.

Future fNIRS studies on cognitive aging should consider the relationship between performance level and prefrontal activation patterns during working-memory performance. For example, groups should be divided into low and high performers or accuracy can be correlated with prefrontal signal change. Recently, this relationship has been considered in fMRI studies [12,13,15,64]. In both young and older adults, differences in prefrontal activation patterns between high and low performers were found. It is suggested that in comparison to low performers, high performers show increased prefrontal activity under high working-memory demands. This might be true for both young and older adults.

To conclude, in this study we examined the effects of aging on prefrontal activation during working-memory performance. The results suggest that young adults are better able to keep the prefrontal cortex recruited over time. Older adults may, already at low levels of working-memory load, recruit both hemispheres possibly in an attempt to compensate for the observed aging-related decline in performance. Also, our study indicates that effects of aging on the time course of hemodynamic processes must

be taken into account in the interpretation of neuroimaging studies that rely on blood oxygen levels, such as fMRI.

Author Contributions

Conceived and designed the experiments: AV AvB JC RK. Performed the experiments: AV AvB. Analyzed the data: AV AvB JC. Contributed reagents/materials/analysis tools: AvB JC. Wrote the paper: AV AvB MOR JC RK.

References

- Salthouse TA, Babcock RL (1991) Decomposing adults age differences in working memory. *Dev Psychol* 27: 763–776.
- Baddeley AD (1992) Working memory. *Science* 255: 556–559.
- Park DC, Lautenschlager G, Hedden T, Davidson NS, Smith AD, et al. (2002) Models of visuospatial and verbal memory across the adult life span. *Psychol Aging* 17: 299–320.
- Grady CL, Craik FIM (2000) Changes in memory processing with age. *Curr Opin Neurobiol* 10: 224–231.
- Reuter-Lorenz PA, Jonides J, Smith EE, Hartley A, Miller A, et al. (2000) Age differences in the frontal lateralization of verbal and spatial working memory revealed by PET. *J Cogn Neurosci* 12: 174–187.
- Mitchell KJ, Johnson MK, Raye CL, D'Esposito M (2000) fMRI evidence of age-related hippocampal dysfunction in feature binding in working memory. *Cogn Brain Res* 10: 197–206.
- Cabeza R, Daselaar SM, Dolcos F, Prince SE, Budde M, et al. (2004) Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cereb Cortex* 14: 364–375.
- Li S-C, Lindenberger U, Sikström S (2001) Aging cognition: from neuromodulation to representation. *Trend Cogn Sci* 5: 479–486.
- Reuter-Lorenz PA, Cappell KA (2008) Neurocognitive aging and the compensation hypothesis. *Curr Dir Psychol Sci* 17: 177–182.
- Meulenbroek O, Kessels RPC, de Rover M, Petersson KM, Olde Rikkert MGM, et al. (2010) Age-effects on associative object-location memory. *Brain Res* 1315: 100–110.
- Mattay VS, Fera F, Tessitore A, Hariri AR, Berman KF, et al. (2006) Neurophysiological correlates of age-related changes in working memory capacity. *Neurosci Lett* 392: 32–37.
- Nyberg L, Dahlin E, Stigsdotter Neely A, Bäckman L (2009) Neural correlates of variable working memory load across adult age and skill: dissociative patterns within the fronto-parietal network. *Scand J Psychol* 50: 41–46.
- Nagel IE, Preuschhof C, Li S-C, Nyberg L, Bäckman L, et al. (2011) Load modulation of the BOLD response and connectivity predicts working memory performance in younger and older adults. *J Cogn Neurosci* 23: 2030–2045.
- Missonnier P, Herrmann FR, Rodriguez C, Deiber M-P, Millet P, et al. (2011) Age-related differences on event-related potentials and brain rhythm oscillations during working memory activation. *J Neural Transm* 118: 945–955.
- Schneider-Garces NJ, Gordon BA, Brumback-Peltz CR, Shin E, Lee Y, et al. (2009) Span, CRUNCH, and beyond: working memory capacity and the aging brain. *J Cogn Neurosci* 22: 655–669.
- Villringer A, Chance B (1997) Non-invasive optical spectroscopy and imaging of human brain function. *Trends Neurosci* 20: 435–442.
- Hoshi Y (2003a) Functional near-infrared optical imaging: utility and limitations in human brain mapping. *Psychophysiology* 40: 511–520.
- Obrig H, Villringer A (2003) Beyond the visible – imaging the human brain with light. *J Cereb Blood Flow Metab* 23: 1–18.
- Liu H, Boas DA, Zhang Y, Yodh AG, Chance B (1995a) Determination of optical properties and blood oxygenation in tissue using continuous NIR light. *Phys Med Biol* 40: 1983–1993.
- Liu H, Chance B, Hielscher AH, Jacques SL, Tittel FK (1995b) Influence of blood vessels on the measurement of hemoglobin oxygenation as determined by time resolved reflectance spectroscopy. *Med Phys* 22: 1209–1217.
- Logothetis NK, Wandell BA (2004) Interpreting the BOLD signal. *Annu Rev Physiol* 66: 735–769.
- Steinbrink J, Villringer A, Kempf F, Haux D, Boden S, et al. (2006) Illuminating the BOLD signal: combined fMRI-fNIRS studies. *Magn Reson Imaging* 24: 495–505.
- Fazli S, Mehnert J, Steinbrink J, Curio G, Villringer A, et al. (2012) Enhanced performance by a hybrid NIRS-EEG brain computer interface. *Neuroimage* 59: 519–529.
- Hock C, Müller-Spahn F, Schuh-Hofer S, Hofmann M, Dirnagl U, et al. (1995) Age dependency of changes in cerebral hemoglobin oxygenation during brain activation: a near-infrared spectroscopy study. *J Cereb Blood Flow Metab* 15: 1103–1108.
- Kameyama M, Fukuda M, Uehara T, Mikuni M (2004) Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. *Neuroimage* 22: 1715–1721.
- Schroeter ML, Zysset S, Kruggel F, von Cramon DY (2003) Age dependency of the hemodynamic response as measured by functional near-infrared spectroscopy. *Neuroimage* 19: 555–564.
- Kwee IL, Nakada T (2003) Dorsolateral prefrontal lobe activation declines significantly with age: functional NIRS study. *J Neurol* 250: 525–529.
- Holtzer R, Mahoney JR, Izzetoglu M, Izzetoglu K, Onaral B, et al. (2011) fNIRS study of walking and talking while talking in young and old individuals. *J Gerontol A Biol Sci Med Sci* 66: 879–887.
- Herrmann MJ, Walter A, Ehlis A-C, Fallgatter AJ (2006) Cerebral oxygenation changes in the prefrontal cortex: effects of age and gender. *Neurobiol of Aging* 27: 888–894.
- Tsujii T, Okada M, Watanabe S (2010) Effects of aging on hemispheric asymmetry in inferior frontal cortex activity during belief-bias syllogistic reasoning: A near-infrared spectroscopy study. *Behav Brain Res* 210: 178–183.
- Jansma JM, Ramsey NF, Coppola R, Kahn RS (2000) Specific versus nonspecific brain activity in an parametric n-back task. *Neuroimage* 12: 688–697.
- Owen AM, McMillan KM, Laird AR, Bullmore E (2005) N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp* 25: 46–59.
- Salthouse TA (1996) The processing-speed theory of adult age differences in cognition. *Psychol Rev* 103: 403–428.
- Cabeza R (2002) Hemispheric asymmetry reduction in older adults: the HAROLD model. *Psychol Aging* 17: 85–100.
- Folstein MF, Folstein SE, McHugh PR (1975) “Mini-mental state”: A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12: 189–198.
- Verhage F (1964) Intelligentie en leeftijd bij volwassenen en bejaarden. Van Gorcum, Assen.
- Schmand B, Lindeboom J, van Harskamp F (1992) De Nederlandse leestest voor volwassenen. Swets & Zeitlinger, Lisse.
- Barch DM, Braver TS, Nystrom LE, Forman SD, Noll DC, et al. (1997) Dissociating working memory from task difficulty in human prefrontal cortex. *Neuropsychologia* 35: 1373–1380.
- Sakatani K, Yamashita D, Yamanaka T, Oda M, Yamashita Y, et al. (2006) Changes of cerebral blood oxygenation and optical pathlength during activation and deactivation in the prefrontal cortex measured by time-resolved near infrared spectroscopy. *Life Sci* 78: 2734–2741.
- van de Ven MJT, Colier WNJM, van der Sluijs MC, Walraven D, Oeseburg B, et al. (2001) Can cerebral blood volume be measured reproducibly with an improved near infrared spectroscopy system? *J Cereb Blood Flow Metab* 21: 110–113.
- van Beek AHEA, Lagro J, Olde Rikkert MGM, Zhang R, Claassen JAHR (2011) Oscillations in cerebral blood flow and cortical oxygenation in Alzheimer's disease. *Neurobiol Aging* 33: 428.e21–428.e31.
- Haussinger FB, Heinzel S, Hahn T, Schecklmann M, Ehlis A-C, et al. (2011) Simulation of near-infrared light absorption considering individual head and prefrontal cortex anatomy: implications for optical neuroimaging. *PLoS ONE* 6: e26377.
- Fukui Y, Ajichi Y, Okada E (2003) Monte Carlo prediction of near-infrared light propagation in realistic adults and neonatal head models. *Appl Optics* 42: 2881–2887.
- Okamoto M, Dan H, Sakamoto K, Takeo K, Shimizu K, et al. (2004) Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. *Neuroimage* 21: 99–111.
- Duncan A, Meek JH, Clemence M, Elwell CE, Fallon P, et al. (1996) Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatr Res* 39: 889–894.
- Claassen JAHR, Colier WNJM, Jansen RWMM (2006) Reproducibility of cerebral blood volume measurements by near infrared spectroscopy in 16 healthy elderly subjects. *Physiol Meas* 27: 255–264.
- Grier JB (1971) Nonparametric indexes for sensitivity and bias: computing formulas. *Psychol Bull* 75: 424–429.
- Hoshi Y, Tsou BH, Billock VA, Tanosaki M, Iguchi Y, et al. (2003b) Spatiotemporal characteristics of hemodynamic changes in the human lateral prefrontal cortex during working memory tasks. *Neuroimage* 20: 1493–1504.
- Herrmann MJ, Walter A, Schreppe T, Ehlis A-C, Pauli P, et al. (2007) D4 receptor gene variation modulates activation of prefrontal cortex during working memory. *Eur J Neurosci* 26: 2713–2718.
- Honma M, Soshi T, Kim Y, Kuriyama K (2010) Right prefrontal activity reflects the ability to overcome sleepiness during working memory tasks: a functional near-infrared spectroscopy. *PLoS ONE* 5: e12923.

51. Petersen SE, van Mier H, Fiez JA, Raichle ME (1998) The effects of practice on the functional anatomy of task performance. *Proc Natl Acad Sci U S A* 95: 853–860.
52. Matsuda G, Hiraki K (2006) Sustained decrease in oxygenated hemoglobin during video games in the dorsal prefrontal cortex: a NIRS study of children. *Neuroimage* 29: 706–711.
53. Heckeren HR, Obrig H, Wenzel R, Eberle K, Ruben J, et al. (1997) Cerebral haemoglobin oxygenation during sustained visual stimulation - a near-infrared spectroscopy study. *Phil Trans R Soc B* 352: 743–750.
54. Fallgatter AJ, Strik WK (1997) Right frontal activation during the continuous performance test assessed with near-infrared spectroscopy in healthy subjects. *Neurosci Lett* 223: 89–92.
55. Fallgatter AJ, Strik WK (1998) Frontal brain activation during the Wisconsin Card Sorting Test assessed with two-channel near-infrared spectroscopy. *Eur Arch Psychiatry Clin Neurosci* 248: 245–249.
56. Hock C, Villringer K, Müller-Spahn F, Wenzel R, Heckeren H, et al. (1997) Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS) -correlation with simultaneous rCBF-PET measurements. *Brain Res* 755: 293–303.
57. Butti M, Contini D, Molteni E, Caffini M, Spinelli L, et al. (2009) Effect of prolonged stimulation on cerebral hemodynamic: a time-resolved fNIRS study. *Med Phys* 36: 4103–4114.
58. D'Esposito M, Deouell LY, Gazzaley A (2003) Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nature Rev Neurosci* 4: 863–872.
59. Farkas E, Luiten PGM (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* 64: 575–611.
60. Marin J (1995) Age-related changes in vascular responses: a review. *Mech Ageing Dev* 79: 71–114.
61. Marchal G, Rioux P, Petit-Taboué MC, Sette G, Travère JM, et al. (1992) Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Arch Neurol* 49: 1013–1020.
62. León-Carrión J, Damas J, Izzetoglu K, Pourrezai K, Martín-Rodríguez JF, et al. (2006) Differential time course and intensity of PFC activation for men and women in response to emotional stimuli: a functional near-infrared spectroscopy (fNIRS) study. *Neurosci Lett* 403: 90–95.
63. León-Carrión J, Martín-Rodríguez JF, Damas-López J, Pourrezai K, Izzetoglu K, et al. (2007) A lasting post-stimulus activation on dorsolateral prefrontal cortex is produced when processing valence and arousal in visual affective stimuli. *Neurosci Lett* 422: 147–152.
64. Nagel IE, Preuschhof C, Li S-C, Nyberg L, Bäckman L, et al. (2009) Performance level modulates adult age differences in brain activation during spatial working memory. *Proc Natl Acad Sci U S A* 106: 22552–22557.