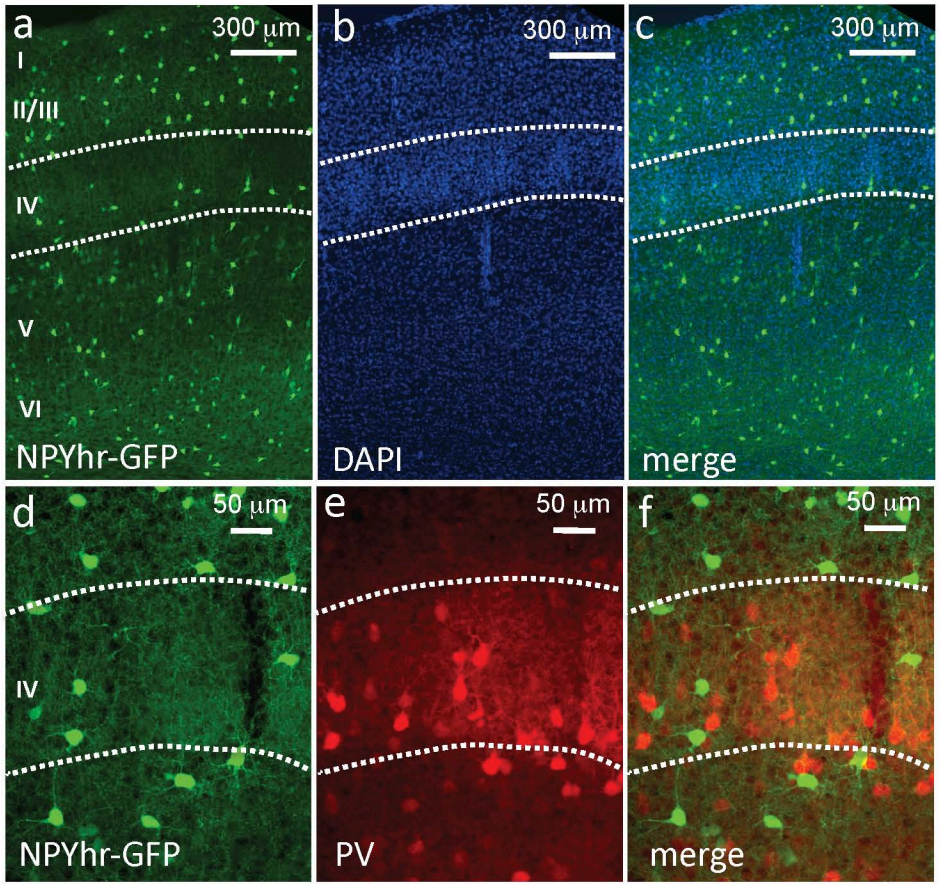
Chittajallu, Pelkey, McBain:
Neurogliaform cells dynamically regulate somatosensory integration via synapse specific modulation



Supplementary Figure 1: NPYhr-GFP transgenic mouse employed to facilitate targeting of layer IV NGFCs. (a-c) Thalamocortical slice of the NPY-hr GFP transgenic mouse showing GFP+ located in layer IV delineated by DAPI staining; dotted lines. **(d-e)**. Layer IV NPY-hrGFP+ cells never co-expressed parvalbumin.

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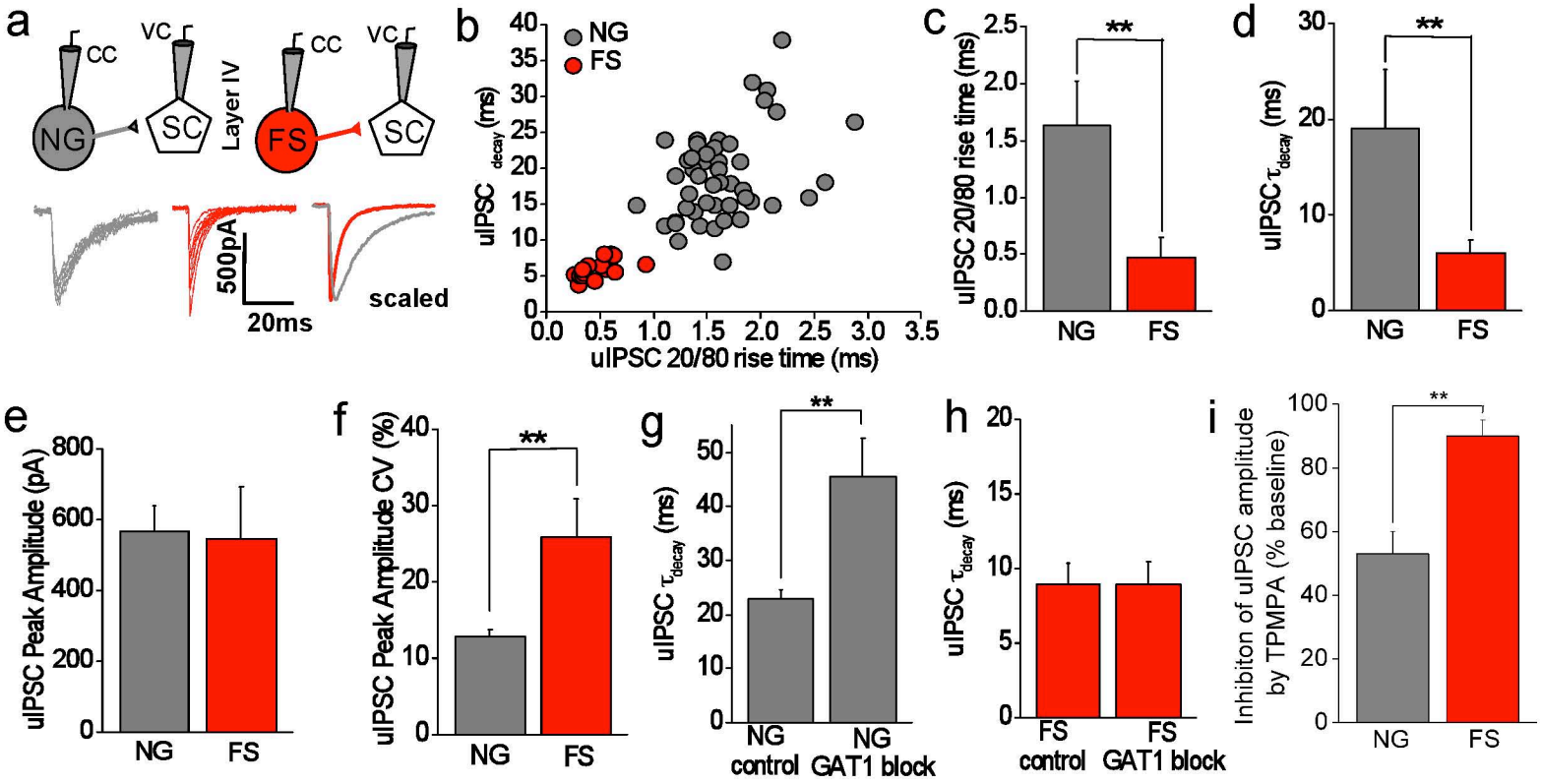

Supplementary Figure 2: Layer IV NGFCs possess similar synaptic properties to NGFCs described in other brain regions yet are distinct to FS INs . (a) Schematic depicting paired recording configuration (top panels). K+- and CsCl-based internal solutions were used for the INs and SCs, respectively (see **Supplementary Methods)**. Representative examples of uIPSCs on SC elicited by NGFC and FS IN (bottom panel; grey and red traces, respectively). Each set of traces is an overlay of 10 consecutive uIPSCs (bottom left and middle panels). Scaled overlay of mean uIPSCs elicited by NGFC (grey) and FS IN (red; bottom right panel). **(b)** Plot of individual rise and decay times of uIPSCs in SCs elicited by NGFCs (grey; n=46) and FS INs (red; n= 15)). **(c,d)** Mean rise and decay times of uIPSCs elicited by NGFCs (grey; n=46) and FS INs (red; n=15). **(e)** Mean uIPSC peak amplitudes elicited by NGFCs (grey; n=46) and FS INs (red; n=15). **(f)** Mean co-efficient of variation of uIPSC peak amplitudes elicited by NGFCs (grey; n=46) and FS INs (red;n=15). **(g,h)** Effect of GAT-1 block with 25mM SFK87796A on decay time of uIPSC on SCs mediated by NGFC (grey; n=4) and FS IN (red; n=3). **(i)** Effect of low affinity $GABA_A$-receptor antagonist on uIPSC peak amplitude on SCs mediated by NGFCs (grey; n=3) and FS INs (red; n=3). **p<0.01; Mann-Whitney U-Test

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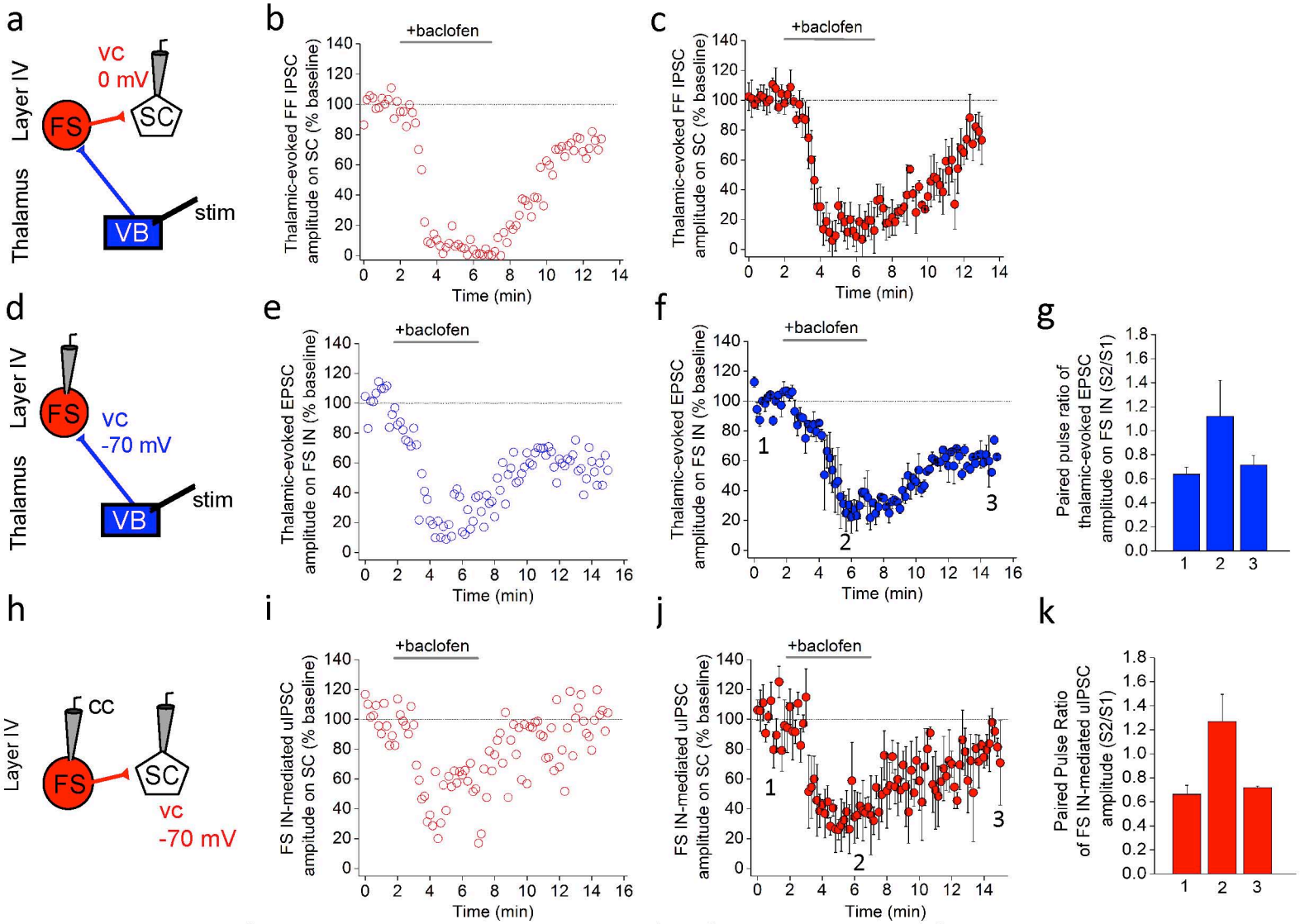

Supplementary Figure 3. Baclofen strongly inhibits thalamic-evoked feed-forward inhibition. (a) Schematic of recording configuration for monitoring of thalamic-evoked FFI on SCs. **(b,c)** Single example and pooled data (n=3) of 10μM baclofen-mediated decrease in the thalamic-evoked FFI on SCs. **(d)** Schematic of recording configuration for monitoring of thalamic-evoked EPSCs on FS INs. **(e,f)** Single example and pooled data (n=4) of 10μM baclofen-mediated decrease in the thalamic-evoked EPSCs on SCs. **(g)** Bar graph of paired pulse ratio of thalamic evoked EPSCs on FS INs at time points denoted by numbers in **(f)** corresponding to baseline, baclofen and washout conditions. **(h)** Schematic of recording configuration of paired recordings for monitoring of FS IN elicited uIPSCs on SCs. **(i,j)** Single example and pooled data (n=4) of 10μM baclofen-mediated decrease in FS In mediated uIPSC on SCs. **(k)** Paired pulse ratio of FS IN mediated uIPSC on SCs at time points denoted by numbers in **(i)** corresponding to baseline, baclofen and washout conditions.

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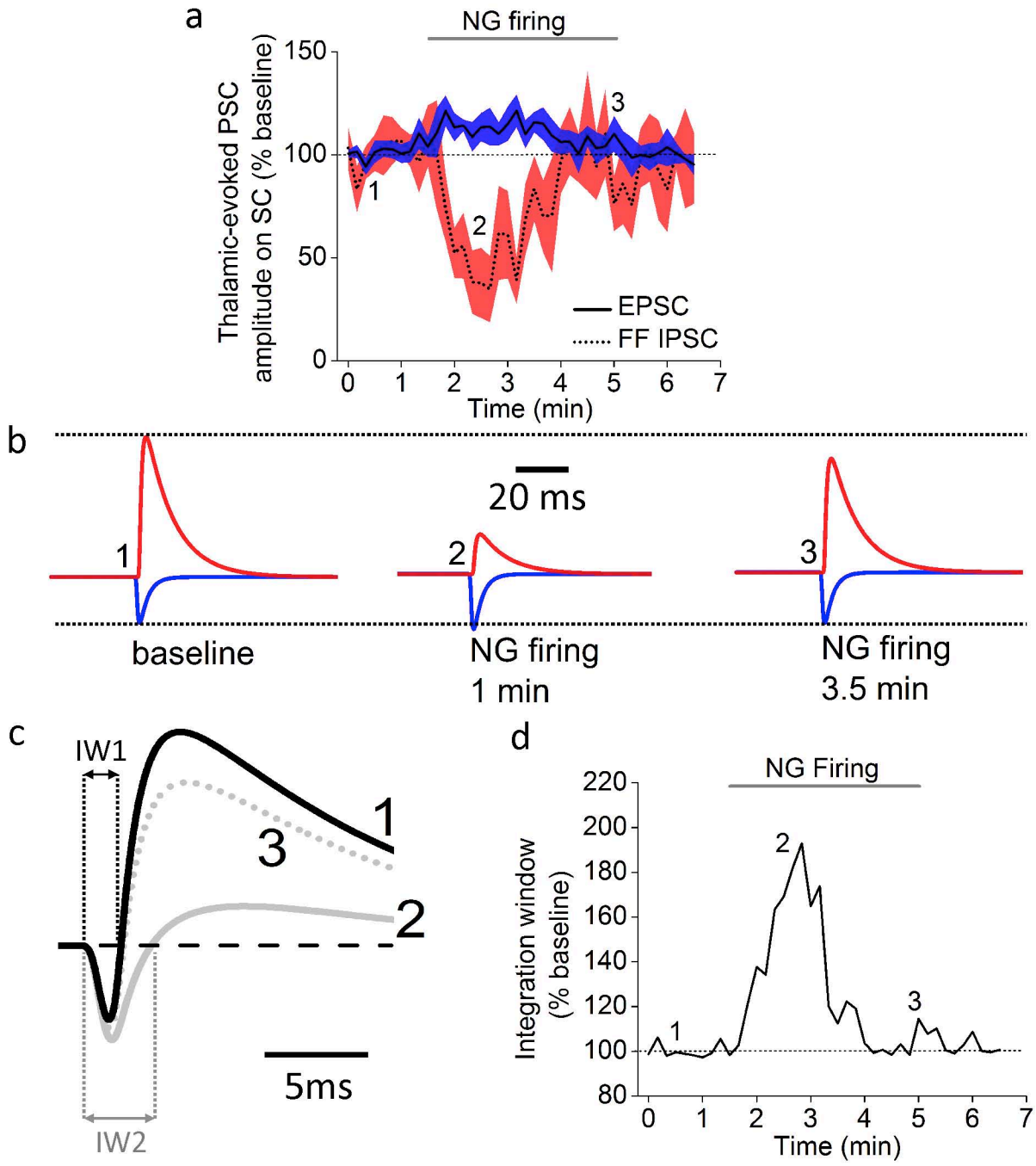
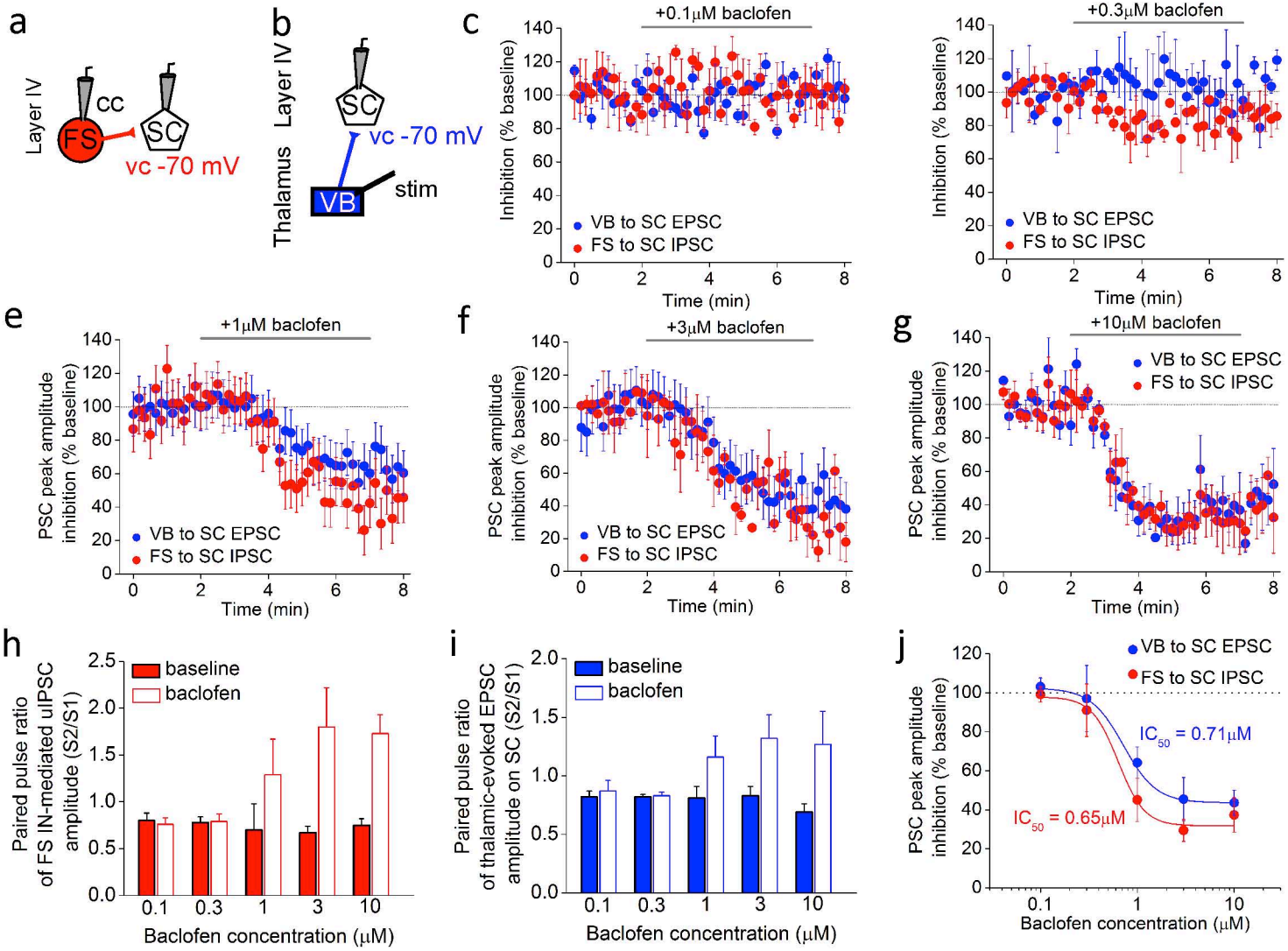


Supplementary Figure 4. NGFC-activity selectively inhibits thalamic-evoked feed-forward inhibition whilst leaving feed-forward excitation intact resulting in a modulation of integration window on SCs. **(a).** Re-plot of data shown in Fig. 2j showing selective modulation of NGFC firing on feed-forward inhibition. **(b)** Modeled thalamic-evoked SC EPSGs (blue) and FF IPSGs (red) scaled according to the mean percentage change in the experimentally determined SC EPSCs and FF IPSCs shown in **(a)** at times indicated by the numbers**. (c)** Linear summation of the modeled conductances at the corresponding time points. Dotted lines depict measurement of the SC IW. **(d)** Percentage change in IW on SCs during the time-course of the modeled experiment. (See **Supplementary Methods** for details on modeling**).**



Supplementary Figure 5. Dose-response comparison of baclofen mediated inhibition of synaptic transmission at thalamic:SC sand FS:SC synapses (a,b) Schematic of recording configuration for monitoring fast-spiking interneuron (FS) mediated IPSCs and thalamic evoked EPSCs on layer IV stellate cells (SC) **(c-g)** Inhibition of the peak amplitude of FS mediated IPSCs (red traces) and thalamic evoked EPSCs (green traces) elicited by varying concentrations of baclofen (0.1, 0.3, 1, 3 and 10 μM). Note that the FS:SC data in g (red trace) is replotted from Supplementary Figure 3j. **(h,i)** Bar graph of paired pulse ratios of FS mediated IPSC and thalamic evoked EPSC peak amplitudes on SCs during baseline (red and green closed bars) and after 5 minutes baclofen (red and green open bars) at varying concentrations. Note that part of the data for 10 μM baclofen are replotted from Supplementary Figure 3k **(j)** Dose response curves for baclofen mediated inhibition of FS mediated IPSCs and thalamic evoked EPSCs on SCs with corresponding IC_{50} values shown. All data are pooled mean values ± SEM (n=3-7).

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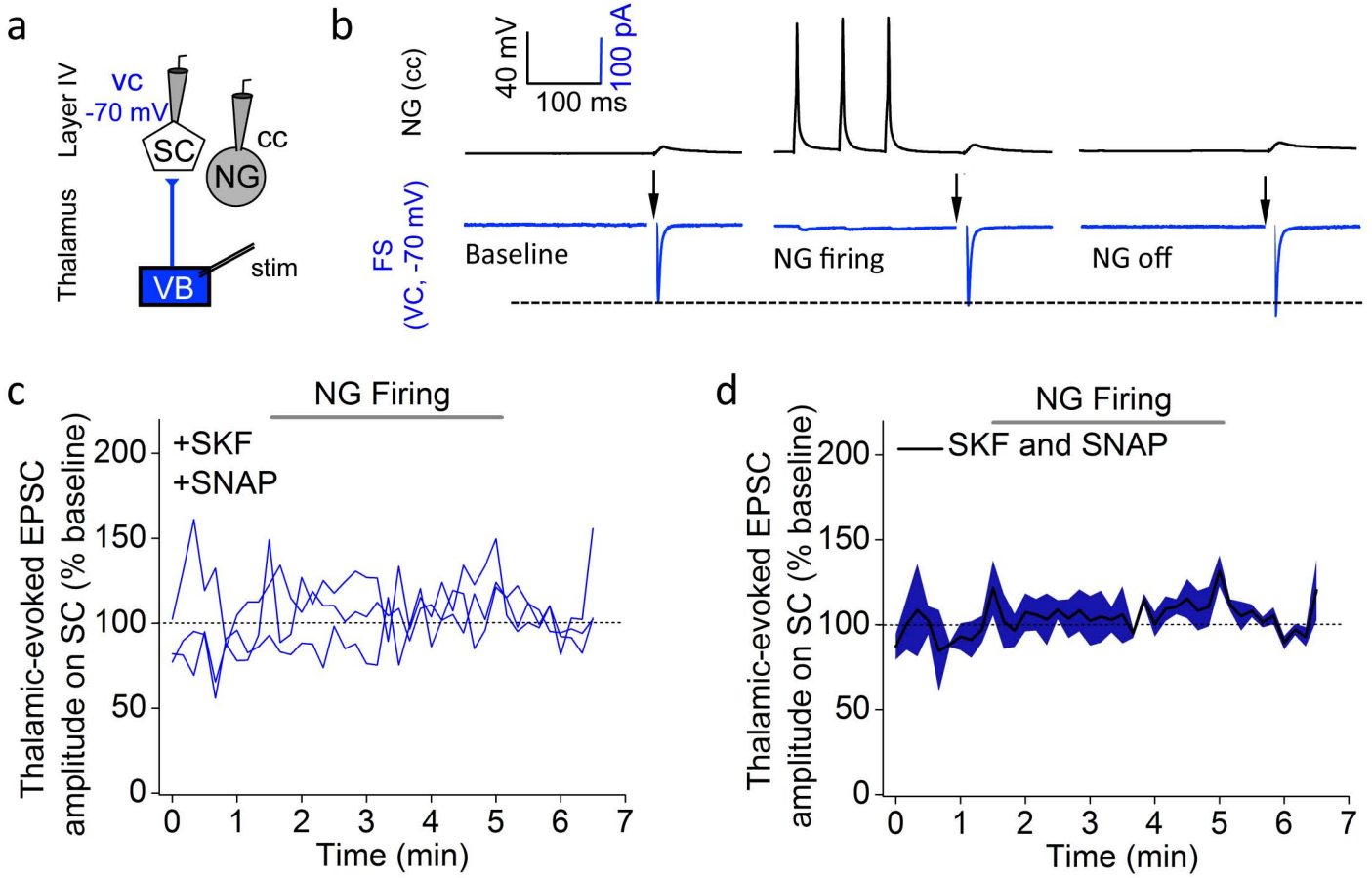

Supplementary Figure 6. Combined GAT1 and GAT3 uptake block does not reveal an NGFC-activity mediated modulation of the thalamic-evoked EPSCs on SCs (a) Schematic of the dual NGFC and SC whole-cell recording configuration. **(b)** Single trace examples of simultaneous current-clamp and voltage-clamp recording in a NGFC (black traces) and SC (Vh=-70mV; green traces), respectively. **(c)** Individual and **(d)** pooled data showing EPSC amplitude on SCs at baseline, during and after NGFC firing in presence of combined GAT-1 and GAT-3 uptake block with 25μM SFK-89976A and 100μM SNAP-5114, respectively (n=3).

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