



Published in final edited form as:

*Hum Brain Mapp.* 2014 September ; 35(9): 4932–4943. doi:10.1002/hbm.22523.

## Respiration phase-locks to fast stimulus presentations: implications for the interpretation of posterior midline “deactivations”

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### Abstract

The posterior midline region (PMR) –considered a core of the default mode network- is deactivated during successful performance in different cognitive tasks. The extent of PMR-deactivations is correlated with task-demands and associated with successful performance in various cognitive domains. In the domain of episodic memory, functional MRI (fMRI) studies found that PMR-deactivations reliably predict learning (successful encoding). Yet, it is unclear what explains this relation. One intriguing possibility is that PMR-deactivations are partially-mediated by respiratory artifacts. There is evidence that the fMRI signal in PMR is particularly prone to respiratory artifacts, because of its large surrounding blood vessels. Since respiratory fluctuations has been shown to track changes in attention, it is critical for the general interpretation of fMRI results to clarify the relation between respiratory fluctuations, cognitive performance, and fMRI signal. Here, we investigated this issue by measuring respiration during word encoding, together with a breath-holding condition during fMRI-scanning. Stimulus-locked respiratory analyses showed that respiratory fluctuations predicted successful encoding via a respiratory phase-locking mechanism. At the same time, the fMRI analyses showed that PMR-deactivations associated with learning were reduced during breath-holding and correlated with individual differences in the respiratory phase-locking effect during normal breathing. A left frontal region – used as a control region- did not show these effects. These findings indicate that respiration is a critical factor in explaining the link between PMR-deactivation and successful cognitive performance. Further research is necessary to demonstrate whether our findings are restricted to episodic memory encoding, or also extend to other cognitive domains.

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## Introduction

The posterior midline region (PMR) is considered a core region of the default mode network (DMN) (Huijbers, et al., 2012). One of the most reliable findings in the neuroimaging literature is that the PMR is activated during rest but deactivated during demanding cognitive tasks (Buckner, et al., 2008). The extent of PMR-deactivations has been shown to correlate with task demands (Gould, et al., 2006; McKiernan, et al., 2003), and has been associated with successful task performance in various cognitive domains including attention, language and memory (Binder, et al., 2009; Daselaar, et al., 2004; Weissman, et al., 2006). For instance, in the domain of episodic memory, several functional MRI (fMRI) studies of episodic encoding have found that PMR-deactivations reliably predict successful encoding as assessed by a subsequent memory test, independent of the specific memory paradigm. While regions such as left ventrolateral prefrontal cortex (VLPFC) typically show greater activity for subsequently remembered (R-items) than forgotten (F-items) items, or in other words a positive *difference in memory* effect (*positive DM*) (Buckner, et al., 1999), the PMR shows the opposite pattern: less activity for R- than F-items, or *negative DM* (Daselaar, et al., 2004; Daselaar, et al., 2009; Otten and Rugg, 2001). Despite the consistency of this finding across cognitive domains, the relation between PMR-deactivations and successful cognitive performance remains unclear.

One intriguing possibility is that PMR-deactivations as measured with fMRI are mediated by changes in respiration. fMRI is a functional brain imaging technique that is based on changes in local oxygen concentrations in the brain, which provide an indirect measure of neuronal activity. When a brain region is activated, its metabolic processes require oxygen that is not stored locally. To meet metabolic demands, oxygen is delivered via a local increase in cerebral blood flow, which is measured by fMRI (Ogawa, et al., 1990). However, because of its dependence on blood flow, the fMRI signal is not only affected by neural changes, but also by physiological variables such as respiration. Respiratory fluctuations affect cerebral blood flow and thereby the fMRI signal by changing the CO<sub>2</sub> blood level (Birn, et al., 2006; Birn, et al., 2008a; Chang and Glover, 2009; Kastrup, et al., 1999). As a powerful vasodilator, any rise in CO<sub>2</sub> will lead to an increase in cerebral blood flow, and thus to an increase in fMRI signal that does not have a neural, but rather a vascular origin.

Resting-state fMRI studies have indicated that the PMR is one of the regions in the brain particularly prone to respiratory artefacts, because of the surrounding, large, blood vessels (Birn, et al., 2006; Birn, et al., 2008a). In resting-state studies, considerable effort has been invested in removing the confounding effects of respiration from the fMRI signal (Birn, et al., 2006; Birn, et al., 2008a; Glover, et al., 2000). Some fMRI studies have also started to examine effects of respiratory fluctuations on signal changes during task performance rather than mere rest (Birn, et al., 2009; Madjar, et al., 2012; Thomason, et al., 2007). However, these studies did not consider potential interactions between stimulus presentation during the task and the respiratory cycle itself. This lack of interest may reflect the assumption that the fast event-related fMRI designs that are typically used are insensitive to slow physiological changes such as respiration, or the assumption that respiration-related changes are constant across conditions and hence, are subtracted out. However, there is evidence that respiratory fluctuations can be affected by stimulus presentations due to attentional orienting processes

(Boiten, et al., 1994; Porges and Raskin, 1969; Walter and Porges, 1976). Attentional orienting can induce respiratory accelerations, decelerations, and arrests (Boiten, 1993; Boiten, 1998; Boiten, et al., 1994). Therefore, it is critical to examine the relation between cognition, respiratory fluctuations, and fMRI signal, even when using fast event-related fMRI paradigms.

In the present study, we investigated the effects of respiratory fluctuations on memory and event-related fMRI by combining a memory encoding task with a breath-holding manipulation. Participants encoded words in the MRI scanner either during 20-second blocks of normal-breathing or during 20-second breath-holding blocks (Figure 1). Respiration was measured with a respiratory belt around the abdomen. The rationale for the study was that, during short periods of breath-holding, participants can continue to perform an encoding task with ensuing neural activity. Because there are no respiratory fluctuations during the breath-holding condition, any difference in fMRI signal between R- and F-items cannot be attributed to respiratory artefacts. Thus, by contrasting R- and F-items during breath-holding and normal-breathing conditions, we can assess the contribution of respiratory fluctuations to the encoding-related fMRI signal in PMR. As we will address in the Discussion section, some caution with this line of reasoning is appropriate given the dominant effect of breath-holding on overall CO<sub>2</sub> levels as compared to the much smaller task-induced changes.

We tested four main predictions. First, in view of previous studies suggesting attentional orienting effects on respiration (Boiten, et al., 1994; Leskovsek, et al.), we predicted that the respiratory cycle would phase-lock to the stimulus presentations (*respiratory phase-locking hypothesis*). Second, given the strong link between attention and successful encoding (Chun and Turk-Browne, 2007), we expected that this phase-locking effect would be stronger for R-than F-items. Third, based on resting-state fMRI studies (Birn, et al., 2006; Birn, et al., 2008a), we expected that the negative DM effect in PMR would be sensitive to our respiratory manipulation. Finally, given that resting-state fMRI studies have not identified left VLPFC as a region particularly prone to respiratory artifacts, we predicted that the breath-hold manipulation would not affect the positive DM effect in VLPFC.

## Materials and Methods

### Participants

Twenty-six subjects (20 female, mean age 22) recruited from the University of Amsterdam community participated in the experiment. All subjects were in good health, and right-handed. Their native language was Dutch and they were paid 35 euro for participation. All subjects gave their informed consent and the study met all criteria for approval of the Academic Medical Center Medical Ethical Committee. The data of one subject was excluded due to excessive motion inside the fMRI scanner and another subject was excluded because of very poor performance on the memory task. Also, because of equipment malfunction, the respiratory data from two subjects was lost. The fMRI data of these subjects were included in the fMRI analysis, but not in the respiratory analysis (see analysis).

**Stimuli**—Stimuli consisted of 840 words (nouns), selected from the MRC Psycholinguistic database ([www.psy.uwa.edu.au/mrcdatabase/uwa\\_mrc.htm](http://www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm)), and subsequently translated to Dutch. Words varied between 5 and 12 letters in length and were of moderate frequency. Half the word referred to living, and the other half to non-living, entities.

## Experiment

The experiment consisted of three parts: (1) a pre-scan training phase, (2) a scan phase including memory encoding, and (3) an immediate post-scan retrieval phase. The pre-scan training was conducted on a separate day, in the week before the participants participated in the fMRI experiment. During the pre-scan training participants were familiarized with the memory and breathing instructions. Specifically, the breath-hold period consisted of a 5 second preparation countdown during which the participants exhaled and then refrained from inhaling for 20 seconds (Figure 1). Only participants who were able to comfortably hold their breath without overt signs of stress as evident from individual reports were included in the actual fMRI experiment. Next, during the scan-phase, participants were instructed to make fast living/non-living judgments, within max.1200 ms, in order to encode words into memory. Words were presented in blocks of 6 items, during normal breathing and during the breath-hold condition. ITI's within a block ranged from 600-3800 ms in such manner that a single block lasted exactly 20 seconds. Each breath-hold block was followed by a 15 seconds normalization period and all blocks were preceded by the 5 seconds preparation phase. In total participants performed 5 encoding sessions, consisting of 7 blocks with breath-hold and 7 blocks with normal breathing. In total participants encoded 420 words. Finally, during the post-scan retrieval phase, participants performed a self-paced recognition test with a 1:1 old/new ratio. Following each recognition judgment participants also rated their confidence on a 4-point scale. Only old items that were recognized correctly with high confidence (rating-4), were considered as remembered (R-items). All old-items incorrectly classified as new were coded as forgotten (F-items).

**Data acquisition**—Functional MRI images were collected with a Phillips Intera 3.0T. using a standard SENSE head coil and a T2\* sensitive echo planar imaging (EPI) sequence (96 × 96 matrix, TR 2000 ms., TE 30 ms., FA 80°, 34 slices, 2.3 mm × 2.3 mm voxel size, 3-mm thick transverse slices). During five encoding sessions 275 sequential images were acquired. During scanning, stimuli were projected on a screen at the front-end of the scanner-table and observed via a mirror mounted on the head coil. The participant's head was fixed by foam and they wore earplugs to reduce scanner noise. The behavioral responses were collected by an MR-compatible four-button box (Lumitouch™). After the functional scans, a high-resolution T1-weighted structural scan was collected (256 × 256 matrix, TR 12 ms, TE 5ms, FOV 24 cm, 68 slices, 1 mm slice thickness). The respiratory signal was measured by an MR compatible air-filled respiration-belt placed around the waist and sampled at 500 Hz. The respiratory belt was an integrated part of the Phillips scanner and the measurements obtained are linearly related to the expansion of the belt. Before the analysis, we down-sampled the respiratory signal to 10 Hz using a 100 ms sliding average.

**Respiratory data analysis**—The respiratory signal was (pre)processed consistent with the descriptions in (Birn, et al., 2008b; Chang and Glover, 2009). First, using software

package Charts 5.5 (ADInstruments Ltd), we screened the signal and removed periods during which the breathing signal was very irregular. After removal, we used a simple algorithm (peak-after threshold), as implemented in Charts 5.5 to determine the minima and maxima of each breathing cycle, outside of a 1 second time-window. The threshold was set separately for each session by manual inspection, ranging within 1.5 and 3 standard deviations. Next, we used the maxima to calculate a time series with both amplitude and respiratory duration for each time point and exported the data to Matlab for further analysis.

To assess the interaction between slow on-going respiratory fluctuation and fast cognitive events, we cut out a 5 seconds respiratory data segment before and after each event of interest. For a given event ( $i = 1, \dots, N$ ), with  $N$  the total number of events, we obtained a series of phase values for different frequencies ( $\theta_i(f)$ ), through Fast Fourier Transforming the Hann-tapered respiratory signal after (or before) the event, (5 sec, 0.2-1.4Hz), and taking the phase of the complex Fourier spectrum. For a given frequency, we then computed the respiratory phase-locking value i.e., the mean resultant length over the input vector of computed phases (e.g. Fisher, 1993; Lachaux, et al., 1999) which we defined as:

$$rPLV(f) = \left| \frac{1}{N} \sum_{i=1}^N \exp(i\theta_i(f)) \right|$$

**fMRI data analysis**—Statistical Parametric Mapping (SPM5; (<http://www.fil.ion.ucl.ac.uk/spm>)) software was used to preprocess and analyze the MRI data. First, the images were preprocessed, using slice-time correction, motion-correction and coregistration to the structural scan. Next, individual normalization parameters were obtained by normalizing the segmented structural scan of each subject using the Montreal Neurological Institute (MNI) T1 template image. These normalization parameters were then applied to the functional images. At the end of preprocessing, the normalized functional images were resliced to a resolution of 3×3×3 mm and spatially smoothed using an 8-mm isotropic Gaussian kernel.

For the subject-level fMRI analysis, the time series were filtered using 128 seconds high-pass filter and normalized using proportional scaling (dividing voxel intensity by the global mean yielding an average signal of 1 for each scan). Next, trial related activity was modeled by convolving a vector of trial onsets with a canonical hemodynamic response function (HRF). The General Linear Model (GLM), as implemented in SPM5, was used to model effects of interest and remove confounding effects. The Statistical Parametrical Maps were identified for each participant by applying linear contrasts to the parameter estimates (beta weight) for the events of interest, resulting in a t-statistic for every voxel. The model included both events and blocks of interest. Subsequent remembered (R-items) and forgotten (F-items) were modeled separately for breath-hold (HOLD) and normal-breathing (NORM) blocks using a canonical HRF. The rest, preparation, and normalization periods were modeled using a boxcar function. Group effects were assessed by applying random effects analyses thresholded at  $P < 0.001$  (uncorrected), cluster size = 25.

## Results

### Behavioral results

Behavioral performance ( $N = 24$ ) was similar during the NORM and HOLD conditions, but we observed some slight differences in terms of response times (RTs) and memory accuracy. Average RTs for the living/non-living classifications, made during memory encoding, were slightly faster during breath-holding as compared to normal breathing and slightly longer for R-items (correctly remembered with highest confidence rating 4) as compared to F-items (all misses). Average reaction times were  $812 \pm 19$  msec. for R-NORM,  $789 \pm 19$  msec. for F-NORM,  $751 \pm 16$  msec. for R-HOLD, and  $732 \pm 19$  msec. for F-HOLD. A repeated measures ANOVA demonstrated main effects of performance (R-/F-item,  $p < 0.001$ ) and condition (HOLD/NORM,  $p < 0.001$ ), but importantly, no significant performance  $\times$  condition interaction ( $P = 0.99$ ).

Memory accuracy, as quantified by  $d'$ -prime –regardless of retrieval confidence— (MacMillan and Creelman, 2005; Murdock Jr, 1965) showed only a non-significant difference (t-test:  $P = 0.074$ ) between NORM ( $1.21 \pm 0.063$ ) and HOLD ( $1.16 \pm 0.057$ ). However, there was a difference ( $p = 0.006$ ) in percentage hits –calculated over all encoded trials— made with the highest level of confidence (level 4) between NORM ( $0.31\% \pm 0.038$ ) and HOLD ( $0.29\% \pm 0.039$ ) conditions. Thus, although on an absolute level memory performance was similar for the NORM and HOLD conditions, there were slight, but consistent, behavioral differences across conditions.

### Relation between respiratory fluctuations and stimulus presentation

**Respiratory phase-locking**—In order to test the *respiratory phase-locking hypothesis*, we first investigated the relation between respiration and stimulus presentation. For this purpose, we focused only on the normal-breathing condition and calculated the respiratory signal time-locked to the period five seconds before the onset of the study words. As shown in Figure 2-A, and in line with the respiratory phase-locking hypothesis, we found a significant difference in the time-locked average of normalized respiratory belt measures after stimulus onset, but not before. If respiration is unrelated to cognitive events, one would not expect a time-locked difference, since random trial presentations relative to the respiratory cycle should cancel each other out, resulting in a flat line. However, we found a significant trough of  $-0.67 \pm 0.18$  ( $P < 0.001$ ) at about two seconds after stimulus onset and a peak of  $1.48 \pm 0.15$  ( $P < 0.001$ ) at four seconds, thus indicating a robust change in respiration following stimulus presentation that was consistent across subjects (Figure 2A).

To further investigate the *respiratory phase-locking hypothesis*, we used Fourier analysis on the respiratory signal during the normal breathing blocks comparing a 5-sec window before and after stimulus presentation. Subsequently, we estimated the phase consistency of the signal with respect to stimulus onset by means of the respiratory phase-locking value, which is defined as the circular resultant length of the respiratory phase (see Materials and Methods), averaged across all events (Fisher, 1993; Lachaux, et al., 1999). The resulting respiratory phase-locking values, ranges between 0 and 1, with a value of 1 indicating maximal phase-locking, and a value of 0 indicating no phase-locking to the stimulus onsets.



To assess whether the respiratory phase-locking was driven by the stimulus presentations, we calculated the difference in respiratory phase-locking values targeting the 5 second periods before and after stimulus onset. As shown in Figure 2-B, respiratory phase-locking values are significantly stronger after stimulus presentation (post-stimulus) than before (pre-stimulus) in the frequency bands that fall within the respiration frequency range (0.20 Hz:  $p = 0.050$ ; 0.40 Hz:  $p = 0.00041$ , and 0.60 Hz:  $p = 0.0017$ ), while those outside the respiratory frequency range showed no significant difference in phase-locking (0.80 Hz  $p = 0.17$ , 1.00 Hz  $p = 0.64$ , 1.20 Hz  $p = 0.68$ , 1.40 Hz  $p = 0.28$ ). These results show that respiratory phase-locking to stimulus-presentation occurs and that respiratory fluctuations are linked to behavioural performance even when using a fast, random event-related design.

**Greater phase-locking for R- than F-items**—The next question we asked is whether there might be a difference in the extent of respiratory phase-locking between R- and F-items. As shown in Figure 3-A, the respiratory belt measures showed, on average, a deeper trough and higher peak for R- than F-items during the post-stimulus period, whereas no differences were found during the pre-stimulus period. Using the respiratory belt signal, we confirmed the difference in respiration for both trial types by comparing the difference of the peak-minus-trough values for R-item and F-items; two-sample t-test:  $p = 0.023$ ). Respiratory phase-locking values were significantly higher for both trial types after (post-stimulus) as compared to before (pre-stimulus) presentation. R-items showed a significant difference at 0.20 and 0.40 Hz ( $p < 0.001$ ), while F-items showed a significant difference at 0.40 Hz ( $p = 0.034$ ) and 0.60 Hz ( $p = 0.035$ ). Again these values fell within the range of the respiratory frequency, while those outside the respiratory frequency range did not show significant phase-locking. Next, we directly compared the respiratory phase-locking difference values for R- and F-items. As shown in Figure 3-B, the pre- and post-stimulus difference in respiratory phase-locking was significantly greater for R-items than F-items at 0.20 Hz ( $p = .00048$ ) and trending at 0.40 Hz ( $p = 0.087$ ), indicating greater phase-locking for post stimulus periods in which R-items were presented. Thus, these results show not only that respiratory phase-locking occurs, but also that this phase-locking process is more pronounced for R- than F-items.

## fMRI results

**Breath-holding manipulation**—The aforementioned results reveal a relation between respiratory fluctuations and memory performance. Given these findings, it is critical to assess the effects of respiration on memory-related fMRI signal. As noted, our fMRI analyses focused on two regions of interest: the left VLPFC, the region generally showing the strongest positive DM effect (Buckner, et al., 1999) and the PMR -a core region of the DMN- generally shows the strongest *negative DM* effect (Daselaar, et al., 2004; Daselaar, et al., 2009). To identify significant voxels within these regions related to encoding success, we first combined the R- and F-items from the breath-holding and normal-breathing blocks to avoid biasing one condition over the other. As expected and as shown in Figure 4-A, we found a large cluster within the left VLPFC (Max T value = 10.39;  $MNI_{(x,y,z)} = -48, 24, 21$ , cluster size = 702) showing a *positive DM* effect (R-items > F-items; normal-breathing/breath-holding combined). Also, as predicted and shown in Figure 4-B, we found a large cluster of activity within the PMR, including precuneus and posterior cingulate cortex (Max

T-value = 6.68;  $MNI_{(x,y,z)} = -9, -66, 30$ ; cluster size = 750) showing a *negative DM* effect (F-items > R-items). To investigate the relation between respiration and memory-related fMRI signal, we extracted average cluster activity separately for R- and F-items and subtracted their values to quantify the size of the *positive* and *negative DM* effects for the breath-holding and normal-breathing conditions. In order to facilitate the comparison between the size of the *positive* and *negative DM* effects, the values of the *negative DM* effects were plotted upwards for both the breath-holding and normal-breathing conditions. Thus, *positive* and *negative DM* effects in Figure 4 are both pointing upwards.

Given the link between respiratory artifacts and resting-state fMRI signal in PMR but not VLPFC, we predicted that the *negative DM* effect in PMR but not the *positive DM* effect in VLPFC would be reduced by the breath-hold manipulation. Confirming this prediction and as shown in Figure 4-A, we found no significant difference in the size of the DM effect between breath-holding and normal-breathing conditions within left VLPFC ( $P = 0.41$ ) but a considerable reduction of about 65% of the *negative DM* effect in PMR during breath-holding ( $P < 0.001$ ; Figure 4-B). A repeated measures ANOVA of region (PMR/VLPFC) by breathing-condition (HOLD/NORM) confirmed the region-specific effect, via the interaction ( $P = 0.0009$ ). Thus, the findings indicate a considerable respiratory contribution to the *negative DM* effect in PMR. Yet, it is important to note that a significant *negative DM* effect remained during the breath-holding condition ( $P = 0.0005$ ), which suggests a respiratory-independent component of the *negative DM* effect in PMR to occur as well.

#### Individual subject correlations between respiratory fluctuations and fMRI—

The foregoing fMRI results indicate a respiratory contribution to memory-related fMRI differences in PMR. However, there are two important issues that need to be addressed. First, as noted, we found some behavioral differences between the breath-holding and normal-breathing conditions. In particular, participants were overall faster to respond in the breath-holding condition. As will be outlined in the discussion section, this difference might relate to dual-task or stress factors occurring during the breath-holding condition. Second, because of our hypercapnic design (breath-hold vs. normal breathing), our data required a correction for global signal differences across the scans in order to obtain sensible GLM results (see Materials and Methods). An example of a dataset with and without global scaling is shown in Supplemental Figure 1. It has been argued that scaling can sometimes result in artefactual increases or decreases in fMRI signal (Aguirre, et al., 1998; Murphy, et al., 2009). Thus, even though we are comparing R- and F- items trials within the breath-hold and normal-breathing blocks respectively, the point could be made that our results are somewhat affected by this data processing step.

To address these possible confounders, we performed an additional fMRI analysis focusing only on the normal-breathing condition, which eliminates any issues associated with the breath-hold blocks. In this analysis, we focused on individual differences in the respiratory effects. If respiration is a factor in the *negative DM* effect in PMR, one would expect that the participants that show the greatest respiratory response difference between R- and F-items also show the greatest *negative DM* effect in PMR, while this should not affect the *positive DM* effect in VLPFC. To test this prediction, we first quantified each participant's respiratory response by subtracting the peak and trough of the respiratory belt values for R-



and F-items separately. Next, we correlated this respiratory response with the size of both the *negative DM* effect in PMR and the *positive DM* effect in left VLPFC. In line with our prediction and as shown in Figure 5, we found that the *negative DM* effect in PMR was significantly correlated with the respiratory difference in memory ( $R = 0.47$ ,  $p = 0.028$ ), whereas the *positive DM* effect in left VLPFC was not ( $R = -0.24$ ,  $p = 0.29$ ). A direct Fisher comparison (Fisher, 1993) of the correlations in PMR and left VLPFC indicated a significant difference between the regions ( $p = 0.037$ ). Together with the previous fMRI results involving the breath-holding condition, these findings provide further support for a considerable respiratory contribution to encoding-related activity in PMR, but not in left VLPFC.

## Discussion

The present fMRI study explored the role of respiratory fluctuations in explaining the link between successful cognitive performance and PMR-deactivations as measured with fMRI. To control respiratory fluctuations, we used a memory encoding task that included a breath-hold condition. The study yielded three main findings. First, we found significant respiratory phase-locking to the presentation of memory items. Second, we found that this phase-locking effect was stronger for items later remembered (R-items) than those that were forgotten (F-items), indicating a link with successful performance. Finally, we found that respiratory fluctuations have substantial effects on encoding-related activity in PMR, but not in left VLPFC as indicated by the breath-hold manipulation and individual subject correlations. Below, we discuss these three findings in separate sections.

### Respiratory phase-locking

This is the first study to use phase-locking analysis methods commonly applied in electrophysiological studies of neural activity (Engel, et al., 2001; Lachaux, et al., 1999; van Wingerden, et al., 2010) to characterize the relation between respiratory fluctuations and stimulus presentation. In line with the *respiratory phase-locking hypothesis*, we found that the respiratory cycle indeed phase-locks to the stimulus presentations, yielding a respiratory response that differs significantly from baseline (Figure 2). Dynamic responses of the respiratory system to stimulus presentations have been reported previously (Leskovsek, et al.; Porges and Raskin, 1969). Using various respiratory measures, several studies found changes in the respiratory cycle following stimulus presentations. As noted in the introduction section, these changes have been generally attributed to attentional orienting processes (Harver and Kotses, 1987; Vlemincx, et al., 2011; Walter and Porges, 1976). In other words, it is assumed that the capturing of attention by the stimulus results in a change in the dynamics of the respiratory cycle leading to transient respiratory phase-locking. In the present study, we adhere to this *respiratory phase-locking account* for the overall interpretation of our findings.

An important difference, though, between previous work and the current study is that previous studies used affective stimuli which tend to evoke strong autonomic responses or manipulated the difficulty in perceptual detection of the stimulus (Gomez, et al., 2005; Stekelenburg and van Boxtel, 2001). In addition, all these studies either used extended

blocks of stimuli (Birn, et al., 2009; Chang, et al., 2009; van Buuren, et al., 2009) or slow presentation of trials (e.g. Gomez and Danuser, 2010; Gomez, et al., 2004; Leskovsek, et al.; Van Diest, et al., 2001). Here – using analysis methods derived from electrophysiological studies – we show for the first time that significant respiratory phase-locking occurs also for affectively neutral conditions. Moreover, we found that this phase-locking effect even occurs when using a fast event-related task (~1 trial every 3 secs). This last finding has important implications for fMRI studies as outlined in the *fMRI and respiration* section below.

### Respiration predicts subsequent memory

Following the *respiratory phase-locking account*, and the known link between encoding and attention, we also predicted that respiratory phase-locking would be stronger for R- than F-items. In line with our predictions, we found that there was significantly greater respiratory phase-locking for R- than F-items. Although both remembered items (R-items) and forgotten items were correlated with respiratory belt measures, R-items showed a more pronounced effect with a deeper trough and a higher peak (Figure 3). These findings show that respiration does not only correlate with immediate responses, but also with later memory performance. Thus, we interpret this finding as greater attentional orienting towards R- than F-items leading to better memory for the former trials. To our knowledge, this is the first study to show correlations between dynamic changes in respiration and memory performance. Although we used an encoding paradigm in this study, we expect that our findings are not exclusive to the episodic memory domain, and future research might indicate a general link between attention, arousal and respiration.

Previous memory studies have also found correlations between physiological measures and memory performance. For instance, Hannula and Ranganath found that eye movements during memory retrieval tracked whether a stimulus was seen before or not, regardless of the participants' conscious awareness (Hannula and Ranganath, 2009). In addition, emotional studies have found that the *emotional enhancement effect* – better memory for emotional than neutral stimuli – is coupled with increases in the galvanic skin response, which presumably reflects greater arousal levels that support memory formation and retrieval (Anderson, et al., 2006). The current study adds to these findings by showing that respiration can also be used as a predictor of memory. Future studies should investigate whether and how these different physiological parameters are coupled and whether their combination leads to a better prediction of memory.

### fMRI and respiration

In addition to the relationship between respiration and memory performance, we also found a region-specific relation between respiratory fluctuations and encoding-related fMRI activations. We predicted that encoding-related fMRI activity would be affected by the breath-hold manipulation, which eliminates respiratory fluctuations, but not in VLPFC. In line with previous studies, we found a robust negative DM effect (*less* activity for R- than F-items) in PMR, and a positive DM effect (*more* activity for R- than F-items) in the left VLPFC (Figure 4). However, in line with our prediction, we found a substantial reduction during breath-holding in the negative DM effect in PMR, while the activation in the VLPFC

remained unaffected. These findings indicate a region-specific interaction between respiration and the fMRI signal associated with successful task performance. The regional interaction is consistent with resting-state studies showing high susceptibility to respiratory artifacts along the posterior midline (Birn, et al., 2006; Van Dijk, et al., 2010). Here, we add to these resting-state findings by showing that respiratory fluctuations can explain changes in task-related fMRI signal, even when using fast event-related designs. The fact that we used a subsequent memory paradigm with fast trial presentation indicates that respiratory contributions to fMRI signal cannot be eliminated by simply contrasting different trial conditions.

An alternative explanation for the difference between NORM and HOLD conditions in the present study is that the large hypercapnic effect of breath-holding overrides subtle fluctuations in fMRI signal associated with R- and F-items, thereby leading to a reduced DM-effect in PMR. However, there are two reasons that support the finding of respiratory fluctuations on event-related fMRI signals in PMR. First, even though the global signal between NORM and HOLD blocks is different, we compared R- and F-items within the respective NORM and HOLD blocks. Thus, any differences in overall signal should be subtracted out. Still, we investigated this issue further by explicitly modeling the effects of hypercapnia with a respiratory response function (RRF; see Supplemental Figures 2-4). Respiratory effects are slower than neuronally induced BOLD signal changes, having longer time-delayed effects ranging unto 30 to 40 seconds. Recently, it was shown that these delayed effects of respiration can be modeled effectively using the respiratory response function (Birn, et al., 2008b; Chang, et al., 2009; Chang and Glover, 2009). As reported in the Supplemental Materials, we applied this approach to the current data and found comparable results to our previous analyses. Together, the regional differences we found indicate that changes in respiratory fluctuations during stimulus presentation have a bigger impact on a large blood-vessel region such as PMR than on VLPFC.

Regarding the regional differences in VLPFC and PMR some cautionary note is also appropriate given that there were differences in the overall T-values (VLPFC: 10.39; PMR: 6.68). There have been reports of a non-linear relationship between BOLD signal as a function of cerebral blood flow (CBF) / cerebral metabolic rate for oxygen (CMRO<sub>2</sub>) associated with the magnitude of activation (Lin, et al., 2008; Vafaei and Gjedde, 2000). These findings may suggest that the differences we found between VLPFC and PMR are not so much reflecting different responsiveness to respiratory artifacts, but rather are due to a difference in the overall amplitude associated with the task stimuli. Regardless of this possibility, our findings demonstrate that there is a clear relation between respiratory fluctuations and PMR signal, and that, in general, it is important to consider non-neural/vascular effects in future task-based fMRI studies.

Another issue is that there might be a higher level of stress and divided attention during the breath-hold condition, which could be considered a dual task. In other words, breath-holding might draw attentional resources or evoke mild stress that may change the default mode network functional dynamics. In line with this alternate explanation, the behavioral results indicated a slight difference in memory performance and response times were overall slightly faster for the HOLD condition. Also, due to the nature of our design, we used global

scaling of the fMRI data (Supplemental Figure 1). It could be argued that this data processing step affected our comparison between breath-hold and normal-breathing blocks (see Results section).

To address these important issues, we conducted a follow-up analysis focusing only on the NORM condition, thereby eliminating any issues associated with the HOLD blocks. To this end, we correlated the size of the DM effects in PMR and VLPFC during normal-breathing with the respiratory amplitude difference between R- and F-items derived from the time-locked averages of the respiratory response (Figure 5). The results indicated that individuals with the largest respiratory difference between R- and F-items also showed a greater negative DM effect in PMR, whereas we did not find this correlation regarding the size of the positive DM effect in VLPFC (Figure 5). This finding is difficult to explain by dual-task/stress or global signal/scaling factors alone. Together with the breath-hold results, these findings indicate a substantial contribution of stimulus-evoked respiratory changes to task-based fMRI signal in PMR even in fast event-related fMRI studies.

Our findings may have important implications for the interpretation of the results of previous fMRI studies that focused on PMR and that did not measure respiration. Here we used event-related fMRI, but most early fMRI studies that focused on PMR deactivations used block designs comparing active to passive conditions. Results from these studies may be even more sensitive to respiratory artifacts, because overall respiratory rate differences cannot be subtracted out when using slow block designs. Several studies have shown that, compared to active externally-oriented tasks, passive internally oriented conditions, such as relaxation, imagery, and daydreaming, are associated with slower breathing rates (Corwin and Barry, 1940; Dudley, et al., 1964; Rehwoldt, 1911; Skaggs, 1930). We actually confirmed this within the present study. Although not described in the results section, we found that the respiratory rate was significantly slower during rest ( $13.9 \pm 0.6$  breaths per minute) than during stimulus presentation blocks ( $14.4 \pm 0.6$  breaths per minute; ( $p = 0.020$ ; Supplemental Figure S2). This within-subject difference in breathing-rate was negatively correlated with the within-subject difference in fMRI signals between task-and rest-block. In other words, individuals showing a relatively large difference in breathing-rate also showed relatively strong task-induced deactivations in PMR. Similar respiratory differences could potentially account for PMR findings reported in previous fMRI studies that compared active and passive conditions. This issue is particularly important for clinical fMRI studies that compare populations with different arousal levels and, hence, are likely to show different respiratory patterns, such as schizophrenic, post-traumatic stress disorder, and autistic patients. Thus collectively, our findings indicate a substantial contribution of stimulus-evoked respiratory changes to the fMRI signal in PMR not only in fast event-related, but also blocked, fMRI studies.

Finally, it is important to note that a significant negative DM effect remained present in PMR during breath-holding. This finding indicates that, although respiratory/vascular factors account for a considerable portion of encoding-related fMRI signal in PMR, there is also a respiratory independent contribution. This interpretation is consistent with findings from intracranial measurements during rest, which have revealed clear correlates between local neuronal activity and BOLD signal fluctuations within PMR (Mantini, et al., 2007;

Schölvinck, et al., 2010). Moreover, electrophysiological recording from both humans and macaque monkeys have indicated that local populations of neurons show decreases in firing-rates consistent with fMRI findings concerning task-related deactivations in PMR (Hayden, et al., 2009; Jerbi, et al., 2010).

Also, one previous blocked-task fMRI study (lexical decision task) used a gas delivery system to directly measure and control end-tidal CO<sub>2</sub>/O<sub>2</sub> levels (Madjar, et al., 2012). Controlled CO<sub>2</sub>- vs. normal breathing yielded clear differences in PMR signal. Interestingly, they found that PMR-deactivations were more significant under controlled conditions due to reduced variability in CO<sub>2</sub> level. Thus, this study demonstrates that the fMRI signal in the PMR cannot be fully explained by respiratory changes. These findings support the interpretation that the remaining negative DM effect in PMR during the breath-hold condition we saw in our study reflects a neuronal contribution to the fMRI signal. However, it is not clear from the Madjar et al. study how respiratory phase-locking during normal breathing affects CO<sub>2</sub> levels and ensuing changes in fMRI signal.

The current findings clearly show that it is critical to incorporate respiratory measures in fMRI analyses to account for respiratory/vascular effects, even when using fast event-related fMRI designs. We interpret our findings as a confounder induced by respiratory effects on the fMRI signal. It should be noted, though, that it remains possible that selective populations of neurons change their firing-rate in phase with physiological fluctuations. In other words, changes in the fMRI signal that seem physiological in origin might also have an underlying neural component. Our experiment cannot distinguish between these alternatives. Nevertheless overall, the current results indicate that measures of respiration need to be considered for an appropriate interpretation of fMRI results, in particular when observing deactivations of the PMR.

## Conclusions

The study yielded three main findings. Our first and most important finding is that respiration phase-locks to the presentation of word stimuli, presumably through an attentional orienting process. Second, we found that this respiratory phase-locking effect was more pronounced for items later remembered (R-items) than those that were forgotten (F-items). In line with an attentional orienting process, this finding indicates a link between respiratory fluctuations and successful cognitive performance, even when using fast event-related designs. Finally, we found that respiratory fluctuations have substantial effects on encoding-related activity in PMR, but not in left VLPFC, as indicated by the breath-hold manipulation. We confirmed this finding by showing a correlation between the respiratory phase-locking effect and *negative* DM effects in the PMR across individual subjects during the normal-breathing condition. Together, these findings have important implications for the interpretation of fMRI results not only for resting-state fMRI studies (Birn, et al., 2006), but also for task-related fMRI studies even when using fast event-related fMRI designs. The fact that this respiratory effect is particularly pronounced in PMR may have important implications for the interpretation of fMRI studies that found differences in PMR between controls and clinical populations, which are likely to have different arousal levels and respiratory patterns, such as patients with schizophrenia or post-traumatic stress disorder

(Bleichert, et al., 2007; Filik, et al., 2006). Further research is necessary to demonstrate that our findings are not restricted to episodic memory encoding, but also extend to other cognitive domains.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

This work was supported by the European Molecular Biology Organization: ALTF 318-2011 [W.H], the Amsterdam Brain Imaging Platform [W.H, S.D], the National Institutes of Health (NIH; R01 AG019731 and R01 AG23770) [R.C], and the Netherlands Organization for Scientific Research, VICI 918.46.609 [C.P] and VENI 916.66.022 [S.D].

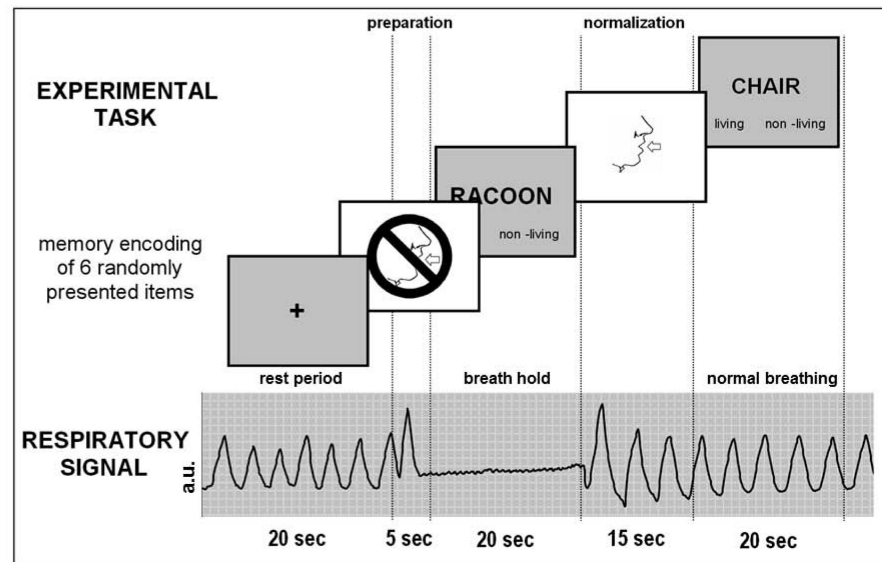
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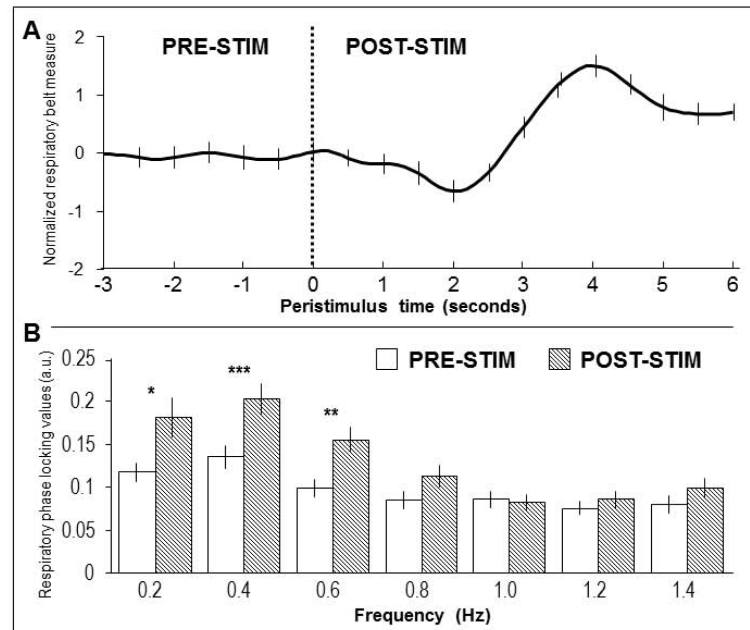
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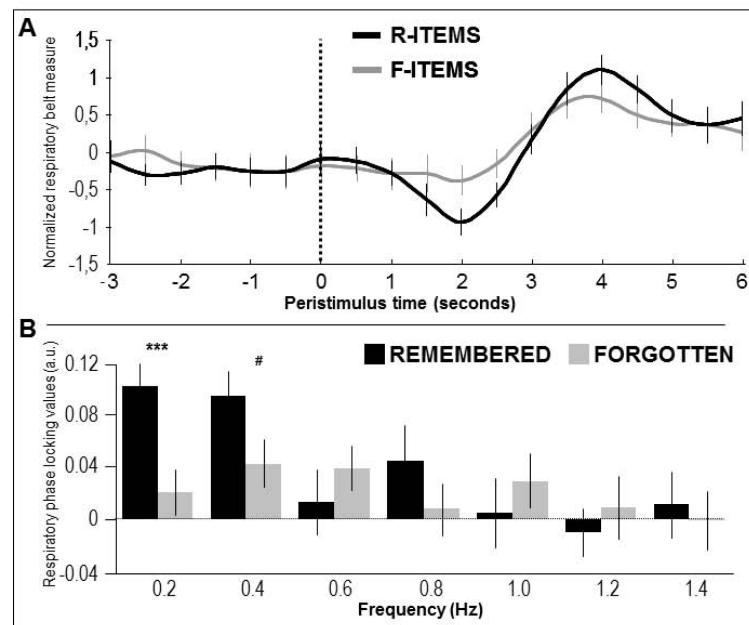
**Figure 1. Task design**

Experiment consisted of 20 second blocks of (1) a rest period with during normal-breathing, (2) encoding during breath-holding, (3) encoding during normal-breathing. During each encoding block, 6 items were randomly presented. Breath-hold blocks were flanked by 5 seconds of preparation and 15 seconds of normalization. Lower panel illustrates an individual time course of the respiratory signal in arbitrary units (a.u.).



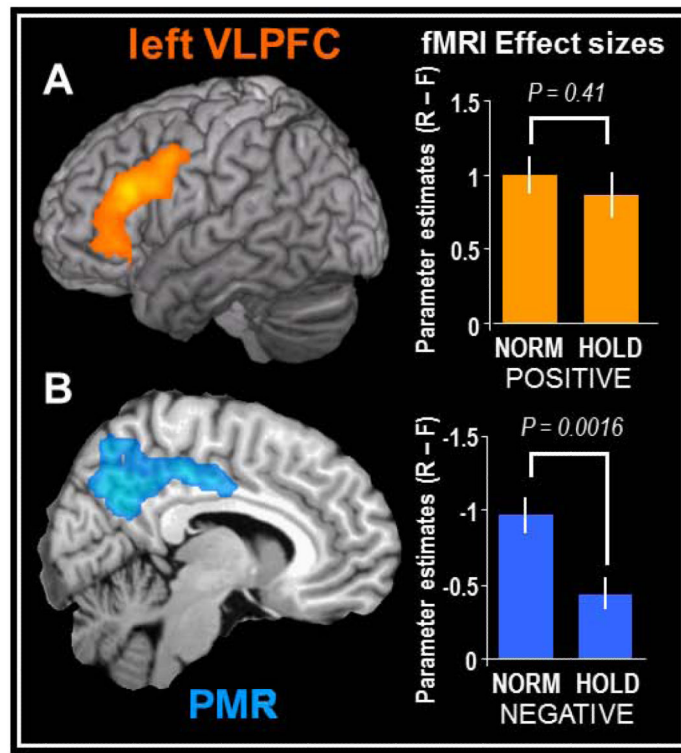
**Figure 2. Respiratory phase-locking and stimulus onset**

Panel A shows the average normalized respiratory signal, time-locked to stimulus presentation, before (PRE) and after (POST) stimulus onset ( $t = 0$ ). Panel B: The bar graphs show the average respiratory phase locking value (i.e. circular resultant length) for the frequencies between 0.20 and 1.40 Hz. In the range of the respiratory cycle (0.20-0.60 Hz), we observed significant respiratory phase-locking differences between pre- and post-stimulus onset. Striped bars represent the respiratory phase-locking values after stimulus presentation and dotted bars before. Vertical lines indicate the standard error of the mean (SEM) and the asterisks denote P-values of t-tests (\* =  $P < 0.05$ , \*\* =  $P < 0.005$ , \*\*\* =  $P < 0.001$ ).



**Figure 3. Respiratory phase-locking and memory encoding**

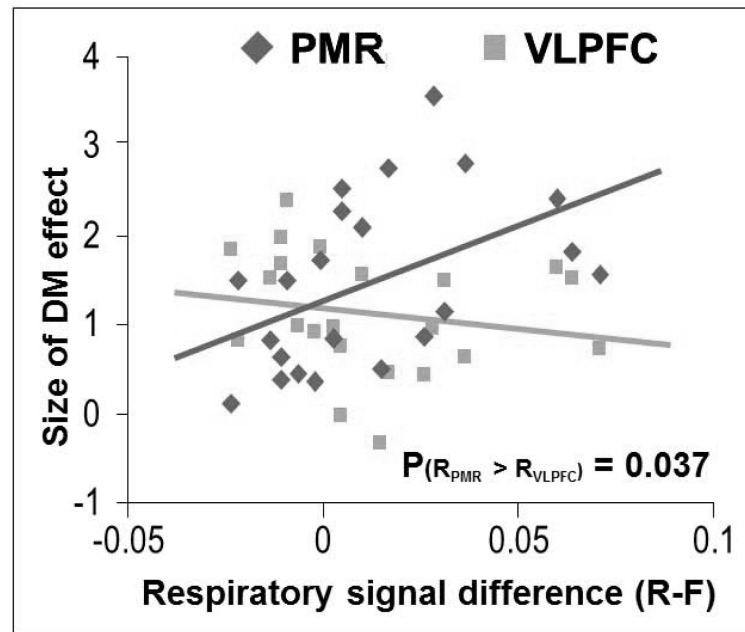
Panel A shows the average normalized respiratory signal, time-locked to stimulus presentation ( $t = 0$ ), separately for subsequently remembered (R-items in black) and forgotten items (F-items in gray). Panel B: the bar graphs show the difference in respiratory phase-locking pre- and post-stimulus presentation for remembered items (black) and forgotten items (gray) separately. Vertical lines indicate the standard error of the mean (SEM) and asterisks denote P-values of t-tests (# =  $p < 0.10$  (trending), \*\*\* =  $P < 0.001$ ).



**Figure 4. Task-related fMRI and respiration**

Panel A shows the positive memory effect in the left ventrolateral prefrontal cortex (VLPFC; orange color; R-items > F-items:  $P < 0.001$ , uncorrected). Panel B shows the negative memory effect in the posterior midline region (PMR; blue color; for F-items > R-items:  $P < 0.001$ , uncorrected). Bars show the average signal differences in memory (R-items - F-items) for the normal (NORM) and the breath-hold (HOLD) conditions. Vertical lines indicate the standard error of the mean (SEM)





**Figure 5. Correlation between normal respiration and memory effect**

Scatter plot showing the average difference in respiratory amplitude between R-and F-items (x-axis) and the average activity difference in memory (DM) between R-and F-items. Each point represents the data from a single subject. The blue diamonds are derived from the PMR ROI and the orange squares from the VLPFC ROI.