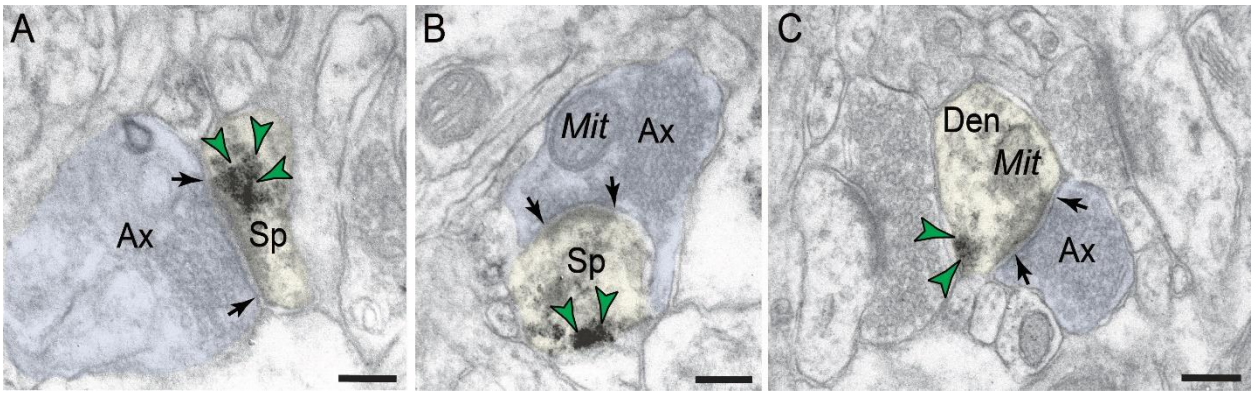
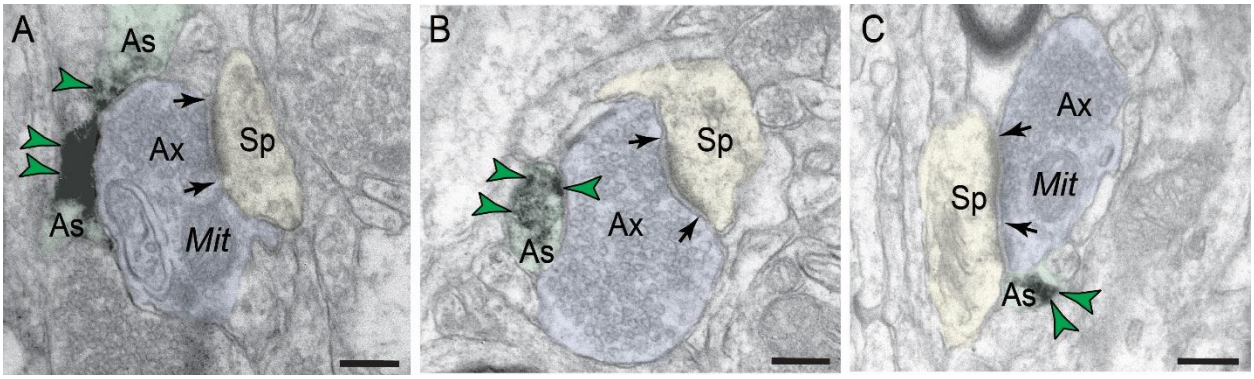
Figure S1: M1R localization in postsynaptic compartments in dlPFC layer III



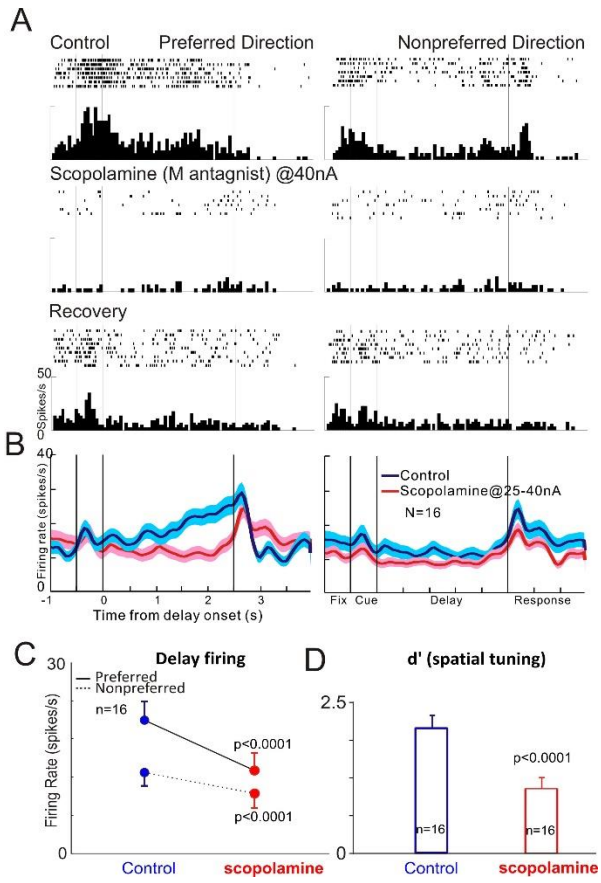
Related to Figure 2. **A-B.** M1R is visualized in dendritic spines in postsynaptic locations in dlPFC layer III microcircuits. The labeling pattern for M1R is typically in association with the postsynaptic density (PSD), and in perisynaptic (panel A) and extrasynaptic (panel B) subcompartments, near axospinous asymmetric glutamate-like synapses. **C.** Delicate labeling for M1R can also be visualized in dendritic shafts receiving asymmetric glutamate-like asymmetric synapses. These are putatively GABAergic dendrites. Synapses are between arrows. Color-coded arrowheads (green) point to M1R immunoreactivity. Profiles are pseudocolored for clarity. Ax, axon; Sp, spine; Den, dendrite; Mit, mitochondria. Scale bars, 200 nm.

Figure S2: Astrocytic expression of M1R in monkey dlPFC layer III



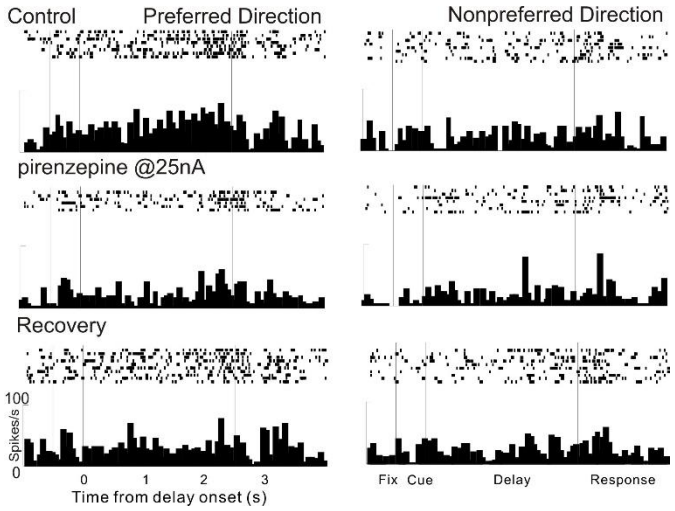
Related to Figure 2. **A-C.** M1R labeling is visualized in astrocytic leaflets ensheathing axospinous asymmetric glutamate-like synapses on dendritic spines. The labeling is predominantly concentrated on the astrocytic plasma membrane. Note that the receptor is not distributed uniformly on the plasma membrane, but occurs in more specific subcompartments, extrasynaptically (A-B), or perisynaptically (C). Sparse M1R intracellular cytosolic labeling in astrocytes is also observed (A). Synapses are between arrows. Color-coded arrowheads (green) point to M1R immunoreactivity. Profiles are pseudocolored for clarity. Ax, axon; Sp, spine; Mit, mitochondria; As, astrocytes. Scale bars, 200 nm.

Figure S3: **General muscarinic receptor antagonist, scopolamine, significantly reduced delay-related firing and spatial tuning of dlPFC Delay cells**.



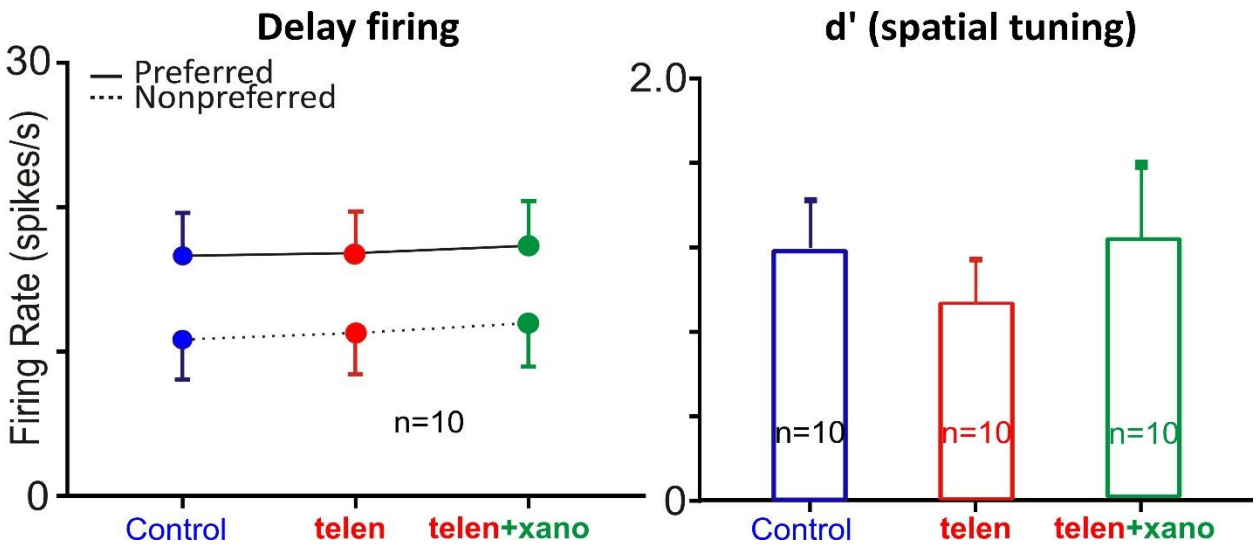
Related to Figure 3. **A.** An example neuron is shown that iontophoresis of the general muscarinic receptor antagonist, scopolamine, markedly reduced delay-related firing for the neuron's preferred and non-preferred directions, and the firing was partially restored when scopolamine was no longer applied (recovery). **B**. population spike density functions for the average of 16 delay cells showing firing for their preferred vs. their non-preferred directions under control conditions (blue) and following iontophoresis of scopolamine conditions (red). **C**. the mean±SEM firing rate of 16 Delay cells during the Delay period of the task. Scopolamine significantly reduced the delay-related firing for both the preferred and the non-preferred direction, with greater reduction for the preferred direction (R-two-way ANOVA, $F_{directionxdrug}(1,15)=29.83$, $p<0.0001$; Sidak's multiple comparisons: preferred direction, $p<0.0001$ and nonpreferred direction, $p<0.0001$). **D**. iontophoresis of scopolamine significantly decreased the spatial tuning of Delay cells by decreasing d' (control vs scopolamine, $t(15)=5.441$, $p<0.0001$, two-tailed paired t test).

Figure S4: M1R antagonist, pirenzepine, reduced delay-related firing and spatial tuning of dlPFC Delay cells.



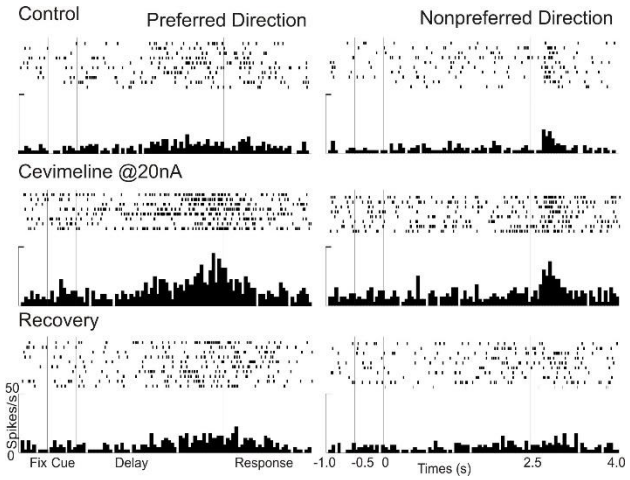
Related to Figure 3. An example neuron is shown that iontophoresis of the M1R antagonist, pirenzepine, markedly reduced delay-related firing for the neuron's preferred direction, and the firing was partially restored when pirlenzepine was no longer applied (recovery).

Figure S5: The M1R antagonist, telenzepine, prevented the enhancing effects of M1R stimulation in population level, consistent with xanomeline actions on M1R.



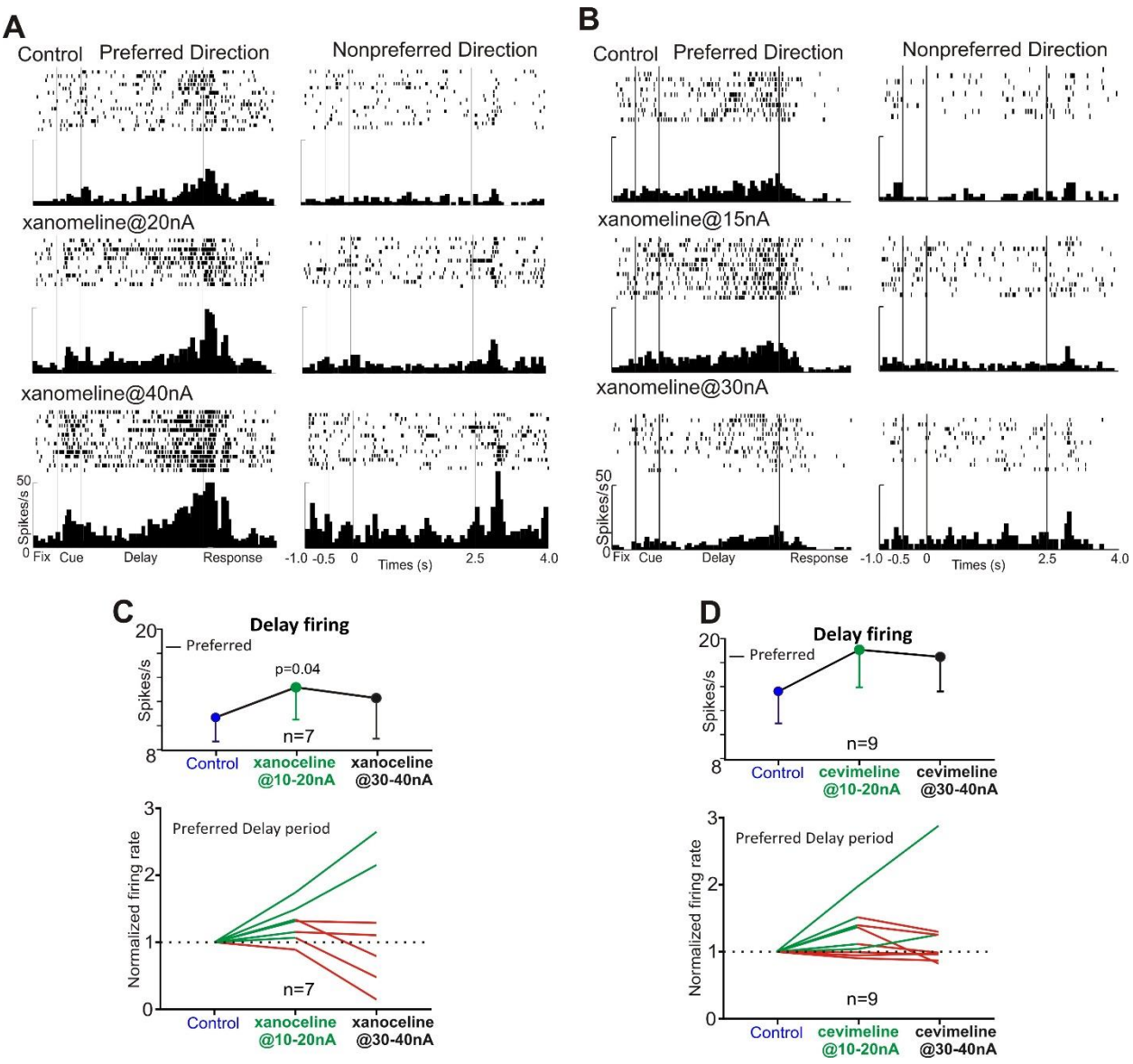
Related to Figure 3 and Figure 4. At the population level (n=10) a low dose of the M1R antagonist telen, at a dose that has no effect on delay firing or spatial tuning alone, prevents the enhancing effect of the M1R agonist xano. See main text for analyses.

Figure S6: M1R agonist, cevimeline, enhanced delay-related firing and spatial tuning of dlPFC Delay cells.



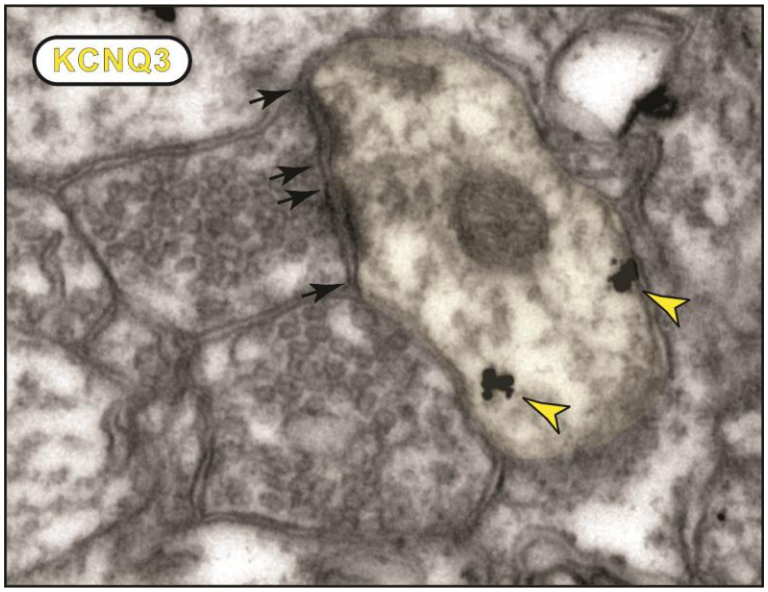
Related to Figure 4. An example neuron is shown that iontophoresis of the M1R agonist, cevimeline, significantly enhanced delay-related firing for the neuron's preferred direction, and the firing was reduced to control level when cevimeline was no longer applied (recovery).

Figure S7: The mixed effects of higher M1R stimulation on delay-related firing of dlPFC Delay cells.



Related to Figure 5. **A**. An example of an individual neuron that showed a linear, dose-related increase in firing. A low dose of xanomeline (20nA) increased delay firing for the preferred direction, while subsequent application of xanomeline at 40nA further increased delay firing. **B.** An example of an individual neuron with an inverted U dose response. xanomeline at 15nA significantly increased delay firing, but subsequent application at 30nA decreased delay firing. **C, D**, The mixed, inverted U effect of higher M1R stimulation at the population level, with 5 of 7 neurons at higher doses of xamomeline, 7 of 9 neurons at higher doses of cevimeline showing decreased firing.

Figure S8: Immunogold localization of KCNQ3 on a dendrite with interneuron characteristics in layer III dlPFC.



Related to Figure 6. KCNQ3 labeling is seen on or near the plasma membrane of a dendrite receiving asymmetric synapses characteristic of an interneuron.