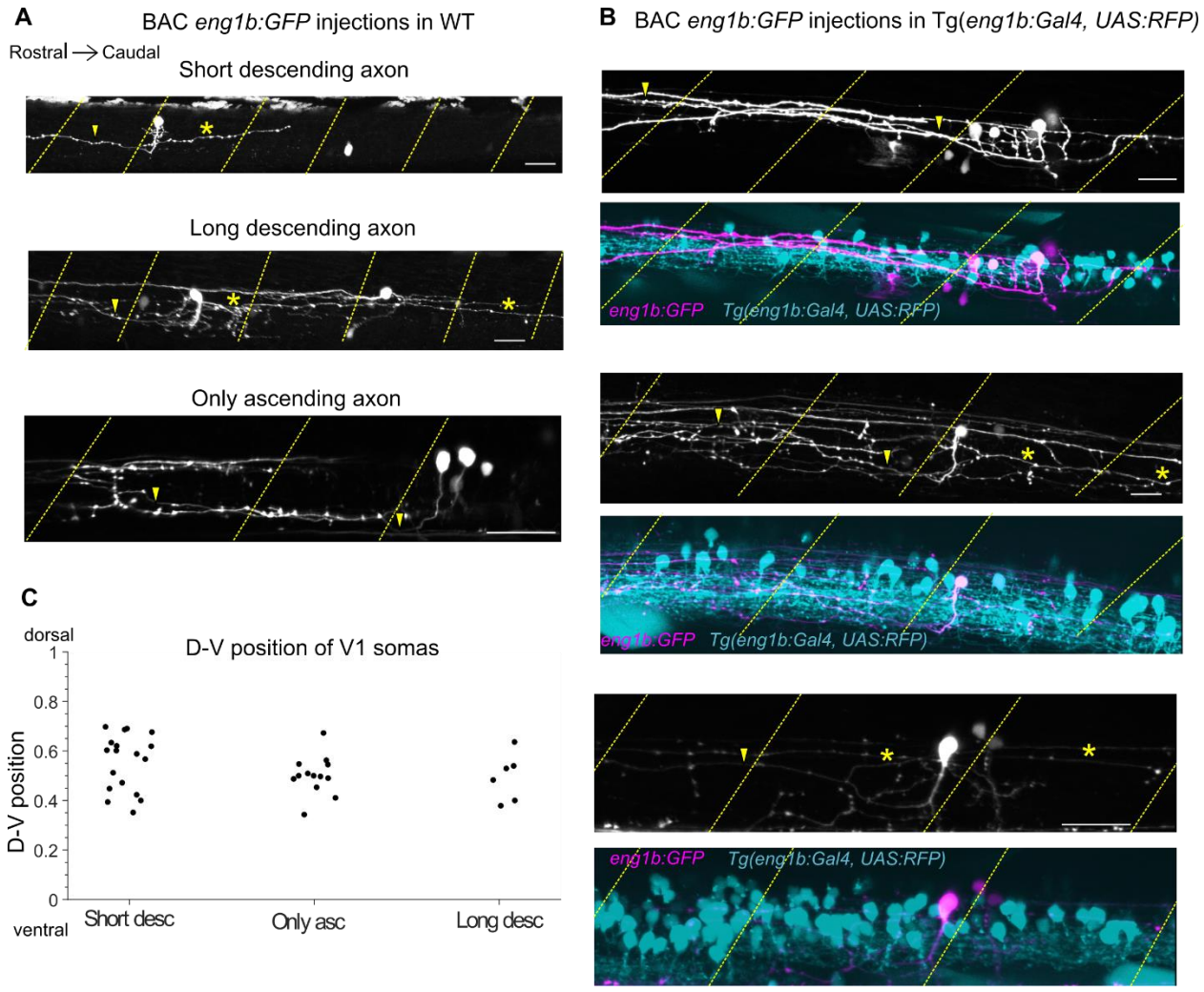

Figure S1: Characterization of V1 anatomy. Related to Figure 1.

A. Three representative examples of V1 neurons labelled by injection of *eng1b:GFP* BAC construct in WT larvae. B. Three representative examples showing *eng1b:GFP*$^{+}$ neurons are also usually labeled in the *Tg(eng1b:Gal4)* line, but not always (bottom image pair). In all images, segment borders are shown in yellow dashed lines. Arrowheads mark the ascending axon, and the asterisk marks the descending axon. Scale bar: 20 µm. C. Scatter plot showing D-V positions of V1 somata are similar across different anatomical groups.

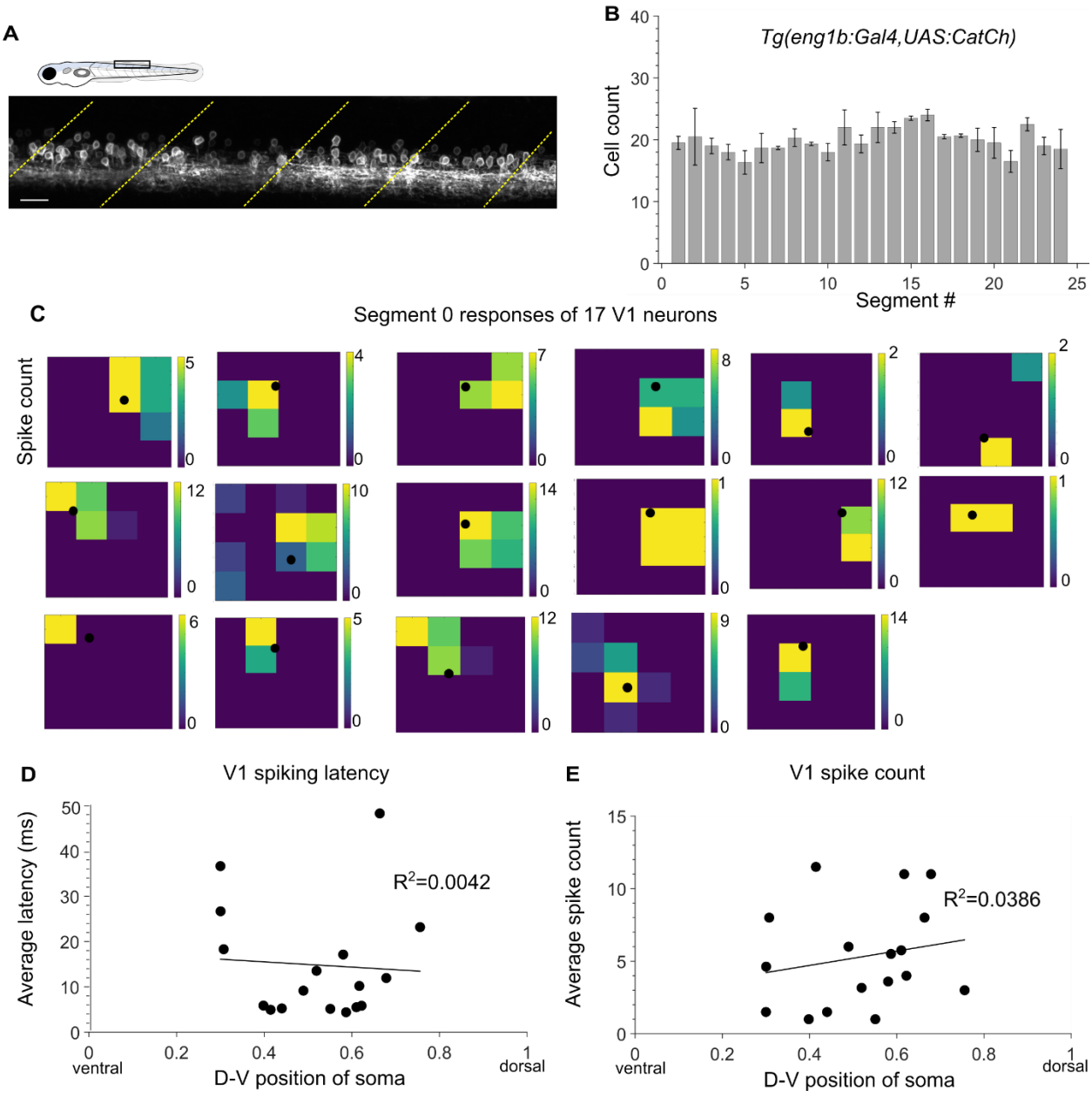

Figure S2: Characterization of *Tg(eng1b:Gal4,UAS:CatCh)* animals and V1 spiking. Related to Figure 2.

A. Representative confocal image (z-projection; 66 µm in depth) of mid body segments 11-14 in a 5 dpf *Tg (eng1b:Gal4,UAS:CatCh)* larva. Scale bar: 20 µm. B. Bar plot showing mean cell count of V1 neurons per segment along the rostro-caudal axis. n = 3 larvae. Error bars represent SEM. C. Heat maps showing segment 0 responses for 17 V1 neurons, measured as in Fig 2C. D. Scatter plot showing no relationship between the mean latencies of the first spike in V1 neurons with respect to the D-V position of their somata. N=17 neurons. E. Scatter plot showing no relationship between mean spike counts per trial of V1 neurons with respect to the D-V position of their somata. N=17 neurons.

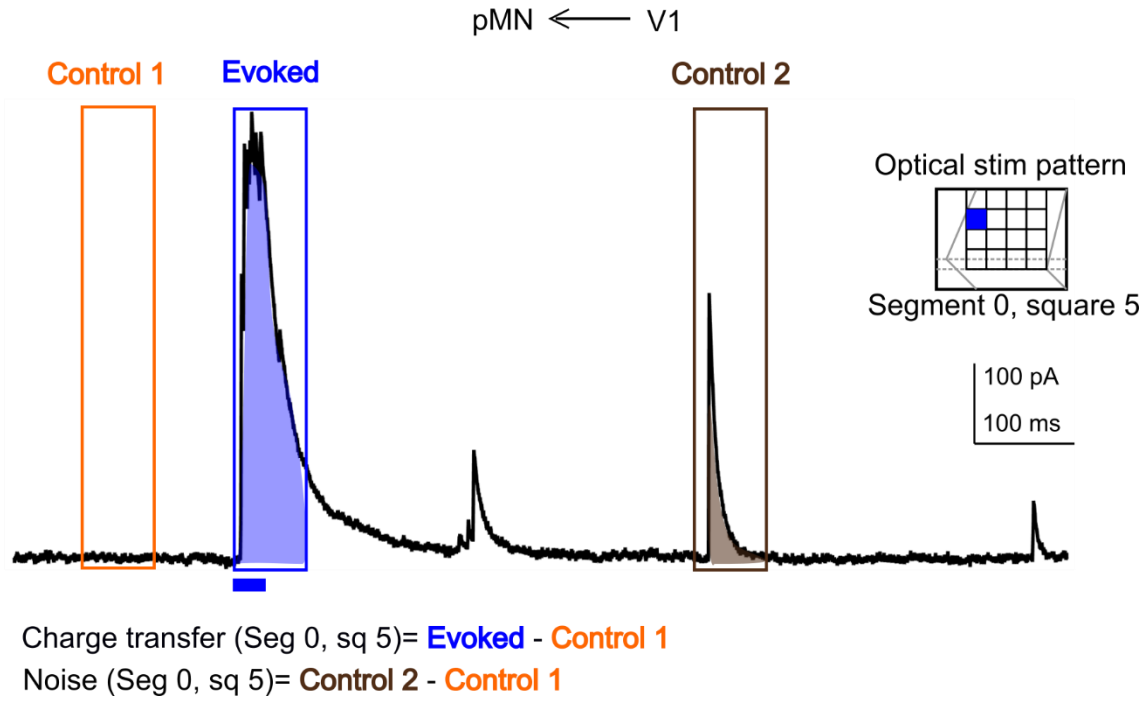

Figure S3: Analysis of charge transfer per segment and calculation of noise. Related to Figure 3.

Representative trace of IPSCs recorded in a single primary motor neuron during illumination of square 5 in Segments 0 (inset). Duration of the optical stimulus is shown as a blue bar. Charge transfer for the evoked response was calculated by integrating the current in a 50 ms window from the onset of the optical stimulus (Evoked, shaded region in the blue block). To account for spontaneous activity, the Evoked response was subtracted from Control 1, a 50 ms window before the onset of the stimulus (Orange block). To calculate noise, the charge transfer in a separate 50 ms window post stimulus was calculated (Control 2, shaded region in the brown block) and this was subtracted from Control 1.

Charge transfer (Seg 0, sq 5) = Evoked - Control 1
Noise (Seg 0, sq 5) = Control 2 - Control 1

Total evoked charge transfer (Seg 0) = Charge transfer (Seg 0, sq 1) + Charge transfer (Seg 0, sq 2) .....+ Charge transfer (Seg 0, sq 16)

Total noise (Seg 0) = Noise (Seg 0, sq 1) + Noise (Seg 0, sq 2) .....+ Noise (Seg 0, sq 16)

For statistical comparisons, total charge transfer per segment was compared to total noise per segment for each neuron.

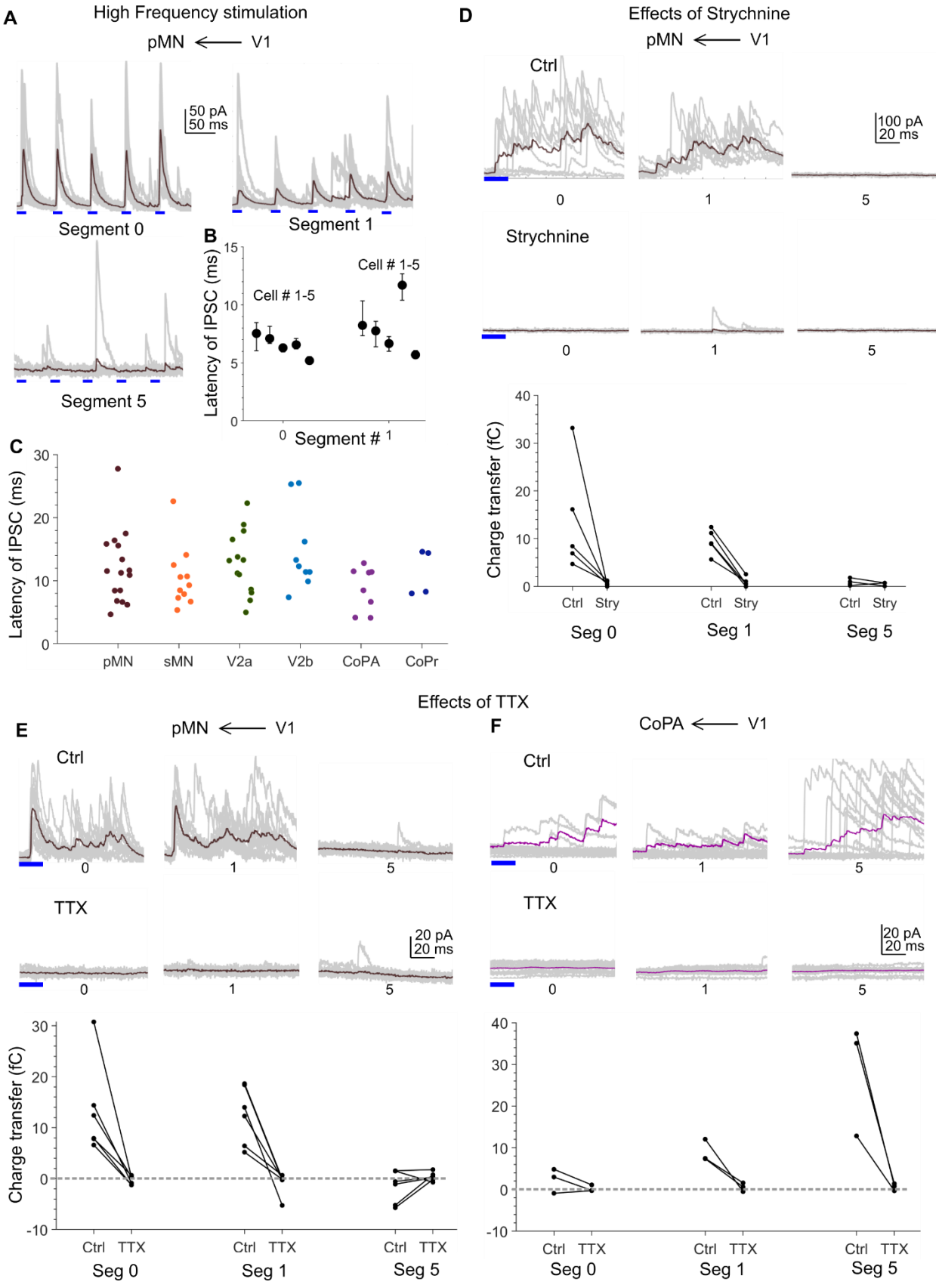
A
High Frequency stimulation
pMN ⟵ V1
50 pA
50 ms
Segment 0
Segment 1
Segment 5
B
Latency of IPSC (ms)
Cell # 1-5
Cell # 1-5
Segment #
C
Latency of IPSC (ms)
pMN
sMN
V2a
V2b
CoPA
CoPr
D
Effects of Strychnine
pMN ⟵ V1
Ctrl
100 pA
20 ms
Strychnine
Charge transfer (fC)
Ctrl Stry
Seg 0
Seg 1
Seg 5
Effects of TTX
E
pMN ⟵ V1
Ctrl
TTX
20 pA
20 ms
Charge transfer (fC)
Ctrl TTX
Seg 0
Seg 1
Seg 5
F
CoPA ⟵ V1
Ctrl
TTX
20 pA
20 ms
Ctrl TTX
Seg 0
Seg 1
Seg 5

Figure S4: Light-evoked IPSCs are monosynaptic. Related to Figure 4.

A. Representative overlay of 15 traces of IPSCs recorded in primary motor neurons (pMNs) during a 20 Hz optical stimulus train (5 pulses, 20 ms) on segments 0, 1, and 5. B. Scatter plot showing median latency of the first IPSC with the train stimulus. Error bars represent 25th and 75th percentiles. Dots represent Cell # 1-5 for each segment. N=5 neurons. C. Scatter plot showing the distribution of latencies of the first IPSC for six postsynaptic targets during illumination of Segment 1. Each dot represents the median value from one cell. D. Strychnine abolished light-evoked IPSCs. Top, Middle-Representative overlay of 15 traces of IPSCs recorded in primary motor neurons during illumination of segments 0, 1 and 5 before (top) and after (middle) bath application of strychnine. Bottom: Scatter plot showing the total charge transfer before and after application of strychnine. N=5 neurons. E, F. Application of TTX abolished light-evoked IPSCs. Top, Representative overlay of 15 traces of IPSCs recorded in primary motor neurons (left) and CoPA neurons (right) during illumination of segments 0, 1 and 5 before and after bath application of TTX. Bottom: Scatter plot showing the total charge transfer before and after application of TTX in pMNs (left) and CoPAs (right). N=5 neurons for pMNs and 3 neurons for CoPAs. For all IPSC plots, colored trace represents mean and duration of the optical stimulus is shown as a blue bar.

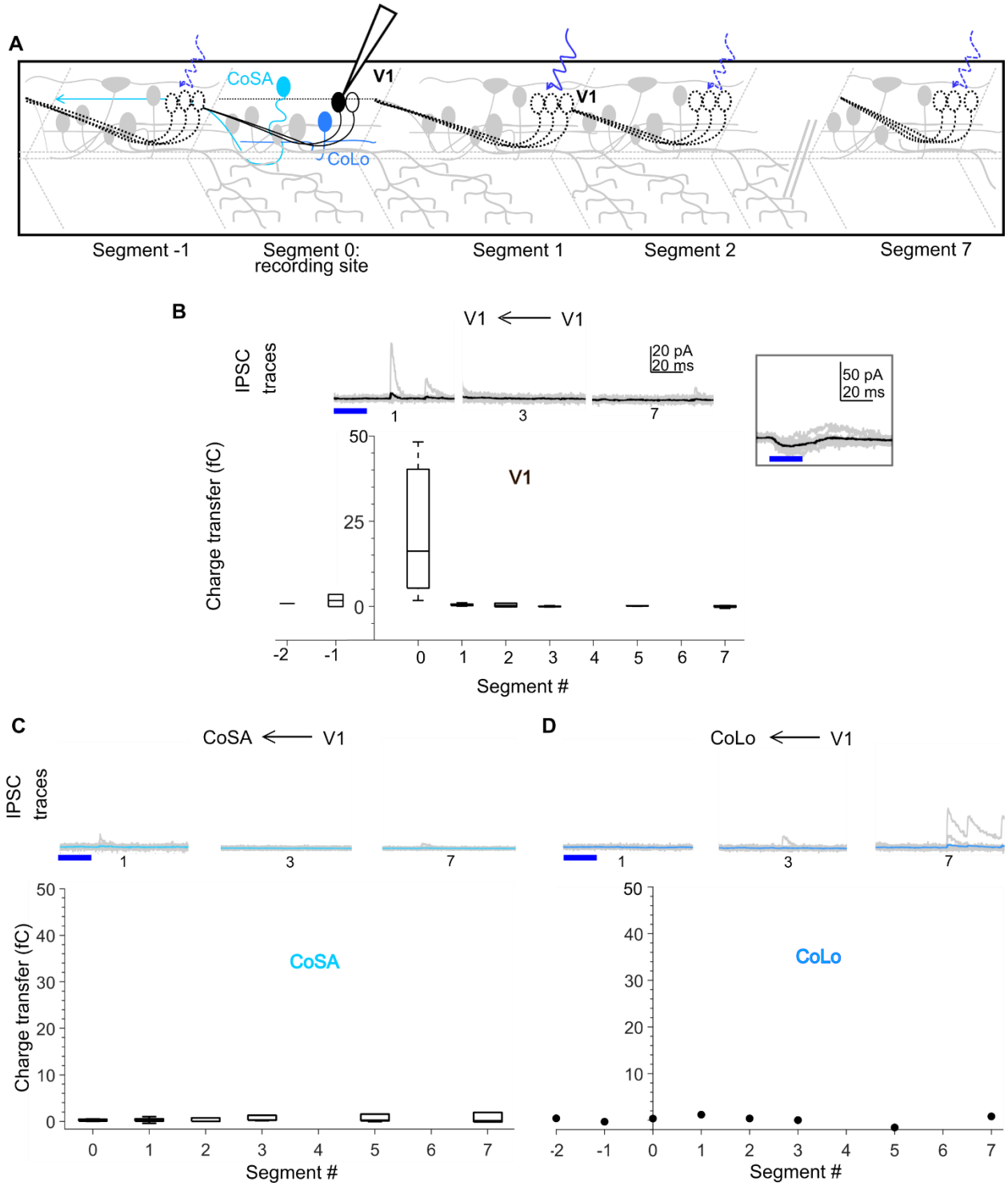

Figure S5: V1 neurons inhibit other V1 neurons only locally. Related to Figure 5.

A. Schematic of the experimental design showing intracellular recordings from V1 neurons paired with optical stimulation of V1 neurons along the rostro-caudal axis. B. Top: Representative overlay of 15 traces of IPSCs recorded in V1 neurons during illumination of segments 1, 3, and 7 caudal to the recorded neuron position. Colored trace represents mean. Duration of the optical stimulus is shown as a blue bar. B. Bottom: Box plots showing the total charge transfer per segment recorded in V1 neurons. N =

5 neurons for each data point. Inset: Recording from the same cell holding at -65 mV showing slow CatCh induced depolarization. Blue bar represents duration of the optical stimulus. C. Same as in B for Commissural Secondary Ascending (CoSA) neurons. N= 4 neurons for each data point. D. Same as in B for Commissural Local (CoLo) neuron. N = 1 neuron.

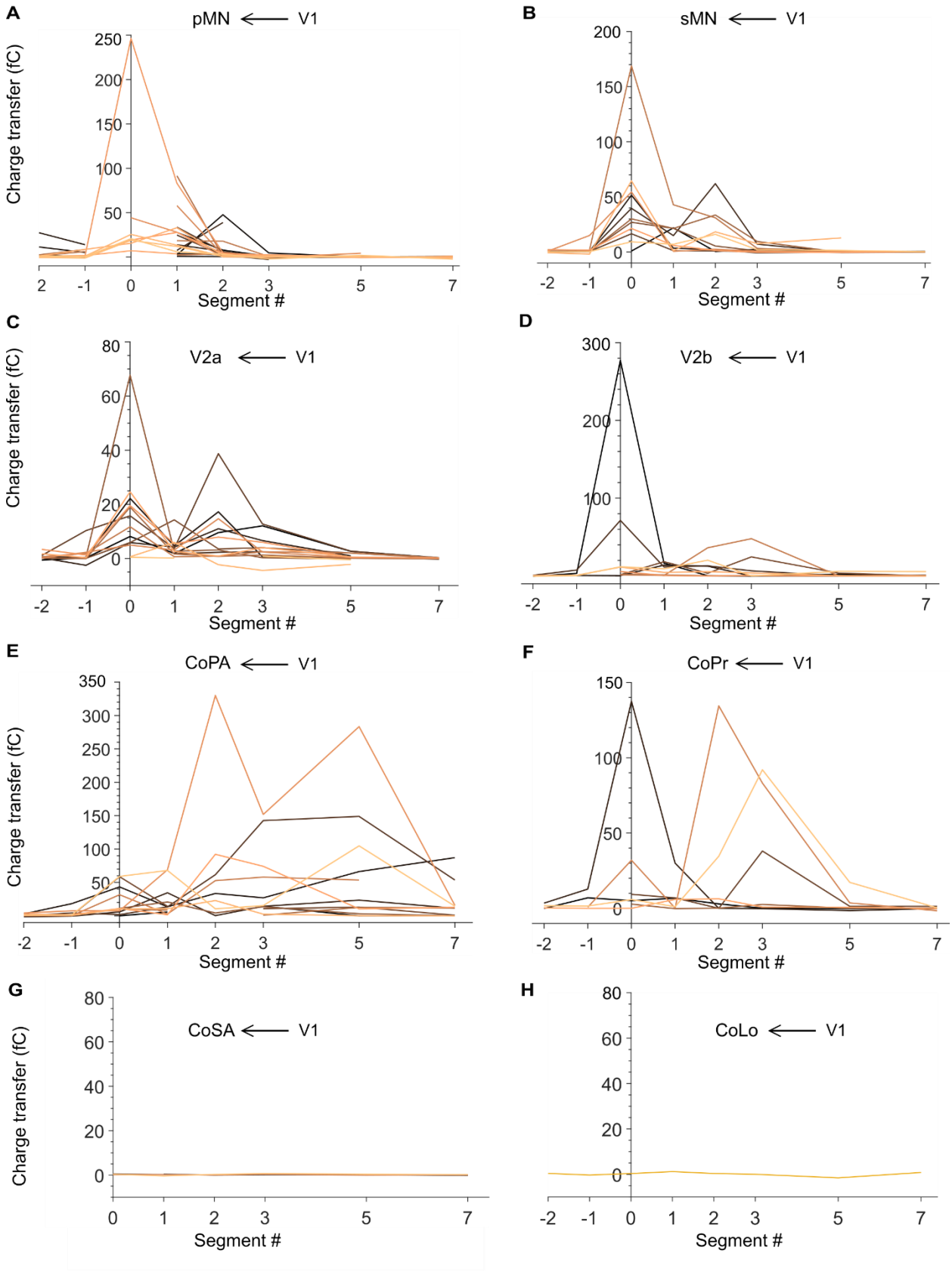
A
pMN ← V1
Charge transfer (fC)
Segment #
B
sMN ← V1
Segment #
C
V2a ← V1
Charge transfer (fC)
Segment #
D
V2b ← V1
Segment #
E
CoPA ← V1
Charge transfer (fC)
Segment #
F
CoPr ← V1
Segment #
G
CoSA ← V1
Charge transfer (fC)
Segment #
H
CoLo ← V1
Segment #

Figure S6: Cell by cell comparison of V1 connectivity to different post synaptic targets. Related to Figure 6.

A-H. Line plots showing total charge transfer across the R-C axis for pMNs (A), sMNs (B), V2as (C), V2bs (D), CoPAs (E), CoPrs (F), CoSAs (G) and one CoLo (H). Each line in each plot represents one recorded cell. Same data has been used for box plots in the respective main figures for each target population.

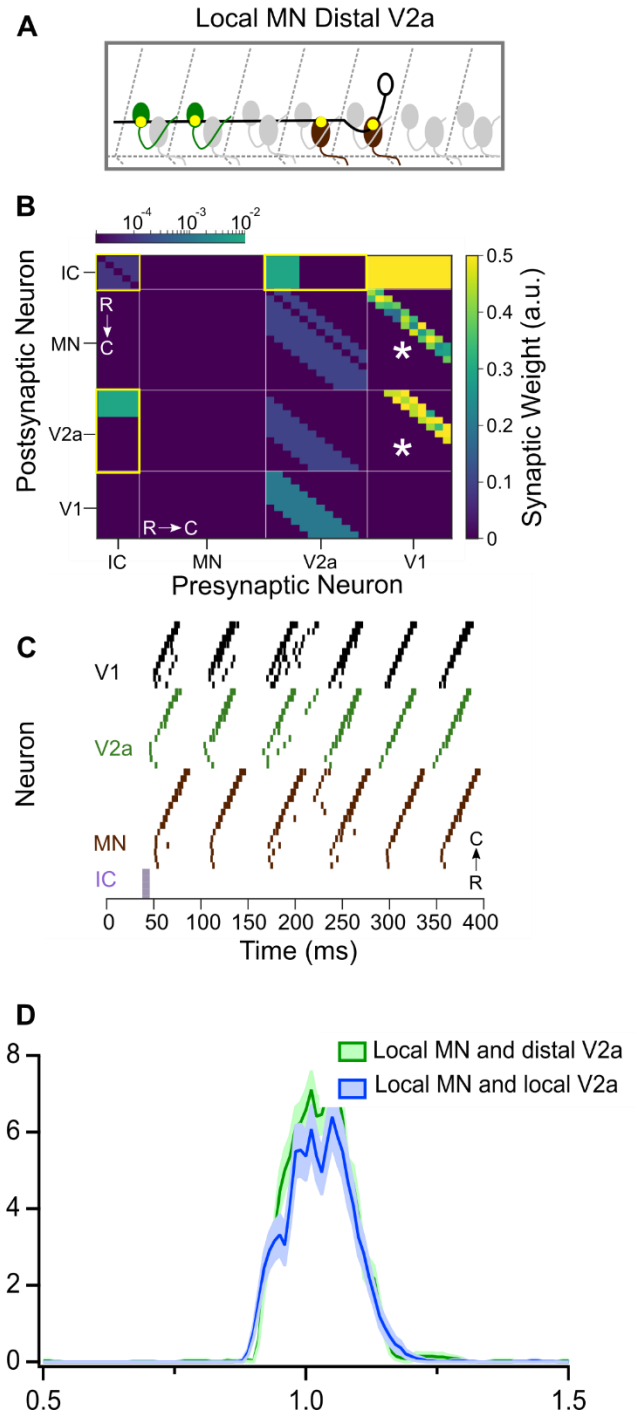

Figure S7: Spinal cord model with distal V1 to V2a and local V1 to MN connectivity. Related to Figure 7.

A. Schematic showing distal V2a and local MN connectivity from V1s. V1s synapse onto MNs 1 to 3 segments away and V2as located within 4 to 6 segments. B. Heatmap showing connectivity weights (scale bar, right). Connections highlighted in yellow are gap junctional and follow a logarithmic scale (top).

Asterisks highlight the portion of the model that was altered, with connectivity shifted from more local to distal positions for the V1 connectivity to V2a neurons only. C. Raster plots of spike times from 1 representative simulation. Rostrally located neurons are at the bottom within each neuron class, and thus the locomotor propagation moves from bottom to top. E. Spiking of MNs with respect to the swim cycle compared between models.

Neuron Type	a	b	c	d	Peak V (mV)
Pacemakers	0.02	0.25	-50	2.0	-10
V2a	0.1	0.25	-53	6.0	0
V1	0.2	0.25	-53	6.0	0
MN	0.1	0.25	-53	6.0	0

Table S1: Izhikevich Parameters for model. Related to Figure 7.

Synapse Type	τ rise	τ decay	Reversal V (mV)
Glycinergic	0.5	3.0	-70
Glutamatergic	0.25	1.0	0

Table S2: Chemical Synapse Parameters for model. Related to Figure 7.