Fig. S1. Electrically and optogenetically evoked DA transients are similar. DA transients evoked by electrical (Left) or optogenetic (Right) stimulation show near identical time courses, CV plots, and voltammograms. (Scale bars, 200 nM and 1 s.)
Fig. S2. Comparison of the effect of muscarinic drugs and cocaine on the decay time constant and amplitude of oDA transients. (A, Left) Time course of the effect of oxo-m and scopolamine on the decay time constant (open) and amplitude (filled) of oDA transients in DAT-cre. (Right) Representative oDA transients before (black), during oxo-m (10 μM, red), and during scopolamine (1 μM, gray). (B and C) Time course of the effect of oxo-m and scopolamine on (B) the decay time constant and (C) the peak of oDA transients without (black) or with 3 μM cocaine (blue). In the presence of 3 μM cocaine, oxo-m produced a smaller increase in the decay time constant compared with when cocaine was not present (17 ± 2% vs. 35 ± 7% increase in time constant in the presence and absence of 3 μM cocaine, respectively; n = 6–8/3–3; P < 0.001 by unpaired t test). These data suggest that oxo-m acts on DAT to increase the tau, and that there are other mechanisms affected by the muscarinic agonist. Furthermore, in the presence of cocaine, oxo-m produced a similar increase in the peak of DA transients (14 ± 3% vs. 18 ± 2% increase in peak in the presence and absence of 3 μM cocaine, respectively; P = 0.425). These results suggest that the muscarinic agonist oxo-m affects the mechanism of DA reuptake to increase the decay time constant of the transients. (D) Cocaine increased the decay time constant and the peak of oDA transients in a concentration-dependent manner (τ = 25 ± 3% by 100 nM and 48 ± 6% by 200 nM cocaine, respectively; n = 9 slices/4 DAT-cre mice; t₈ > 9.087; P < 0.001; peak = 8 ± 2% by 100 nM and 13 ± 3% by 200 nM cocaine, respectively; t₈ > 4.9; P < 0.002). (Left) Time course of the effect of increasing cocaine concentration on the decay time constant (open) and peak amplitudes (filled) of oDA transients. (Right) Representative DA transients before (black) and during increasing concentrations of cocaine (blue). (E) Percentage increase in the peak of oDA transients was plotted as a function of the percentage increase in the decay time constant for cocaine, mAChR agonists, and other cholinergic drugs. Although the relationship was linear for cocaine when varying the concentration, the data points for the mAChR agonists/cholinergic drugs were found to be above the line, as they showed a larger increase in the peak relative to the decay time constant compared with cocaine.
**Fig. S3.** Depression of eDA transients by oxo-m is intact, but potentiation of eDA transients is absent in M₅KO mice in the presence of a nAChRs blocker. (A and B) Effect of oxo-m and scopolamine on eDA transients evoked by electrical stimulation in M₅KO mice (A) in the absence and (B) in the presence of mecamylamine (20 μM). (Top) Representative traces before (black), during oxo-m (10 μM, red), and during scopolamine (1 μM, gray). (Bottom) Time course of the effect of oxo-m and scopolamine on the normalized DA transient amplitudes (mean ± SEM). (Scale bars, 200 nM and 2 s in A; 100 nM and 2 s in B.)

**Fig. S4.** Summary of pharmacologic effects of muscarinic/cholinergic drugs on oDA transients and oEPSCs. (A and B) Percentage change in the amplitude of oDA transient (A) and oEPSC (B) induced by the muscarinic agonists oxo-m (10 μM) and carbachol (10 μM), and the acetylcholine esterase inhibitors physostigmine (10 μM) and ambenonium (1 μM) in three different mouse strains. All data are mean ± SEM. *Significant difference determined by one-sample t test for each drug, or unpaired t test of oxo-m effects between DAT-cre and vGluT₂-cre mice.