**SI Fig. 1. Examples of Delay cells.** Individual examples of task-related firing of DELAY neurons in the dorsolateral prefrontal cortex (DLPFC) of young (left, 7-y old), middle-aged (middle, 12-y old), and aged (right, 21-y old) monkeys. Raster plots show the spike trains during individual trials and spike density functions (σ=50 ms), with the average activity separated for each direction. Neurons were selected by the computer on the basis of their similarity to the population-averaged spike density functions shown in Figure 1F. Colors indicate the activity during the trials in which the cue was presented in the neuron's preferred (blue) and anti-preferred (red) directions; the darker gray background refers to the cue period; the lighter gray background to the delay period.
**SI Fig. 2. Firing rate histograms.** Histograms of average firing rates during the delay period in a spatial working memory task in young (black bars), middle-aged (dark gray bars), and aged (light gray bars) monkeys for the neuron’s preferred spatial direction (left) and for its anti-preferred (null) directions (right). Neurons from young monkeys had a broad range of firing rates, while those in aged monkeys were restricted to lower firing rates (ANOVA on firing rates with the age group as the factor, $p<10^{-10}$, for both preferred and anti-preferred directions).
SI Fig. 3. Relationship between accuracy and firing rate. A, Firing rate data from Correct vs. Error trials during test sessions when the oldest monkey made errors on the task. The left graph shows the average firing rate ± standard error of the mean (SEM) during the 2.5s Delay period for 12 neurons recorded during test sessions when the aged monkey made more than 3 errors on trials where the cue occurred in the neurons’ preferred direction. The average firing rate during the Delay period was significantly higher on Correct trials (n=116 trials) than for Error trials (n=81 trials) (p<0.0001). The right graph shows the average firing rate of one of these 12 neurons on correct vs. error trials. B and C, An individual example of a neuron recorded from the oldest monkey during a test session when delays were raised from 2.5s to 5.0s. When the delay was raised to 5.0s, the aged monkey performed 13 trials with an accuracy of only 61.5% correct. She then quit testing, apparently frustrated by the difficulty of the task under these conditions. However, the firing rates on these 13 trials were of interest. As shown in SI Fig. 3b, the 8 trials which she performed correctly were associated with sustained firing throughout the 5s epoch, while the error trials were associated with a significant decline in firing during the later portion (2.5-5.0s) of the delay epoch (2-way repeated measures ANOVA with within-subjects factors of Epoch and Accuracy, significant Epoch x Accuracy interaction, p<0.001). Results represent mean firing rate during the Delay period for the neuron’s preferred direction ± SEM.
SI Figure 3c shows the reduction in task-related firing on correct trials when the delay was raised from 2.5s (left graph) to 5.0s (right graph) in the oldest monkey. D, In contrast to the oldest monkey, the middle-aged monkey performed accurately when delays were raised to 5s, and firing rates during the Delay epoch remained high in this animal. Thus, firing rates during the Delay epoch for trials with 2.5s delays (left graph) and trials with 5.0s delays (right graph) were similarly high.
SI Fig. 4. Computational modeling. Model simulation that explores the impact of age-related changes of effective synaptic connectivity in the PFC on task-related firing under conditions when the delay period is 5s. The model is a biologically based recurrent (attractor) circuit model of spiking neurons. The normalized synaptic strength refers to the ratio of the connectivity parameter $w_+$ relative to the control value ($w_+^*=1.9$). Blue: preferred direction, red: anti-preferred direction. C: cue period, D: 5 second delay period, R: response period. Dashed vertical line: half way (2.5sec) into the delay period. A. Sample traces with decreasing synaptic strength, leading to reduced firing rate of persistent activity (left: 55 Hz, middle: 38 Hz, right (before the spontaneous switching off): 20 Hz). When $w_+$ falls below a threshold level, delay activity is no longer sustained for a long time (right panel), similar to what is observed in the monkey experiment. B. $d'$ prime ($d'$) as a function of normalized synaptic strength, akin to the observation of $d'$ as a function of increased age in the physiological experiment (Fig. 2D).

Model simulations were carried out using the model of Wang (2002)\textsuperscript{31}, which is a spiking local circuit capable of working memory and decision-making computations. Briefly, the model circuit consists of selective neural pools of spiking neurons, excitatory cells within each neural pool interact with each other strongly through horizontal connections that depend on the NMDA receptors. Different neural pools compete with each other through shared GABA mediated feedback synaptic inhibition. Simulations used the same parameter values as in Wang (2002), with the following modifications. First, the key assumption is that experimentally observed changes of mnemonic persistent activity from young, middle to old age, results from multiple cellular and synaptic alternations that end up diminishing the effective connectivity in the PFC circuitry. Therefore, the parameter that controls the strength of within-pool recurrent excitation $w_+$ is varied to mimic the presumed changes in the PFC. Second, to generate a reasonable amount of heterogeneity across cells, the leak voltage varies from cell to cell according to a Gaussian distribution with a standard deviation of 1 mV\textsuperscript{32}. Third, the Poisson rate for the background synaptic noise is 2kHz. Fourth, during the cue and response periods, 500 ms long pulse of increased and decreased, respectively, Poisson inputs are applied. There is no external input during the delay period.
SI Fig. 5. cAMP signaling effects on task-related firing. A, The effects of increasing cAMP signaling in PFC via iontophoresis of the phosphodiesterase 4 (PDE4) inhibitor, etazolate (25 nA), on the task-related firing patterns of aged DLPFC DELAY neurons. In contrast to agents that inhibit cAMP signaling and enhance delay-related firing, iontophoresis of the PDE4 inhibitor, etazolate, suppressed delay-related firing for the neuron’s preferred direction (n=4 neurons, all suppressed by etazolate p<0.001). This figure shows an individual example from these neurons under control conditions (left), and following iontophoresis of etazolate (25 nA). The blue traces represent the neuron’s average firing on trials when the cue appeared in its preferred spatial direction; the red traces the average firing for its anti-preferred direction. Note the difference in scale from Figure 1f. B-E, Bar graphs showing the average firing rate during the delay period for the individual neurons shown in Figure 4e-h in the main text under baseline conditions and in response to drugs that inhibit cAMP signaling (B, C) or block HCN channels (D) or KCNQ channels (E). Blue bars: preferred direction; Red bars: anti-preferred direction; open bars: control conditions; filled bars: iontophoresis of compound as indicated. Error bars represent the standard error of the mean (SEM). * p<0.05 ** p<0.01 and *** p<0.001 significant difference between drug vs. control for the neuron’s preferred direction.
SI Fig. 6. Behavioral performance. Short delays (2.5s) were used to ensure that the aged monkeys could perform as well as the young monkeys in the study. Although the aged monkeys often rested more between trials, their performance during the task was similar to the young monkeys. 

A, On trials with 2.5s delays, there were no significant differences in accuracy between young and aged monkeys (p=0.48) during training or during the study. 

B, Aged monkeys performed fewer trials per session than young monkeys (left graph), and were unable to work as long as young monkeys (right graph). 

C, on trials with 2.5s delays, there were no significant differences in reaction time (p=0.25, left graph) during training or during the study, although aged monkeys had a broad range of reaction times (right graph). 

D, there were no significant differences in fixation break rate during the delay period (p=0.4). Results represent mean ± SEM.
SI Fig. 7. Isolation and stability of single units. Raw spike traces of the neurons recorded by carbon fiber electrodes used for iontophoresis. Spikes for each neuron were from correct trials, which were chosen randomly, for the preferred direction. (A) Example raw spike traces of a single DLPFC neuron. The carbon fiber electrode recorded spikes with an excellent signal-to-noise ratio. The average spike amplitude was 0.32 mV for this example neuron, whereas the background noise was typically less than 0.04 mV. (B) Raw spike traces (left) and the average waveform (right) of the neuron shown in Figure 4G. Left: top and bottom panels represent the spike traces before (control condition) and during ZD 7288 iontophoresis, respectively. The spike shape and amplitude were not altered during ZD 7288 application. The spike shape and amplitude were not altered when any other drugs in the present study were applied iontophoretically. Error bars represent SEM. Note that previous research has shown that iontophoresis of saline has no effect on neuronal response. 

\[33\]
Supplementary Data

Effects of potential sampling bias on age-related changes in delay activity-
Several lines of evidence suggest that the age-related decline in the DLPFC activity during the delay period is unlikely to result from a sampling bias that led to more neurons with weaker activity in old animals than in young animals.

First, the data from young (7 and 9 years old) and middle-aged (12 and 13 years old) monkeys were collected as part of our standard research at a time before we started an aging study. Thus, there was no room for unconscious bias while we collected this subset of the data. We thus reanalyzed this subset of the data to see if there were significant effects of age that were still apparent under these “blind” conditions. Our re-analysis shows that there is still a significant effect of age even if we only compare young vs. middle-aged neurons. Similar to the results from the regression analysis applied to the entire data set, the effect of aging was highly significant when only the neurons in the young and middle-aged monkeys were included (t-test on the age regressor, p<0.001).

Second, the effect of age on the average firing rate during the delay period for preferred directions in a regression analysis remained highly significant even when neurons with low firing rates (<5, <10, or <15 spikes/sec) were removed from the analysis (see Table S1 below).

Third, the fact that the age-related decline in DLPFC activity was specific to the delay cells but not found in the cue-cells also makes it unlikely that the effect of age was caused by a sampling bias.

Table S1. Statistical significance for aging effect on a subset of neurons with relatively high firing rates during the delay period for preferred direction.

<table>
<thead>
<tr>
<th>Minimum firing rate (spikes/s)</th>
<th>p-value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.7853e-013</td>
</tr>
<tr>
<td>10</td>
<td>1.6278e-008</td>
</tr>
<tr>
<td>15</td>
<td>0.0001046</td>
</tr>
</tbody>
</table>

Relationship of age-related changes in physiology to working memory impairment-
The current study was performed with brief, 2.5s delays so that aged and young animals could all perform well. However, there were a few pilot sessions when the aged monkey had errors on the task, and these gave us the opportunity to examine possible differences in firing rates on Correct vs. Error trials. In such cases, the average firing rate during the Delay period was significantly reduced during error trials (Fig. S3A). We also tried to examine the effects of raising the delay from 2.5s to 5.0s within a single test session in a middle-aged monkey and in the oldest monkey. We were only able to have one recording session with 5.0s delays with the oldest monkey, as she quit testing due to poor performance (61.5% correct) when the delay was raised. However, the results from this single session were illuminating, as there was a significant reduction in the firing rate during the later portion of the delay period on error trials compared to correct trials (Fig. S3B). Even on correct trials, the firing rate was substantially lower on 5.0s delay trials compared to 2.5s delay trials (Fig. S3C). In contrast, the middle-aged monkey was able to
maintain normal, very high rates of performance and firing rates when delays were raised from 2.5s to 5.0s (Fig. 3SD). These results are consistent with age-related declines in DELAY cell firing contributing to impairments in working memory performance at longer delays.

As we were only able to observe the changes in neuronal firing with a longer delay in a single neuron in the oldest monkey, we used computational modeling to examine how increasing delay length alters delay-related firing when PFC network strength is systemically weakened. This work used an established model of PFC spatial working memory circuits in dorsolateral PFC, based on the same ODR paradigm\textsuperscript{31,32}. Similar to the results shown in Figure S3, the computational model showed that firing rate decreased when the network strength was weakened, and that these effects were particularly apparent during the 2.5-5s epoch (Fig. S4).

**Supplementary Discussion**

**Working memory abilities decline with advancing age**

It has been known for more than 30 years that aging monkeys show marked impairments on spatial working memory tasks. The pioneering studies of Bartus and colleagues first revealed age-related deficits on the spatial delayed response task\textsuperscript{34}. They found that aged monkeys showed deficits in working memory performance that were unrelated to levels of motivation or changes in sensory processing. The performance of aged monkeys resembled that of young monkeys with lesions to the dorsolateral PFC; both groups were especially susceptible to interference from distractors introduced after the cue during the delay period\textsuperscript{35,36}. These landmark findings were later replicated and developed by other labs. For example, Rapp and Amaral showed that aged monkeys were impaired on performance of a two-well, spatial delayed response task even at delays of 5 sec, while they still retained normal levels of object recognition memory\textsuperscript{37}. The labs at Boston University and Yerkes Primate Center later found that aged monkeys were impaired on two related tasks, spatial span\textsuperscript{38} and a monkey version of the Wisconsin Card Sorting Task\textsuperscript{39}, that require spatial working memory and attentional set-shifting, respectively. Attentional set-shifting tasks also rely on dorsolateral PFC\textsuperscript{40}, and recent evaluations across the lifespan in monkeys have discerned deficits in set-shifting ability starting in middle age\textsuperscript{41}. Similar results have been seen in humans, in that there are prominent working memory deficits in aged individuals that are related to reduced, top-down suppression of interference\textsuperscript{42}, and deficits on the Wisconsin Card Sorting Task that emerge in middle age\textsuperscript{43}. Thus, the aged monkey is an excellent animal model of human cognitive aging.

**The role of cAMP signaling**

Increasing cAMP signaling by inhibiting phosphodiesterase catabolism reduced task-related firing in aged DELAY cells (Fig. S5). In contrast, decreasing cAMP signaling with guanfacine or Rp-cAMPS improves working memory performance in aged animals. The current study found that iontophoresis of compounds that inhibit cAMP signaling (guanfacine, Rp-cAMPS) or that block HCN or KCNQ channels (ZD7288, XE991) increase the delay-related firing of aged PFC neurons. The iontophoresis technique delivers a minute amount of drug- enough to alter neuronal firing for a small number of cells- but not enough to alter behavioral performance. Thus, we were unable to observe whether these treatments improved behavioral performance in these individual animals. However, previous studies have shown that guanfacine and Rp-cAMPS are particularly
effective in improving working memory performance in aged animals. Three separate labs have shown that the systemic administration of the $\alpha_2A$ adrenoceptor agonist, guanfacine, improves working memory performance in aged monkeys$^{44-46}$. Guanfacine was particularly helpful in rescuing delayed response performance under distracting conditions when aged monkeys perform most poorly$^{46, 47}$. Guanfacine can also improve working memory performance when infused directly into the PFC of aged rats$^{48}$. These enhancing effects were reversed by a cAMP agonist, Sp-cAMPS$^{48}$, and mimicked by the cAMP antagonist, Rp-cAMPS$^{49}$, consistent with guanfacine having beneficial effects via inhibition of cAMP signaling pathways. Guanfacine can also improve the performance of young adult monkeys when administered systemically$^{50}$, or infused directly into the dorsolateral PFC$^{51}$. However, its effects are more subtle in young monkeys who already have strong working memory performance, likely due to ceiling effects. Similar effects are seen at the cellular level, whereby guanfacine and Rp-cAMPS have only subtle enhancing effects on Delay cell firing in young monkeys when task-related firing is already strong under control conditions$^{52}$. However, Delay cells with weak task-related firing in young monkeys do show robust increases in task-related firing following iontophoresis of guanfacine, Rp-cAMPS, or ZD7288$^{52}$, similar to that seen in aged neurons.

We chose not to infuse guanfacine into the PFC of the aged monkeys in the current study, as the large infusions needed to alter behavioral performance can cause lesions that impede further research. The physiological data from the current study suggest that guanfacine restores working memory performance in aged animals by increasing the delay-related firing of PFC neurons. Based on the positive behavioral data in aged animals, guanfacine is currently being tested as a potential cognitive enhancer in elderly humans.

**Supplementary References**


