SUPPLEMENTAL FIGURES

Suppl. Fig. 1. (Associated with Fig.3). Renshaw cells receive VGluT1+ synapses originating in corticospinal neurons. (A_{1-2}) Experimental protocol for labelling corticospinal synapses (A_1). Mice injected at birth (P0) with AAV9-GFP bilaterally in the cortex (A_2). At P10, the L1 and L2 spinal segments were examined with immunohistochemistry. **(B_{1-4}, C_{1-4})** Single plane confocal images of a wild type (B_{1-4}) and a SMA (C_{1-4}) Renshaw cell labelled with calbindin (blue, B_1 and C_1), AAV9-GFP (green, B_2 and C_2), VGluT1 (red, B_3 and C_3) antibodies. Merged images are shown in B_4 and C_4. Insets are areas indicated by the dotted boxes, showing GFP+ and VGluT1+ synapses on the soma (yellow arrows) of Renshaw cells. **($D_{1,2}$)** Neurolucida reconstruction of a wild type (D_1) and a SMA (D_2) Renshaw cell with cholinergic (VAChT+) synapses marked by red dots. **(E)** Number of cholinergic (VAChT+) synapses on the soma (left graph) and dendrites (right graph) of Renshaw cells in wild type (blue) and SMA (red) without spinal cord transection at P10. Differences were significant on cell bodies (** p=0.0018 unpaired two-tailed t-test) but not on dendrites. (n=20 or 10 Renshaw cells per animal; N=2 WT and 2 SMA mice) **(F)** Number of cholinergic (VAChT+) synapses on the soma (left graph) and dendrites (right graph) of Renshaw cells in wild type (blue) (n=14, N=3) and SMA (red) two days after T4 spinal cord transection at P10 (n=17, N=3). Differences are non-significant (unpaired two-tailed t-test).

Suppl. Fig. 2. (Associated with Fig.4). No electrophysiological differences in SMA spinal interneurons that do not receive proprioceptive synapses. (A) Superimposed voltage responses (top traces) following current injection (bottom traces) in spinal interneurons that do not receive direct proprioceptive synapses in wild type and SMA mice at P4. **(B)** Resting membrane potential (RMP), input resistance (R_{IN}), voltage threshold (V_{Th}), time constant (τ) and capacitance of spinal interneurons without direct proprioceptive activation in wild type (blue, n=15 neurons, N=15 mice) and SMA (red, n=14 neurons, N=14 mice) mice at P4.

Suppl. Fig. 3. (Associated with Fig.5). Validation of mIPSCs in wild type and SMA motor neurons. Current recordings from voltage clamp experiment in wild type **(A)** and SMA **(B)** motor neurons in which mIPSCs (top traces) were abolished by application of bicuculine and strychnine (bottom traces).

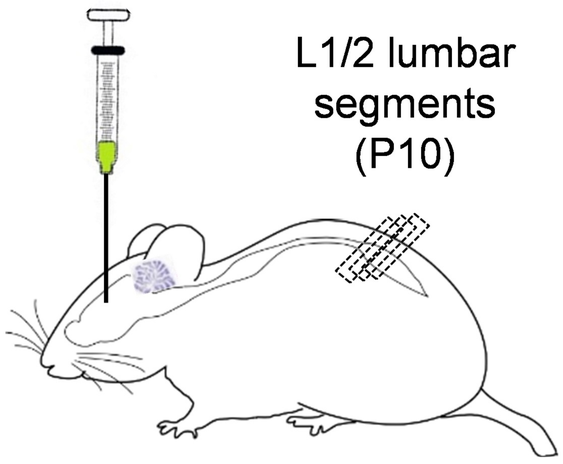
Suppl. Fig. 4. (Associated with Fig.6). Validation of gephyrin knockdown; no difference in NKCC1 or KCC2 between wild type and SMA mice; behavioral phenotype injected with AAV9-Geph$_{RNAi}$ or treated *in vivo* with Org25543. (A) Map of the plasmid for gephyrin knockdown. **(B_{1-3})** Confocal images from the ventral spinal cord of a wild type mouse at P4 showing ChAT (red, B_1), AAV9-Gephyrin$_{RNAi}$-GFP (green, B_2) immunoreactivity and their merged image (B_3). **(C)** Percentage of motor neurons (MNs) transduced by AAV9-Gephyrin$_{RNAi}$-GFP in wild type (n=295 MNs, N=6) and SMA (n=191 MNs, N=7) mice at P11. Each data point represents one mouse. **(D)** GFP (green) and gephyrin (red) in a wild type (left) and a SMA (right) motor neuron at P11. Images at the bottom are higher magnification areas from the dashed boxes, respectively. **(E)** Number of gephyrin clusters per µm of motor neuron membrane in wild type mice (blue; n=15 MNs, N=3 mice), wild type mice injected with AAV9-Geph$_{RNAi}$ (cyan; n=15 MNs, N=3 mice), SMA mice (red; n=17 MNs, N=3 mice) and SMA mice injected with AAV9-Geph$_{RNAi}$ (green, n=18 MNs, N=3 mice). ** p=0.002, WT vs WT+Geph$_{RNAi}$; ** p=0.0063, WT vs SMA; *** p<0.0001, SMA vs SMA+Geph$_{RNAi}$; OneWay ANOVA, Tukey's *post hoc* test. "ns": not significant. Relative expression of *nkcc1* and *kcc2* in medial L5 motor neurons **(F)** and lateral L5 motor neurons **(G)** in wild type (N=3) and SMA (N=3) mice. **(H)** Average life span in SMA mice injected with AAV9-GFP (as controls, N=9 mice) or with AAV9-Gephyrin$_{RNAi}$-GFP (N=15 mice). **(I)** Body weight gain in wild type (blue, N=17 mice), wild type mice treated with Org25543 (cyan, N=7 mice), SMA mice (red, N=7 mice) and SMA mice treated with Org25543 (purple, N=9 mice).

Supplementary Figure 1 (associated with Fig.3)

A1

Cortex
(bilateral inj P0)
AAV9-GFP

L1/2 lumbar
segments
(P10)



WT

B1 Calbindin

B2 AAV9-GFP

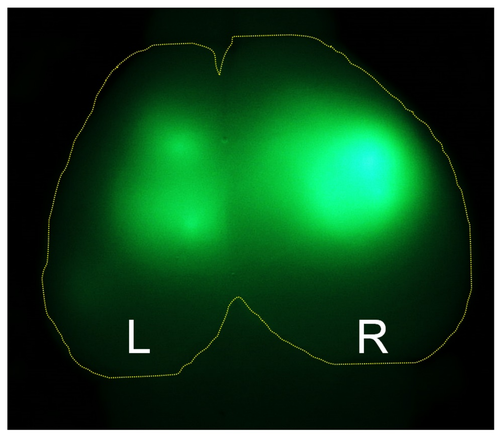
B3 VGluT1

B4 Merged

20μm

A2

Cortex (P10)



SMA

C1 Calbindin

C2 AAV9-GFP

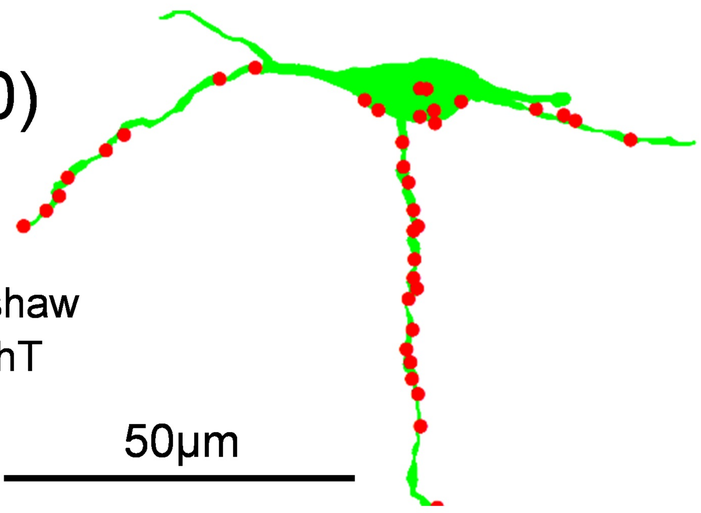
C3 VGluT1

C4 Merged

20μm

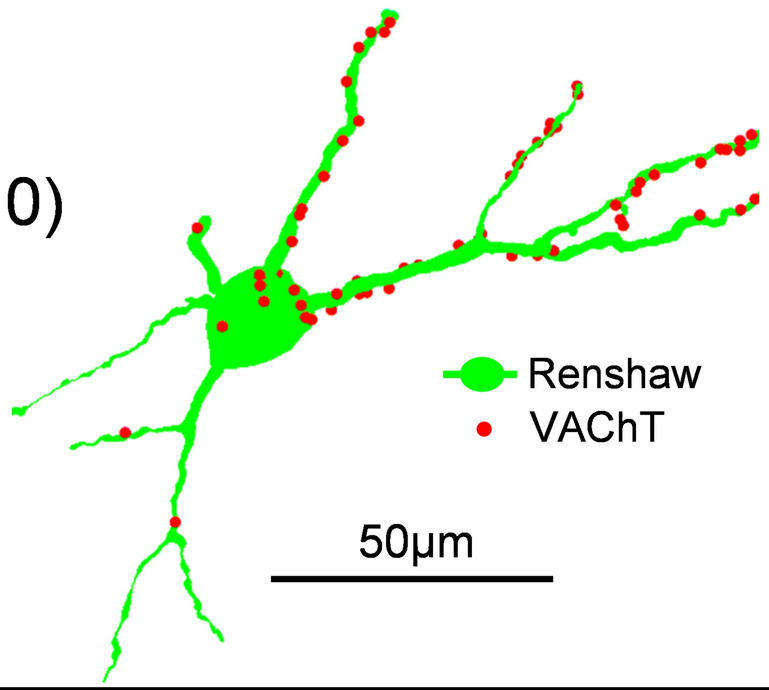
D1

WT (P10)



D2

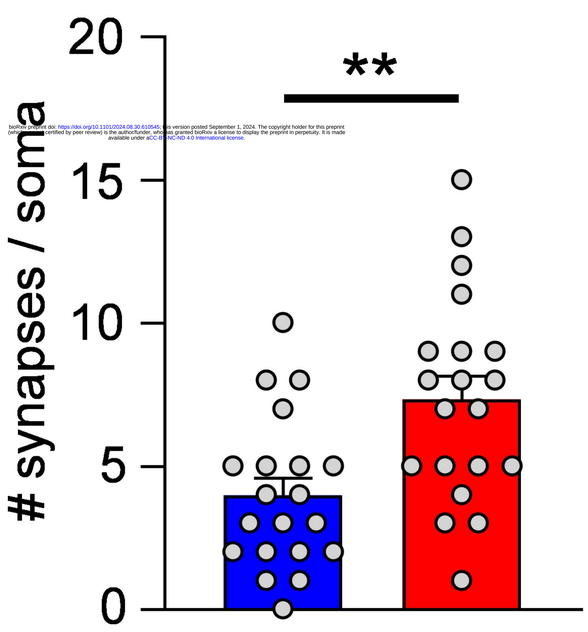
SMA (P10)



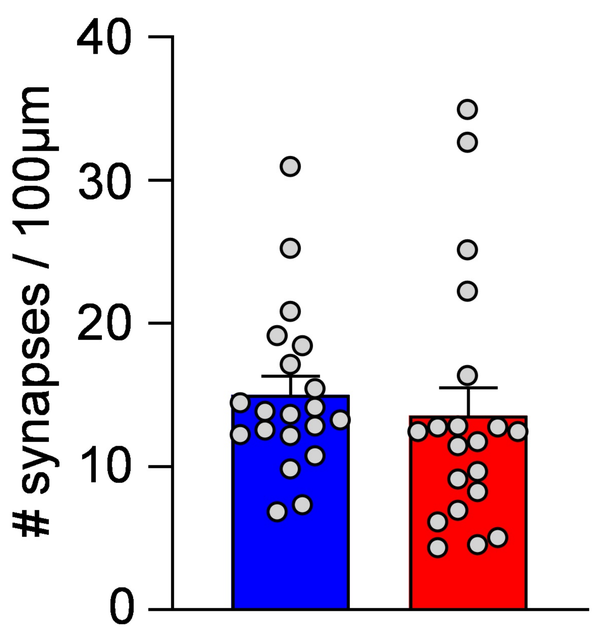
E

Without transection

VAChT soma density



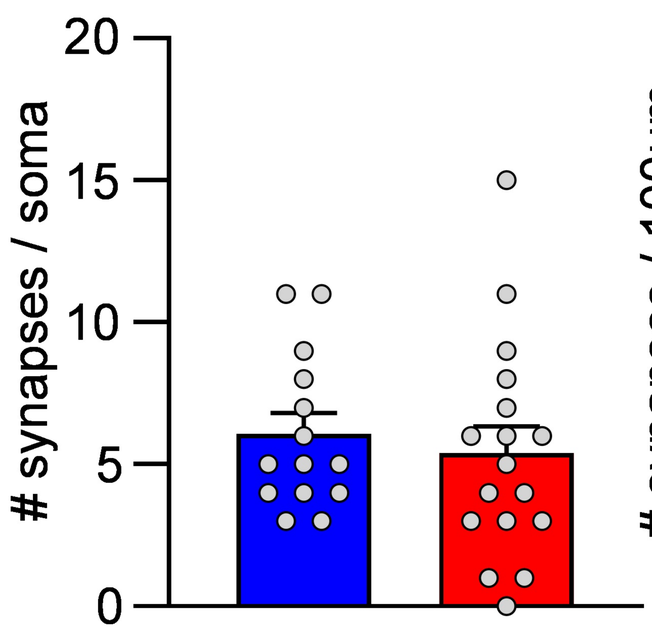
VAChT dendritic density



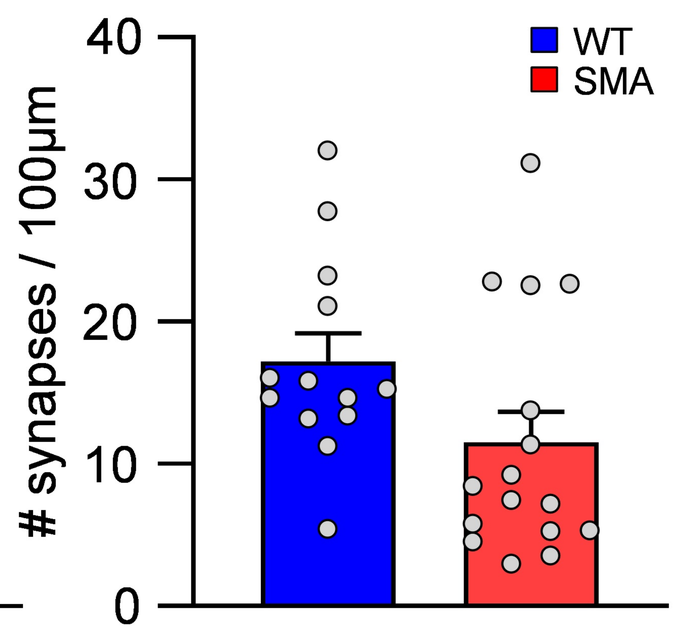
F

After transection

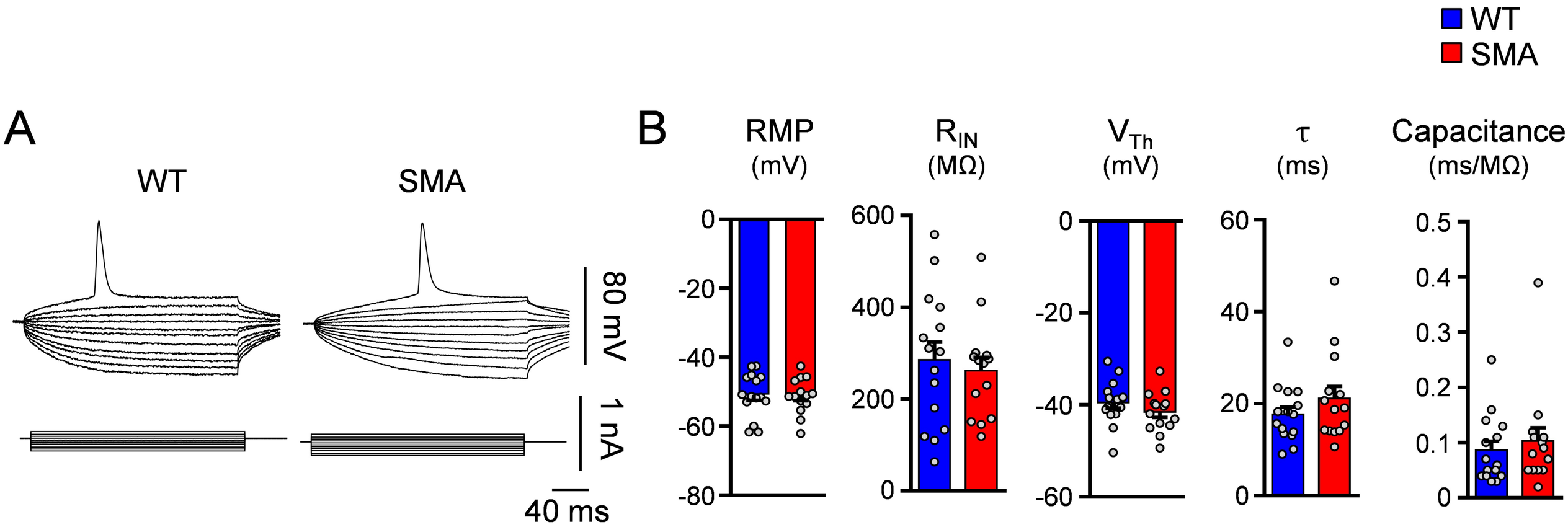
VAChT soma density



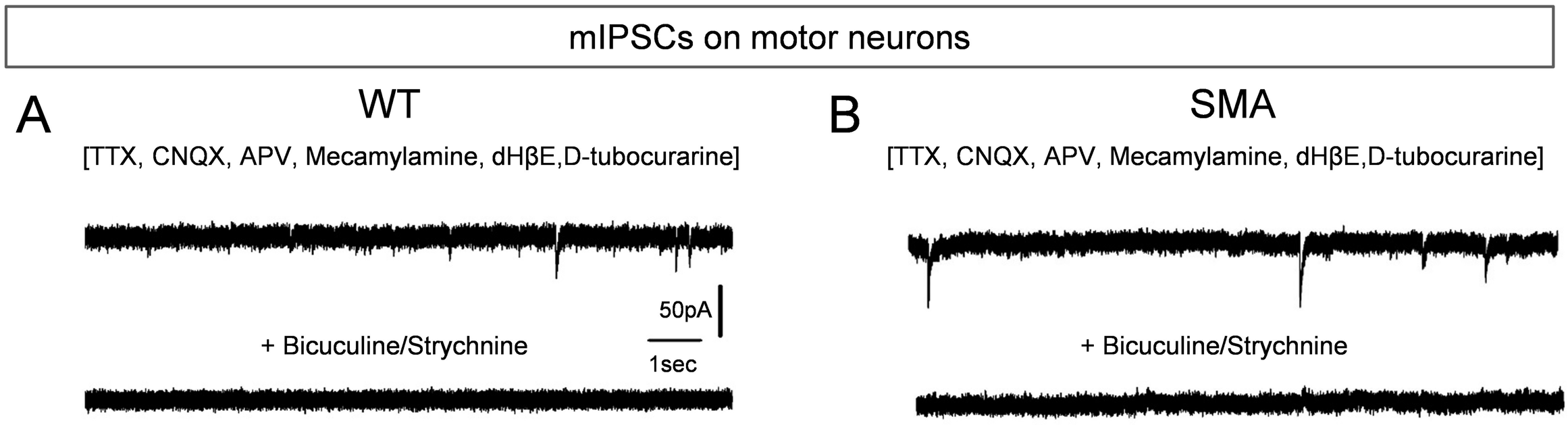
VAChT dendritic density



Spinal interneurons (no DR or VR monosynaptic activation)



Supplementary Figure 3 (associated with Fig.5)



Supplementary Figure 4 (associated with Fig.7)