Age related improvement in visuospatial working memory is associated with increased activity in task relevant areas: corroborating evidence from longitudinal and cross-sectional data

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Abstract

Visuospatial working memory (VSWM) is central for a wide range of cognitive functions and continues to develop throughout childhood and adolescence. The neural processes supporting VSWM development have previously mainly been studied cross-sectionally. A limitation of developmental cross-sectional findings is potential confounds by inter-individual differences unrelated to age. Here we performed both longitudinal, cross-sectional and mixed model analysis of fMRI data to detect developmental changes of the neural underpinnings of VSWM . 138 subjects between 6 and 27 years were scanned while performing a VSWM task. 56 subjects were scanned twice, two years apart. Overlapping results of longitudinal and cross-sectional analyses revealed increased working memory (WM) activity in frontal and parietal regions with increasing age. Additionally,WM capacity correlated with increased activity in a largely overlapping set of regions. Age related WM improvements are associated with increased WM related brain activity in a subset of the areas where both age and WM capacity predict WM activity. These include bilateral superior parietal- and intraparietal cortex, bilateral superior frontal sulci and anterior caudate nucleus. Neither age nor WM capacity were correlated with decreased WM activity anywhere in the brain, supporting the idea that VSWM maturation is associated exclusively with increased WM activity.

Key words: Working memory, development, neural correlates, fRMI

Age Related Improvement in Visuospatial Working Memory is Associated with Increased Activity in Task Relevant Areas: Corroborating Evidence from Longitudinal and Cross-sectional Data

Working memory (WM) refers to the ability to maintain and process goal-relevant information for short periods. WM predicts school performance (Hitch, Towse, & Hutton, 2001) and is central to a wide range cognitive of functions such as reading compression (Just, Carpenter, & Keller, 1996), reasoning (Miyake, Friedman, Rettinger, Shah, & Hegarty, 2001), and mathematical abilities (Samuelsson, 2010).The development of WM from childhood throughout adulthood is well documented (e.g. Hitch, Towse, & Hutton, 2001; Luna, Garver, Urban, Lazar, & Sweeney, 2004). Studying the neural processes underlying development of WM memory is important for several reasons. Firstly, prevalent neurodevelopment disorders such as ADHD and autism are associated with working memory deficits and deviant neural signatures of WM (Westerberg, Hirvikoski, Forssberg, & Klingberg, 2004; Koshino et al., 2005; Koshino et al., 2008; Luna et al., 2002). Increased knowledge of WM development could improve characterization of neurocognitive endophenotypes and facilitate diagnosis of psychopathologies. Secondly, identifying the neural substrate of WM maturation could shed light on the causal mechanisms of how the brain implements WM. Finally, identification of factors that influence the development of WM and its associated neural processes has potential use for planning and evaluation of WM training.

Several studies on the developmental changes of the neural basis of WM have been conducted during the last decade (for reviews, see e.g. Luna, Padmanabhan, & O'Hearn, 2010 and Paus, 2005). The studies vary e.g. with regard to the cohorts and tasks that were examined,

whether they focused on structural or functional brain changes, and whether the experimental designs were cross-sectional- or longitudinal, block- or event related. In this study we used functional magnetic resonance imaging (fMRI) to identify the neural correlates of maturation of visuospatial working memory (VSWM). In a developmental context, studying VSWM is advantages to verbal WM since VSWM is easier to compare among ages in a un-counfounded way, due to the fact that VSWM tasks can be designed to relay minimally on long term memory or verbal strategies (Luna, Padmanabhan, & O'Hearn, 2010). Since the first developmental WM study fifteen years ago (Casey et al., 1995), most subsequent studies has focused on VSWM (Bunge & Wright, 2007).

The study by Thomas et al. (1999), one of the earliest studies to include both children and adults in a fMRI VSWM study, revealed a largely overlapping pattern of cortical activations between age groups, a finding supported by subsequent studies (Klingberg, Forssberg, & Westerberg, 2002; Kwon, Reiss, & Menon, 2002). Age related increased of WM-activity was found in parietal areas in all three studies and frontal increases in the latter two. Klingberg et al. (2002) localized bilateral activity increases primarily in superior frontal sulci, superior parietal cortex, and intraparietal sulcus. Kwon et al. (2002) reported, in addition to bilateral intraparietal cortex and superior frontal sulci, also bilateral dorsolateral prefrontal cortex, superior frontal sulci and left ventral prefrontal cortex. The to our knowledge only longitudinal VSWM fMRI identified a rather different pattern of age related changes (Durston et al., 2006). These consisted mainly of decreased activity in frontal and parietal areas (including frontal and precentral gyrus). Inferior frontal gyrus was the only area where activity increased with age. The reasons for discrepant findings above are unclear, but will be discussed later in this paper.

The neural basis of WM maturation is not satisfactory captured in terms of only age related changes. Changes in the neural correlates of WM in adult and elderly populations without corresponding improvement of WM performance should not count as WM maturation. A more satisfactory account of maturation should also take improvement in performance into account. A complement to merely correlate age with WM activity is to correlate brain activity with WM capacity or adult like performance. One such study reported effects in left superior frontal sulcus, left intra parietal and left inferior parietal cortex (Klingberg et al., 2002). However, since inter-individual differences in WM capacity can be unrelated to age is also this approach limited. Rather, maturation refers to age-related WM improvement, and the neural processes subserving this have to our knowledge not yet been explicitly studied.

The goal of this study is to identify the neural correlates of WM development. To accomplish this we used fMRI to study VSWM related brain activity among subjects with ages ranging from 6 to 27 years. Several questions relating to maturation are explored. Firstly, do cross-sectional and longitudinal findings of age related changes in the neural correlates of WM overlap? Secondly, how does WM capacity predict brain activity? Thirdly, how does longitudinal (i.e within subject) *change* of WM capacity predict longitudinal *change* in brain activity? Finally, to what extent do activity predicted by age and WM capacity coincide?

2. Methods

2.1 Subjects

The data reported in current study was collected as a part of a comprehensive longitudinal research project called Brain Child. Data were acquired at two time points two years apart. In the first round of data collection, subjects were recruited using random sampling from a population registry from the town Nynäshman. 1012 individuals in nine age groups (ranging from 6 to 25

years) were contacted. Exclusion criteria were (1) neurological or psychiatric diagnosis other than ADHD and dyslexia, (2) mother tongue different from Swedish and (3) hearing or vision impairments that could affect test-performance. 88 randomly selected subjects gave informed consent to participate in the fMRI experiment.

Two years later, 58 subjects from round one agreed on participating in an identical fMRI experiment a second time. To replace the drop out, another 26 randomly selected subjects agreed on partake in the fMRI experiment.

After dismissing sessions with movement artifacts (8 sessions in the first round and 6 in the second) we used scans for 56 subjects for the longitudinal analysis of age related changes in WM activity (mean age at time one = 12.75 years, *SD*= 4.80, 27 females). The same subjects were included in the comparative cross-sectional analysis of age predicted WM activity. Due to absent WM estimates, only 51 of these subjects were included in the longitudinal analysis correlating change in WM capacity with change in WM related brain activity (mean age at time one = 12.41 years, *SD*= 4.33 , 25 females). Mean time between scan one and two was 21.9 months (*SD*= 1.5). For the mixed model analyses, we dismissed another 9 scans belonging to subjects that performed below chance level in the scanner paradigm, yielding a total of 138 scans (mean age at scanning = 13.9 years, *SD*= 5 years, 72 females).

2.2 WM Task

The VSWM paradigm, scanner and scanning parameters were the same in the first and second round of data collection. In both rounds, each subject completed two 5 minutes runs of the VSWM task. Every run consisted of 16 WM trials and 16 control trials in a pseudo randomized order. Each working memory trial consisted of a display of a series of 2 or 4 yellow circles within a 4x4 grid, with the circles being presented sequentially after each other for 500 ms

separated by a 500 ms inter-item interval. During the subsequent delay period was a black screen displayed for 1500 ms. Finally, the grid reappeared with a number in one of the 16 positions, and subjects were asked to respond with button presses (yes/ no) whether the displayed order number and grid position matched. In the visually matched control trials, 2 or 4 red circles were displayed, followed by delay and ending with reappearance of the grid, with a question mark in a random position. In the control trials, subjects were asked to press yes regardless of the position of the question mark (see fig 1).

[insert figure 2 about here]

2.3 Estimates of WM Capacity

Working memory capacity was estimated outside the scanner using a VSWM test called the Dot matrix, that is part of the Automated Working Memory Assessment (AWMA) test. The Dot matrix is a standardized computer based grid task that was run on a 15 inch laptop (Alloway, 2007)

2.4 fMRI Acquisition

FMRI data were collected using a 1.5 T Siemens Avanto scanner. T2*- weighted, gradient echo-planar images (EPI) were acquired with TR =3000ms, TE=50ms, flip angle = 90˚. Thirty slices 4.5 mm thick and FOV was 64 ×64 rendered voxels of 3.44 ×3.44 ×4.5 size. The functional runs consisted of 96 functional scans and two dummy scans that allowed for longitudinal equilibration but were excluded from further analysis. Structural T1-weighted image were obtained using spin echo pulse sequence (FOV=256 x 256).

2.5 Data Analysis

The fMRI data were analyzed using SPM5 (Welcome Department of Imaging Neuroscience, London; UK). First the functional volumes were corrected for slice timing. The

volumes were then realigned to the first volume in each run by using six realignment parameters for rigid body transformations estimated by minimizing the differences between the first volume and the subsequent volumes. The mean image of the realigned functional volumes was coregistered to the anatomical images, and the obtained coregistration parameters were used to coregister remaining functional images. Structural images were normalized to the MNI template, and the estimated normalization parameters were used to normalize the functional images to the MNI template. Finally, in order to increase inter-individual overlap of activations and reduce noise, spatial smoothing was performed by convolving the images with a 12 mm 3D Gaussian kernel.

Fixed factor general linear models were specified for each subject and contained a set of regressors constituted by four experimental events convolved with hemodynamic response function with associated temporal- and dispersion derivative, six movement parameters and a global mean. The four experimental events were correct WM trials for load 2, load 4, and control trials with load 2 and 4. The duration of the events equaled the inter trial interval, i.e. included the time of memory item display, maintenance and response (8 seconds in load 2 conditions and 10 seconds in load 4 conditions).

From each subject, contrast images were obtained by pooling correctly responded load 2 and load 4 WM trials and subtracting the pooled control conditions. This contrast revealed what in the following will be referred to as WM related activity. An additional first level contrast image were created for subjects that was scanned twice by subtracting WM related activity in round one data from corresponding activity in round two data.

Subject specific contrast images where then used for mixed effect analysis in order to infer effects to population level. Age related changes in WM related brain activity were identified

using longitudinal, cross sectional and mixed model analyses. In order to allow comparison of cross-sectional and longitudinal results, only scans from round one that were used in the longitudinal analysis were included in the cross-sectional analysis, in which age was used as a covariate. Results from the longitudinal and cross-sectional analysis were projected on to the same template in order to detect overlapping age predicted WM activity.

In order to be able to include all subjects in the second level analysis, we created a mixed model by using the flexible factorial design option in SPM. This allowed inclusion of subjects that had data from only one time point and specification of repeated measures for the subjects that had been scanned twice. Due to high covariance between age and WM capacity (see paragraph 3.1), age predicted WM activity and WM activity predicted by WM capacity were analyzed using two separate mixed models, precluding conjunction analysis.

3. Results

3.1 Behavioral Result

Performance on WM test outside the scanner were significantly correlated with age (r (136)= 0.529, p<0.001, see fig. 2). Subjects that were included in the longitudinal analysis showed a significant higher WM capacity in the second round (M=30.41, SD= 7.10), compared to the first round (27.41, SD= 7.29), t (50) =4.30, p<0.001.

[insert figure 2 about here]

3.2 Imaging Results

3.2.1 Main effect of WM. Contrasting correct working memory trials with control trials resulted in activation maps with significant BOLD signal increase that extended widely distributed frontal and parietal areas. Particularly strong effects were observed in left and right superior parietal - and intraparietal cortex, left and right middle frontal gyri and right and left

insula, but activity extended over large parts of parietal and frontal lobes (see fig 3.). (In the following, all reported effects are FDR corrected at the significance level of $p< 0.05$, if not specified otherwise.)

[insert figure 3 about here]

3.2.1 Age. For the longitudinal analysis, WM activity in round one was subtracted from WM activity in round two on a subject basis. These contrast images were analyzed at the group level using a one sample t-test. Higher activity in round two compared to round one was found particularly in bilateral superior parietal and intraparietal cortex, bilateral frontal superior gyri and right caudate nucleus (see table 1). In a mixed model analysis we pooled round one- and round two data and used age as a continuous covariate for WM related activity. This revealed greatly distributed cortical activations, replicating the mentioned longitudinal finding of bilateral superior parietal-, intraparietal- and superior/middle frontal gyrus activations. Additionally it also revealed e.g. posterior areas of cingulate cortex and supra marginal gyrus. Compared to the mixed model analysis, the cross-sectional analysis of round one data revealed fewer and less extended activations, but greater activations compared to the longitudinal finding. Overlap between the longitudinal and the cross-sectional analysis were found in bilateral superior parietal-, inferior parietal, intraparietal and mid/superior frontal gyri, and in right caudate nucleus (see fig 4).

No decreases of WM activity were observed anywhere in the brain between round one and two. Similarly, cross-sectional analysis of negative correlation between age and WM activity did not reveal any areas that were more activated in younger subjects.

[insert figure 4 about here]

3.2.2 WM and WM combined with age. AWMA scores were positively correlated with WM activity in a wide range of regions including bilateral middle frontal gyrus, bilateral superior parietal and mainly left intraparietal cortex (see table 1 and fig 5).

Using intra-individual *change* in AWMA score from round one to round two as a covariate to predict *change* in WM related activity from round one to round two did not reveal any significant results even at a low significance threshold (p=0.001, uncorrected).

AWMA and age are highly correlated. By projecting WM related brain activity predicted by age and by AWMA on a common template, significant overlap was observed in bilateral superior parietal sulcus, bilateral precentral gyrus and right caudate nucleus.

[insert figure 5 and table 1 about here]

4. Discussion

The main goal of this study was to characterize the blood-oxygen-level dependent (BOLD) markers of VSWM maturation. We performed several analyses to accomplish this. Age- and WM related increases in brain activity will first be mentioned separately, and subsequently discussed in a common context.

4.1 Age Related Increases

We have identified several brain areas in which WM related brain activity increases with age. The longitudinal analysis revealed greater activity in parietal and frontal areas, such as bilateral superior parietal-, intraparietal- and superior frontal cortex. The finding was supported by a cross-sectional analysis of data from round one, rendering strong corroborating evidence of age related activity increase in superior parietal lobe, intraparietal sulcus, superior frontal sulcus, and caudate nucleus. These results are in line with and extend upon findings reported by earlier studies (Klingberg et al. 2002; Kwon, Reiss, & Menon, 2002; Thomas et al., 1999; Scherf,

Sweeney, & Luna, 2006). However, the most sensitive analysis was the mixed model analysis that allowed inclusion of the greatest number of scans while taking advantage of the repeated nature of the data from subjects scanned at both round one and two. The mixed model analysis identified more distributed areas that included and extended upon the longitudinal and cross-sectional findings. Among developmental VSWM studies, current study has unprecedently large set of data yielding greater statistical power, which can explain our unique findings of age effects in for instance the right caudate nucleus and right supra marginal cortex.

4.2 WM Capacity Related Increases

WM capacity as estimated outside the scanner was positively correlated with WM related brain activity in widespread areas including superior parietal cortex and bilateral frontal gyrus. An earlier study using a nearly identical paradigm found that WM activity in left inferior parietal, left intraparietal and left superior frontal sulcus areas were predicted by WM capacity (Klingberg et al, 2002). Comparably low power of that study (n=14) might explain why only a subset of current findings were reported. Todd and Marois (2004) showed that parametrically increased VSWM load correlated with increased activity in bilateral intraparietal sulcus during correct trials trial-by trial performance. Thus, intraparietal activity predicts both trial by trial performance and inter-individual VSWM capacity.

4.3 Age and WM

Our attempt to directly examine brain changes associated with age-related WM trough correlating gain in WM capacity with change in brain activity between the first and second round did not yield any significant results. We believed that this analysis would give the most adequate measure of the neural correlate of WM maturation, and the reasons for the null-result were puzzling. One possible explanation could be that our measure of WM was too noisy relative the

small inter-individual differences in WM change. A more sensitive measure of WM improvement would then be needed, although the VSWM measure we used is to our knowledge one of the most reliable.

WM capacity and age both correlate positively with bilateral superior parietal sulcus, bilateral postcentral sulcus and right caudate nucleus. These overlapping areas *encompassed* but did not necessarily *constitute* the neural correlates of age related WM improvements. Rather, the neural correlates of WM maturation were possibly only a subset of the overlapped areas.

Neither age nor WM correlated with any deactivations. Although in line with several earlier findings (Klingberg et al., 2002; Kwon et al., 2002) this finding contrast several other (Durston et al., 2006; Scherf et al., 2006), and brings up the enigmatic question of how to interpret developmental changes in brain activity.

4.4 Interpretations of Developmental BOLD Signal Change

Is VSWM maturation associated with increased or decreased magnitude and extent of the task evoked BOLD signal? Durston et al. (2006) found age related activity reductions in prefrontal regions during a target detection task. Scherf et al. (2006) found decreases in basal ganglia, anterior insula and cerebellum during a memory guided saccade task. The discrepancy between our study and the above mentioned studies could possibly be due to the use of different paradigms and control conditions rendering different main effects of VSWM. Current study revealed a main effect of VSWM in wide spread frontal and parietal areas similar to what have been reported in previous studies (Klingberg et al., 2002; Courtney, Petit, Maison, Ungerleider, & Haxby, 1998;Ricciardi et al., 2006; Thomason et al., 2009). A partly different set of regions for the main effect of WM were found by both Scherf et al, (2006) and Durston et al. (2006), which included more anterior regions and encompassed areas were WM activity decreased with

age. These regions were proposed to compensate for developing networks of more specialized and localized frontal and parietal regions found in mature systems.

Similarly, studies of the neural effect of WM training report rather inconsistent results of the activity changes that accompany WM improvements. Olesen, Westerberg, & Klingberg (2004) correlated behavioral gain on a trained VSWM task with increased WM related brain activity in e.g bilateral superior, intraparietal and inferior parietal cortex, caudate nucleus and in left middle fronal gyrus. Other WM training studies using considerable shorter periods of training commonly report decreased activity in parietal and frontal areas (Garavan, Kelley, Rosen, Rao, & Stein, 2000; Landau, Garavan, Schumacher, & D'Esposito, 2007). Klingberg (2010) hypothesized that initial training effects such as priming, habituation, shorter time on task and strategy learning could explain reductions in task evoked activity in certain areas. Long term practice effects, on the other hand, seems to imply increased recruitment of task relevant brain areas. Long term increases could occur in what Durston et al. (2006) refer to as crucial areas, i.e. areas in which activity correlate with task performance in all ages. Training induced short term deactivations would mainly occur in areas not critical for carrying out the task but that support compensatory functions in developing or elderly subjects (Mattay et al., 2006). Cortical changes that depend on the duration of WM training are analogous to what have been observed in training studies of motor functions, in which short term training effects included decreased activity in frontal and primary motor cortex, whereas long term changes included increase magnitude and extent of activity in primary motor cortex (Floyer-Lea & Matthews, 2005; Karni et al., 1998).

Current findings show that activity predicted by age and WM capacity to a large extent overlap with the main effect of WM, whereas corresponding overlaps seem less obvious in above mentioned studies were WM maturation were associated with decreased activity. This could be

interpreted as if current experimental task was less vulnerable to age dependent strategies or compensatory cognitive processes. According to this line of reasoning, studies employing tasks that exclusively engage task-relevant areas would characterize maturation as increased activity. Contrary, tasks that allow for compensatory processes (e.g. attentional and mental effort that are likely to rely on more anterior pre-frontal and dorsolateral areas) might yield maturational decreases in corresponding regions (similar to what has been proposed by e.g. Casey, Tottenham, Liston, & Durston, 2005). However, to substantiate explanations of how BOLD-signal changes relates to development of WM, it would be informative to examine the neuronal changes that subserve WM maturation.

4.5 Cellular Underpinnings of Changed BOLD

Cellular changes that are likely to support WM maturation include pruning and myelination (Luna, Padmanabhan, & O'Hearn, 2010; Toga et al., 2006). Synaptic pruning is the eliminations of excessive neuronal connections and myelination refers to growth of the isolating myelin sheets that surround axons. Both processes occur during development (Giorgio et al., 2010; Paus, 2001). However, the relationship between developmental cellular changes and the neural correlates of WM maturation is not clear. Pruning implies fewer synapses, and may result in reduced energy consumption that decreases task evoked BOLD signals. This interpretation is supported by studies that associate cognitive development with a narrowed, more focused neural network (Durston et al. 2006). On the contrary, current and other studies (e.g. Kwon et al. 2002) that report increased task evoked brain activity during development, support an alternative explanation of how pruning affect BOLD. By making neural communication more efficient and directed, pruning could allow for stronger recruitment of task relevant brain areas e.g. in parietal and frontal areas where increased brain activity has been observed. Similarly, myelination could

lead to either regional increase or decrease of BOLD signal. By increasing local and global connectivity, myelination could facilitate engagement of specialized regions e.g. in the parietal cortex. Myelination could thus unburden regions supporting general functions, by allowing task related activity to transfer from general and diffuse to specialized networks. As clear from the reasoning above, neither pruning nor myelination lend themselves to unequivocal and refutable predictions of the developmental changes of WM related BOLD. In a unique attempt to understand how neuronal development relates to development of WM related brain activity, Edin et al (2007) used a computational neural model of VSWM. According to this study, neither myelination nor pruning, but only strengthening of fronto-parietal synaptic connections between cells coding for a stimulus, could directly account for the observed changes of WM related brain activity during development. However, myelinisation could indirectly change the pattern of WM related brain activations by strengthening such inter-regional connections (Giorgio et al., 2010).

4. 6 Methodological considerations

There are several methodological issues to consider when planning and comparing developmental WM studies. Firstly, different performance among different ages could, if not controlled for, confound age related differences in WM related brain activity. Several developmental WM studies compare age groups by using block designs that do not account for whether performance are different in different groups. Since younger subjects generally make more mistakes, comparison of brain activity among age groups could imply comparing WM related activity in older adults with neural correlates of e.g. thought wandering in younger subjects. In this study differential performance were accounted for by using an easy WM task in which a high ceiling effect was obtained. Secondly, for our mixed model analyses we removed sessions were subjects performed at or below chance level, thus minimizing inclusion of non-task

related activity. Finally and most importantly, using an event related rather than a blocked design allowed us to separately analyze correct and erroneous trials.

Assumed that differential performance among ages is controlled for, different ages could use different cognitive strategies for solving the same task, rendering comparison of the neural correlates of the task execution meaningless. To the extent that subjects with mature and immature WM employ different cognitive faculties when performing a WM task, the different strategies would confound observed WM maturation and undermine any attribution of an eventual BOLD difference to improved WM capacity per see. For instance, if older people use verbal strategies to a larger extent than younger subjects, increased activations in language areas should not be interpreted as neural correlates of WM maturation. Although interesting in their own right, age dependent cognitive strategies should be avoided in order to render valid comparison between ages. Even though it seems difficult to fully ensure that subjects use the same cognitive functions during the experimental task, our visuospatial WM task is likely less prone to evoke age specific cognitive strategies than e.g. a verbal WM task, that to a greater extent relies on long term memory and verbalization.

A third concern when comparing hemodynamic correlates of WM across ages is developmental differences in brain anatomy. Structural brain changes are known to occur from childhood until adulthood, although the gross anatomical organization is at place in mid childhood (Giedd et al., 1996;White, Su, Schmidt, Kao, & Sapiro, 2010). Additionally, vascular changes during development could possibly contribute to differences in BOLD signals among different ages. However, Kang et al. (2003) compared time course, magnitude and loci of the BOLD signal during a simple cognitive task among children and adults, and concluded that

between group differences were neglectible and that direct comparisons of task evoked activity among ages are feasible.

4.7 Limitations and Future Directions

In order to detect age related changes of the neural correlates of WM we used age as a continuous covariate which had the advantage of avoiding arbitrary age categories (Scherf et al. 2006). However, using age as a covariate rather than comparing age groups has the drawback of being less sensitive to developmental trends that follow inverted U shapes, which are frequently observed in structural development where e.g. grey matter of important WM related brain regions peak at early adolescence (e.g. Giedd et al., 1999).

In current study we looked at the averaged activity of both encoding, maintenance and retrieval of memory items. These different cognitive processes are likely subserved by separate neural structures. Analyzing these processes separately would increase sensitivity and result in a more detailed picture of the neural processes underlying WM maturation. Furthermore, in our analysis we pooled the two levels of load in the WM- and control condition, respectively, making us blind to interaction effects of age and load such as found in earlier studies (see Schleepen & Jonkman, 2010; Thomason et al., 2009). Finally, considering known developmental changes of structural connectivity among WM relevant brain regions, studying developmental changes in functional connectivity would likely be a fruitful approach.

4.8 Conclusions

We have, by using both longitudinal and cross sectional data, localized brain regions were both WM capacity and age positively correlate with WM activity. These regions include bilateral superior parietal- and intraparietal cortex, bilateral superior frontal cortex and anterior caudate nucleus. Thus, the neural substrate of age related WM improvements are to be found within a

subset of these areas. Neither age nor WM capacity correlate negatively with WM activity, supporting the proposal that WM maturation is associated with increased neuronal activity, and particularly so in WM related areas. Future studies should investigate how activity in these areas are altered in neurodevelopmental disorders and influenced by clinical interventions.

Figure 1. Experimental paradigm. In WM trials (top row), either two or four yellow dots were sequentially displayed for 0.5 s. After a 1.5 s delay period, the subjects were asked whether the displayed number of serial position and location matched by button press (yes/no). In the control condition (bottom row), subjects were asked to press yes regardless position of the probe.

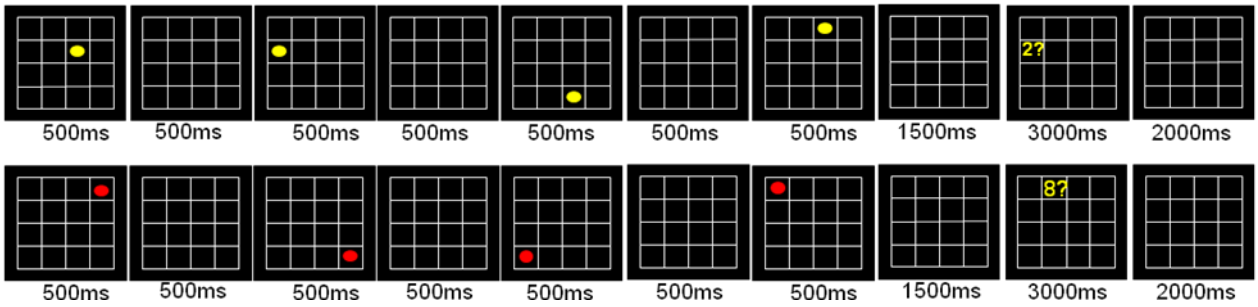

Figure 2. Improved WM with age. WM capacity (AWMA score outside scanner) as a function of age (in years) of the subjects that were scanned to obtain the 138 images included in the mixed model analyses of age- or WM capacity related WM activity.

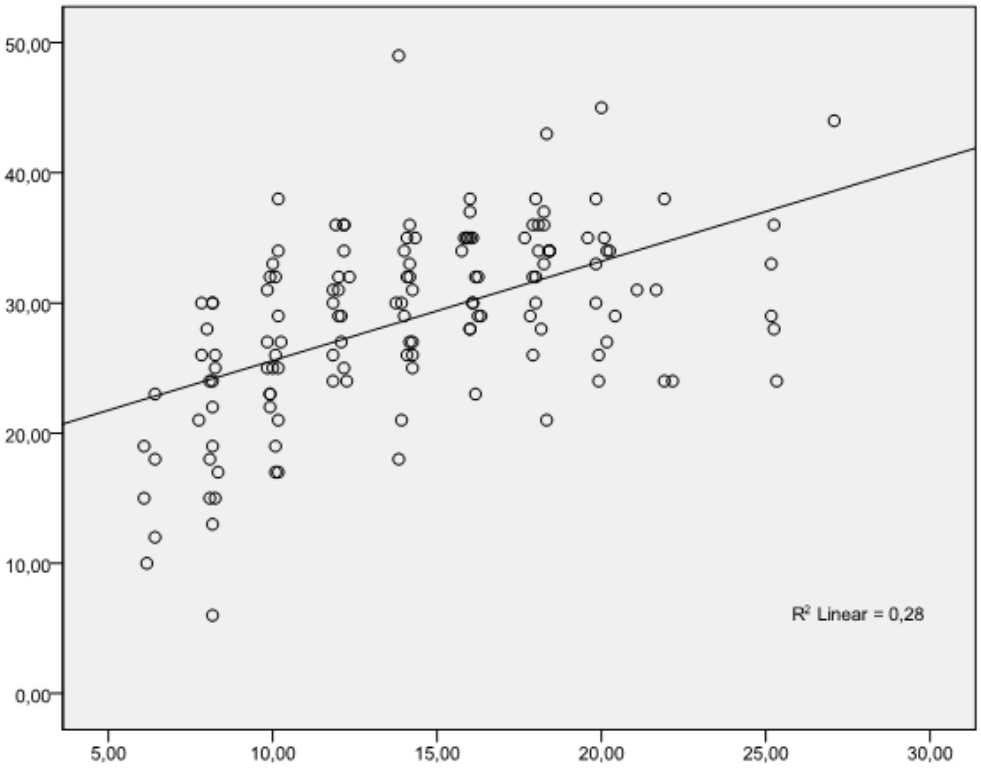

Figure 3. Main effect of WM (WM> control). (FDR corrected at $p < 0.05$.)

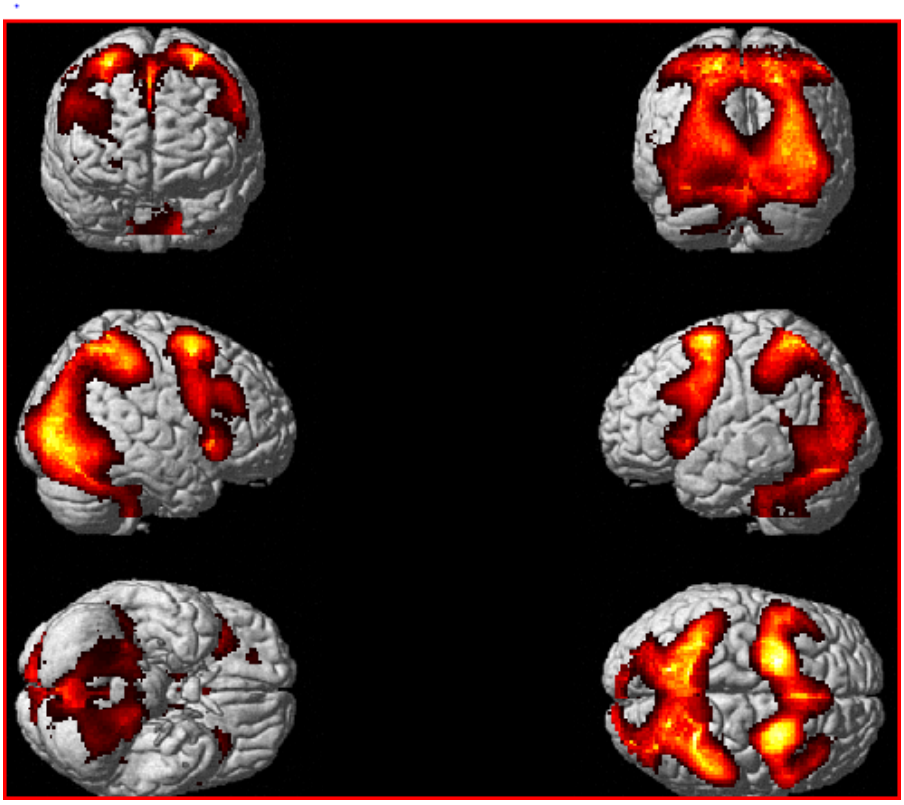

Figure 4. Age related increase in WM activity as revealed by longitudinal (red) and cross sectional analysis (blue). Overlapping findings (pink) included activity in bilateral posterior parietal gyri, bilateral superior frontal gyri and anterior caudate nucleus. (N= 56, $p < 0.05$, FDR corrected.)

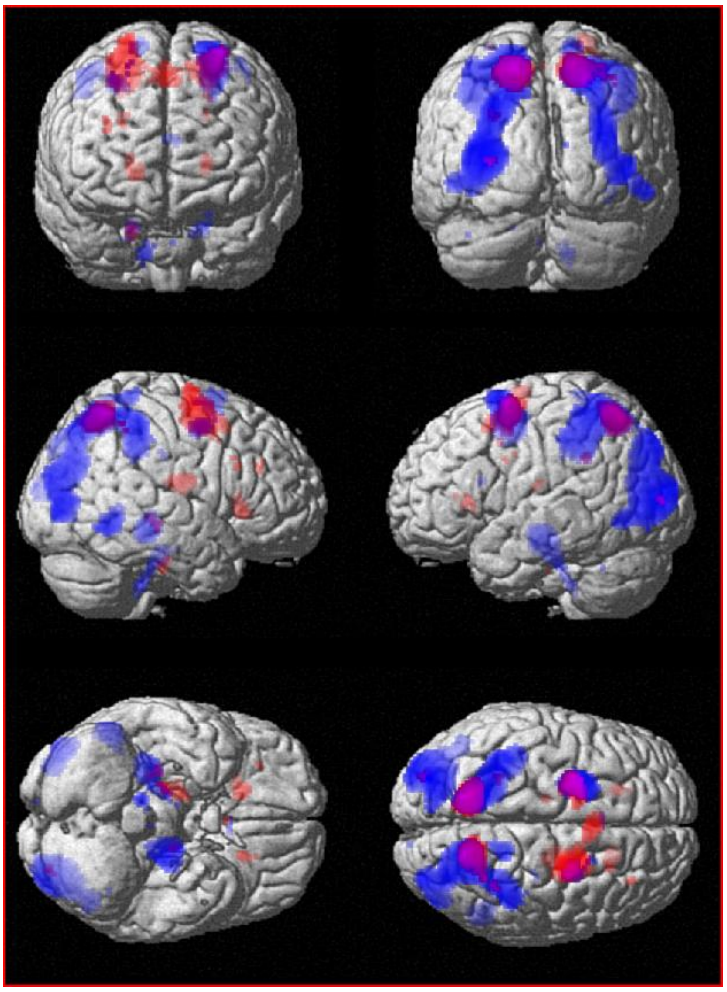

Figure 5. Areas were age (red) and WM capacity (blue) positively correlates with WM activity, as revealed by the mixed model analyses. Overlapping areas (pink) are found in widespread parietal and frontal areas, including superior parietal- and intraparietal cortex, and superior frontal cortex. (N=138, $p< 0$ .05, FDR corrected.)

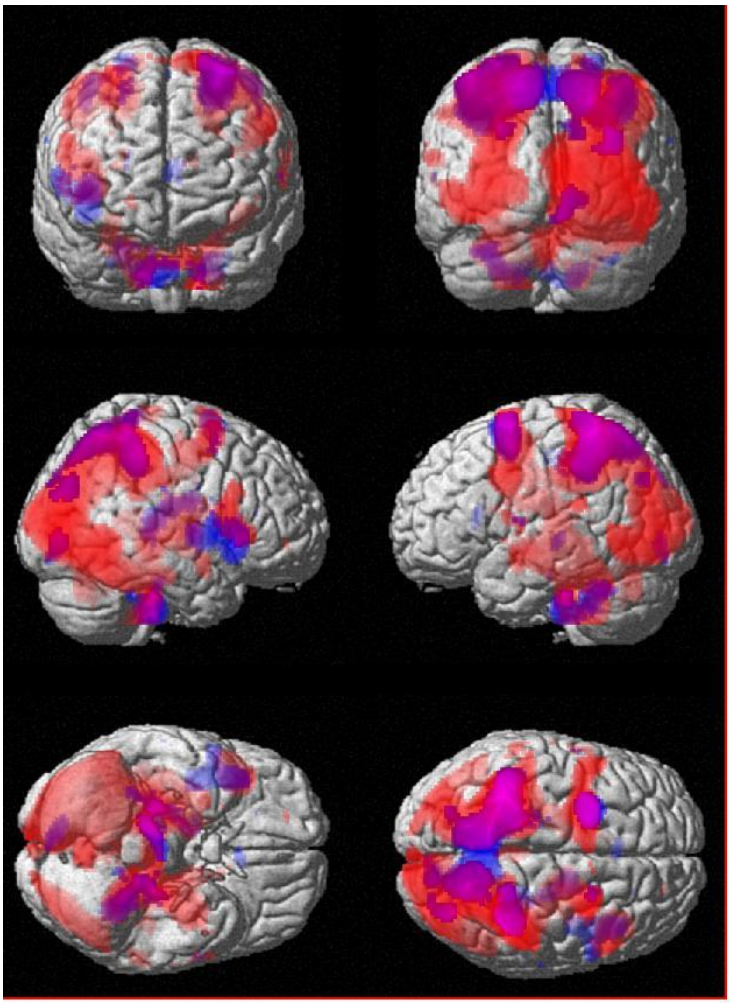

Table 1. Result table of all significant results. Specified regions contain the peak voxel, but significant activity extend in most cases beyond reported regions. L= left, R= right.

Analysis	Region	MNI coordinates, x,y,z (cluster peak)	Cluster size (≥5)	Z-value	P-value (FDR corrected, < 0.05)
WM>control, flexible factorial	R. superior parietal gyrus	18 -62 60	72870	Inf	0
Age positively correlating with WM activity, (mixed model)	L. inferior parietal gyrus	-28 -48 48	29793	6.74	0
	R. posterior cingulated gyrus	4 -40 8	507	3.91	0.001
	R. inferior frontal gyrus	40 18 8	646	3.38	0.005
	L. superior temporal gyrus	-60 -38 18	42	2.81	0.02
	L. precentral sulcus	-60 0 10	18	2.67	0.027
	R. putamen	26 12 14	6	2.55	0.033
WM related activity in round 2 > round 1	L. superior parietal gyrus	-18 -62 58	396	4.28	0.041
	L. superior frontal sulcus	-22 0 58	283	4.22	0.041
	R. superior frontal sulcus	24 -6 62	543	4.22	0.041
	R. anterior caudate nucleus	18 -10 20	115	4.04	0.041
	R. posterior caudate nucleus	16 24 -2	45	3.85	0.041
	R. superior parietal sulcus	16 -58 60	348	3.81	0.041
	R. supplementary motor area	4 10 54	98	3.67	0.041
		-20 24 6	17	3.62	0.041

		20 -20 -28	13	3.54	0.042
	R. hippocampus	28 -28 -6	7	3.45	0.044
	R. middle frontal sulcus	32 34 26	5	3.42	0.045
	R. inferior frontal sulcus	24 20 26	5	3.4	0.046
	L. thalamus	-14 -16 18	7	3.38	0.046
	L. middle occipital gyrus	-28 -88 8	5	3.32	0.047
Age positively correlating with WM activity (cross sectional analysis)	L. middle occipital gyrus	-28 -82 6	2151	5.23	0.003
	L. middle frontal sulcus	-24 2 60	613	4.5	0.004
	R. superior parietal sulcus	20 -70 56	2387	4.38	0.006
	R. para-hippocampus	30 -30 -14	252	4.34	0.006
	L. inferior frontal sulcus	-38 -40 44	1874	4.11	0.009
		-18 -16 -12	489	3.92	0.012
	R. inferior temporal gyrus	56 -56 -10	166	3.57	0.018
	R. postcentral sulcus	14 -42 68	76	3.36	0.023
	R. cerebellum	20 -26 -26	58	3.34	0.024
	R. precentral gyrus	28 -2 48	69	3.31	0.025
		12 -36 -42	26	3.23	0.028
		-4 18 16	13	3.11	0.033
WM capacity positively correlated with WM activity	L. precuneus /L. sup. parietal sulcus	-4 18 16	3580	5.09	0.003
	L. middle frontal sulcus	-24 6 62	544	4.8	0.003
	R.thalamus	26 -28 10	328	4.02	0.008

R. supramarginal gyrus	38 -36 44	564	4.01	0.009
R. insula	42 22 -4	721	3.96	0.009
	-26 -44 -40	255	3.64	0.014
R. postcentral sulcus	22 -40 70	85	3.62	0.015
	6 -24 -36	220	3.51	0.018
R. cerebellum	26 -24 -28	42	3.43	0.02
	-26 -24 -28	19	3.37	0.023
R. middle occipital sulcus	36 -76 28	119	3.34	0.024
R. cerebellum	34 -38 -34	14	3.29	0.026
L. superior occipital sulcus	-24 -76 32	38	3.22	0.029
R. lingual sulcus	14 -82 0	119	3.21	0.03
L. cerebellum	-10 -48 -40	7	3.2	0.03
L. caudate	-4 20 14	30	3.16	0.032
L. middle frontal sulcus	24 6 50	19	3.12	0.034

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