mining whether the bacterium could replicate extracellularly in that organ in living mice. To localize the bacteria in the gall bladder, we used image-guided histology of infected gall bladders (Fig. 2D). The bacteria were found extracellularly in the lumen of the organ, as has been previously described for the murine bile duct of infected animals (13). The bacterial cells were most often in chains, suggesting replicative forms. The methods employed in staining are capable of revealing intracellular bacteria, but none were observed. No signs of inflammation or tissue damage were observed. Because signal intensity depends on many parameters, possibly leading to a decreased ability to detect the bacteria, photon flux was correlated with cfu counts from the gall bladder lumen (fig. S2).

The results show photons and cfu counts to be related, with a correlation coefficient of 0.86 (fig. S2B).

Mutants of LLO (Δhly) are greatly attenuated in mice, primarily because of their inability to replicate intracellularly (14). To examine the effect of the Δhly mutation on the ability of L. monocytogenes to grow in the gall bladder, we transduced the 2C lux insertion (15) into an L. monocytogenes Δhly 10403S strain. Logarithmic phase–dependent BLI signals from the gall bladder would be consistent with bacterial growth in that organ. Only growing L. monocytogenes emitted detectable signal (fig. S3), indicating that metabolically active bacteria were producing the signal in vivo.

The bioluminescently labeled 2CΔhly (LLO-negative) strain was then used to infect female 6-week-old Balb/c mice. Strong signals were detected from the gall bladder, beginning 3 to 4 days post-inoculation (Fig. 3, A and B). The absence of internalins A and B does not affect the ability of L. monocytogenes to grow in the gall bladder, as a Δhly ΔinlA/B mutant retained this ability (Fig. 3C). The number of Δhly cfu recovered from gall bladders of animals with strong signals (3 × 10⁷ cfu per gall bladder) was much greater than that recovered for Δhly bacteria in the entire liver after 24 hours (14).

Together, the growth-phase dependence of BLI signal, the high number of bacteria recovered from the gall bladder luminal contents, the persistent presence of the signal in deletion mutants of hly, and the histological localization of the bacteria in the lumen indicate that L. monocytogenes grows extracellularly in the murine gall bladder. L. monocytogenes is known to express a bile salt hydrolase that affects virulence when deleted (7). These and other adaptations may help the bacteria to grow transiently in the gall bladder, possibly escaping the immune system and gaining spread from the secretion of bile into the intestine, perhaps to reinfest the same animal or to be transmitted (13). It is unknown whether L. monocytogenes replicates in the gall bladder of asymptomatic humans; cases of listerial cholecystitis, inflammation of the gall bladder, have been reported (16), but only in patients with severe disease. If healthy individuals support growth of L. monocytogenes in the gall bladder, listeriosis could be spread unknowingly, in a manner similar to that of typhoid fever.

References and Notes


Selective D2 Receptor Actions on the Functional Circuitry of Working Memory

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Prefrontal neurons engaged by working memory tasks express a sequence of phasic and tonic activations linked to a train of sensory, mnemonic, and response-related events. Here, we report that the dopamine D2 receptor selectively modulates the neural activities associated with memory-guided cascades in oculomotor delayed-response tasks yet has little or no effect on the persistent mnemonic-related activity, which is instead modulated by D1 receptors. This associates the D2 receptor with a specific component of working memory circuitry and fractionates the modulatory effects of D1 and D2 receptors on the neural machinery of a cognitive process.

The D2 family of dopamine receptors has long been of interest because of its involvement in a wide range of affective, motor, and cognitive functions, many of which are the output neurons of the cortex. This suggests that D2 actions in cortical circuits might be associated with particular motor commands (11), or motor feedback mechanisms such as corollary discharge (14, 15).

In the present study, we combined single-neuron recording in nonhuman primates performing an eight-target spatial oculomotor delayed-response (ODR) task with iontophoretic application of selective D2 agonists and antagonists to examine the role of D2 family receptors in the functional properties

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of prefrontal neurons. Behavioral training, neurophysiological recording, and iontophoretic application of drugs were as described previously (16–18) (Materials and Methods). The dorsolateral prefrontal cortex is an excellent site to study D2 actions, because neurons in this area express different profiles of activity that correspond to distinct behavioral events in spatial delayed-response tasks, related respectively to (i) the registration of a stimulus (cue-related), (ii) the maintenance of the trace of the foregoing stimulus (delay-related), and (iii) the execution of a response at the end of the delay (response-related) (19, 20). The interrelated actions of these neurons have been proposed as the circuit basis of working memory (21). In the present study of 102 task-related neurons from the dorsolateral prefrontal cortex in two monkeys, 10 neurons were classified as pure cue-related, 47 as sustained delay-related, and 45 as pure response-related (saccade-related) by two-way analysis of variance (ANOVA), with task epoch versus baseline as factors (P < 0.01).

Given the low level of D2 receptors in cortex and preferential expression in the output layer of the cortex, we expected that D2 receptors might have distinct effects on the firing properties of prefrontal neurons engaged in working memory tasks. We found that, in contrast to the D1 receptor, which alters the delay-related activity of prefrontal neurons (17), the D2 receptor is reliably associated with saccade-related activity at the end of the trial. These findings indicate that actions of different dopamine receptors in the prefrontal cortex differ not only pharmacologically but substantially with respect to cellular circuit and functional specificity.

The selective D2 antagonists, raclopride or eticlopride, were iontophoretically applied to 20 saccade-related neurons in the prefrontal cortex as an initial drug condition. Both drugs clearly attenuated saccade-related activity when applied at 25- to 40-nA ejection current in 18 of 20 cases (one-way ANOVA for each neuron; P < 0.05). In Fig. 1A, raclopride (40 nA) significantly decreased saccade-related activity for the neuron’s preferred direction (P < 0.01) but had no effect on activity recorded during trials for nonpreferred targets (P > 0.05) nor during other periods of the task (P > 0.05). This attenuation for the preferred directional targets during the saccade period was consistently reversed by iontophoresis of the selective D2 agonist, quinpirole, at 25 to 40 nA (9 of 12 cases; P < 0.01). Conversely, D2 receptor stimulation significantly augmented saccade-related neuronal activity in 10 of 13 neurons examined with quinpirole as the first drug condition (example shown in Fig. 1C, left; P < 0.05). Population analysis of 26 saccade-related neurons selected on the basis of their postsaccadic response profile and comparable dosing in the range of 25 to 40 nA confirmed these results and demonstrated their robustness (Fig. 2A). Moreover, the effects of D2 blockade and stimulation were dose-dependent; higher doses of the D2 antagonist induced larger reduction of the saccade-related activity for the preferred direction. Similarly, higher doses of the D2 agonist produced greater enhancement of the saccade-related activity (Fig. 1B).

Most saccade-related neurons (34 of 45) exhibited postsaccadic activation; that is, the peak activity was present immediately after the termination of the saccade. However, 11 neurons (24%) exhibited presaccadic activity that began before the initiation of the eye movement. Figure 1C shows rasters aligned at the initiation of saccades in the response period for one presaccadic neuron (H208), which began firing 50 ms before the initiation of the saccade to its preferred target, and one postsaccadic neuron (H224), which began firing 100 ms after the completion of the saccadic eye movement. D2 blockade or stimulation had specific effects on both pre- and postsaccade-related activity.

We also examined the effects of the D2 antagonist and agonist on 47 neurons that expressed spatially tuned persistent activity in the delay period of the ODR task. In contrast to the effect of the D2 agents on saccade-related activity, neither raclopride nor quinpirole had significant effects on tonic delay-related (mnemonic) activity in 43 out of 47 neurons. This finding was clear both for individual cells (P > 0.05) (Fig. 3A) and at the population level (Fig. 2B). Similarly, these drugs (25 to 40 nA) were without effect on any of the 10 neurons that exhibited directionally selective phasic cue-related activity in 43 out of 47 neurons. This observation was made both for individual cells (P > 0.05) (Fig. 3A) and at the population level (Fig. 2B).

### Fig. 1. Effects of D2 receptor blockade and stimulation on directionally selective saccade-related activity.

(A) Rasters and average histograms of unit H113 during the control condition (top) and during iontophoresis of the D2 antagonist, raclopride (middle), and the D2 agonist, quinpirole (bottom). Recordings for preferred and nonpreferred directions are shown. Quinpirole reversed the raclopride’s depression of the phasic postsaccadic response. (B) Dose-dependent effects of raclopride (left) and quinpirole (right) on saccade-related activity. (C) Examples of presaccadic (neuron H208) and postsaccadic (neuron H224) neuronal activity, respectively. Rasters and histograms are aligned at saccade initiation in the response period (saccade initiation at time t = 0).
Finally, to examine whether the D2-mediated modulation of saccade-related activities in prefrontal neurons was pharmacologically specific, we examined SCH39166, a selective D1 antagonist, in 12 experiments, 6 of which also tested neurons with a D2 antagonist. Iontophoretic application of SCH39166 (25 to 40 nA) failed to depress saccade-related activity in any of these neurons. The neuron shown in Fig. 4A was tested successively with a D1 antagonist and a D2 antagonist at 25 nA. The saccade-related activity of this cell was impervious to the D1 antagonist ($P > 0.05$) and dramatically attenuated by the D2 antagonist ($P < 0.001$). Dissociable effects of D1 and D2 receptors were also evident in the effects of agonists on delay-related activity. The delay-specific activity of the neuron shown in Fig. 4B was sharply reduced by the D1 agonist, SKF38393, at 40 nA ($P < 0.0001$), whereas the D2 agonist, quinpirole (40 nA), applied after recovery was ineffective in altering the cell’s firing profile ($P > 0.05$). We thus conclude that neurons with saccade-related activity either have a high density of postsynaptic D2 receptors or receive an external input from neurons that do, whereas cells expressing memory-related persistent activation are preferentially modulated by D1 receptors (17).

The present study dissociates the actions of D1 and D2 receptors on cortical circuit elements engaged in a distinctly cognitive function: working memory. Such independence is consistent with a number of recent studies of mice lacking D2 receptors or isoforms of this receptor. D1-mediated functions in these animals are generally preserved, whereas D2-mediated behaviors, particularly locomotor behaviors, are disturbed (2, 3). D2 stimulation enhanced neural activity, whereas D1 receptor stimulation attenuated it, indicating that the actions of these two receptors differ not only in their signaling transduction pathways (22) but also in their physiological actions and the nature of the component neural processes they target.

The question naturally arises as to whether the saccadic activities observed are related to reward. This does not seem to be the case. In our paradigm, reward is given for the monkey’s eye movements when they are made to all directions, yet the saccade-related activities are restricted to trials in which responses are made to preferred targets. Another possible explanation for the selective postsaccadic activation is that it represents the preparation of the monkey to perform a return eye movement to the fixation spot. This is unlikely, however, because the...
same postsaccadic signal was observed whether the animal kept its eyes on the target for 0.5 to 1.5 s once it was acquired (monkey H) or moved its eyes randomly around the screen as soon as the saccade was completed (monkey M).

An attractive possibility is that the phasic saccadic responses modulated by dopamine receptors may be a corollary discharge that informs the prefrontal network that a motor command has been completed. Recent studies in the primate oculomotor system provided evidence that the projections from the superior colliculus (27) could also provide this signal. It is of considerable interest that a deficit in corollary discharge has been proposed as a mechanism for explaining positive symptoms in schizophrenia (13, 14).

It has been reported that D2 receptor agonists improve working memory performance in both monkeys (29) and humans (4, 5). Further, quinpirole has been shown to induce hallucinatory-like behaviors in monkeys (29), and hallucinations in human patients have been related to weak corollary discharge from prefrontal cortex (15). The modulation of the saccadic signal by D2 selective drugs in the present study provides a cellular basis for this proposition and possible dysfunctional manifestations in schizophrenia.

References and Notes
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Materials and Methods
Fig. 5
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