Supplementary figures

Figure S1 Method of confocal spectral imaging

A, A lambda stack of confocal images of SNC neurons from an α4YFP Hom mouse, 500-596 nm at 10 nm intervals obtained with a 488 nm excitation line of an Argon laser.

B, Emission spectra of α4YFP from a Hom mouse and from autofluorescent background from a brain section from a WT mouse. Note the signature peak at ~527 nm for α4YFP, while autofluorescence from fixed brain tissue has a broad spectrum. Spectral unmixing, using YFP and WT tissue as reference spectra, deconvolves a lambda stack of brain section images of an α4YFP mouse into pure α4YFP signal (C) and autofluorescent signal (D).

Figure S2 Whole-cell patch clamp and fura-2 recordings of cultured ventral midbrain neurons from α4YFP mice

A) Whole-cell electrophysiological recordings show similar magnitudes of ACh induced nicotinic currents between WT and HOM α4YFP cultured midbrain neurons at low (3 μM) and high (300 μM) ACh concentrations.

B) Fura-2 recordings show nearly identical ACh dose-response relations for WT and HOM α4YFP cultured midbrain neurons.

Figure S3 Hot-plate assay to assess nicotine-induced antinociception

A, Control baseline responses on the hot-plate for α4 KO, WT, Het, and Hom α4YFP mice.

B, Antinociception responses on the hot-plate to various doses of nicotine (0, 1.0 and 1.5

mg/kg s.c.) for KO, WT, Het, and Hom α4YFP mice.

Figure S4 α4YFP localization in the ventral and dorsal striatum

A, α4YFP expression in a coronal section of the caudate putamen.

B, Spectrum confirming true α4YFP fluorescence.

C, Sagittal section of the mouse brain(Paxinos and Franklin, 2004). Pink circle shows the approximate location imaged in the caudate putamen of the brain section

(D-F). Green circles indicate approximate locations imaged showing the nigrostriatal pathway and the dopaminergic pathway terminating in the nucleus accumbens (C, G-L). D-F, A sagittal brain section of the caudate putamen showing double labeling of α4YFP (green) and tyrosine hydroxylase (TH, red). α4YFP is expressed in both dopaminergic and nondopaminergic fibers coursing through the caudate putamen. This example shows, unusually, somewhat higher expression in nondopaminergic fibers.

G-I, A sagittal section showing α4YFP expression in TH+ dopaminergic neurons (red) in the SNC and VTA region. Dopaminergic fibers can be seen originating from this region.

J-L, A sagittal section showing very low but detectable α4YFP expression along dopaminergic fibers (red) terminating in the nucleus accumbens.

Figure S5 α4YFP localization in the thalamus and internal capsule

A-C, α4YFP localization in reticular thalamic neurons, which are the only GABAergic neurons in the mouse thalamus. The section is double labeled with MAP-2 (red), a somatodendritic marker. The unresolved haze in the background includes α4YFP -

containing axonal fibers, shown in higher power in E,F.

D, Spectrum with a distinct peak at ~527 nm confirms true α4YFP located in the neurons.

E, F, High and lower power images of thalamic axons labeled with α4YFP. G, Internal capsule showing α4YFP localized in axons.

Figure S6 Electrophysiological and pharmacological properties of SN neurons

A, Typical averaged action potential traces from a putative SNR GABAergic neuron and a putative SNC DA neuron.

B, Putative SNR GABAergic neurons fire more rapidly than putative SNC DA neurons. Each point corresponds to a single neuron.

C, An exemplar putative SNC DA neuron (left trace), but not an exemplar putative SNR GABAergic neuron (right trace), expresses inward currents in response to hyperpolarizing voltage steps (to test potentials of -60 to -160 mV at increments of -20 mV).

D, Typical traces (upper) and time courses (lower) of firing rates in an exemplar putative SNC DA (left panels) and SNR GABAergic (right panels) neurons before, during and after bath application (5 min) of 1 μM DAMGO, a μ-opioid receptor agonist.

E, Typical traces (upper) and time courses (lower) of firing rates in a putative SNC DA neuron (left panels) and a putative SNR GABAergic neuron (right panels) before, during and after the bath application (5 min) of 0.2 mM Quinpirole, a D2-like dopamine receptor agonist.