

# Neuronal basis of age-related working memory decline

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Many of the cognitive deficits of normal ageing (forgetfulness, distractibility, inflexibility and impaired executive functions) involve prefrontal cortex (PFC) dysfunction<sup>1–4</sup>. The PFC guides behaviour and thought using working memory<sup>5</sup>, which are essential functions in the information age. Many PFC neurons hold information in working memory through excitatory networks that can maintain persistent neuronal firing in the absence of external stimulation<sup>6</sup>. This fragile process is highly dependent on the neurochemical environment<sup>7</sup>. For example, elevated cyclic-AMP signalling reduces persistent firing by opening HCN and KCNQ potassium channels<sup>8,9</sup>. It is not known if molecular changes associated with normal ageing alter the physiological properties of PFC neurons during working memory, as there have been no *in vivo* recordings, to our knowledge, from PFC neurons of aged monkeys. Here we characterize the first recordings of this kind, revealing a marked loss of PFC persistent firing with advancing age that can be rescued by restoring an optimal neurochemical environment. Recordings showed an age-related decline in the firing rate of DELAY neurons, whereas the firing of CUE neurons remained unchanged with age. The memory-related firing of aged DELAY neurons was partially restored to more youthful levels by inhibiting cAMP signalling, or by blocking HCN or KCNQ channels. These findings reveal the cellular basis of age-related cognitive decline in dorsolateral PFC, and demonstrate that physiological integrity can be rescued by addressing the molecular needs of PFC circuits.

Our society is rapidly ageing, with the number of seniors in the United States expected to double by 2050 (United States census, <http://www.census.gov/population/www/pop-profile/elderpop.html>). At the same time, the information age requires increasing organizational skills to deal with even basic needs such as medical care and paying bills. However, executive and working memory functions decline early in the normal ageing process<sup>10–13</sup>, beginning in middle age<sup>14,15</sup>. Thus, cognitive changes with advancing age may be costly, forcing retirement from demanding careers and jeopardizing the ability to live independently in an increasingly complex society. Ageing monkeys provide an ideal model to reveal the neurobiology of normal ageing, as they have a highly developed PFC, but are not subject to age-related dementias<sup>16</sup>. Thus, one can be certain that cognitive changes are the result of normal ageing and not incipient Alzheimer's disease. Like humans, monkeys begin to develop deficits in executive function as early as middle age<sup>17</sup>. Both aged monkeys<sup>18,19</sup> and humans<sup>3,20</sup> are impaired on working memory tasks that require constant updating of the contents of memory (Supplementary Information), bringing to mind information from longer-term stores (for example, where did I leave my car keys this time?), or keeping in mind a recent event (for example, remembering a new phone number).

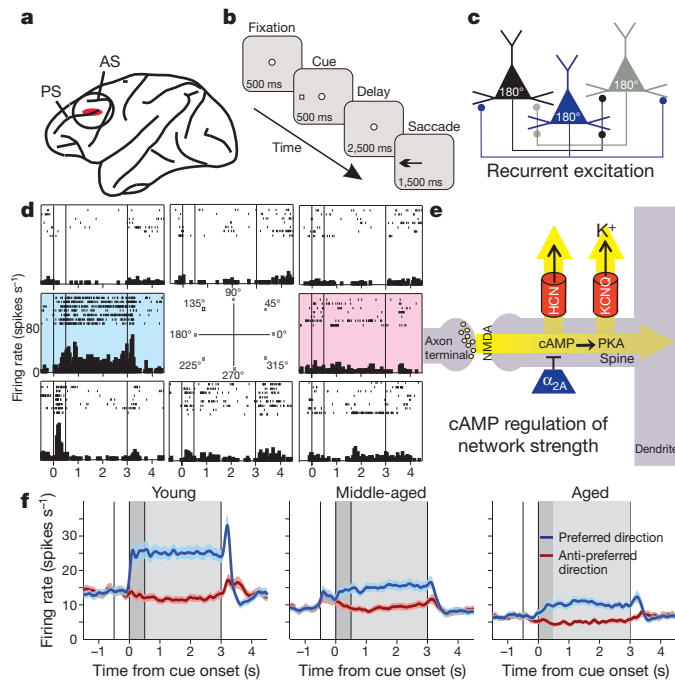
In primates, spatial working memory depends on the highly evolved dorsolateral PFC<sup>6</sup> (Fig. 1a). Spatial working memory performance (Fig. 1b) relies on networks of pyramidal neurons that interconnect at dendritic spines (Fig. 1c), and excite each other to keep information 'in mind', that is, generating persistent spiking activity over a delay

period in a working memory task<sup>6</sup> (Fig. 1d). This ability to maintain information that is no longer in the environment is a fundamental process needed for abstract thought and flexible responding<sup>6</sup>. Intracellular signalling pathways modulate the physiological strength of these recurrent, excitatory PFC network connections<sup>9</sup>. Recent data show that increased cAMP signalling weakens network connectivity by opening potassium channels, whereas inhibiting cAMP signalling and/or closing these channels strengthens connectivity and cognitive ability<sup>9</sup> (Fig. 1e). Specifically, cAMP signalling seems to weaken persistent firing and impair working memory by increasing the open state of HCN (hyperpolarization-activated cyclic nucleotide-gated) channels that are localized on spines where networks interconnect<sup>8</sup>. Recent data indicate that HCN channels may also gate synaptic inputs through interactions with KCNQ channels, whose open state is increased by cAMP-activating protein kinase A (PKA)<sup>21</sup>. Studies indicate that cAMP signalling is disinhibited in the aged PFC<sup>22</sup>. Noradrenergic  $\alpha_{2A}$  receptor inhibition of cAMP may be reduced from loss of  $\alpha_{2A}$  receptors in the aged PFC<sup>23</sup>, and decreased excitation of noradrenergic neurons<sup>24</sup>.

There have been few electrophysiological recordings from aged PFC neurons owing to the demanding nature of this procedure. Recordings from rat orbital PFC found reduced flexibility in aged neurons<sup>25</sup>. However, there have been no *in vivo* recordings from the aged dorsolateral PFC, even though behavioural data indicate that this region is particularly vulnerable to normal ageing. *In vitro* recordings from dorsolateral PFC neurons found relatively subtle changes in excitability with advancing age<sup>26</sup>, but their consequences for executive function must be observed in a cognitively engaged circuit. Here we perform the first physiological characterization of PFC neuronal response during a working memory task in young adult, middle-aged and aged monkeys.

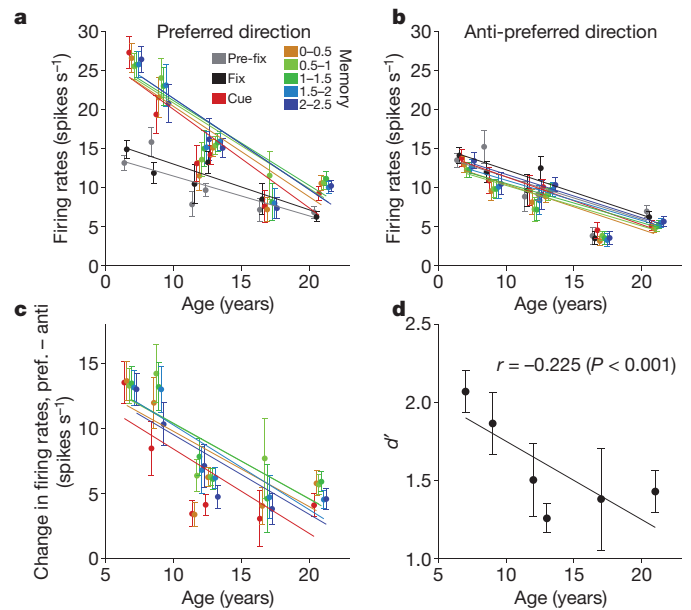
Monkeys (*Macaca mulatta*,  $n = 6$ ) were trained to perform a spatial working memory task in which they have to remember a spatial location over a brief delay period; the spatial location changes randomly on each trial (Fig. 1a). Two animals were young adults (7- and 9-year-old males), two were middle-aged (12- and 13-year-old males), and two were aged (17-year-old male, 21-year-old female). Short delays (2.5 s) were used in all age groups to ensure similar performances (>85% per cent correct) across age groups. Neurons ( $n = 301$ ) were recorded from area 46, the dorsolateral PFC subregion most needed for visuospatial working memory (Fig. 1a). Neurons were characterized based on task-related firing as responsive during (1) the visuospatial cue period, (2) the delay period when the spatial position was being remembered, and/or (3) the motor response period. Some neurons fired only during cue presentation (CUE cells,  $n = 28$ ), whereas most neurons fired during the delay period as well as to the cue and/or response periods (DELAY cells,  $n = 273$ ). Persistent firing during the delay period is of particular interest, as it is required for working memory<sup>6</sup>. Many PFC DELAY neurons increased their activity during the memory of one spatial location (its preferred direction), but not other locations (the 'anti-preferred' direction, 180° away from the preferred direction; Fig. 1d).

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**Figure 1 | Age-related changes in the PFC networks that subserve working memory.** **a**, The region of the dorsolateral PFC most needed for spatial working memory, and the site of recordings. AS, arcuate sulcus; PS, principal sulcus. **b**, The oculomotor delayed response (ODR) spatial working memory task. The monkey fixates on the central stimulus and maintains fixation for the duration of the trial. A cue is briefly presented in one of eight locations, followed by a delay period (2.5 s) in which no spatial information is present. At the end of the delay period, the fixation spot disappears, and the monkey makes an eye movement (saccade) to the remembered location for a juice reward. The cue position randomly changes on subsequent trials. **c**, A diagram of the recurrent excitatory networks subserving working memory. Pyramidal cells with similar spatial tuning excite each other to maintain persistent firing across the delay period<sup>6</sup>. These networks are concentrated in deep layer III<sup>6</sup>. Spatial tuning is enhanced by GABAergic lateral inhibition (not shown). **d**, An example of a dorsolateral PFC DELAY neuron with spatially tuned, persistent firing during the delay period. This neuron shows increased firing for the cue, delay and response for the neuron's preferred direction (highlighted in blue), but not for nonpreferred directions (white backgrounds). The anti-preferred direction opposite to the neuron's preferred direction is shown in red; note that subsequent figures show only the preferred and anti-preferred directions for the sake of brevity. **e**, Pyramidal cells synapse on spines where cAMP–PKA signalling regulates the open state of HCN and KCNQ channels, and thus modulates the strength of network connections<sup>9</sup>. **f**, Population average activity for the dorsolateral PFC DELAY neurons recorded in each age group (102, 101 and 70 neurons for young, middle-aged and old monkeys, respectively). Colours 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 grey background refers to the cue period; the lighter grey background to the delay period. Error envelope represents s.e.m.

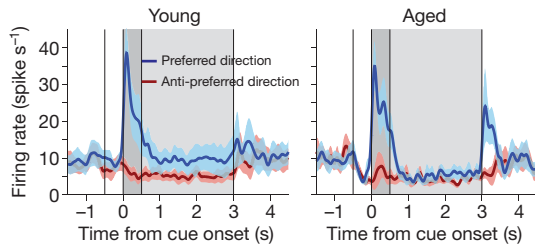
The firing of DELAY cells was markedly reduced with advancing age (Figs 1f and 2). Figure 1f portrays the differences in firing rates across the population of DELAY neurons in young, middle-aged and aged animals (for individual examples of DELAY neurons in young, middle-aged and aged monkeys, see Supplementary Fig. 1). There was a significant decline in the spontaneous firing rate of DELAY cells, as well as a marked decline in task-related firing. Figure 2 shows a steep decline in the firing rates of DELAY cells across the age span (*t*-test on age variable in regression analysis,  $P < 10^{-4}$  for all epochs), with older animals showing a restricted range of lower firing rates (Supplementary Fig. 2). This age-related activity decline persisted throughout the 2.5-s delay period (without main effect of epoch (0.5 s) or age  $\times$  epoch interaction in repeated measures ANOVA,  $P > 0.25$ ). Additional control analyses showed that age-related decline in the firing rate of



**Figure 2 | Age-dependent decline in the spatially tuned, persistent firing of dorsolateral PFC DELAY neurons.** **a**, Marked reduction of dorsolateral PFC DELAY activity for the neurons' preferred direction with advancing age. Activity of individual neurons of each animal was averaged separately for the last 0.5 s during the intertrial interval (pre-fix, grey), the fixation period (fix, black), the cue period (cue, red), and the delay period for the neuron's preferred direction. Firing during the delay period is represented in a successive series of 0.5 s intervals (colour coded from yellow to blue). Lines were obtained using linear regression. **b**, Firing rates during the delay period for the anti-preferred direction of the same neurons shown in **a**. There was a significant age-related decline in all epochs, but it was less prominent than the decline in firing for the preferred direction during the delay period. Colour coding for each 0.5 s interval is as in **a**. **c**, Age-related decline in spatial tuning, whereby the difference between firing for the preferred versus anti-preferred directions during the delay period declines with advancing age. Colour coding is as in **a**. **d**, Age-related decline in  $d'$ , that is, the ability to distinguish preferred from anti-preferred spatial directions based on firing rate patterns during the entire delay period. Error bars represent s.e.m.

DELAY cells is not due to a sampling bias during the recording experiment (see Supplementary Information). The age-related decline in firing rate was particularly prominent during the cue and delay periods for the neuron's preferred direction (Fig. 2a); the decline in firing for the anti-preferred direction (Fig. 2b) or before target onset (Fig. 2a) was less pronounced. Consequently, the difference in delay-related firing for the neuron's preferred direction versus its anti-preferred direction eroded with increasing age (*t*-test,  $P < 10^{-5}$ , for cue period and every 0.5 s epoch in the delay period; Fig. 2c), largely due to reduced firing for the neuron's preferred direction (Fig. 2a). This led to a reduction in  $d'$  with advancing age (*t*-test on age versus  $d'$  correlation coefficient,  $P < 0.0001$ ), that is, a reduced ability to distinguish the preferred from anti-preferred directions during the delay period when spatial information was held in working memory (Fig. 2d). These results are consistent with studies showing impairment in spatial working memory in aged monkeys at relatively short (for example, 5 s) delays<sup>18</sup>, and single-unit data as well as neural circuit modelling indicate that inadequate PFC recurrent network firing underlies the deficits in PFC cognitive function observed in ageing monkeys and humans (Supplementary Figs 3, 4 and Supplementary Information).

In contrast to DELAY neurons, which showed prominent decline in firing with advancing age, there were no age-related changes in the firing rates of PFC CUE cells that responded specifically to the spatial cue (Fig. 3). The average firing rate of these neurons for the preferred direction during the cue period was  $26.7 \pm 4.4$  spikes  $s^{-1}$  in young monkeys ( $n = 12$  neurons), and  $25.3 \pm 3.7$  spikes  $s^{-1}$  in old monkeys (11 neurons; *t*-test,  $P > 0.8$ ; significant age  $\times$  cell-type interaction in



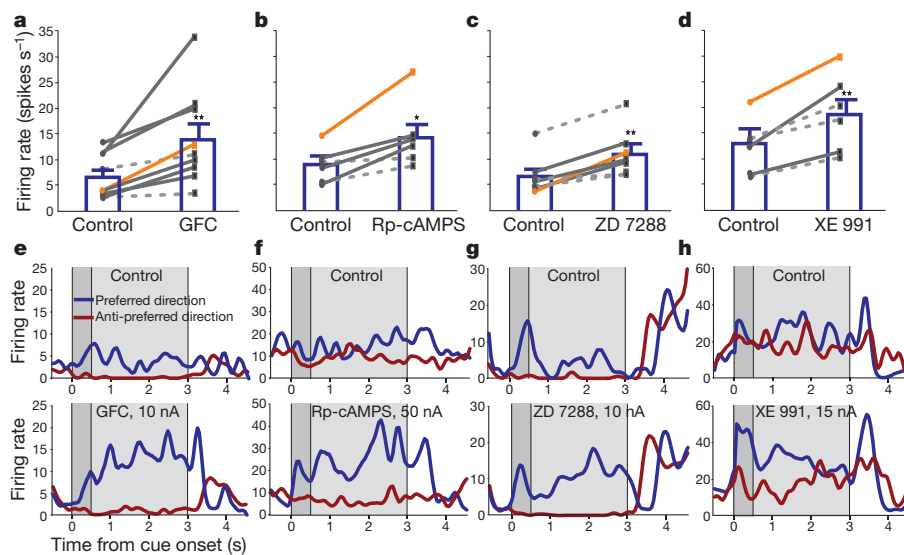
**Figure 3 | Firing rates of dorsolateral PFC CUE cells remain stable in aged monkeys.** The average firing rates of CUE cells in young monkeys (left graph; 10 neurons from a 7-year-old and 2 neurons from a 9-year-old monkey), did not differ from the firing rate in the oldest monkey (right graph; 11 neurons from a 21-year-old monkey), or from the firing rate averaged for both middle-aged (5 neurons from 13-year-old monkey, not shown separately) and old monkeys ( $t$ -test,  $P > 0.7$ ). CUE cells may receive direct, 'bottom-up' excitation from parietal association cortex<sup>6</sup>, which may be less vulnerable to subtle molecular changes with advancing age. A subset of CUE cells recorded in the aged monkey showed high firing rates during the response period. Error envelope represents s.e.m.

two-way ANOVA,  $P < 0.05$ ). These data indicate that reductions in memory-related firing rate do not arise from generalized changes with advancing age affecting all neurons but, rather, are especially evident in recurrent circuits that must maintain firing in the absence of 'bottom-up' sensory stimulation.

It is important to identify which changes in the ageing brain contribute to reduced firing during the delay period. There are many brain alterations associated with advancing age<sup>27</sup>, including decreased PFC grey matter volume<sup>28</sup>, focal changes in white matter<sup>29</sup> and dendritic spine loss<sup>30</sup>, all of which correlate with cognitive decline. Importantly, spine loss is especially prominent in layer III—the layer where the recurrent excitatory networks reside—and thin-type spines are the most vulnerable in the aged PFC<sup>30</sup>. Immunoelectron microscopy indicates that thin spines have the greatest concentration of cAMP–HCN-channel signalling proteins, indicating that disinhibition of cAMP

signalling with advancing age may weaken thin spines in particular<sup>9</sup>. Thus, we tested whether inhibition of cAMP signalling in the PFC could partially restore the working-memory-related firing of aged neurons, or whether reductions in firing were irreversible owing to immutable architectural changes in the aged brain. Drugs were applied near the recorded neurons using iontophoresis, whereby a small electrical current is applied to extrude charged molecules from glass pipettes attached to the recording electrode. Only a minute amount of drug was released, sufficient to alter the firing of nearby neurons, without altering behavioural performance.

Agents that inhibit cAMP signalling or block HCN or KCNQ channels restored persistent firing during the delay period of the working memory task (Fig. 4). For example, iontophoresis of the  $\alpha_{2A}$  agonist, guanfacine (Fig. 4a, e), or the cAMP–PKA inhibitor, Rp-cAMPS (Fig. 4b, f), significantly increased firing during the delay period on trials when the cue had appeared at the neuron's preferred direction. In contrast, the PDE4 inhibitor, etazolate—which increases cAMP signalling—further decreased neuronal firing in aged neurons ( $P < 0.001$ ; Supplementary Fig. 5). We also tested whether blockade of HCN or KCNQ channels could restore firing, given that cAMP–PKA signalling increases the open state of these ion channels. As shown in Fig. 4c and g, a low dose of the HCN channel blocker ZD7288 significantly enhanced the delay-related firing rates of neurons in aged monkeys. KCNQ channels were also of interest, as *in vitro* physiological characterizations of PFC neurons in aged primates have found increases in the slow afterhyperpolarization, which is mediated in part by KCNQ channels<sup>26</sup>. As shown in Fig. 4d and h, blockade of KCNQ channels with XE991 increased delay-related firing in aged PFC neurons. Thus, agents that reduced cAMP opening of HCN or KCNQ channels all restored firing rates to levels resembling those observed in younger monkeys. These findings are consistent with behavioural data showing that guanfacine and Rp-cAMPS can enhance working memory performance in aged animals when administered systemically (guanfacine) or directly into the rat PFC (guanfacine or Rp-cAMPS)<sup>22</sup> (see Supplementary Information). On the basis of these data, guanfacine



**Figure 4 | Iontophoresis of compounds that inhibit cAMP–PKA signalling, or block HCN or KCNQ channel signalling, strengthens delay-related firing in aged PFC DELAY neurons.** a–d, A summary of the results showing a significant increase in population-average firing rate for the neuron's preferred direction compared to control conditions (paired  $t$ -test,  $P < 0.01$  for a, c and d, and  $P < 0.05$  for b) following iontophoresis of the  $\alpha_{2A}$  adrenergic agonist guanfacine applied at 10 nA (GFC; a, significant effects in 7 out of 9 neurons,  $t$ -test,  $P < 0.05$ , indicated by solid lines); the cAMP inhibitor Rp-cAMPS at 50 nA (b, significant in 4 out of 6 neurons); the HCN channel blocker, ZD7288 at 15 nA (c, significant in 4 out of 7 neurons); and the KCNQ channel blocker

XE991 at 15 nA (d, significant in 3 out of 6 neurons). In all cases, significant effects were found more frequently than expected by chance (binomial test,  $P < 0.005$ ). The orange lines represent the individual neurons shown in e–h. e–h, Individual examples of neurons under control conditions (top) firing to their preferred (blue trace) or anti-preferred (red trace) directions, compared to their firing patterns following iontophoresis of guanfacine (e), Rp-cAMPS (f), ZD7288 (g) or XE991 (h). The orange lines in a–d indicate the individual neurons shown in e–h. Error bars are s.e.m. \* $P < 0.05$ , \*\* $P < 0.01$  significant difference between drug versus control for the neuron's preferred direction.



is currently being tested in elderly humans with PFC cognitive deficits (<http://www.clinicaltrials.gov>: trial number NCT00935493).

The current study revealed a physiological basis for age-related working memory decline in the primate brain, with a reduction of memory-related firing beginning in middle age and worsening with advancing age. This marked change in network physiology may render higher cortical circuits especially vulnerable to neurodegenerative processes such as Alzheimer's disease. However, these studies also uncovered more hopeful data showing that restitution of the proper neurochemical environment can partially restore physiological integrity. These data establish that cognitive changes with advancing age are malleable, and that there is potential to restore at least some cognitive abilities in the elderly. Maintaining strong PFC physiology into advanced age will be an important advantage in an increasingly complex, ageing society.

## METHODS SUMMARY

All experiments were performed in accordance with National Institutes for Health guidelines for animal research and were approved by the Yale Institutional Animal Care and Use Committee.

**Behavioural training.** Monkeys were trained on the oculomotor delayed response (ODR) task (Fig. 1b) as reported elsewhere<sup>8</sup>, with special care to minimize all stress. The aged monkeys took longer to learn the task, rested more frequently than young monkeys during the testing sessions, and performed fewer trials each day than younger animals. A brief delay period (2.5 s) was chosen to ensure that all monkeys in the study performed above 85% correct during training, and could maintain high levels of performance during the study (Supplementary Fig. 6).

**Physiology and iontophoresis.** Single unit recordings were made from the dorsolateral PFC surrounding the caudal portion of the principal sulcus (Fig. 1a). The recording procedures and iontophoresis methods have been described previously<sup>8</sup>. Single neuron activity was readily isolated (Supplementary Fig. 7). Denoting the average activity during the 0.5 s cue period and that during the 2.5 s delay period during the trials in preferred directions as *C* and *D*, neurons were classified as CUE cells, when the ratio *D/C* was >0.5, and as DELAY cells otherwise. Statistical analyses, including ANOVA and regression analyses, were performed using Matlab (MathWorks). The effect of age on firing rate during a series of 0.5 s epochs was tested using a regression model, and its statistical significance with a *t*-test.

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Author Contributions** M.W. and X.-J.W., J.A.M., D.L. and A.F.T.A. designed the experiments. M.W. carried out all the physiology experiments, with the help of Y.Y., N.J.G., L.E.J. and J.A.M. Data analyses were performed by M.W., D.L., J.A.M. and M.L. Computational modelling was performed by X.-J.W. All authors participated in the writing of the paper.

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## METHODS

**Oculomotor delayed response task.** Studies were performed on four adult male rhesus monkeys (*Macaca mulatta*) trained on the spatial oculomotor delayed response (ODR) task as previously described<sup>8</sup>. This task requires the monkey to make a memory-guided saccade to a remembered visuospatial target. Each trial began when the subject fixated at the central spot for 0.5 s (fixation period). Subsequently, a cue was illuminated for 0.5 s at one of eight peripheral targets (cue period). After the cue was extinguished, a 2.5 s delay period followed. During the cue and delay periods, the monkey was required to maintain central fixation. At the end of the delay, the fixation spot was extinguished, instructing the monkey to make a memory-guided saccade to the previously cued location (saccade period). The monkey was rewarded with fruit juice immediately after every successful response. The position of the stimulus was randomized over trials such that it had to be remembered on a trial-by-trial basis. The subject's eye position was monitored with the ISCAN Eye Movement Monitoring System, and the ODR task was generated by the TEMPO real-time system (Reflective Computing). The aged monkeys took longer to learn the task, rested more frequently than young monkeys during the testing sessions, and performed fewer trials each day than younger animals. A brief delay period (2.5 s) was chosen to ensure that all monkeys in the study performed above 85% correct during training, and could maintain high levels of performance during the study (Supplementary Fig. 6).

**Recording locus.** Before recording, the animal underwent a magnetic resonance image (MRI) scan to obtain exact anatomical coordinates of brain structures, which guided the placement of the chronic recording chambers. MRI-compatible materials were used for the implant so that the position of the recording chambers could be confirmed by MRI after implantation. The site of recordings in the present study were located in an area ranging from 0–5 mm anterior to the caudal end of the principal sulcus and –2–2 mm medial to the principal sulcus.

**Pharmacology, physiology and data acquisition.** Guanfacine, XE991 and ZD7288 (Tocris) and etazolate (Sigma) were dissolved at 0.01 M in triple-distilled water (adjusted with HCl to pH 3.5–4.0). Rp-cAMP (Sigma) was dissolved at 0.01 M in triple-distilled water (adjusted with NaOH to pH 9).

Iontophoretic electrodes were constructed with a 20- $\mu$ m pitch carbon fibre (ELSI) inserted in the central barrel of a seven-barrel non-filamented capillary glass (Friedrich and Dimmock). The assembly was pulled using a multipipette electrode puller (MicroData Instrument) and the tip was bevelled to obtain the finished electrode. Finished electrodes had impedances of 0.3–1.0 M $\Omega$  (at 1 kHz) and tip sizes of 30–40  $\mu$ m. The outer barrels of the electrode were then filled with 3 drug solutions (two consecutive barrels each) and the solutions were pushed to the

tip of the electrode using compressed air. A Neurophore BH2 iontophoretic system (Medical Systems) was used to control of the delivery of the drugs. The drug was ejected at currents that varied from 5–50 nA. Retaining currents of –3 to –5 nA were used in a cyclical manner (1 s on, 1 s off) when not applying drugs. Drug ejection did not create noise in the recording, and there was no systematic change in either spike amplitude or time course at any ejection current.

The electrode was mounted on a MO-95 micromanipulator (Narishige) in a 25-gauge stainless steel guide tube. The dura was punctured using the guide tube to facilitate access of the electrode to cortex. Extracellular voltage was amplified using an AC/DC differential amplifier (A-M systems; Model 3000) and band-pass filtered (180 Hz–6 KHz, 20 dB gain, 4-pole Butterworth; Kron-Hite). Signals were digitized (20.83 kHz, micro 1401, Cambridge Electronics Design) and acquired using the Spike2 software (CED). Neural activity was analysed using waveform sorting by a template-matching algorithm, which made it possible to isolate more than one unit at the same recording site. Post-stimulus time histograms and rastergrams were constructed online to determine the relationship of unit activity to the task. Unit activity was measured in spikes per second. If the rastergrams displayed task-related activity, the units were recorded further and pharmacological testing was performed.

Data were first collected from the cell under a control condition in which at least eight trials at each of eight cue locations were obtained. On establishing the stability of the cells' activity, this control condition was followed by the drug application. Dose-dependent effects of the drug were tested in two or more consecutive conditions. Drugs were continuously applied at a relevant current throughout a given condition. Each condition had ~8 (typically 10 or more) trials at each location for statistical analyses of effects.

**Data analysis.** For purposes of data analysis, each trial in the ODR task was divided into four epochs: fixation, cue, delay and response (saccade). The fixation epoch lasted for 0.5 s. The cue epoch lasted for 0.5 s and corresponds to the stimulus presentation phase of the task. Delay lasted for 2.5 s and reflects the mnemonic component of the task. The response phase started immediately after the delay epoch and lasted ~1.5 s.

Two-way analysis of variance, ANOVA, was used to examine the spatial tuned task-related activity with regard to (1) different periods of the task (cue, delay, response versus fixation); and (2) different cue locations. One-way ANOVAs were used to assess the effect of the drug application on cells showing delay-related activity. Statistical analyses, including ANOVA and regression analyses were performed using Matlab (MathWorks). The effect of age on firing rate during a series of 0.5 s epochs was tested using a regression model, and its statistical significance with a *t*-test.