Supplementary Figure 1

Selectivity of D2 and α1A CNiFERs characterized in vitro.

(a) D2-CNIFER FRET response to 20 nM DA alone or in the presence of D1-receptor antagonist SCH 23390 (100 nM, blue, n = 5; p = 0.89, t-test) or D2-receptor antagonist eticlopride (50 nM, red, n = 5, p = 0.0003, t-test). (b) α1A-CNIFER response to 50 nM NE alone or in the presence of β-adrenergic receptor antagonist sotatol (5 µM, blue, n = 4; p = 0.17, t-test), or α1A-receptor antagonist WB4101 (50 nM, red, n = 4, p = 0.0001, t-test). CNIFER response to agonist alone normalized to one, ** p < 0.001.
Supplementary Figure 2

*In vitro* characterization of CNiFERs to repeated pulses of agonist.

(a) Single-trial response of seven individual D2 CNiFERs and (b) seven individual α1A CNiFERs to a single 2.5 s pulse of 100 nM DA (left) or 100 nM NE (right).
Supplementary Figure 3

Identification of dopaminergic and noradrenergic projections to frontal cortex.

(a) Immunostaining for tyrosine hydroxylase (green), Fluorogold™ tracer (magenta) injected ~ 200 µm deep into frontal cortex (+1.5 mm A/P, +1.5 mm M/L), and NeuroTrace®*, a Nissl stain that labels neurons (blue). Coronal sections including substantia nigra (SN) (left, A/P -3.5 mm) or locus coeruleus (LC) (right, A/P -5.6 mm). (b) Co-labeling of tyrosine hydroxylase (green) and Fluorogold™ (magenta) in SN (left) or LC (right), magnified from cyan boxes in (a). (c) Position of co-labeled cell bodies in SN (left) or LC (right) indicated by magenta dots imposed on three-dimensional reconstructions as outlined by grey in (a).
Supplementary Figure 4

Individual mouse FRET onset times plotted as a function of conditioning day.

Error bars represent standard error (n = 13). (a) Licking onset times during conditioning trials (CS, grey bar; US, dashed red line) across five days of conditioning. (b) D2-CNIFER FRET response onset times during conditioning. FRET onset times are measured relative to CS onset (n = 13). (c) α1A-CNIFER onset times during conditioning (n = 7). (d) M1-CNIFER onset times during conditioning (n = 4). (e) Example of M1-CNIFER FRET response in a trial where the animal engaged in high frequency licking but there was no CS or US presentation.