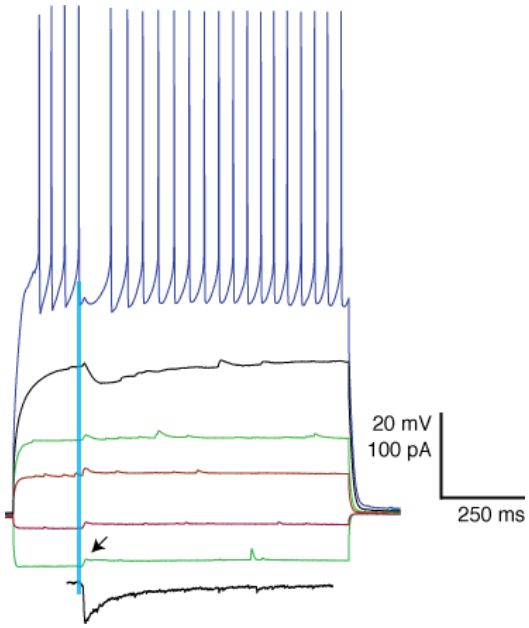
English et al.

Supplementary information

Novel GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons

Daniel F. English, Osvaldo Ibanez-Sandoval, Eran Stark, Fatuel Tecuapetla, Gyorgy Buzsaki, Karl Deisseroth, James M. Tepper and Tibor Koos.

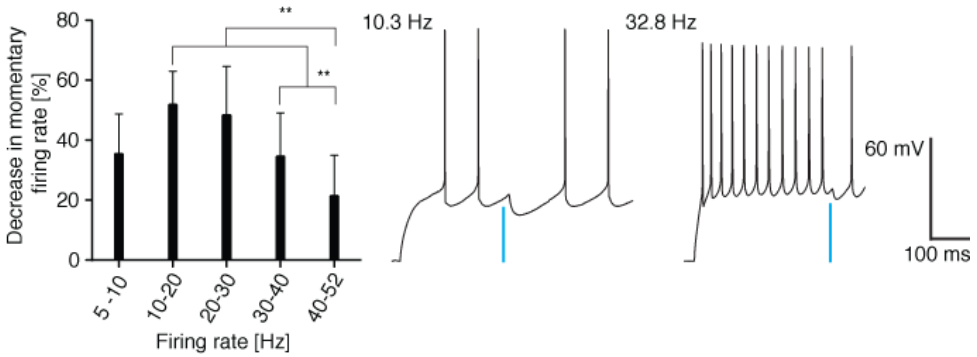
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 1. The optically induced IPSP/C in SPNs persists in the presence of AMPA receptor blockade

Top traces: Responses of an SPN to optical stimulation during hyperpolarizing and depolarizing current injection. V_{rest} = -88 mV. Arrow indicates first depolarizing IPSP. *Bottom traces:* IPSC recorded in a different SPN using $CsCl^-$ internal solution. The neuron is voltage clamped at -90 mV. 20 μM CNQX is present in the aCSF in both recordings. Blue bar represents 5 ms blue light optical stimulus. Both experiments were performed in slices from animals expressing ChR2-EYFP in ChAT interneurons.

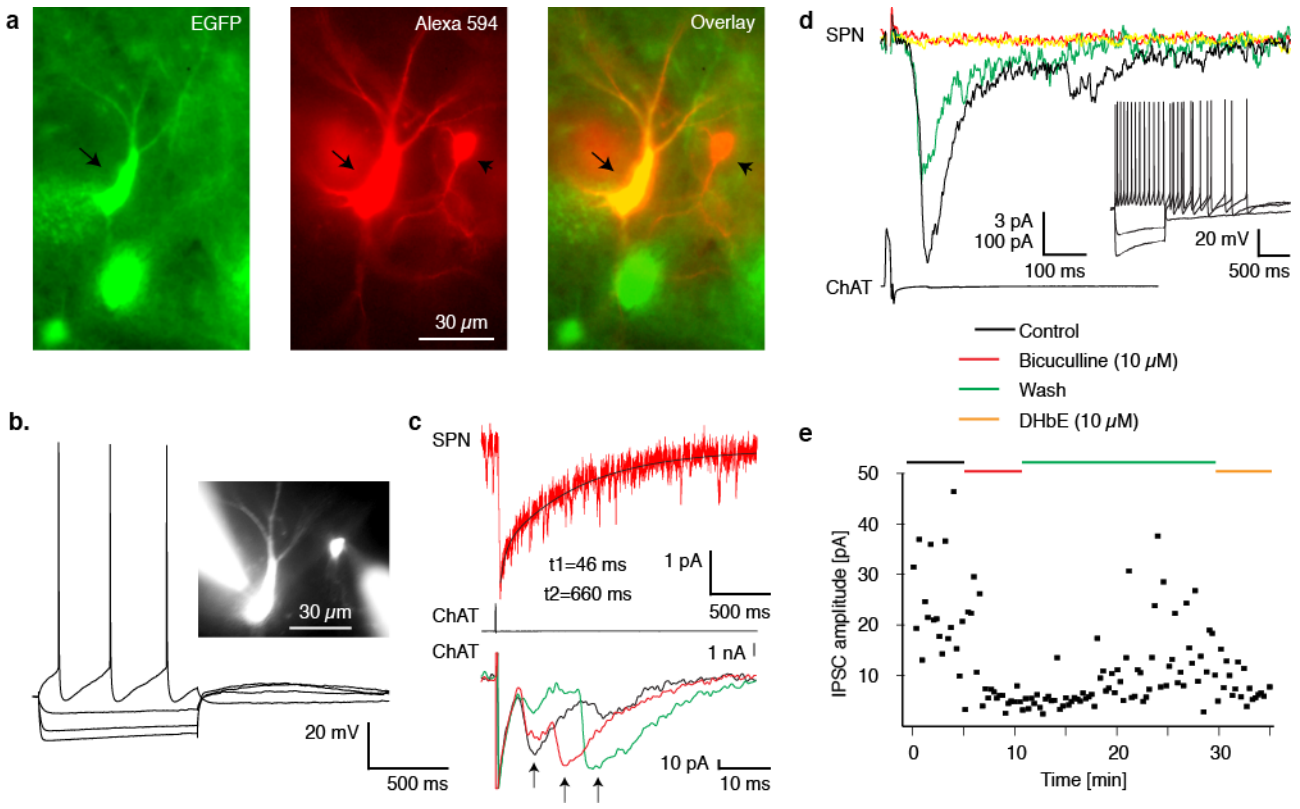
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 2. The magnitude of the inhibition of the evoked firing of SPNs depends on the momentary postsynaptic firing rate.

Left. The magnitude of reduction in momentary firing rate as a function of the initial rate (one-way ANOVA, $p<.05$, Tukey post-hoc test, $^{**}p<.01$). *Right.* Representative examples of inhibition at low and high rates of firing. Blue bars represent 5 ms optical stimuli.

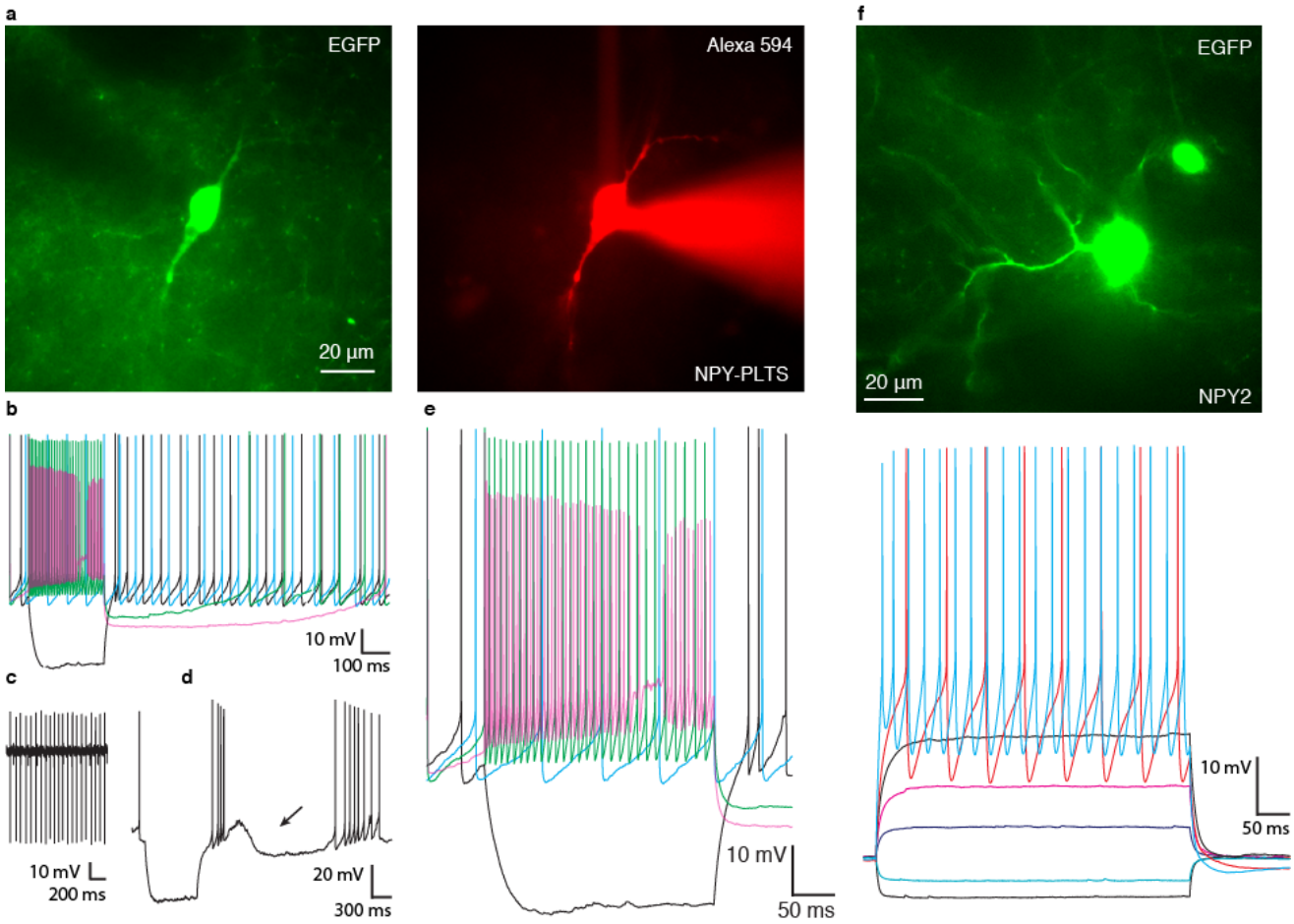
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 3. GABAergic IPSCs are elicited in SPNs by activation of single ChAT interneurons.

a. Photomicrograph of an EGFP expressing ChAT interneuron (*left*, arrow) recorded and labeled with Alexa-594 (*middle*, arrow). A simultaneously recorded SPN is also labeled (*right* arrowhead). Overlay is on the right. **b.** Whole cell current clamp recording of the EGFP positive neuron demonstrates characteristic responses to current injection (same as in *a*). Photomicrograph taken during recording (inset). **c.** IPSCs elicited in the SPN (*top*, same as in **a**) and the ChAT interneuron (*bottom*, arrows, same as in **a** and in Supplementary Fig. 7a) to single spikes of the ChAT interneuron elicited in voltage clamp (middle trace, black). Double exponential fit to the IPSC in the SPN is shown. **d.** Another paired recording demonstrates that the IPSC in the SPN (black) is blocked by bicuculline (red, block; green, partial recovery), and by DHβE (yellow). Inset shows the membrane potential responses of the presynaptic interneuron to injected current. **e.** Scatter plot of individual IPSC amplitudes during pharmacological challenge.

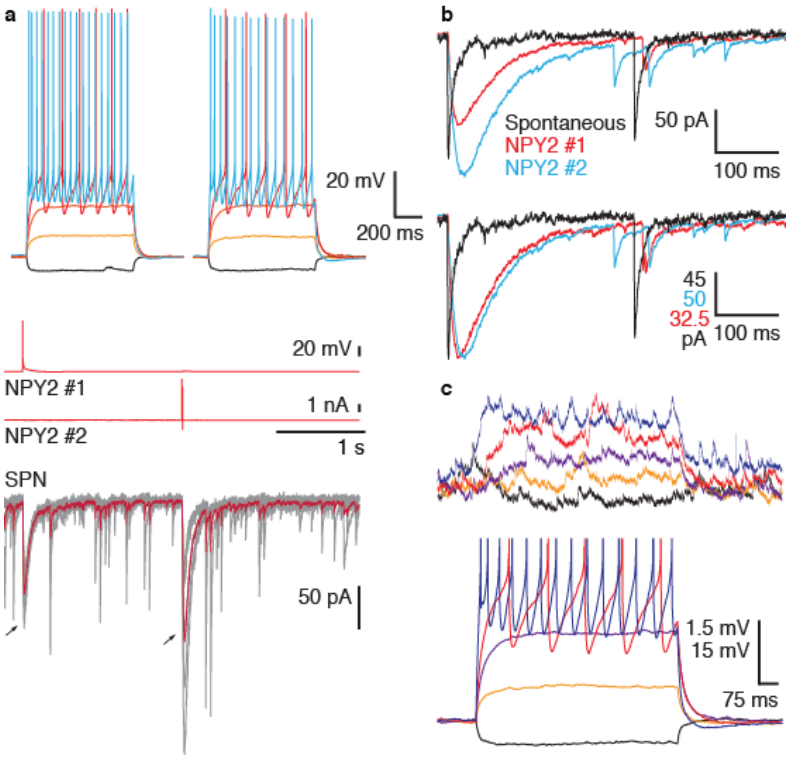
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 4. Comparison of the electrophysiological and morphological properties of NPY-PLTS and NPY-NGF interneurons.

a. Photomicrographs of an NPY-PLTS interneuron targeted in NPY-EGFP mice (*left*) intracellularly labeled with Alexa-594 (*right*). **b-e.** Electrophysiological characteristics of NPY-PLTS neurons. Characteristic high maximal firing rate of an NPY-PLTS interneuron in response to current injection pulses (**b, e)**, and typical *in vitro* spontaneous activity of the same interneuron (whole cell recording, **b**; cell attached recording, **c**). Note that spontaneous activity is seen only in some NPY-PLTS neurons. **d.** A hyperpolarizing current pulse elicits rebound firing driven by an LTS followed by a long lasting plateau depolarization (arrow) illustrating defining characteristics of these neurons (different neuron from that in *a*). **f.** Photomicrograph (*top*) and membrane potential responses (*bottom*) of a typical NPY-NGF interneuron. Note the striking electrophysiological and morphological differences from NPY-PLTS interneurons. Note the extensive branching of the dendritic arborization resembling that of neurogliaform neurons in the cortex and hippocampus.

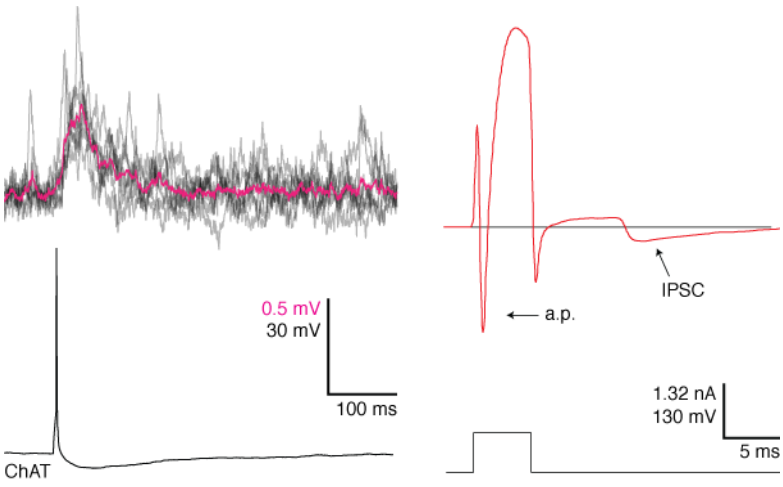
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 5. Synaptic connections and electrotonic coupling of NPY-NGF interneurons.

a. Triple recording from 2 NPY-NGF interneurons (*top* panels, responses of the neurons to current pulses) and an SPN (*bottom*). Note the striking similarity of the firing responses of these 2 neurons as well as those of the NPY-NGF neuron in Supplementary Fig. 4., illustrating the highly consistent and characteristic nature of these responses. Spikes elicited in the 2 NPY-2 interneurons (*middle*, red traces) evoke IPSCs in the SPN (*bottom*, arrows; individual traces, gray; average, red). The IPSCs exhibit very little amplitude variance and no transmission failures from either neuron. Note that one of the NPY-NGF interneurons is stimulated in voltage clamp (*middle*). **b.** Overlay of the average IPSCs elicited in the SPN (*top*) by the 2 interneurons (*bottom* panel: peak-scaled IPSCs). Note the nearly identical and characteristic slow time course of the IPSCs (blue and red). A regular, fast spontaneous IPSC is shown on the same scale (black) for comparison to illustrate the kinetic difference between the responses. **c.** Electrotonic coupling of the same 2 NPY-NGF interneurons shown by membrane potential deflections in one neuron (*top* traces) induced by hyperpolarizing (black) and depolarizing potentials in the other neuron (*bottom* traces).

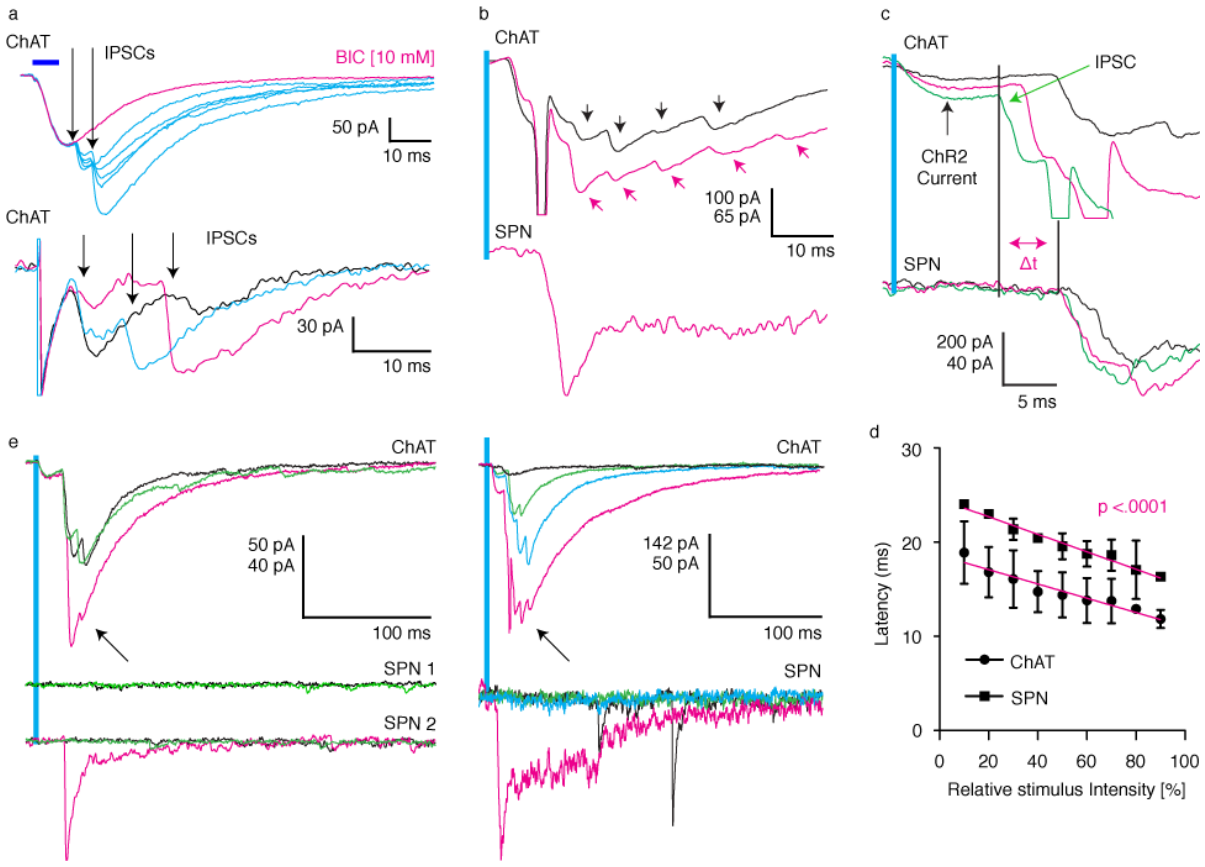
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 6. Stimulation of ChAT interneurons in current and voltage clamp.

Left: An action potential elicited in a ChAT interneuron with current injection in current clamp (bottom trace) elicits depolarizing postsynaptic potentials in a simultaneously recorded NPY-NGF interneuron. The postsynaptic response is closely similar to the nEPSP elicited in similar pairs with presynaptic spikes elicited in voltage clamp (Fig. 2). *Right:* An unclamped action potential was elicited in a ChAT interneuron in voltage clamp with a large 100 mV command potential and is seen as a fast inward current response (a.p., arrow). The response triggers a recurrent IPSC ("IPSC", arrow). Same recording as in Fig. 2. The corresponding nEPSPs elicited in an NPY-NGF interneuron is shown in Fig. 2b.

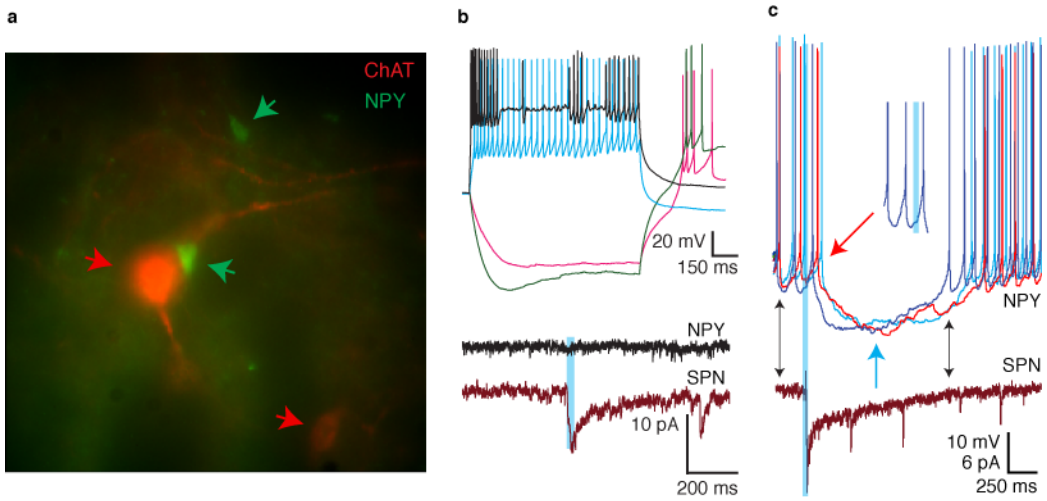
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 7. The feed-forward inhibition of SPNs and the recurrent inhibition of ChAT interneurons are mediated by distinct mechanisms.

a. *Top:* Recurrent IPSCs (arrows) elicited by optogenetic stimulation (blue bar) are mediated by $GABA_A$ receptors (top; red trace shows the ChR2 current after blocking the IPSCs). *Bottom:* Single spikes evoked in voltage clamp in a ChAT interneuron elicit a train of recurrent IPSCs (arrows point to onsets of the IPSCs). Note that over successive trials the onset latencies remain approximately constant (arrows) while the IPSC amplitudes vary significantly. This observation strongly suggests that the recurrent IPSCs originate from stereotypical burst firing of one or more interneurons that is/are driven by a regenerative intrinsic ionic mechanism triggered by nicotinic depolarization. **b.** Feed-forward inhibition in an SPN and recurrent inhibition in a simultaneously recorded ChAT interneuron exhibit distinct temporal patterns. **c-d**. The different onset latency of the recurrent and feed-forward IPSCs indicate the involvement of different circuits. **c.** Simultaneously recorded recurrent IPSCs (top) and feed-forward IPSCs (bottom) exhibit different onset latencies at 3 different optical stimulus intensities. Note that the onset latencies in the SPNs at low stimulus intensities are significantly longer then the latency at maximum intensity reported in the main text. **d.** Cumulative comparison of the onset latency difference in 3 ChAT-SPN pairs at 9 different stimulus intensities. The latencies are statistically significantly different (paired t-test, $p<.0001$). Note that the average difference in onset latency (4.8 ± 1.7 ms), far exceeds the typical total conduction delay among local circuit neurons strongly suggesting that the responses cannot originate from the same source. **e.** Triple (left) and paired recording (right) examples in which the recurrent inhibition in ChAT interneurons could be activated within a specific range of stimulus intensities (green, blue and black traces) without triggering a response in the simultaneously recorded nearby SPNs. Note that sub-maximal, but higher stimulation intensities (pink traces) can elicit IPSCs in the SPNs demonstrating that the feed-forward circuit to SPNs is not compromised. The IPSC is shown for one of the SPNs in the left panel (pink trace). Independent activation of these circuits with wide-filed optical stimuli strongly suggests that the IPSCs originate from distinct sources exhibiting different activation thresholds.

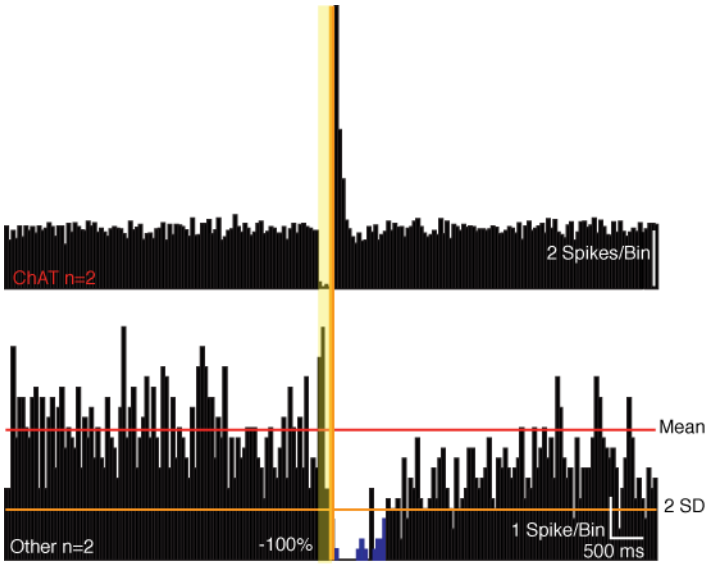
Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 8. NPY-PLTS interneurons do not mediate the inhibition in SPNs

a. Photomicrograph of a brain slice from a double transgenic ChAT-Cre/NPY-EGFP mouse expressing Cre-recombinase in ChAT interneurons and EGFP in NPY expressing neurons. Viral mediated expression of a Cre/lox controlled ChR2-mCherry transgene in these mice allowed selective optogenetic stimulation and targeted whole cell patch clamp recording of ChAT interneurons (red arrows) as well as targeted recording from EGFP expressing NPY+ neurons (green, arrows). NPY expressing neurons were identified as NPY-PLTS interneurons and distinguished from NPY-NGF interneurons based on their membrane potential responses to injected current (*top*, see Supplementary Fig. 4). **b.** Simultaneous whole-cell recording from an NPY-PLTS interneuron and a SPN demonstrates that optogenetically elicited IPSC in the SPNs is not accompanied by any detectable postsynaptic currents in the NPY-PLTS interneuron (*bottom*). The lack of a postsynaptic response in this neuron representative of the majority of NPY-PLTS interneurons. **c.** A spontaneously active NPY-PLTS interneuron (top traces) exhibits a long-lasting inhibition in response to optogenetic stimulation of ChAT interneurons (blue bar). Note the accompanying IPSC in a nearby SPN (*bottom*, red trace). Also note that the optical stimulus does not trigger spikes or elicit EPSPs in the NPY-PLTS interneuron. Also note the lack of monosynaptic responses in the SPN in response to spiking in the NPY-PLTS neuron (double arrows)

Nature Neuroscience: doi:10.1038/nn.2984



Supplementary Figure 9. Optogenetically elicited pause-excitation sequence of ChAT interneurons inhibits putative neostriatal interneurons *in vivo*

Top: 200 ms optical stimulus (yellow bar) elicits pause-excitation sequences in ChAT interneurons (same as in Fig. 6). *Bottom:* Short waveform, fast firing units classified as "Other" neurons (distinct from SPN and ChAT units, see Methods) are silenced by the excitation phase of the ChAT population activity. Orange bar indicates the end of stimulus. 50 ms bins. Horizontal lines in bottom trace are average (red) and 2SD (orange) of the firing rate. Bins in blue exhibit less than mean-2SD firing rates.

Nature Neuroscience: doi:10.1038/nn.2984