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Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging

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Aging has independently been associated with regional brain atrophy, reduced slow wave activity (SWA) during non–rapid eye movement (NREM) sleep and impaired long-term retention of episodic memories. However, whether the interaction of these factors represents a neuropatholgical pathway associated with cognitive decline in later life remains unknown. We found that age-related medial prefrontal cortex (mPFC) gray-matter atrophy was associated with reduced NREM SWA in older adults, the extent to which statistically mediated the impairment of overnight sleep–dependent memory retention. Moreover, this memory impairment was further associated with persistent hippocampal activation and reduced task-related hippocampal-prefrontal cortex functional connectivity, potentially representing impoverished hippocampal-neocortical memory transformation. Together, these data support a model in which age-related mPFC atrophy diminishes SWA, the functional consequence of which is impaired long-term memory. Such findings suggest that sleep disruption in the elderly, mediated by structural brain changes, represents a contributing factor to age-related cognitive decline in later life.

A recognized and problematic feature of human aging is cognitive decline[1], including impaired long-term retention of episodic memories[2]. These cognitive changes are paralleled by two prominent, albeit independently considered, signatures of aging. The first is structural brain atrophy, pronounced in midline frontal lobe regions[3]. The second is disrupted electroencephalographic (EEG) quality of NREM slow-wave sleep (SWS)[4,5], evidenced in decreased SWA. Despite these coinciding features, whether age-related structural changes are associated with disrupted sleep physiology, and whether such structural and physiological changes are associated with age-related memory impairment remain unknown. We sought to determine whether these independently recognized features of aging are inter-related and, if so, determine the precise nature in which their interaction predicts overnight memory retention.

Independent of aging, an emerging body of evidence in healthy young adults continues to support a role for NREM SWS physiology in the long-term consolidation of episodic memories[6,7]. Relative to equivalent time awake, NREM SWS beneficially limits the offline decay of episodic memory representations over time, resulting in superior retention[8]. Furthermore, electrical facilitation of SWA over prefrontal cortex (PFC) causally enhances retention of episodic memories[9]. Mechanistically, these findings have been considered in a postulated hippocampal-neocortical framework of memory consolidation[10,11], whereby NREM SWS promotes the transformation of episodic representations from an initially hippocampal-dependent to an increasingly hippocampal-independent (and potentially more semanticized) state[6,7,12]. In doing so, older (more hippocampally independent) memory representations[11] are proposed to be less vulnerable to interference by ongoing encoding of new hippocampal-dependent episodic information[7]. Consistent with this framework, human neuroimaging findings suggest that NREM SWS may be associated with the degree of increasing hippocampal independence during post-sleep memory retrieval[8]. Conversely, sleep deprivation after learning impairs long-term declarative memory retention, potentially resulting in a greater reliance of memory retrieval on the hippocampus[13]. Such data suggest that one potential mechanism by which SWA promotes consolidation is the transformation of episodic memories from a labile, hippocampal-dependent state, to an increasingly hippocampal-independent state[6,7,10,11].

These findings predict that the known age-related reduction of NREM SWA should result in impoverished hippocampal memory consolidation and next-day retention in the elderly. In support of these predictions, NREM SWS correlates with overnight memory retention across young and middle-aged adults, although, to date, examinations of this association have not controlled for age[2]. Furthermore, NREM SWA has been associated with the degree of overnight memory retention in healthy older adults and patients with amnestic mild cognitive impairment[14]. These findings support a role for NREM SWS in age-related memory decline, an effect that is potentially exacerbated in older individuals with memory disorders[14]. However, a preceding issue is the nature of age-related deficits in NREM SWS and whether such a deficit is an inevitable consequence of the aging brain[15]. One candidate is structural brain atrophy in regions that promote NREM SWA. Specifically, the mPFC not only expresses some of the greatest

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gray matter reductions in older adults[1,3], but is a region in young adults that has been identified as a prominent EEG electrical generator of NREM slow waves[16]. Furthermore, NREM slow waves show a marked preponderance in origin and density over mPFC EEG derivations, as does the expression of associated NREM SWA[17]. Such data further predict that the extent of age-related atrophy of mPFC should negatively affect the ability to generate NREM SWA in older adults.

These findings, and the role of prefrontal SWA in promoting episodic memory retention in young adults, suggest that age-related reductions in SWA, mediated by reduced prefrontal gray matter atrophy, represent a neuropathological pathway that is associated with impaired long-term memory retention in older adults. Conversely, this collection of co-occurring changes in brain structure, sleep physiology and episodic memory may, instead, represent independent processes associated with aging that do not influence each other. Seeking to discriminate between these two possibilities, we examined whether age-related reductions in mPFC gray matter volume statistically mediate the effects of age on impaired NREM SWA and whether this age-related interaction between brain atrophy and NREM SWA consequently predicts age-related failure of overnight episodic memory retention, and, with it, the persistent reliance (rather than increasing independence) of memory retrieval on the hippocampus.

RESULTS

A group of cognitively normal older adults and a group of healthy young adults performed an episodic associative (word pair) memory task sensitive to sleep-dependent episodic memory retention (**Fig. 1**, **Table 1** and **Supplementary Table 1**)[6]. All participants were initially trained to criterion on a set of word pairs in the evening, pre-sleep, and then subjected to two separate recognition memory tests. The first (short delay) recognition memory test occurred 10 min after the initial study session, where a subset of the studied word pairs were tested. Following the short-delay recognition test, participants were given an 8-h sleep period in accordance with habitual sleep-wake habits, recorded using polysomnography with full-head EEG coverage. The next morning, participants performed the second (long delay) post-sleep recognition test, in which the remaining subset of originally studied word pairs was tested. The long-delay recognition testing was performed during a functional magnetic resonance imaging (fMRI) scan session to assess differences in retrieval activity, focused *a priori* on the hippocampus[18]. An additional structural MRI scan followed the fMRI session, allowing for assessment of gray matter differences between young and older adults. The measure of overnight memory retention was calculated by subtracting short-delay recognition performance from long-delay recognition performance[2,13].

Age effects on sleep and gray matter volume

We first sought to characterize SWA (0.8–4.6 Hz[19]) in older and young adults. As predicted, and consistent with previous findings[16,17], SWA was dominant over prefrontal EEG derivations in both the young and older adult groups (**Fig. 2a**). Also consistent with prior reports[4], older adults exhibited marked and significant reductions in SWA relative to young adults. This was true globally, averaged across the entire head ($P < 0.001$; **Fig. 2b**), and when focusing locally over prefrontal EEG derivations exhibiting the highest SWA ($P < 0.001$, **Fig. 2b**); the latter associated both with the dominant EEG source region of cortical slow waves[16] and where experimental manipulations of SWA causally modulate episodic memory retention[9]. Similar age effects were detected for global ($P < 0.001$) and prefrontal ($P < 0.001$) absolute SWA and time spent in slow wave sleep (**Fig. 2c**,**d** and **Supplementary Table 2**).

Given the described homology between prefrontal dominance in both slow-wave generation and age-related brain atrophy, we next examined group differences in gray matter volume, focusing *a priori* on mPFC. Consistent with previous findings[1,3], the peak significant difference in gray matter volume between young and older adults, when examining across the whole brain, was detected in mPFC ($P < 0.05$, family-wise error (FWE) corrected; **Fig. 3**). Significant reductions in gray matter volume in older relative to young adults were also detected in bilateral insula and posterior cingulate cortex ($P < 0.05$, FWE corrected; **Supplementary Fig. 1**). This is notable, as these regions have also been associated with slow-wave source generation, albeit to a lesser degree than the mPFC[16].

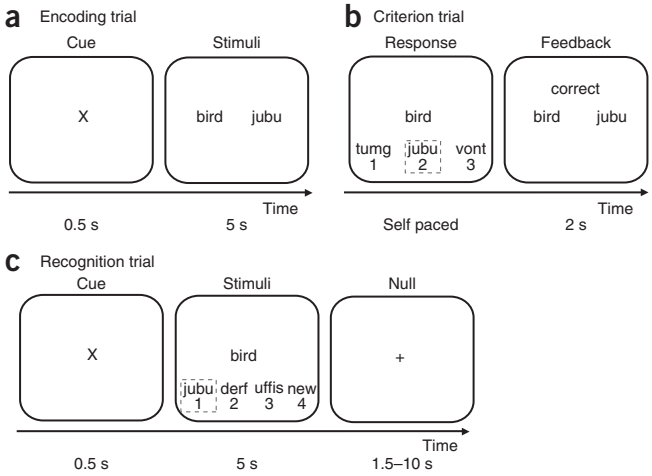

Figure 1 The sleep-dependent episodic word-pair task[6,9] used word-nonsense word pairs to maximize the novel episodic and associated hippocampal-dependent demands of the task[33] and minimize the semantic, and therefore hippocampal-independent, demands of the task[11,12,33]. Words were 3–8 letters in length and drawn from a normative set of English words[34]. Nonsense words were 6–14 letters in length, derived from groups of common phonemes[33]. The word-pair task began with an encoding phase composed of 120 word-nonsense word trials. (**a**) During each encoding trial, a word-nonsense word pair was shown for 5 s. Criterion training occurred immediately after encoding. (**b**) During each self-paced criterion trial, a previously studied probe word was presented with its original nonsense word associate from encoding (outlined in the gray box) and two new nonsense words not previously shown. Following responding, the participant was given feedback for 1 s, with incorrect responses resulting in trial repetition at random intervals. (**c**) During recognition trials, either a previously studied probe word or a new (foil) probe word was shown for 5 s with four response options presented below. When a previously studied probe word was presented, the following response options were presented below: (1) the nonsense word originally paired with that probe word at encoding (hit), (2) a previously studied nonsense word, but one presented with a different previously studied probe word (lure), (3) a new nonsense word never seen during encoding, and (4) an option designating the shown probe word as new. New nonsense words were only presented once during the entire experiment, whereas previously studied nonsense words were presented twice during recognition testing, always in the same session, as a lure on one trial and the correct paired associate on another. When a foil probe word was presented, the four response options consisted of three new nonsense words never presented during learning (which, if chosen, would designate a false alarm), and an option designating this foil probe word as new (which, if chosen, would designate a correct rejection). We interspersed 45 null events, consisting of a fixation display (1.5–10 s), throughout long-delay recognition testing during fMRI acquisition, jittering trial onsets.

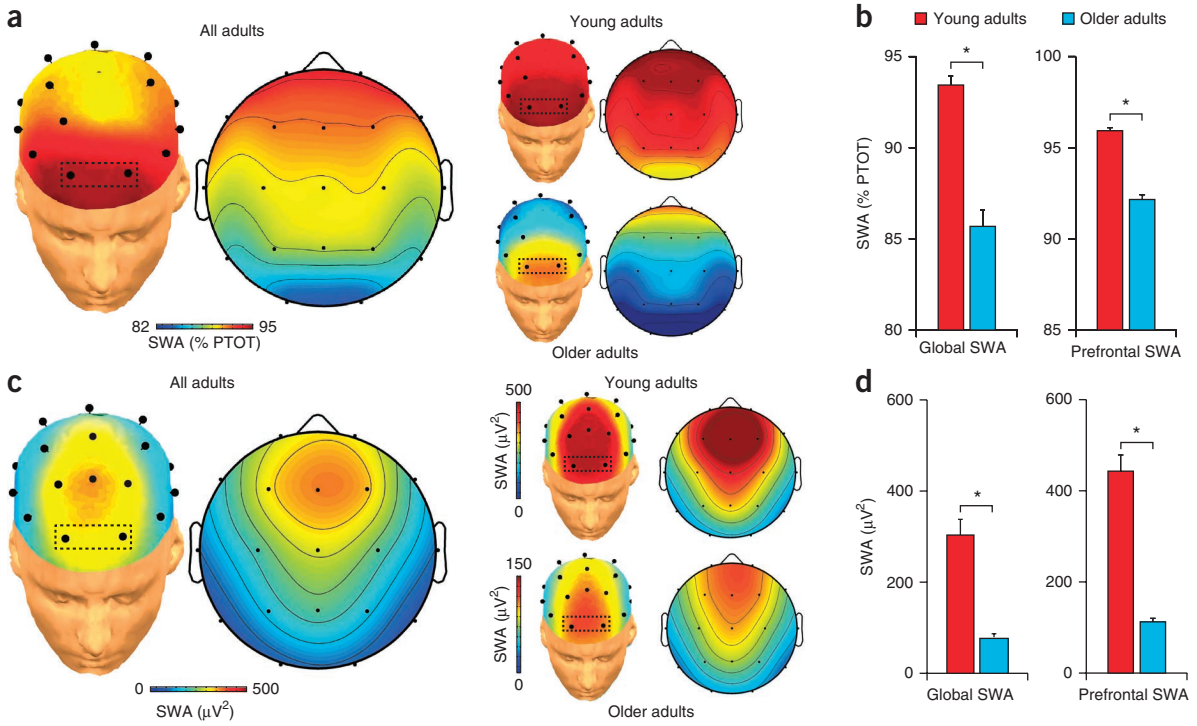

Figure 2 Age differences in SWA (**a**,**b**) EEG topographic plots of relative SWA (0.8–4.6 Hz, **a**) during SWS in young and older adults and the relative SWA difference (**b**) between young and older adults, both averaged across all electrode sites for a metric of global SWA (left) and averaged across prefrontal electrode sites (right), outlined in the box in **a** (electrode derivations Fp1 and Fp2), exhibiting peak relative SWA in both groups. (**c**,**d**) EEG topographic plots of absolute SWA (0.8–4.6 Hz, **c**) during SWS in all adults collapsed and young (top plot) and older (bottom plot) adults separately, and the SWA difference (**d**) between young and older adults both averaged across all electrode sites for a metric of global SWA (left) and averaged across prefrontal electrode sites (right), outlined in the box in **a** (electrode derivations Fp1 and Fp2), exhibiting prefrontal derivations where absolute SWA was averaged and then compared between groups. Error bars indicate s.e.m. $^*P < 0.001$. % PTOT denotes percentage of total spectral power (0.4–50 Hz).

Given that age was associated with both reduced SWA and prefrontal gray matter atrophy, we next sought to determine whether age effects on SWA were statistically mediated by changes in mPFC gray matter volume using mediation analyses. Older age was associated with decreasing global SWA ($r = -0.86$, $P < 0.001$) and decreasing mPFC gray matter volume ($r = -0.94$, $P < 0.001$). However, age no longer significantly predicted the extent of global SWA when mPFC gray matter volume was included in the statistical model (mPFC, $P = 0.009$; age, $P = 0.446$), reflected in a significant mediation effect (Sobel test[20], $P = 0.005$). This association between mPFC gray matter volume and global SWA ($r = 0.89$, $P < 0.001$; **Fig. 2b**) was present in young ($r = 0.60$, $P = 0.01$) and older ($r = 0.52$, $P = 0.05$) adults separately. Thus, age was not independently associated with reduced mPFC gray matter and reduced global SWA. Instead, the SWA decrease with age was mediated by the reduction in mPFC gray matter. To examine whether the mediating role of gray matter in age-related changes in SWA was specific to mPFC, we repeated the mediation analyses in other regions associated with age-related memory decline: the precuneus, hippocampus and temporal lobe[1,21]. The precuneus, hippocampus and temporal lobe all showed age-related atrophy, with negative associations between age and gray matter volume (precuneus: $r = -0.74$, $P < 0.001$; hippocampus: $r = -0.42$, $P = 0.014$; temporal lobe: $r = -0.83$, $P < 0.001$) and significant gray matter volume reductions in older relative to young adults (precuneus, $P < 0.001$; hippocampus, $P = 0.003$; temporal lobe, $P < 0.001$). However, precuneus ($P = 0.157$, age $P < 0.001$), hippocampus ($P = 0.586$, age $P < 0.001$) and temporal lobe ($P = 0.679$, age $P < 0.001$) gray matter volume did not predict SWA when age was

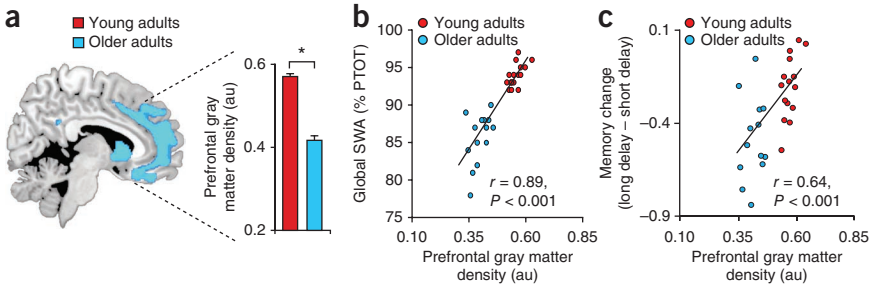

Figure 3 Age differences in gray matter volume and associations with SWA and memory. (**a**) Age effects (older < young adults) in gray matter volume, with medial prefrontal gray matter volume in young (red) and older (blue) adults plotted to the right. (**b**,**c**) Regression between prefrontal gray matter volume and global SWA (**b**), defined as the average relative SWA across all electrode sites, and associative episodic memory change (**c**). Activations are displayed and considered to be significant at the voxel level of $P < 0.05$ FWE corrected for multiple comparisons across the whole brain volume. Cool colors represent the extent of reduced gray matter volume in older relative to young adults. Error bars indicate s.e.m. $^*P < 0.05$ FWE, whole brain corrected. au, arbitrary units. % PTOT denotes percentage of total spectral power (0.4–50 Hz).

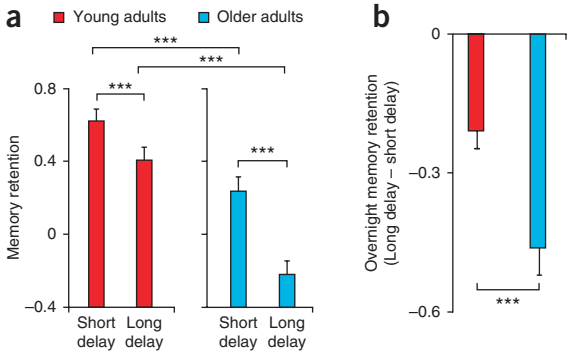

Figure 4 Age differences in memory retention (**a**,**b**) Recognition performance (hit rate for originally studied word pairs – lure rate to originally studied word pairs – false alarm rate to new, unstudied words) in young (red) and older (blue) adults both at pre- (short delay) and post-sleep (long delay) testing (**a**), and the change in recognition performance (long delay – short delay; **b**), reflecting a measure of associative episodic memory consolidation. Age differences in recognition performance were the result of changes in hit rate ($P < 0.001$) and not lure rate ($P = 0.16$) or false alarm rate ($P = 0.17$), suggesting that age differences in recognition performance were driven by differences in memory and not response bias. Error bars indicate s.e.m. ***$P < 0.001$.

included in the statistical model, suggesting that gray matter atrophy in these regions did not significantly mediate the effects of age on SWA (all Sobel test[20], $P > 0.13$) or the effects of SWA on long-term memory retention (see below; all $P < 0.001$ for SWA in models including age and gray matter). Thus, age-related changes in SWA are statistically mediated by regionally specific changes in gray matter volume that include the mPFC.

Age effects on memory

We examined whether the overnight change in episodic memory retention was impaired in older adults relative to young adults (**Fig. 4a** and **Table 1**). A two-way, repeated-measures ANOVA revealed significant main effects of group (young versus older, $P < 0.001$) and testing session (short delay (10 min, pre-sleep) versus long delay (10 h, post-sleep), $P < 0.001$). Most relevant, there was also a group × testing session interaction effect ($P = 0.001$). Specifically, the decline from pre- to post-sleep episodic memory, expressed as the memory savings difference between short (10 min, pre-sleep) and long (10 h, post-sleep) delay recognition performance, was significantly impaired (that is, greater) in older than in young adults ($P = 0.001$; **Fig. 4b** and **Table 1**). These findings indicate that memory performance in older adults was worse than that in young adults, that both groups showed a deterioration in retention of memory overnight, relative to short delay (10 min) recognition testing, and that older adults exhibited a significant impairment in sleep-dependent memory retention compared with young adults.

That differences in overnight memory retention were not driven by differences at encoding was explored by two additional analyses. First, recognition performance at the short delay (10 min) did not predict the amount of overnight memory change across all subjects combined or within subgroups (all $r^2 < 0.02$, $P \geq 0.35$). Second, and to further ensure that differences in memory encoding between young and older adults did not drive differences following sleep, we performed a subset analysis matching the highest older adult memory performers at the short delay ($n = 8$, 0.42 ± 0.05) with the lowest young adult memory performers at the short delay ($n = 8$, 0.40 ± 0.08). The scores were not different between these subgroups ($P = 0.855$). The highest performing older adults at the short delay test, similar to the overall older adult cohort, exhibited a significantly greater decrease in memory between the short- and long-delay tests than the lowest performing young adults (-0.54 ± 0.06 versus -0.21 ± 0.05, respectively; $P < 0.001$). To further ensure differences in overnight memory retention were not driven by encoding differences, we trained participants to criterion. Specifically, participants were tested until they correctly identified all paired associates. To examine differences in criterion accuracy, defined as the number correct divided by the total number attempted, across age or experimental groups, we performed a two-way ANOVA with age (young versus older) and experimental (sleep versus wake) group as between subject factors. No significant effects were detected (all $P > 0.1$). Taken together, these findings suggest that overnight differences in the memory retention between the young and older adult groups are not likely to be accounted for by differences in memory at the initial short-delay test.

To determine whether diminished memory retention in older relative to young adults was unique to offline periods of sleep (rather than simply time), we asked two separate groups of young and older adult participants to perform the same word-pair memory task, but spanning a delay period of wakefulness during the day (**Supplementary Table 1**). A three-way, repeated-measures ANOVA was performed with test session (short delay versus long delay) as a within subject factor, and age group (young versus older) and experimental group (sleep versus wake) as between subject factors. Significant main effects of test session ($P < 0.001$) and age group ($P < 0.001$) were detected, suggesting that recognition performance was lower at long-delay (10 h) than at short-delay (10 min) testing and worse in older than in young adults. Notably, however, significant age group × testing session ($P < 0.001$) and experimental group × test session ($P = 0.005$) interaction effects were detected. Older adults expressed significantly worse offline retention relative to young adults across periods including sleep (-0.46 ± 0.06 in older versus -0.21 ± 0.04 in young adults, $P = 0.001$) and periods including only wake (-0.62 ± 0.09 in older versus -0.40 ± 0.04 in young adults, $P = 0.031$). However, and consistent with prior reports[6,9,13], only young adults demonstrated superior offline memory retention following sleep compared with wake ($P = 0.003$), whereas older adults showed no such sleep relative to wake memory retention benefit ($P = 0.161$).

Table 1 Demographic, behavioral and neuropsychological measures (mean ± s.d.)

Variable	Young ($n = 18$)	Older ($n = 15$)
Demographic and behavioral measures		
Age (years)	20.4 ± 2.1	72.1 ± 6.6***
Gender	10 female	12 female
Education (years)	14.3 ± 1.8	17.8 ± 1.6***
MMSE	29.6 ± 0.8	29.3 ± 0.8
Mean bed time	0:28 ± 0:48	22:28 ± 1:09***
Mean wake time	8:34 ± 0:48	6:55 ± 1:03**
Mean prestudy time in bed (h)	8.10 ± 0.61	8.46 ± 0.81
Mean prestudy sleep time (h)	7.79 ± 0.63	6.91 ± 1.07*
Mean prestudy sleep latency (min)	14.8 ± 9.1	46.2 ± 56.1
Mean prestudy sleep efficiency (%)	96.3 ± 2.2	82.6 ± 13.2**
Scan time relative to pre-study wake time	1:21 ± 1:16	1:33 ± 0:57
Short delay recognition (HR-LR-FAR)	0.62 ± 0.07	0.24 ± 0.08***
Long delay recognition (HR-LR-FAR)	0.41 ± 0.07	–0.22 ± 0.07***
Memory change (long-short delay)	–0.21 ± 0.04	–0.46 ± 0.06***
Neuropsychological measures		
CVLT (long delay, number of free recalled)		11.5 ± 3.2
WMS (visual reproduction %)		83.3 ± 12.2
Trailmaking B (s)		69.8 ± 42.4
Stroop (number correct in 60 s)		54.3 ± 13.0

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. FAR, false alarm rate; HR, hit rate; LR, lure rate; MMSE, Mini-Mental State Examination; CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale.

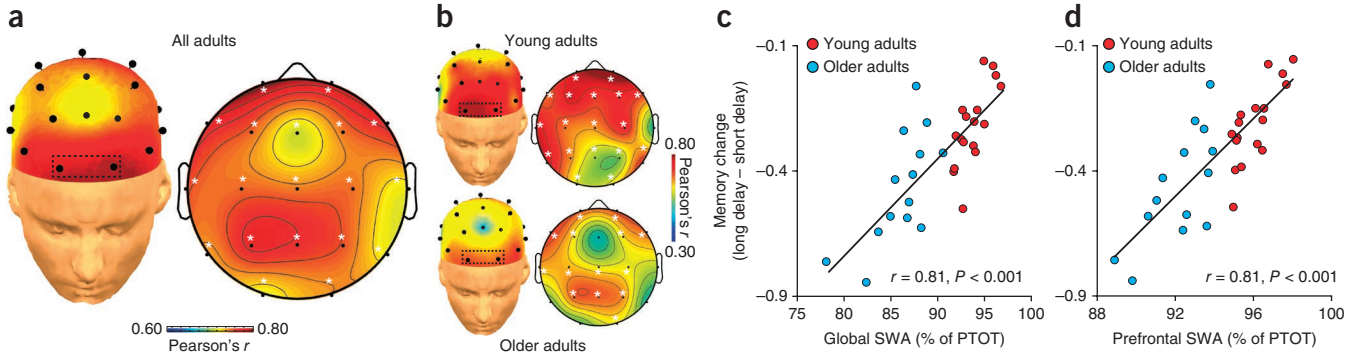

Figure 5 SWA predicts overnight memory change in young and older adults (**a**–**d**) Topographic plots of the association between relative SWA (0.8–4.6 Hz) during SWS and associative episodic memory change in all participants collapsed (**a**) and in young (top plots) and older (bottom plots) adults (**b**), with corresponding regressions for young (red) and older (blue) adults plotted for global relative SWA (**c**), defined as the average relative SWA across all electrode sites, and prefrontal SWA (**d**), defined as the average at prefrontal electrodes outlined in the box in **a** and **b** (Fp1 and Fp2), exhibiting peak relative SWA in both groups. Associations were specific to SWA, as measures of subjective sleepiness and alertness, objective alertness, circadian preference, neurocognitive status, fast spindle density during stage 2 sleep (**Supplementary Fig. 2**), TST, wake after sleep onset time, and sleep efficiency did not correlate with episodic memory change in either young or older adults separately (**Supplementary Table 3**). Similar to relative SWA, both global absolute SWA ($r = 0.57$, $P = 0.001$) and prefrontal absolute SWA (Fp1 and Fp2 mean; $r = 0.60$, $P < 0.001$) predicted episodic memory change across all participants. Although not significant in all cases, a similar association was detected in each group separately for both global (young adults, $r = 0.49$, $P = 0.045$; older adults, $r = 0.36$, $P = 0.21$) and prefrontal (young adults, $r = 0.56$, $P = 0.019$; older adults, $r = 0.24$, $P = 0.41$) absolute SWA. White asterisks, $P < 0.05$ corrected[22]. % PTOT denotes percentage of total spectral power (0.4–50 Hz).

Sleep associations with memory

Having established disruptions in prefrontal gray matter volume, SWA and episodic memory change in older adults, we next tested the hypothesis that the extent of this SWA disruption significantly and statistically mediated the effects of age and reductions in mPFC gray matter on the success of overnight memory retention. Both global ($r = 0.81$, $P < 0.001$; for young adults only, $r = 0.77$, $P = 0.001$; for older adults only, $r = 0.71$, $P = 0.006$) and local ($r = 0.81$, $P < 0.001$; for young adults only, $r = 0.80$, $P < 0.001$; for older adults only, $r = 0.69$, $P = 0.009$) relative SWA over prefrontal EEG derivations were positively and significantly associated with the success of overnight memory change across all participants combined (**Fig. 5**). In addition, this significant positive relationship was identified in young and older adult groups separately, with peak correlations over frontal electrodes (**Fig. 5b**); significant when corrected for multiple comparisons[22]. These findings establish that SWA, especially over prefrontal cortex, predicted the success of memory consolidation, such that deficits in the degree of overnight memory retention were proportional to the extent of SWA impairment.

Building on these associations, and to formally examine the interrelationships between age, mPFC gray matter, SWA and overnight memory consolidation, we performed mediation analyses. Age and mPFC gray matter were significantly associated with SWA, both globally, averaged across all EEG derivations (age, $r = -0.86$, $P < 0.001$; mPFC gray matter, $r = 0.89$, $P < 0.001$), and locally, over prefrontal EEG derivations (age, $r = -0.83$, $P < 0.001$; mPFC gray matter, $r = 0.86$, $P < 0.001$). Furthermore, age and mPFC gray matter were also significantly associated with deficits in episodic memory retention (age, $r = -0.61$, $P = 0.001$; mPFC gray matter, $r = 0.64$, $P = 0.001$). However, neither age nor mPFC gray matter significantly predicted the extent of overnight episodic memory retention when SWA was included in the statistical model. This was the case for global SWA (SWA $P < 0.001$, age $P = 0.178$; SWA $P < 0.001$, mPFC gray matter $P = 0.186$) and prefrontal SWA (SWA $P < 0.001$, age $P = 0.249$; SWA $P < 0.001$, mPFC gray matter $P = 0.357$), reflected in significant mediation effects for both measures (Sobel test[20], all $P < 0.001$). These findings indicate that, although both age and prefrontal gray matter changes were independently associated with overnight memory retention, such effects were significantly mediated by SWA when this variable was added to the same statistical model. Such data support the hypothesis that offline episodic memory consolidation deteriorates with age, in part, as a result of mPFC gray matter atrophy that reduces NREM SWA (**Fig. 6**). It should be noted that these data alone do not establish causality. Furthermore, they do not prove that the disruption in NREM SWA directly causes impaired memory retention in older adults, as there may be unmeasured factors beyond the collection of co-factors that we controlled for that could account for the statistical associations between each of these variables. However, our findings nevertheless make clear that these three *a priori* targets (mPFC brain atrophy, NREM SWA and delayed memory retention) are not independent of each other and that other potentially unaccounted for factors would themselves be associated with these specific sleep and atrophy changes in predicting memory.

Influence of co-factors on memory

To determine whether age and sleep effects on memory were explained by the influence of circadian rhythms, alertness, neurocognitive status, or hippocampal volume, we performed follow-up analyses. First, recognition performance at short delay did not differ

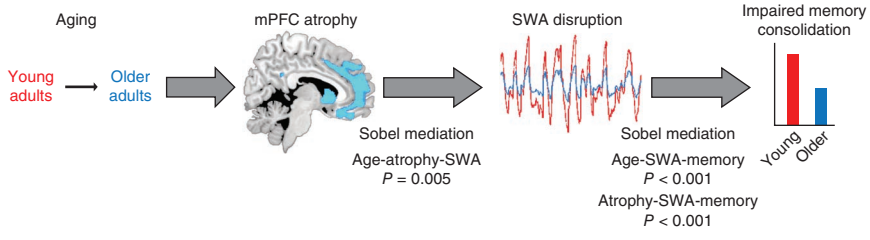

Figure 6 Model schematic of mediation findings. Aging is associated with gray matter atrophy, which mediates the degree of SWA disruption, with SWA in turn mediating the degree of impaired memory retention.

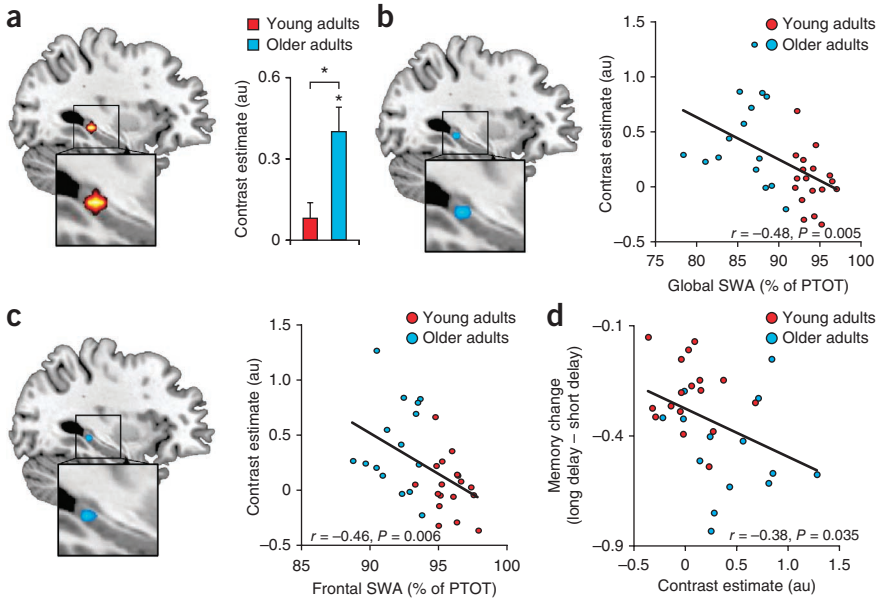

Figure 7 Differences in hippocampal activation and hippocampal-prefrontal task-related functional connectivity are associated with SWA and memory change. (**a**) Age effects (older > young adults) in left hippocampal activation greater during successful associative episodic retrieval than correct rejection of novel words (hits – correct rejections, 6-mm sphere region of interest (ROI): x = –33, y = –32, z = –7)[18]. No age differences in activation were detected outside the hippocampus when employing FWE correction across the whole brain. (**b**,**c**) Regressions between global SWA (**b**), defined as the average relative SWA across all electrode sites, and prefrontal SWA (**c**), defined as the average at prefrontal electrodes exhibiting peak relative SWA in both groups and left hippocampal activation (hits – correct rejections, 6-mm sphere ROI: x = –33, y = –32, z = –7)[18]. (**d**) Regression between overnight memory retention and left hippocampal activation (hits – correct rejections) at the peak of both SWA correlations. Activations are displayed and considered to be significant at the voxel level of $P < 0.05$ FWE corrected for multiple comparisons within the *a priori* hippocampal regions of interest[18]. Hot colors represent the extent of increased activation in older relative to young adults, and cold colors represent the extent of the negative correlation between hippocampal activation and SWA. Although the 6-mm sphere ROIs used to correct for multiple comparisons did extend outside the hippocampus, no effects were detected or presented outside the hippocampus. Error bars indicate s.e.m. *$P < 0.05$ FWE corrected. au, arbitrary units. % PTOT denotes percentage of total spectral power (0.4–50 Hz).

significantly between evening (sleep) and morning (wake) groups in young adults (0.62 ± 0.07 versus 0.62 ± 0.08, $P = 0.942$) or older adults (0.24 ± 0.08 versus 0.40 ± 0.08, $P = 0.175$). Second, a measure of circadian preference[23] did not predict memory change scores in young or older adults (all $r^2 < 0.063$, $P > 0.35$). Third, testing time relative to habitual wake time did not differ between groups ($P = 0.798$) or predict memory change in either group (all $r^2 < 0.11$, $P > 0.245$). Fourth, subjective sleepiness and alertness did not differ by age group (all $P > 0.25$)[24,25] or explain memory change in young or older ($r^2 < 0.16$, $P > 0.16$) adults. Fifth, reaction times during short- and long-delay recognition testing, and the change in reaction times, did not predict memory at short or long delay, or memory change in young or older (all $r^2 < 0.10$, $P > 0.15$) adults. Sixth, episodic memory change revealed no significant association with any of the neurocognitive metrics of assessment described in **Table 1** (all $P > 0.45$). Finally, hippocampal volume also did not predict memory change ($P = 0.454$). These data suggest that age and sleep effects on memory are not parsimoniously explained by the influence of circadian rhythms, alertness, neurocognitive status or hippocampal volume. However, these data also do not suggest that these factors cannot influence memory.

Furthermore, no association was detected between fast spindle density (13.5–15 Hz, calculated as described previously[26]) during stage 2 NREM sleep and episodic memory change when examined across all participants or within each group separately. Stage 2 sigma power (12–15 Hz) also did not predict memory change at any 1 of the 19 electrode derivations (all $r^2 < 0.04$, $P > 0.30$). Moreover, no sleep stage metric predicted episodic memory change in either young or older adults (all $r^2 < 0.12$, $P > 0.16$; **Supplementary Table 3**). When examined across participants, total sleep time (TST, $r = 0.40$, $P = 0.027$), sleep efficiency ($r = 0.40$, $P = 0.028$) and percent stage 1 sleep ($r = -0.36$, $P = 0.045$) significantly predicted memory change. However, when age was included in the model, none of these variables significantly predicted memory (TST, $P = 0.79$; sleep efficiency, $P = 0.98$; percent stage 1 sleep, $P = 0.94$). Furthermore, when SWA and age were included in the model with these other variables (TST, sleep efficiency and percent stage 1 sleep time), neither age nor these other variables remained significant (all $P > 0.2$), whereas SWA (both global and prefrontal) remained a significant predictor of memory change (all $P < 0.001$). Taken together, these data support the conclusion that SWA during SWS specifically predicts overnight memory consolidation in young and older adults.

Hippocampal association with memory and SWA

We tested the hypothesis that this age-related impairment in overnight memory retention, statistically mediated by deficient SWA, was associated with persistent (greater) hippocampal activation[6–8,10,13], and diminished hippocampal-neocortical functional connectivity[13] during memory retrieval. Consistent with this prediction, age effects were detected in the hippocampus, such that older adults exhibited greater (rather than progressively less) post-sleep hippocampal-dependent retrieval activation (**Fig. 7a**). In addition, older adults also revealed significantly impaired retrieval-related hippocampal-prefrontal functional connectivity relative to young adults (**Supplementary Fig. 3**). As predicted by the sleep-dependent hippocampal memory transformation model[7,8,10,11,13], both global SWA (**Fig. 7b**) and prefrontal SWA (**Fig. 7c**) negatively correlated with hippocampal activation during successful retrieval, such that greater SWA was associated with significantly less hippocampal-dependent activity. Notably, hippocampal voxels that correlated negatively with SWA also correlated negatively with the offline measure of episodic memory change (**Fig. 7d**), indicating that activity in this region of the hippocampus was not simply sensitive to retrieval, but was sensitive to the change in overnight memory retention itself. Finally, and also congruent with these above associations and model predictions, retrieval-related hippocampal-prefrontal functional connectivity was also positively associated with SWA and episodic memory change (**Supplementary Fig. 3**).

DISCUSSION

Together, our results support a framework in which deficits in age-related prefrontal gray matter predict the extent of disrupted NREM SWA, the degree to which represents a contributing factor (alongside other established pathways[1,3,21,27]) to impaired long-term memory in older adults. These findings establish that the degree of medial prefrontal gray matter atrophy is associated with the extent of impoverished SWA in older adults. Moreover, the extent of impaired SWA is in turn associated with the degree of impaired episodic memory consolidation and, furthermore, when included in the same statistical model, accounts for the effects of age and medial prefrontal gray matter atrophy on such memory failure. Finally, our findings suggest that the extent of age-related impairment in overnight memory retention is also associated with the persistence (rather than progressive independence) of hippocampal activity at later post-sleep retrieval. This, together with reduced hippocampal-mPFC functional connectivity during memory retrieval, is indicative of impoverished overnight memory transformation in aging.

Although other factors, such as circadian rhythms and alertness, can also influence memory change across time, these factors did not appear to strongly account for the memory changes that we observed. Furthermore, young adults exhibited a sleep-dependent stabilization of memory (stabilization in the context of superior memory retention over time asleep relative to time awake), whereas older adults did not. In addition, the degree of this overnight memory retention benefit was predicted by the amount of intervening NREM SWA. Moreover, that NREM SWA negatively predicted hippocampal activation and positively predicted hippocampal-neocortical functional connectivity during retrieval conforms to model predictions of systems-level hippocampal-neocortical memory consolidation[11].

Our data build on and extend previous evidence that middle-age and older adults experience impaired long-term episodic memory across offline time periods that include sleep[2], associated with reductions in time spent in NREM SWS[2]. However, the underlying neuropathological factors predicting these canonical reductions in NREM SWS and associated SWA have remained unknown. Furthermore, whether these reductions in SWA account for age and brain atrophy effects on episodic memory instead of age and brain atrophy independently impairing both sleep and memory has similarly remained unclear. Finally, the neural correlates of sleep-dependent memory impairment in aging have remained unexplored.

Addressing these issues, the current findings suggest that impaired sleep-dependent memory consolidation potentially contributes to diminished long-term memory retention in old age and is associated with canonical reductions in SWA in the elderly. Depending on the task and the nature of the memory assessment, some reports found no effect of age on long-term memory retention[28], whereas others, particularly those that examined memory retention after delays long enough to include periods of sleep, found pronounced age effects[1,29]. Congruent with these findings, our results indicate that sleep offers a greater overnight memory retention benefit in young adults than in older adults. Notably, this is not to suggest that sleep does not benefit memory retention in older adults, which is supported by the fact that SWA still predicted memory retention in older adults. Instead, this benefit was diminished in older adults, the degree to which was proportional to the extent of impoverished SWA.

In light of our findings, we offer a neuropathological model in which age-related prefrontal atrophy may partially explain the prominent and well-documented reductions in NREM SWA in aging[4,5]. Specifically, age-sensitive changes in gray matter atrophy in the mPFC consequently mediate the canonical age-related decline in SWA. Our data do not, by themselves, prove that prefrontal atrophy causes SWA decline in aging. However, this remains a parsimonious interpretation when considering previous evidence in young adults that the cortical origin of NREM slow waves is predominantly localized to mPFC regions[16] similar to those that we identified as having maximal gray matter atrophy in older adults (and mediation of age-related changes in SWA), along with evidence linking NREM SWA changes with prefrontal brain structure development across ontogeny[30].

In addition, our results and proposed framework offer a potential mechanistic basis supporting an association between sleep disruption and age-related cognitive decline[2]. However, our findings suggest that it is not age, *per se*, that independently governs these sleep and memory impediments[2]. Instead, the influence of age and concomitant prefrontal atrophy on impoverished overnight memory retention is statistically mediated by the degree of deficient SWA. Thus, age alone does not independently reduce prefrontal gray matter, SWA and memory retention. Rather, our data suggest these three canonical signatures of the aging brain are significantly inter-related, with the deficiency in SWA mediating the degree of compromised offline episodic memory retention in the aging, atrophied brain. Given these results, it appears tenable that the reductions in prefrontal SWA in older adults, associated with medial prefrontal lobe atrophy, compromise the offline transformation and consolidation of episodic memories. Consistent with this functional relationship, the degree of prefrontal SWA impairment and overnight memory change was associated with persistent next-day hippocampal retrieval activation, as well as reduced hippocampal-mPFC functional connectivity. Such findings conform to predictions made by the model of sleep-dependent hippocampal memory transformation, in which SWA is reduced[6,7,10] in a similar manner to experimental manipulations of SWA in young adults[9].

Building on these findings, it will be important to determine whether such sleep-related changes represent an early predisposing factor to, or accelerant of, cognitive decline in the elderly[27], and further, what role, if any, similar NREM SWA disruption has in degenerative diseases with comorbid sleep abnormalities[14,15]. These findings further relate to the emerging proposal that factors such as sleep are important features determining healthy aging, beyond age *per se*[15]. At a translational level, and in light of this literature, our data endorse the possibility that improvements of SWA in older adults, through physiological, behavioral or pharmacological means[9,31,32], may represent a treatment target for minimizing the cognitive decline associated with deficient long-term memory retention in later life.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

B.A.M. designed the study, conducted the experiments, analyzed the data and wrote the manuscript. V.R. aided in data analysis and manuscript preparation. B.L. aided in study screening procedures and manuscript preparation. J.M.S. provided data analytic tools, aided in data analysis and manuscript preparation. J.R.L. aided in conducting the experiment and manuscript preparation. S.A.-I.

aided in study design and manuscript preparation. W.J. provided the elderly subject pool and data analytic tools, and aided in study design and manuscript preparation. M.P.W. designed the study, aided data analysis and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Thirty-six healthy adult participants completed the study (n = 18 young adults, 10 females, 20.4 ± 2.1 years; n = 18 older adults, 14 females, 72.4 ± 6.1 years). The study was approved by the institutional review board at the University of California, Berkeley (Committee for Protection of Human Subjects), with all participants providing written informed consent. Exclusion criteria included presence of neurologic, psychiatric or sleep disorders, current use of antidepressant or hypnotic medications, or being left handed. Two older participants were excluded because of the presence of sleep apnea, whereas another was excluded because of technical difficulties during data collection. Young and older adults were free of depressive symptoms[35,36] and performed similarly on the mini mental state exam[37] (P = 0.43). Furthermore, and in addition to neuroradiological assessments and medical interviews (obtained within 0–6 months of study entry)[21], participants performed within two s.d. of their age-matched control group on tests of episodic memory[38,39] and frontal function[40,41] (**Table 1**). Prior to study entry, older adults underwent sleep disorders screening with a polysomnography (PSG) recording night (described below) reviewed by a board-certified sleep medicine specialist (author B.L.). Participants were excluded if they displayed evidence of a parasomnia or an Apnea/Hypopnea Index ≥ 15 (ref. 42).

All participants abstained from caffeine, alcohol and daytime naps for the 48 h before and during the entire course of the study. Participants kept normal, habitual sleep-wake rhythms and averaged 7–9 h of reported time in bed per night before study participation, verified by sleep logs (**Table 1**).

General experimental design. Participants entered the lab in the evening and trained to criterion on a sleep-dependent episodic memory task (described below), followed by a short-delay (10 min) recognition test. Participants were then given an 8-h sleep opportunity, measured with PSG, starting at their habitual bed time (**Table 1**) to minimize the influence of age-related circadian differences[43]. Approximately 2 h post-awakening, participants performed an event-related fMRI scanning session while performing a long-delay (10 h) recognition test.

Episodic memory task. The word-pairs task had an intentional encoding phase immediately followed by a criterion phase (**Fig. 1**), which was then followed by a short delay recognition test (10 min, 30 studied trials and 15 foil trials) and a long delay recognition test (10 h, occurring 2 h post-awakening in the MRI scanner, 90 studied trials and 45 foil trials) testing.

Associative recognition memory was calculated by subtracting both the false alarm rate (proportion of foil words endorsed as previously studied) and the lure rate (proportion of previously studied words recognized but erroneously paired with the lure and hence incorrect nonsense word associate) from the hit rate (proportion of previously studied probe words accurately paired with the correct nonsense word associate)[33]. Episodic memory change was subsequently calculated as the difference in short- and long-delay recognition memory performance (long delay – short delay)[44]. Two participants, one young and one older adult, were excluded from analysis as outliers (memory performance more than 1.5 s.d. below the mean).

MRI scanning. Scanning was performed on a Siemens Trio 3 Tesla scanner equipped with a 32-channel head coil. Functional scans were acquired using a susceptibility-weighted, single-shot echo-planar imaging method to image the regional distribution of the blood oxygenation level–dependent signal (repetition time/echo time, 2000/23 ms; flip angle, 90°; field of view, 224 mm; matrix, 64 × 64; 37 3-mm slices). Three functional runs were acquired (159 volumes, 5.3 min). Following functional scanning, two high-resolution T1-weighted anatomical images were acquired using a three-dimensional MPRAGE protocol with the following parameters: repetition time, 1,900 ms; echo time, 2.52 ms; flip angle, 9°; field of view, 256 mm; matrix, 256 × 256; slice thickness, 1.0 mm; 176 slices.

fMRI analysis. fMRI data were analyzed using SPM8 (Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/software/) beginning with standardized preprocessing (realignment, slice timing correction and co-registration), and with normalization accomplished using a mixed young-old template generated from 400 individual T1-weighted anatomical images[45], consistent with recommendations for use in studies comparing young and older adults[45,46].

Following preprocessing, retrieval trials were sorted into hits (correct word-nonsense word recognition), lures (selection of the incorrect, previously studied, nonsense word), misses (incorrect selection of never studied nonsense word or endorsement of word as new), correct rejections (novel words correctly endorsed as new), false alarms (novel words incorrectly endorsed as studied) and omissions (trials with no subject response)[47,48], with each trial modeled using a canonical hemodynamic response function. To generate a validated contrast for retrieval-related activity, hit events were contrasted with correct rejection events (hits – correct rejections)[47,48]. Activation maps at the subject level were then taken to a second-level random effects analysis to examine group effects. Activations were assessed at the voxel level of $P < 0.05$ FWE[49], corrected for multiple comparisons in an *a priori* hippocampal ROI (6-mm sphere, x = −33, y = −32, z = −7)[18].

To determine whether hippocampal involvement during delayed recognition was inversely associated with the degree of preceding SWA[8,13], regression models relating global and prefrontal SWA to hippocampal activation (hits – correct rejections) were examined.

Finally, to examine the role of SWA on hippocampal-neocortical connectivity expressly during the act of memory retrieval, we employed psychophysiologic interaction analysis[50,51] using SPM8 (Wellcome Department of Imaging Neuroscience), with an *a priori* focus on coupling with ventromedial prefrontal cortex (vmPFC)[6–8,10,11,13,52]. Specifically, within each individual, psychophysiologic interaction effects were calculated using a hippocampal seed region defined by the peak hippocampal activation SWA correlations (6mm-sphere ROI: x = −34, y = −35, z = −4), detected in the above described hippocampal ROI[18]. General linear models were constructed for each subject and included (i) a regressor for the deconvolved blood oxygen level–dependent signal from the hippocampal seed region, (ii) a regressor accounting for the cognitive context of successful memory retrieval (hits relative to correct rejections), (iii) the psychophysiologic interaction term of the first and second regressors, and (iv) movement regressors to account for noise relating to head motion. The contrast images for the psychophysiologic interaction term were then forwarded to the second level and regressed against global SWA. In order to ensure effects were also related to group differences, effects were inclusively masked ($P < 0.05$). Connectivity values were assessed at the voxel level of $P < 0.05$ FWE corrected for multiple comparisons, targeting the vmPFC *a priori* (8-mm sphere ROI: x = −2, y = 32, z = −10)[8]. The cluster average of significant voxels was extracted using Marsbar[53] and regressed against memory change to determine whether the interaction between the hippocampus and vmPFC, in the context of memory retrieval, was associated with the metric of successful memory retention.

Structural MRI analysis. To measure gray matter volume, we performed optimized voxel-based morphometry using SPM8 (Wellcome Department of Imaging Neuroscience) with the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) and the Diffeomorphic Anatomical Registration through Exponentiated Lie algebra (DARTEL) toolbox to improve registration of older brains to the normalized MNI template[54,55]. To enhance signal to noise ratio, two T1-weighted MPRAGE images were first co-registered and averaged. Averaged images were then segmented applying the Markov random field approach[56], and then registered, normalized and modulated using DARTEL. Grey matter and white matter segmentations were inputted into DARTEL and used to create a study specific template, which was then used to normalize individual brains into MNI space. Modulated gray matter maps were then smoothed using an 8-mm Gaussian kernel. Gray matter maps were then forwarded into a general linear model. Measures of total intracranial volume for each participant were estimated from the sum of gray matter, white matter and cerebrospinal fluid (CSF) segmentation, and then included as a nuisance regressor. Due to data collection error, anatomical scans from two younger participants and one older participant were excluded from analysis, leaving 14 older and 16 younger adults for all related analyses. An independent samples t test was used to determine the effects of age on gray matter volume, assessed and displayed at the voxel level of $P < 0.05$ FWE corrected for multiple comparisons across the whole brain. Mean gray matter density in medial prefrontal cortex, defined using Brodmann's definition[57], exhibiting significant age effects was extracted using the Marsbar toolbox[53] and used in the mediation analyses.

Measures of subjective and objective alertness. Subjective measures of sleepiness and alertness were measured using a validated visual analog scale[24] collected every 2 h throughout the study while subjects were awake. Subjective ratings were compared from before to after sleep to assess the change in subjective sleepiness and alertness. The after sleep assessment occurred approximately 2 h post awakening and immediately before scanning, to best estimate subjective sleepiness and alertness immediately preceding delayed recognition in the scanner.

Overall reaction time during short-delay and long-delay recognition, and the change in reaction time from short-delay to long-delay (long-delay–short-delay) testing were used to determine whether objective alertness predicted memory change in young and older adults.

Sleep monitoring and EEG analysis. PSG sleep monitoring on the experimental night was recorded using a Grass Technologies Comet XL system (Astro-Med), including 19-channel EEG placed using the standardized 10–20 system, electrooculography recorded at the right and left outer canthi (right superior, left inferior), and electromyography. Reference electrodes were recorded at both the left and right mastoid (A1, A2). Data were digitized at 400 Hz, and stored unfiltered (recovered frequency range of 0.1–100 Hz), except for a 60-Hz notch filter. Sleep was scored using standard criteria[58].

Sleep monitoring on the screening night was recorded using a Grass Technologies AURA PSG Ambulatory system (Astro-Med), similar to as described above save for the following exceptions: EEG was recorded at nine derivations (F3, FZ, F4, C3, CZ, C4, P3, P4, OZ) and data were digitized at 200 Hz. During full PSG screening nights, nasal/oral airflow, abdominal and chest belts, and pulse oximetry were also monitored to screen for the presence of sleep apnea.

EEG data from the experimental night were imported into EEGLAB (http://sccn.ucsd.edu/eeglab/) and epoched into 5-s bins. Epochs containing artifacts were rejected, and the remaining epochs were filtered between 0.4 and 50 Hz. A fast Fourier transform was then applied to the filtered EEG signal at 5-s intervals with 50% overlap and employing Hanning windowing. Analyses in the current report focused, *a priori*, on SWA, defined as absolute and relative spectral power between 0.8–4.6 Hz during SWS[19]. Spectral power during SWS was chosen because staging requires absolute amplitude above a standard threshold[58] requiring, by definition, slow-wave detection. Relative spectral power was chosen because it accounts for individual differences in overall absolute spectral power potentially resulting from differences in brain to scalp distance, skull thickness, impedance, head size and the potential effects of different sleep recording systems[59], standardizing spectral power across subjects.

Statistical analysis. A two-way, repeated-measures ANOVA was used to compare episodic memory retention between young and older adults, with group (young versus older) as a between subjects factor and testing session (immediate versus delayed) as a within subjects factor. To assess whether this effect was specific to sleep, a three-way, repeated-measures ANOVA was used with group (young versus older) and condition (sleep versus wake) as between subjects factors and testing session (short delay, 10 min versus long delay, 10 h) as a within subjects factor. Group differences in sleep variables were assessed using independent, two-sample *t* tests. Associations between prefrontal atrophy, sleep measures and episodic memory change were assessed using Pearson's correlations. SWA and episodic memory change correlations were examined and considered to be significant if they were significant at $P < 0.05$ false discovery rate (FDR) corrected across the electrode array[22].

To test the hypothesis that the effects of age on prefrontal atrophy mediated the effects of age on relative SWA and the effects of age on relative SWA mediated the effects of age and prefrontal atrophy on episodic memory change, mediation analyses were performed using established methods[20,21,60]. In short, these analyses determine whether the influence of independent variable x on dependent variable y is accounted for by mediator M, that is, is the value of the direct path coefficient between x and y reduced by the inclusion of M? Reduction to 0 is interpreted as mediation, partial reduction is interpreted as partial mediation, and nonsignificant reduction is interpreted as no evidence for mediation. Effects were formally tested using the Sobel test of mediation[20,60]. Analyses were completed using SPSS version 18.0 (SPSS).

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