Delay activity of specific prefrontal interneuron subtypes modulates memory-guided behavior

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Memory-guided behavior requires maintenance of task-relevant information without sensory input, but the underlying circuit mechanism remains unclear. Calcium imaging in mice performing a delayed Go or No-Go task revealed robust delay activity in dorsomedial prefrontal cortex, with different pyramidal neurons signaling Go and No-Go action plans. Inhibiting pyramidal neurons by optogenetically activating somatostatin- or parvalbumin-positive interneurons, even transiently during the delay, impaired task performance, primarily by increasing inappropriate Go responses. In contrast, activating vasoactive intestinal peptide (VIP)-positive interneurons enhanced behavioral performance and neuronal action plan representation. Furthermore, while endogenous activity of somatostatin and parvalbumin neurons was strongly biased toward Go trials, VIP neurons were similarly active in Go and No-Go trials. Somatostatin or VIP neuron activation also impaired or enhanced performance, respectively, in a delayed two-alternative forced-choice task. Thus, dorsomedial prefrontal cortex is a crucial component of the short-term memory network, and activation of its VIP neurons improves memory retention.

In this study, we used cell-type-specific calcium imaging to measure the delay-period activity of pyramidal neurons and three major interneuron subtypes in the PFC of mice performing a delayed-response task. Bidirectional optogenetic manipulations were used to test their causal roles in memory maintenance. We found that suppression of pyramidal neuron activity through activation of somatostatin (SST)- or parvalbumin (PV)-positive interneurons, even transiently during an early delay period, severely impaired task performance. In contrast, activation of VIP-positive interneurons enhanced both the neuronal coding of action plans and the animal’s task performance. Furthermore, the endogenous delay activity of SST and PV neurons was strongly biased toward Go trials, whereas VIP neurons were similarly active in both Go and No-Go trials. Our results reveal the functional distinction among three interneuron subtypes in working memory maintenance, and they point to the potential for improving memory performance by activating VIP interneurons in the PFC.

RESULTS

Delay activity of different pyramidal neurons signals Go and No-Go action plans

To examine the function and mechanism of delay-period activity in the PFC, we trained head-fixed mice on a delayed Go versus No-Go auditory discrimination task (Fig. 1a, Supplementary Video 1 and Online Methods). Each trial consists of sample, delay and test periods. During the sample period (2 s) a target (8 kHz) or nontarget (2 kHz) auditory stimulus was presented (the corresponding trial was referred to as a 'Go' trial or 'No-Go' trial, respectively), followed by a 5-s delay period during which water was inaccessible. The test period began when the water port was presented; licking in Go trials within the 2-s response window (Hit) was rewarded, and licking in No-Go trials (False Alarm) was punished. Mice learned this task within several weeks, licking...
mostly during the test period (Fig. 1a and Supplementary Fig. 1); their correct response rate stabilized at 84 ± 2.6% (s.e.m., n = 9 mice; Fig. 1b). Pupillometry during the task suggests similar levels of arousal during Go and No-Go trials (Supplementary Fig. 2). However, the great majority of errors were False Alarms (Fig. 1c), indicating that the task performance depends mainly on the successful suppression of inappropriate Go responses.

To image task-related neuronal activity, we injected Cre-inducible adeno-associated virus (AAV) expressing the calcium indicator GCaMP6f30 into the dorsomedial PFC (dmPFC) of CaMKIIα-Cre mice to specifically label pyramidal neurons27. Imaging was performed through a gradient refractive index lens coupled to a miniaturized integrated fluorescence microscope31, which allowed monitoring of neuronal activity across cortical layers (Fig. 2a).

We found strong task-related activity in dmPFC pyramidal neurons. In addition to the calcium transients time-locked to the sensory stimuli and trial outcomes37, many neurons exhibited sustained activity during the 5-s delay period (Fig. 2b–h). While some neurons were selectively activated in Go trials (Go-prefering neurons; Fig. 2b,d,f,h), others were much more active in No-Go trials (NG-prefering neurons; Fig. 2c,e,g,h), as in a previous observation in mouse M132. Notably, we found a higher fraction of NG-prefering neurons in deeper cortical layers (Fig. 2i; see Supplementary Fig. 3 for analyses at different dorsal–ventral positions), suggesting a transformation of action plan representation across cortical layers10. To quantify the separability between the two action plans encoded in dmPFC activity, we computed the Euclidean distance between the population activity in Go and No-Go trials (Online Methods). The distance increased rapidly after the onset of auditory stimulus and persisted throughout the delay and test periods (Fig. 2j). Decoding analysis also showed that the delay activity in individual trials was highly predictive of the motor behavior in correct response (Hit and Correct Rejection) trials (Supplementary Fig. 4), indicating that information on the action plan was maintained in dmPFC activity.

Delay activity of pyramidal neurons is required for memory maintenance

Previous studies showed that the encoding of future actions improves with behavioral learning in both rats18 and monkeys33. Here the Euclidean distance between the Go and No-Go delay activity was also positively correlated with the task performance (Fig. 2k; r = 0.50, P < 0.0081, Pearson's correlation coefficient, bootstrap), suggesting a functional contribution of this activity to behavioral control. To directly test the causal relationship between dmPFC activity and memory maintenance, we suppressed pyramidal neuron spiking by optogenetically activating inhibitory interneurons9–12,20 (Supplementary Fig. 5). Multielectrode recordings showed that channelrhodopsin-2 (ChR2)-mediated SST neuron activation strongly suppressed dmPFC spiking in both Go and No-Go trials (Supplementary Figs. 5, 6 and 7a), leading to a marked reduction in their Euclidean distance (Supplementary Fig. 7b). Previous studies showed that activation of PV neurons is also highly effective in suppressing pyramidal neuron activity9–11,20. Behaviorally, activating either SST or PV neurons caused a strong impairment in task performance, with a substantial increase in False Alarm rate and a small decrease in Hit rate (Fig. 3a–c). Thus, dmPFC pyramidal neuron activity was important for the delayed response task, especially for the suppression of inappropriate motor responses in No-Go trials.

Suppression of dmPFC activity during the whole trial could affect encoding of the auditory stimulus, retention of the action plan and/or execution of the motor response. To specifically test the role of dmPFC activity in memory retention, we applied optogenetic suppression briefly within the delay period8,12–20. Notably, even a 2-s laser stimulation of SST or PV neurons during early delay caused a strong reduction in behavioral performance (Fig. 3d–f), indicating that the delay activity played a crucial role in maintaining the Go or No-Go action plan after the auditory stimulus ceased. In contrast, activating SST or PV neurons in the primary somatosensory cortex, during either the whole trial or the early delay period, had no significant effect on performance (P = 0.91 and 0.81 for SST and PV, respectively, during the whole trial; P = 0.81 and 0.44 for SST and PV, respectively, during the early delay; Supplementary Fig. 8), indicating that behavioral impairment was not a general side effect of the laser stimulation.

VIP neuron activation improves task performance and neural coding of action plans

Unlike SST or PV neuron activation, which powerfully suppressed pyramidal neuron activity (Supplementary Fig. 7a), VIP neuron activation causes both inhibition and disinhibition of pyramidal neurons22,25,26,34. We next tested their role in memory-guided behavior. Optogenetic activation of VIP interneurons in dmPFC—either throughout the whole trial or only during the early delay period—caused a marked improvement in task performance (Fig. 4a,b). Conversely, archaerhodopsin (Arch)-mediated silencing of VIP neurons caused a significant behavioral impairment (Fig. 5a,d), indicating that their normal activity is important for performing the task. Both the ChR2-mediated increase and Arch-mediated decrease in correct response rate were due primarily to changes in...
the False Alarm rate (Figs. 4a and 5a,d). This suggests that the main effect of VIP neuron activation was to improve the suppression of inappropriate Go responses, in opposition to the effect of SST or PV neuron activation (Fig. 3). In control experiments in the primary somatosensory cortex, VIP neuron manipulation caused no significant change in performance (P = 0.50 and 0.64 for whole trial and early delay, respectively, in VIP-ChR2 mice and P = 0.58 for whole trial in VIP-Arch mice; Supplementary Figs. 9 and 10), indicating a specific role for the dmPFC.

Previous studies have shown that VIP neuron activation disinhibits pyramidal neurons by inhibiting SST and PV interneurons.25,26,34,35 Arch-mediated optogenetic inhibition of SST or PV neurons, either throughout the whole trial or during the early delay period, significantly improved task performance (Fig. 5b,c,e,f), suggesting
that the behavioral effect of VIP neuron activation could indeed be mediated in part by inhibiting SST and PV neurons. On the other hand, direct optogenetic activation of dmPFC pyramidal neurons impaired behavioral performance (Supplementary Fig. 11), indicating that a nonselective increase in their firing rate is detrimental.

To further examine the mechanism by which VIP neuron activation improves the behavior, we performed multielectrode recordings in the dmPFC (Fig. 6a). For the recorded Go-preferring neurons, laser stimulation significantly decreased their delay activity in No-Go trials \( (P = 0.00055, \text{paired } t\text{-test}) \) but not in Go trials \( (P = 0.18; \text{Fig. 6a}) \).

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3}
\caption{Optogenetic activation of dmPFC SST and PV neurons impaired behavioral performance. (a) Schematic of optogenetic stimulation during the whole trial (top) and an example session of SST neuron stimulation (bottom). Each tick indicates one lick (as in Fig. 1a). (b) SST neuron stimulation (SST-ChR2) during the whole trial decreased behavioral performance \((n = 8 \text{ mice}; P = 0.0078, \text{Wilcoxon signed-rank test})\), with a substantial increase in FA rate \((P = 0.016)\) and a small decrease in Hit rate \((P = 0.0078)\). Gray lines, individual mice; black, mean \( \pm \text{s.e.m.} \). Circles, individual mice. \( \Delta \text{rate (laser on – off)} \). (c) PV neuron stimulation (PV-ChR2) also decreased the correct response rate \((n = 7 \text{ mice}; P = 0.031)\) with a substantial increase in FA rate. All error bars denote \( \pm \text{s.e.m.} \). \* \( P < 0.05 \) and \** \( P < 0.01 \); Wilcoxon signed-rank test.}
\end{figure}
Supplementary Fig. 12), resulting in an increased difference between their Go and No-Go activity (Fig. 6b). For the NG-preferring neurons, laser decreased the activity in Go trials ($P = 0.034$) but not in No-Go trials ($P = 0.58$), leading to a decreased Go – No-Go value (Fig. 6b). For the remaining unmodulated neurons, the activity decreased in both Go ($P < 0.001$) and No-Go trials ($P < 0.001$). Thus, while VIP neuron activation caused an overall decrease in dmPFC activity, the magnitude of the effect depended on both the trial type (Go versus No-Go) and the functional property of the cell. Such differential modulations resulted in an increased Euclidean distance between Go and No-Go delay-period activity (Fig. 6c), which may have contributed to the improved behavioral performance (Fig. 2k).

Endogenous delay-period activity of SST, PV and VIP interneurons

Having demonstrated their distinct impacts on memory retention using optogenetic manipulations, we next measured the endogenous task-related activity of the interneuron subtypes to assess their physiological contributions to memory-guided behavior. Cre-inducible AAV expressing GCaMP6f was injected into the dmPFC of SST-Cre, PV-Cre or VIP-Cre mice, and calcium imaging was performed during the delayed-response task (Fig. 7a–c).

While the activity of SST and PV neurons was biased strongly toward Go trials (Fig. 7a,b), the VIP neuron population showed similar activity in both Go and No-Go trials (Fig. 7c). Of the three interneuron subtypes plus the pyramidal neurons, VIP neurons exhibited the highest relative activity in No-Go trials (Fig. 7d; $P < 0.001$ for all comparisons, Tukey-Kramer post hoc test following one-way ANOVA). Since optogenetic activation and inactivation of VIP neurons in No-Go trials improved and impaired the suppression of inappropriate motor responses, respectively (Figs. 4 and 5a,d), the endogenous activity of VIP neurons in these trials may play an important role in enabling high performance of the delayed Go or No-Go task.

**Figure 4** VIP neuron activation improved behavioral performance. (a) Optogenetic activation of VIP neurons during the whole trial increased the correct response rate with a large reduction of FA rate and a small increase in Hit rate ($n = 10$ mice). (b) VIP neuron activation for 2 s during the early delay period also increased the correct response rate ($n = 9$ mice). All error bars denote ± s.e.m. **$P = 0.0020$, ***$P = 0.00098$; Wilcoxon signed-rank test.

**Figure 5** Behavioral effects of optogenetic silencing of VIP, SST or PV neurons in the dmPFC. (a) Arch-mediated silencing of VIP neurons (VIP-Arch) in the dmPFC during the whole trial decreased the correct response rate ($P = 0.031$, Wilcoxon signed-rank test) due primarily to an increase in the FA rate ($P = 0.031$) rather than the Hit rate ($P = 0.16$). (b) Arch-mediated inactivation of SST neurons (SST-Arch) during the whole trial increased the correct response rate ($P = 0.031$) rather than the Hit rate ($P = 0.58$), leading to a decreased Go – No-Go value ($P = 0.58$), resulting in an increased difference between Hit and FA rates ($P < 0.001$) and No-Go trials ($P < 0.001$). For the NG-preferring neuron subtype plus the pyramidal neurons, VIP neurons exhibited the highest relative activity in No-Go trials (Fig. 7d; $P < 0.001$ for all comparisons, Tukey-Kramer post hoc test following one-way ANOVA). Since optogenetic activation and inactivation of VIP neurons in No-Go trials improved and impaired the suppression of inappropriate motor responses, respectively (Figs. 4 and 5a,d), the endogenous activity of VIP neurons in these trials may play an important role in enabling high performance of the delayed Go or No-Go task.

**Figure 6** Optogenetic activation of VIP neurons during the whole trial increased the correct response rate with a large reduction of FA rate and a small increase in Hit rate ($n = 10$ mice). (a) VIP neuron activation for 2 s during the early delay period also increased the correct response rate ($n = 9$ mice). All error bars denote ± s.e.m. **$P = 0.0020$, ***$P = 0.00098$; Wilcoxon signed-rank test.

**Figure 7** Endogenous delay-period activity of SST, PV and VIP interneurons

Having demonstrated their distinct impacts on memory retention using optogenetic manipulations, we next measured the endogenous task-related activity of the interneuron subtypes to assess their physiological contributions to memory-guided behavior. Cre-inducible AAV expressing GCaMP6f was injected into the dmPFC of SST-Cre, PV-Cre or VIP-Cre mice, and calcium imaging was performed during the delayed-response task (Fig. 7a–c).

While the activity of SST and PV neurons was biased strongly toward Go trials (Fig. 7a,b), the VIP neuron population showed similar activity in both Go and No-Go trials (Fig. 7c). Of the three interneuron subtypes plus the pyramidal neurons, VIP neurons exhibited the highest relative activity in No-Go trials (Fig. 7d; $P < 0.001$ for all comparisons, Tukey-Kramer post hoc test following one-way ANOVA). Since optogenetic activation and inactivation of VIP neurons in No-Go trials improved and impaired the suppression of inappropriate motor responses, respectively (Figs. 4 and 5a,d), the endogenous activity of VIP neurons in these trials may play an important role in enabling high performance of the delayed Go or No-Go task.

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The difference in the Euclidean distance was significant for all analysis periods except for the baseline period. The analysis periods, including baseline (1 s before the beginning of sample), sample, delay and post-delay (0.5 s after the end of delay) periods.

Interneuron activation during delayed two-alternative forced-choice task
Performance of the delayed Go or No-Go task depends mainly on the successful suppression of inappropriate Go responses (Fig. 1c), and the effects of interneuron manipulations reported above were due primarily to changes in the False Alarm rate (Figs. 3–5). These observations point to a crucial role of the dmPFC in response inhibition.2,3,36 However, even transient optogenetic manipulations during an early delay period significantly affected the performance (Figs. 3d–f, 4b and 5d–f), arguing against a simple effect on motor suppression during the response period (although it is possible that some ChR2-mediated and Ach- or halorhodopsin-mediated changes in cortical activity outlasted the laser stimulation by several seconds). In addition to changes in the False Alarm rate, several manipulations also had small but significant effects on the Hit rate (Figs. 3b,e and 4a,b), which cannot be accounted for purely by response inhibition.

To further examine the roles of dmPFC SST and VIP interneurons in memory-guided behavior separately from response inhibition, we trained mice to perform a delayed two-alternative forced-choice (2-AFC) task, in which they licked either the left or right water port in response to a tone stimulus after a variable delay period (1.5–2 s; Fig. 8a). We found that ChR2-mediated bilateral activation of SST neurons, during either the whole trial or an early delay period, significantly impaired the behavioral performance, whereas VIP neuron activation improved the behavior (Fig. 8b–e), consistent with the findings using the delayed Go or No-Go task (Figs. 3 and 4). These results further demonstrated the functions of the dmPFC interneurons in memory-guided behavior beyond response suppression.

DISCUSSION
Using a combination of calcium imaging and bidirectional optogenetic manipulations during memory-guided behavior, we revealed distinct functional roles of three major subtypes of dmPFC GABAergic interneurons in short-term memory maintenance. While activation of SST and PV interneurons impairs behavioral performance by suppressing the delay-period activity of pyramidal neurons, activation of VIP interneurons improves their representation of the action plans and the animal’s task performance. These results indicate that the dmPFC is a crucial component of the short-term memory network, and activity of its VIP neurons plays a key role in memory retention. The strong behavioral impairment caused by a transient dmPFC inactivation in well-trained mice (Fig. 3) is reminiscent of the finding in rat frontal orienting field, and it suggests a crucial role for the delay activity in maintaining memory for the action plan.37–39 However, neuronal activity in rats’ frontal orienting fields during error trials reflects motor responses better than instructing sensory stimuli, whereas mouse dmPFC activity in False Alarm trials was more similar to that seen in Correct Rejection trials than that seen in Hit trials (Fig. 2d–g and Supplementary Fig. 4). The difference between these observations could be due to the difference in the brain regions studied or to the difference in task designs.
(2-AFC versus Go or No-Go). Unlike the 2-AFC task, which involves symmetric left- and right-motor responses, Go or No-Go tasks are intrinsically asymmetric, with far more False Alarm than Miss errors (Fig. 1c). It is thus possible that when the animal is motivated to lick, even a slight deviation of dmPFC activity from the pattern required for Correct Rejection could lead to an inappropriate Go response. The Miss errors, on the other hand, increased dramatically near the end of each behavioral session (likely due to water satiety), suggesting that a significant fraction of them are related to the animal's decreased motivation compared to earlier in the session. A full understanding of how dmPFC activity is decoded during this behavior will require observing neuronal activity in the downstream circuits. Our finding

Figure 7 Calcium imaging from each interneuron subtype. (a) Left: field of view in an example imaging session of SST cells. Brown outline, the example region of interest (ROI) shown in the middle panels. M, medial; L, lateral; P, posterior; A, anterior. Middle: raw fluorescence trace and trial-averaged ∆F/F (z-scored) for the example ROI. Format is the same as in Figure 2b. Right: distribution of Go–NG preference during the delay period in all SST cells (n = 230). Scale bar, 100 µm. (b) As in a but for PV cells (n = 387). (c) As in a but for VIP cells (n = 406). The upper and lower panels show two example ROIs with significant Go- and NG-preferring activity, respectively. Gray shading, delay period; blue and orange stripes, sample periods with target and nontarget tones, respectively. Dashed line, end of response window in CR trials. Black tick on top, lick response. Blue and orange arrowheads, delivery of reward and punishment, respectively. (d) Cumulative distribution of Go–NG preference in the three interneuron subtypes and pyramidal cells (Pyr). Of the three interneuron subtypes and pyramidal neurons, VIP neurons exhibited the highest relative activity in No-Go trials (P = 3.3 × 10^{-75}, F_{3,1426} = 131.9, one-way ANOVA with Tukey-Kramer post hoc test).
Figure 8 Optogenetic activation of SST and VIP neurons during a delayed 2-AFC task. (a) Schematic for task design. (b) Schematic of optogenetic stimulation during the whole trial (top) and behavioral performance (bottom). Bilateral SST neuron stimulation during the whole trial decreased the correct rate (number of trials with correct responses divided by the total number of trials for each side) in both left and right trials, and it increased the incorrect response rate but not the miss rate (n = 6 mice). (c) Bilateral VIP neuron stimulation during the whole trial increased the correct rate in both left and right trials, and it decreased the incorrect response rate but not the miss rate (n = 6 mice). (d,e) As in b and c but with 1-s laser stimulation during the early delay period. Gray lines, individual mice; black, mean ± s.e.m. *P = 0.031; Wilcoxon signed-rank test.
is also different from a recent study using a delayed non-match-to-sample task, in which the delay activity of a more ventral region of the medial PFC was found to be important for learning of the task but not for its performance after the mice were well trained\(^\text{20}\). In addition to the differences in brain region (ventral versus dorsal mPFC) and sensory modality (olfactory versus auditory), there is an important difference in the memory requirement for the two tasks. While the delayed non-match-to-sample task requires short-term memory of the sensory cue, the delayed-response task is likely to require maintenance of the action plan.

Besides the frontal cortex, delay-period activity has also been observed in other brain regions, including the parietal cortex, superior colliculus and temporal lobe\(^\text{8,40–43}\). These regions are anatomically connected to the PFC\(^\text{44}\), and during working-memory tasks their activity is often synchronized with PFC activity, with the strength of synchronization predictive of the animals’ performance\(^\text{42,45,46}\). Such inter-areal interactions are likely to be important for the maintenance of delay-period activity without sensory input, although the relative importance of the different regions in various memory-guided tasks remains to be investigated.

Consistent with previous studies based on muscimol inactivation\(^\text{32,47}\), our manipulations of mouse dmPFC activity strongly affected the False Alarm rate (Figs. 3 and 4), indicating a key role for this circuit in suppressing inappropriate motor responses. NG-prefering pyramidal neurons were found to be much more abundant in deep cortical layers (Fig. 2i), raising the possibility that these cells project to distinct downstream targets\(^\text{48}\). One potential target is the subthalamic nucleus, which is selectively activated when subjects inhibit their motor responses; this activity is correlated with PFC activity\(^\text{36,49}\). Other targets of the PFC include neuromodulatory circuits such as the dorsal raphe nucleus, which contains serotoninergic neurons that are important for impulse control. A recent study showed that optogenetic activation of dorsal raphe serotoninergic neurons in the mouse suppresses premature responses\(^\text{50}\). Notably, these serotoninergic neurons also project to the PFC and can activate its VIP neurons through ionotrophic serotonin receptors\(^\text{25}\). Such reciprocal interactions between the PFC and the dorsal raphe nucleus may boost the signals in both circuits for impulse suppression.

We found that SST neuron activation caused a stronger behavioral impairment than PV neuron activation (Fig. 3), consistent with a recent study using a delayed non-match-to-place behavioral model\(^\text{1,2}\). SST neurons also exhibited stronger Go-prefering delay activity than PV neurons (Fig. 7), which may be related to Kim and colleagues’ observation that SST cells show stronger target-dependent delay activity. The most striking difference observed in our study, however, was between VIP and SST or PV interneurons. Although VIP neuron activation caused an overall decrease in dmPFC activity, the magnitude of the effect depended on both the functional property of the cell (Go- versus NG-prefering) and on the trial type (Go versus No-Go; Fig. 6b and Supplementary Fig. 12), leading to an increased Euclidean distance between the Go and No-Go trials (Fig. 6c). The overall decrease in activity could be mediated by direct inhibition of pyramidal neurons by VIP interneurons\(^\text{25,26,35}\), whereas the context-dependent firing rate modulation could be partly mediated by disinhibition of pyramidal neurons through suppression of SST or PV neurons\(^\text{22,25,26,34,35}\). Notably, while optogenetic stimulation of pyramidal neurons impaired task performance (Supplementary Fig. 11), presumably because the indiscriminate activation of Go- and NG-prefering neurons disrupted the specific activity patterns important for behavioral control, activating the VIP neuron population improved the performance (Fig. 4). This suggests that overall VNIP neuron activity, which is likely regulated by the degrees of arousal and task engagement, plays a key role in modulating the behavioral performance. VIP neurons receive numerous glutamatergic inputs from other cortical areas\(^\text{25,34}\) and neuromodulatory inputs from subcortical regions\(^\text{22}\). Thus, these interneurons may serve as a point of convergence for multiple inputs to regulate memory maintenance by the PFC.

**METHODS**

Methods, including statements of data availability and any associated access codes and references, are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

T.K. performed all the experiments and analyzed the data. T.K. and Y.D. conceived and designed the experiments and wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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1. Baddeley, A.D. *Working Memory* (Clarendon Press; Oxford University Press, 1986).
2. Fuster, J.M. *The Prefrontal Cortex* (Academic Press/Elsevier, 2008).
3. Miller, E.K. & Cohen, J.D. An integrative theory of prefrontal cortex function. Annu. Rev. Neurosci. 24, 167–202 (2001).
4. Bauer, R.H. & Fuster, J.M. Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys. J. Comp. Physiol. Psychol. 90, 293–302 (1976).
5. Buckley, M.J. et al. Dissociable components of rule-guided behavior depend on distinct medial and prefrontal regions. Science 325, 52–58 (2009).
6. Funahashi, S., Bruce, C.J. & Goldman-Rakic, P.S. Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic “scotomas”. J. Neurosci. 13, 1479–1497 (1993).
7. Erlich, J.C., Bialek, M. & Brody, C.D. A cortical substrate for memory-guided orientation in the rat. Neuron 72, 330–343 (2011).
8. Kope, C.D., Erlich, J.C., Brunton, B.W., Deisseroth, K. & Brody, C.D. Cortical and subcortical contributions to short-term memory for orienting movements. Neuron 88, 367–377 (2015).
9. Guo, Z.Y. et al. Flow of cortical activity underlying a tactile decision in mice. Neuron 81, 179–194 (2014).
10. Li, N., Chen, T.W., Guo, Z.V., Gerfen, C.R. & Svoboda, K. A motor cortex circuit for motor planning and movement. Nature 519, 51–56 (2015).
11. Li, N., Daie, K., Svoboda, K. & Druckmann, S. Robust neuronal dynamics in premotor cortex during motor planning. Nature 532, 459–464 (2016).
12. Kim, D. et al. Distinct roles of parvalbumin- and somatostatin-expressing interneurons in working memory. Neuron 92, 902–915 (2016).
13. Fuster, J.M. & Alexander, G.E. Neuron activity related to short-term memory. Science 173, 652–654 (1971).
14. Funahashi, S., Chafield, M. & Goldman-Rakic, P.S. Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. Nature 365, 753–756 (1993).
15. Miller, E.K., Erickson, C.A. & Desimone, R. Neural mechanisms of visual working memory in prefrontal cortex of the macaque. J. Neurosci. 16, 5154–5167 (1996).
16. Rigo, R., Brody, C.D., Hernández, A. & Lemus, L. Neuronal correlates of parametric working memory in the prefrontal cortex. Nature 399, 470–473 (1999).
17. Mante, V., Sussillo, D., Shenoy, K.V. & Newsome, W.T. Context-dependent computation by recurrent dynamics in prefrontal cortex. Nature 503, 78–84 (2013).
18. Baeg, E.H. et al. Dynamics of population code for working memory in the prefrontal cortex. Neuron 40, 177–188 (2003).
19. Fujisawa, S., Amarasingham, A., Harrison, M.T. & Buzsáki, G. Behavior-dependent short-term assembly dynamics in the medial prefrontal cortex. Nat. Neurosci. 11, 823–833 (2008).
20. Liu, D. et al. Medial prefrontal activity during delay period contributes to learning of a working memory task. Science 346, 458–463 (2014).
21. Courtin, J. et al. Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature 505, 92–96 (2014).
22. Fu, Y. et al. A cortical circuit for gain control by behavioral state. Cell 156, 1139–1152 (2014).
23. Kim, H., Ahrlund-Richter, S., Wang, X., Deisseroth, K. & Carlén, M. Prefrontal parvalbumin neurons in control of attention. Cell 164, 208–218 (2016).
24. Kvitkowska, D. et al. Distinct behavioral and network correlates of two interneuron types in prefrontal cortex. Nature 498, 363–366 (2013).
25. Lee, S., Kruglikov, I., Huang, Z.J., Fishell, G. & Rudy, B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat. Neurosci. 16, 1662–1670 (2013).
26. Pi, H.J. et al. Cortical interneurons that specialize in disinhibitory control. Nature 503, 521–524 (2013).
27. Pinto, L. & Dan, Y. Cell-type-specific activity in prefrontal cortex during goal-directed behavior. Neuron 87, 437–450 (2015).
28. Sparta, D.R. et al. Activation of prefrontal cortical parvalbumin interneurons facilitates extinction of reward-seeking behavior. J. Neurosci. 34, 3699–3705 (2014).
29. Lee, S.H. et al. Activation of specific interneurons improves V1 feature selectivity and visual perception. Nature 488, 379–383 (2012).
30. Chen, T.W. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300 (2013).
31. Ghosh, K.K. et al. Miniaturized integration of a fluorescence microscope. Nat. Methods 8, 871–878 (2011).
32. Zagha, E., Ge, X. & McCormick, D.A. Competing neural ensembles in motor cortex gate goal-directed motor output. Neuron 88, 565–577 (2015).
33. Meyers, E.M., Qi, X.L. & Constantinidis, C. Incorporation of new information into prefrontal cortical activity after learning working memory tasks. Proc. Natl. Acad. Sci. USA 109, 4651–4656 (2012).
34. Zhang, S. et al. Selective attention. Long-range and local circuits for top-down modulation of visual cortex processing. Science 345, 660–665 (2014).
35. Pfeffer, C.K., Xue, M., He, M., Huang, Z.J. & Scanziani, M. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nat. Neurosci. 16, 1068–1076 (2013).
36. Aron, A.R. & Poldrack, R.A. Cortical and subcortical contributions to Stop signal response inhibition: role of the subthalamic nucleus. J. Neurosci. 26, 2424–2433 (2006).
37. Wong, K.F. & Wang, X.J. A recurrent network mechanism of time integration in perceptual decisions. J. Neurosci. 26, 1314–1328 (2006).
38. Machens, C.K., Romo, R. & Brody, C.D. Flexible control of mutual inhibition: a neural model of two-interval discrimination. Science 307, 1121–1124 (2005).
39. Wimmer, K., Nykamp, D.Q., Constantinidis, C. & Compte, A. Bump attractor dynamics in prefrontal cortex explains behavioral precision in spatial working memory. Nat. Neurosci. 17, 431–439 (2014).
40. Harvey, C.D., Coen, P. & Tank, D.W. Choice-specific sequences in parietal cortex during a virtual-navigation decision task. Nature 484, 62–68 (2012).
41. Yamamoto, J., Suh, J., Takeuchi, D. & Tonegawa, S. Successful execution of working memory linked to synchronized high-frequency gamma oscillations. Cell 157, 845–857 (2014).
42. Salazar, R.F., Dotson, N.M., Bressler, S.L. & Gray, C.M. Content-specific fronto-parietal synchronization during visual working memory. Science 338, 1097–1100 (2012).
43. Miyashita, Y. & Chang, H.S. Neuronal correlate of pictorial short-term memory in the primate temporal cortex. Nature 331, 68–70 (1988).
44. Euston, D.R., Gruber, A.J. & McNaughton, B.L. The role of medial prefrontal cortex in memory and decision making. Neuron 76, 1057–1070 (2012).
45. Liebe, S., Hoeber, G.M., Logothetis, N.K. & Rainer, G. Theta coupling between V4 and prefrontal cortex predicts visual short-term memory performance. Nat. Neurosci. 15, 456–462, S1–S2 (2012).
46. Jones, M.W. & Wilson, M.A. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol. 3, e402 (2005).
47. Narayanan, N.S. & Laubach, M. Top-down control of motor cortex ensembles by dorsomedial prefrontal cortex. Neuron 52, 921–931 (2006).
48. Harris, K.D. & Shepherd, G.M. The neocortical circuit: themes and variations. Nat. Rev. Neurosci. 18, 170–181 (2015).
49. Schmidt, R., Leventhal, D.K., Mallet, N., Chen, F. & Berke, J.D. Canceling actions involves a race between basal ganglia pathways. Nat. Neurosci. 16, 1118–1124 (2013).
50. Fonseca, M.S., Murakami, M. & Mainen, Z.F. Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. Curr. Biol. 25, 306–315 (2015).
Online Methods

Animals. All experimental procedures were approved by the Animal Care and Use Committee at the University of California, Berkeley. Calcium imaging experiments were performed on CaMKII-Ire-Cre (Jackson lab stock 005359)13, PV-Cre (008669), SST-Cre (013044) and VIP-Cre (010908) mice. For optogenetic activation and electrophysiology experiments in the delayed Go/No-Go task, SST-Cre, PV-Cre or VIP-Cre mice were crossed withloxP-flanked-Chr2-eYFP mice (102569). For pyramidal cell activation, wild-type mice (C57) injected with AAV2/2-CaMKII-Ire-CreHChR2(H134R)-EYFP were used. Optogenetic inactivation experiments were performed using PV-Cre, SST-Cre and VIP-Cre mice injected with AAV2-CAG-FLEX-ArchT-dTomato and using PV-Halo mice (PV-Cre mice crossed withloxP-flanked-Halo-EYFP mice (104539)). Optogenetic activation experiments in the delayed 2-AFC task were performed using SST-Cre or VIP-Cre mice injected with AAV2-EF1α-FLEX-Chr2-eYFP. Animals were housed on a 12-h dark/12-h light cycle (light on between 7:00 and 19:00). All the experiments were carried out between 8:00 and 17:00. Imaging, optogenetic and electrophysiology experiments were performed on adult mice (2–8 months old, including both genders, singly housed and naive before experiments). Data collection and analysis were not performed blindly.

Surgery. Adult mice (2–8 months old) were anesthetized with isoflurane (3% induction and 1.5% maintenance) and placed on a stereotaxic frame (David Kopf Instruments). Temperature was kept stable throughout the procedure using a temperature controller (Thermo Fisher Scientific). After surgery, the mouse was given free water rewards (~4 µL) for each lick. In the conditioning phase, the mouse was trained to lick in response to a target tone stimulus (8 kHz, 65 dB). Each trial started with a target tone for 2 s, followed by a water port presentation within reach of the tongue. If a lick was detected within the first 2 s after the water port presentation (response window), the mouse was rewarded with ~4 µL of water. If no lick was detected during the response window, water reward was given after the end of the response window during this conditioning phase. Once the number of licks exceeded 150 within 30 min, the mouse was advanced to the next phase. In the next discrimination phase, each trial had the same temporal structure, but the auditory stimulus was either the target or non-target (2 kHz, 65 dB) tone (in Go or No-Go trials, respectively). The Go and No-Go trials were randomly interleaved, but the same auditory stimulus was never presented more than five consecutive times. Initially the temporal delay between the end of the auditory stimulus and the water port presentation was zero, and it was increased in 500-ms steps when behavioral performance reached ~70% correct in a 50-trial block. In this experiment, licks were detected by an infrared beam-break lick-meter to monitor licking even during the delay period when the water port was retracted. Comparison of the licks detected by the lickometer with those seen in video recording showed that the infrared detector was highly reliable.

After behavioral performance with the 5-s delay reached ~70% correct for more than 3 consecutive days, we started the main experiments, including calcium imaging, optogenetic and electrophysiology experiments. If the mice failed to reach the criterion performance, they were not further used for the experiments. At the start of the trial, mice were presented with an auditory stimulus for ~4 s (target or non-target tones, sample period). The sample period was followed by a 5-s delay period, and then a water port was presented (test period). Licking in response to a target tone within the first 2 s after the water port presentation (response window) was rewarded with a drop of water delivered through the water port (Hit), while licking in response to a nontarget tone triggered a combination of airpuff to the cheek (15–20 psi, 200 ms), electric shock at the tip of the tongue (less than 1 mA) and 7-s timeout period (False Alarm, FA). Water reward was delivered immediately after the first lick during the response window in Hit trials. The intertrial intervals was 7.5 s. No licking in response to a target or nontarget tone within the 2 s response window was regarded as a Miss or Correct Rejection (CR), respectively. The water port was retracted after 2 s from the first lick during the response window in Hit and FA trials or 2 s after the end of the response window in Miss and CR trials. Even though no water was delivered in No-Go (including CR and FA) trials, animals tended to lick the empty water port, which remained for 2 s after the response window (Fig. 1a). In some cases the mice continued to lick even after getting a shock, consistent with the general observation that in the Go/No-Go licking task, resisting the temptation to lick is a major challenge for the mice.

Delayed two-alternative forced choice (2-AFC) task. Each trial consisted of sample, delay and test periods. During the sample period (2 s) an auditory stimulus (either 2 kHz or 8 kHz tone, 65 dB) was presented (the corresponding trial was referred to as a ‘Left’ or ‘Right’ trial, respectively), followed by a variable delay period (1.5 to 2 s) during which water was inaccessible. At the beginning of the
test period, both the left and right water ports were simultaneously presented; licking left in Left trials within the 2-s response window was rewarded (Correct), and licking right was punished with a combination of airpuff to the cheek and a 7-s timeout period (Error); vice versa for Right trials. The intertrial interval was 7.5 s. No licking within the 2-s response window was also regarded as Error, although no punishment was applied. The water port was retracted 2 s after the first lick during the response window in both Correct and Error trials. In this experiment, licks were detected by each of the two water ports via an electrically coupled circuit board.

Microendoscopic calcium imaging. The procedure has been described in detail elsewhere\(^ {27} \). Briefly, we performed cellular-resolution microendoscopic calcium imaging from genetically defined cell types in the right dmPFC using a miniaturized integrated fluorescence microscope (Inscopix; 20x objective; LED power: 0.2–0.7 mW; CMOS sensor resolution: 1,440 × 1,080 pixels) coupled to a GRIN lens\(^ {31} \). Images were acquired at 20 frames/s using nVista HD (Inscopix). Behavioral events were synchronized with imaging by acquiring analog voltage output from both the imaging acquisition and behavioral control software using custom code in LabVIEW (National Instruments).

Optogenetic stimulation. To activate or silence each cell type during the behavioral task, we applied blue laser (473 nm; CrystaLaser, step pulses, 5 mW at fiber tip) or yellow laser (593 nm; step pulses, 15–20 mW at fiber tip) through an optic fiber (200-µm diameter) under the control of a stimulator (Grass). For optogenetic activation/inactivation experiments, an optic fiber mounted on a micromanipulator (Narishige) was inserted through the implanted guide cannula to target the dmPFC or the vS1 at a depth of 0–0.5 mm from the surface. For electrophysiology experiments, the optic fiber was placed < 1 mm from the recording site. For the delayed Go/No-Go task, in the whole-trial condition, laser stimulation started with the presentation of an auditory stimulus and continued for 9.3 s, from the sample period to the end of the response window in each trial. In the early-delay condition, laser stimulation started with the delay period onset and lasted for 2 s, thus covering the early portion of the delay period in each trial. For the delayed 2-AFC task, in the whole-trial condition, laser stimulation started with tone onset and continued for 6 s to cover from the sample period to the end of the response window in each trial. In the early-delay condition, laser stimulation started with the delay period onset and lasted for 1 s, thus covering the early portion of the delay period in each trial. Laser stimulation was applied in 50% of pseudorandomly selected trials, but the same condition never occurred in more than 5 consecutive trials.

Electrophysiology. For electrophysiology recording from behaving mice, the body of the mouse was placed in a metal tube (2.9 cm inner diameter) and the headplate was fixed on a holder. While the animal was under gas anesthesia (1.5% isoflurane in oxygen), a craniotomy (~1 mm diameter) was made over the target area in the dmPFC (center AP +1.8–2.1 mm, ML 0.3–0.5 mm). A silicon probe coated with DiI was inserted to mark the recording tract. We used 5 VIP-ChR2 mice and 6 SST-ChR2 mice for these experiments.

Pupillometry. We monitored the pupil during the delayed Go/No-Go task using an infrared camera placed approximately 2 cm away from the animal’s eye. Images were acquired at a frame rate of 30 Hz. Each frame was converted to a binary image and the pupil was fitted with an ellipse to measure its diameter using custom-written software in Matlab. For each session, pupil diameter was normalized by the mean value during the 3-s intertrial interval immediately preceding the sample period, averaged across all the Go and No-Go trials.

Imaging data analysis. Image processing was performed as previously described\(^ {27} \). The acquired images were first spatially downsampled by a factor of 4. Image stacks were then corrected for lateral motion using an algorithm based on a previous study\(^ {52} \). Regions of interest (ROIs) were drawn manually using a custom-written Matlab program, based on visual inspection of the fluorescence signals recorded in each session; we selected cells that showed at least one fluorescence transient in the session. We also compared our method with a commonly used method based on a pixel-wise activity map\(^ {27,53} \) and found that the ROIs corresponded closely to the activity map. To correct for potential contamination from out-of-focus neuropil fluorescence, for each ROI we subtracted from the raw fluorescence (\(F_{raw}\)) the average fluorescence over a 20-µm ring surrounding that ROI (\(F_{roi}\)): \(F_{roi}(t) = F_{raw}(t) - c \times F_{roi}(t)\), where \(c\) is a correction factor estimated as the ratio between the average fluorescence over a blood vessel and the neuropil adjacent to that blood vessel, both subtracted by a DC offset given by the intensity of off-lens pixels. The obtained \(c\) value was 0.77 ± 0.01 (mean ± s.e.m.), compatible with other reports that used similar methods\(^ {20,51} \). To correct for decreases in baseline fluorescence due to bleaching of the calcium indicator we subtracted slow fluctuations in baseline according to the expression \(F_{corrected}(t) = F_{roi}(t) - G(t) + [F_{roi}(t)\] \(\times G(t)\), where brackets indicate the time average over the entire recording session and \(G(t)\) is the average of \(F_{roi}(t)\) over a 300-s sliding window. Finally, average fluorescence for each ROI was calculated as \(\Delta F(t) = (F_{corrected}(t) - [F])/[F]\), where \([F]\) is the average fluorescence across the entire recording. Unless otherwise stated, \(\Delta F\) was \(z\)-scored using the fluorescence signal during the baseline period (1 s period immediately before the start of the sample period) for each trial type.

To assess whether a cell was significantly modulated by the task, we performed a one-way ANOVA with a factor of trial types (Hit or CR) for the delay activity. In each cell, \(z\)-scored \(\Delta F\) was averaged over the delay period. A cell was defined as a significant ‘Go-prefering’ cell if \(P < 0.05\) with higher activity in Hit trials than CR trials or as a significant ‘No-Go (NG)-prefering’ cell if \(P < 0.05\) with higher activity in CR trials. To further assess the task-related activity in Figures 2d–g and 7a–c, \(z\)-scored \(\Delta F\) was averaged across trials for each trial type and then smoothed with a 500-ms boxcar filter (step size 50 ms). The trial-averaged traces in Miss trials were not displayed, as the numbers of Miss trials were zero or very small in most cases.

For the analysis in Figure 2i, we estimated the absolute laminar position (i.e., distance from pial surface) of each cell by adding its relative mediolateral position in the field of view to (i) the distance between the medial border of the field of view and the medial border of the GRIN lens (determined by registration with a reference image collected on the first day of imaging); and (ii) a fixed offset for each animal, corresponding to the distance between the medial border of the lens and the cortical surface, estimated histologically after the imaging experiments. We then plotted the fraction of significant Go-prefering and NG-prefering cells against the laminar position, binned at 200 µm.

To quantify the separability between the population activity in Go trials and No-Go trials in Figure 2j, we computed the Euclidean distance for trial-averaged \(\Delta F\) traces of a specified set of cells. In each imaging session, 20 cells were randomly selected, and trial-averaged \(\Delta F\) traces were \(z\)-transformed using the entire fluorescence trace. We included both FA and CR trials in the No-Go trials. The Euclidean distances in these 20 cells were then computed for each time point (50 ms resolution) between Go trials and No-Go trials. In each session, the Euclidean distance was normalized by the mean distance during the baseline period. To examine the statistical significance of the Euclidean distance in Figure 2j, a bootstrapped permutation was performed. We randomly relabeled each trial as Go or No-Go trial and computed the Euclidean distance in each permutation. This was repeated 10,000 times to obtain the null distribution. The \(P\) value of the observed Euclidean distance was calculated as the percentile of the null distribution. To test the reliability of the correlation between the animals’ performance and log (Euclidean distance) in Figure 2k, we performed sample-with-replacement from the sessions with 10,000 resamples to obtain the distribution of Pearson’s correlation coefficient \(R\). The \(P\) value was calculated as the fraction of the resampled \(R < 0\) (one-sided test).
For Supplementary Figure 4, we performed a decoding analysis using pyramidal cell activity measured in the imaging experiments and a correlation coefficient-based classifier. The activity in each trial (z-scored ΔF/F) was aligned to sample onset, and decoding was performed for each sliding 500-ms bin (step size: 100 ms). In each imaging session, we selected 80% of Hit and CR trials as training data and the remaining 20% of each type for testing; all Miss and FA trials were used for testing. The activity templates for Go and No-Go were computed by averaging all the training data in Hit and CR trials, respectively. For decoding, the Pearson’s correlation coefficient (CC) was computed between the activity in each test trial and each of the two templates; a test trial was classified as Go/No-Go if the CC was higher/lower for the Hit than for the CR template. We repeated this cross validation procedure 100 times and then computed the proportion of each type of test trials classified as Go.

Electrophysiology data analysis. Task-related activity was examined in each cell by performing a two-way ANOVA with factors of trial types (Go or No-Go trials) and laser (on or off conditions). A cell was deemed a significant ‘Go-preferring’ cell if \( P < 0.01 \) with higher activity during delay in Go trials than No-Go trials or as a significant ‘NG-preferring’ cell if \( P < 0.01 \) with higher activity in No-Go trials. To further analyze the activity, spike trains were smoothed by convolution with a normal Gaussian kernel (\( \sigma = 50 \) ms) to obtain spike density functions (SDFs).

To examine whether the optogenetic stimulation affected the separability between the population activity in Go and No-Go trials in Figure 6c and Supplementary Figure 7b, we computed the Euclidean distance between Go trials and No-Go trials both for laser-on and laser-off conditions using trial-averaged SDFs of all the recorded cells (\( n = 529 \) cells from 5 VIP-ChR2; \( n = 226 \) cells from 6 SST-ChR2 mice). The Euclidean distance was then normalized by the mean distance during the baseline period (1 second immediately before the start of the sample period) in both conditions. Statistical significance of the difference between laser conditions was determined using bootstrap test. In each round of bootstrapping, the same numbers of cells were resampled with replacement and the normalized Euclidean distance was computed on the resampled data set. By repeating this procedure 10,000 times, we obtained a distribution of the laser-induced Euclidean distance changes and the confidence intervals. The \( P \) value of the observed difference was calculated as the fraction of the resampled data < 0 for VIP-ChR2 or > 0 for SST-ChR2 (one-sided tests). The statistical significance of the difference between laser conditions was also examined using a bootstrapped permutation. We randomly relabeled each trial as laser-on or laser-off and computed the Euclidean distance in each permutation. This was repeated 10,000 times to obtain the null distribution of the laser-induced Euclidean distance changes. The \( P \) value of the observed difference was calculated as a percentile of the null distribution.

Behavioral data analysis. For the analysis of behavioral data, correct rate, Hit and FA rates were quantified as follows:

- Correct rate = (number of Hits + number of CRs)/total number of trials
- Hit rate = number of Hits/(number of Hits + number of Misses)
- FA rate = number of FAs/(number of FAs + number of CRs)
- Based on the Hit and FA rates, auditory discriminability (d’) was quantified by:
  \[
  d' = \text{norminv}(\text{Hit rate}) - \text{norminv}(\text{FA rate})
  \]
  where ‘norminv’ is the inverse of the cumulative normal function. Higher \( d' \) values indicate better behavioral performance.

Wilcoxon signed-rank test was performed to determine the statistical significance of the behavioral effects caused by optogenetic stimulation.

Randomization. For all optogenetic stimulation experiments, laser stimulation was applied in 50% of pseudorandomly selected trials, but the same condition never occurred in more than 5 consecutive trials. In both the delayed Go/No-Go and 2-AFC tasks, two kinds of auditory stimulus (either 2 kHz or 8 kHz tone) were randomly interleaved, but the same auditory stimulus was never presented more than 5 consecutive times. In the delayed 2-AFC task, the duration of the delay period was chosen randomly from a uniform distribution between 1.5 and 2 s.

Statistics. Statistical analysis was performed using Matlab. All statistical tests were two-sided unless otherwise stated. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications.

To assess whether a cell was significantly modulated by the task, one-way ANOVA was used for imaging experiments and two-way ANOVA was used for electrophysiology experiments. To test whether the fraction of significant Go- and NG-preferring cells changed depending on the distance from pia, we used one-way ANOVA (5 levels of distance). Wilcoxon signed-rank tests were performed to determine the statistical significance of the behavioral effects caused by optogenetic stimulation. The significance of the correlation between the animals’ performance and log (Euclidean distance) was tested using a bootstrap test (one-sided test). The statistical significance of the difference in the Euclidean distance between laser conditions was determined using a bootstrap test (one-sided test) and a bootstrapped permutation test.

A Supplementary Methods Checklist is available.

Data and code availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. Matlab codes can be requested from the corresponding author.

51. Tsien, J.Z. et al. Subregion- and cell type-restricted gene knockout in mouse brain. Cell 87, 1317–1326 (1996).
52. Ziv, Y. et al. Long-term dynamics of CA1 hippocampal place codes. Nat. Neurosci. 16, 264–266 (2013).
53. Ahrens, M.B. et al. Brain-wide neuronal dynamics during motor adaptation in zebrafish. Nature 485, 471–477 (2012).
54. Kerlin, A.M., Andermann, M.L., Berezovskii, V.K. & Reid, R.C. Broadly tuned response properties of diverse inhibitory neuron subtypes in mouse visual cortex. Neuron 67, 898–971 (2010).
55. Meyers, E.M., Freedman, D.J., Kreiman, G., Miller, E.K. & Poggio, T. Dynamic population coding of category information in inferior temporal and prefrontal cortex. J. Neurophysiol. 100, 1407–1419 (2008).
56. Stokes, M.G. et al. Dynamic coding for cognitive control in prefrontal cortex. Neuron 78, 364–375 (2013).
Erratum: Delay activity of specific prefrontal interneuron subtypes modulates memory-guided behavior

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In the version of this article initially published online, a duplicate of the panel title “Cell 2” was overlaid across the image in Figure 6a. The error has been corrected in the print, PDF and HTML versions of this article.