Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Material

Microchannel device tailored to laser axotomy and long-term microelectrode array electrophysiology of functional regeneration

Rouhollah Habibey,[a] Asiyeh Golabchi,[a] Shahrzad Latifi, [a,b] Francesco Difato*[a] and Axel Blau *[a]

[a] *Department of Neuroscience and Brain Technologies (NBT), Italian Institute of Technology (IIT), via Morego 30, 16163 Genoa, Italy*
[b] *Department of Neurology, David Geffen School of Medicine at UCLA, 635 Charles E. Young, Los Angeles, CA 90095*
** Corresponding authors: axel.blau@iit.it & francesco.difato@iit.it*

Part I. Supplementary Figures S1-S4

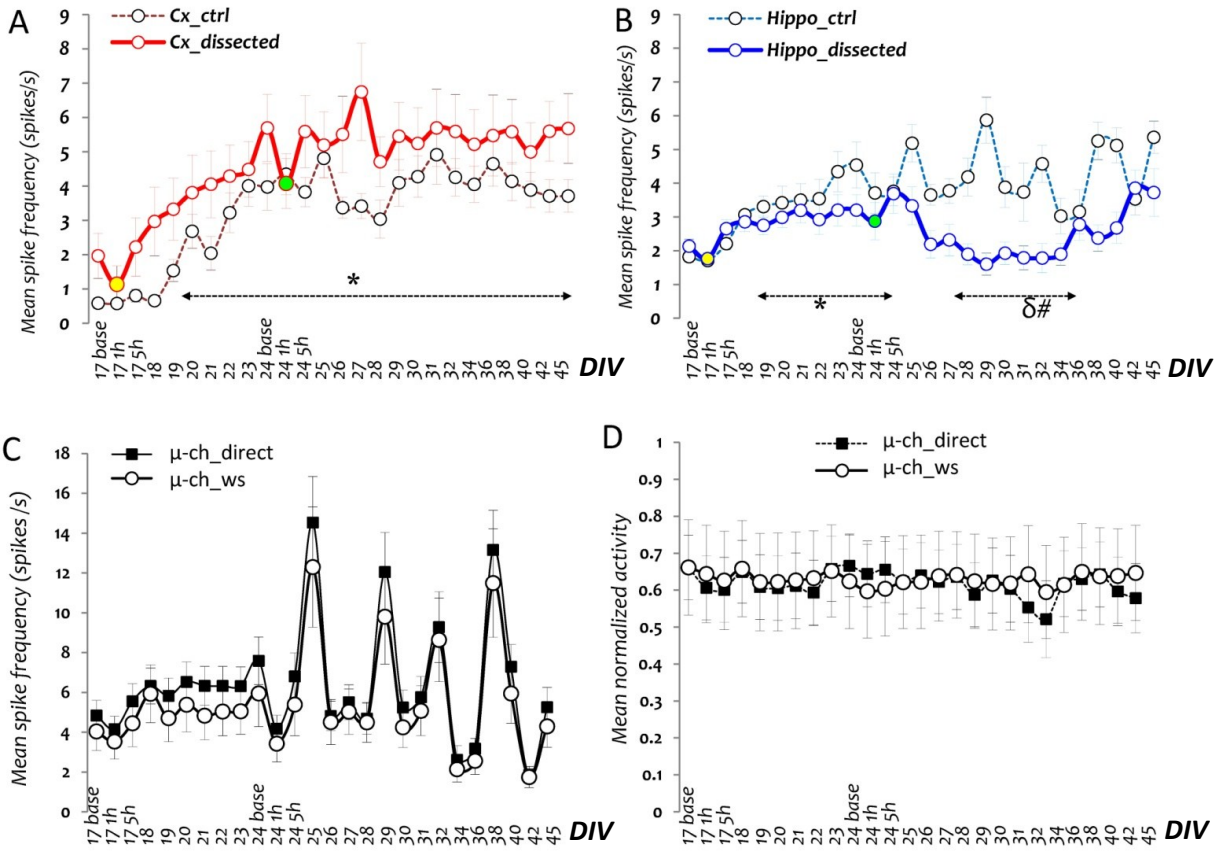

Fig. S1. Activity in dissected *vs.* control cortical or hippocampal cultures. At each dissection DIV, three activity recordings were acquired: baseline (17 base and 24 base), just after dissection (17 1h and 24 1h), and three to five hours after dissection (17 5h and 24 5h). **A**) Mean spike frequency (spikes/s) on electrodes recording from control (Cx_ctrl) and dissected (Cx_dissected) cortical cultures. **B**) Mean spike frequency (spikes/s) on electrodes recording from control (Hippo_ctrl) and dissected (Hippo_dissected) hippocampal cultures. Data are represented as the mean ± SEM of the spike frequency on all active electrodes of the same group (dissected or control) on each day. A mixed ANOVA followed by a *post hoc* Tukey test was performed to compare dissected groups with control groups at different time points. # $p < 0.05$ *vs.* ctrl group. A repeated measure ANOVA was applied to compare the mean spike frequency in all other sessions with the pre-dissection baseline activity (* $p < 0.05$ *vs.* 17 base, δ $p < 0.05$ *vs.* 24 base). **C**) Mean spike frequency and **D**) normalized activity in microchannels without working station (µ-ch; black squares) *vs.* microchannels with working stations (µ-ch_ws; circles) in all hippocampal control cultures. The mean spike frequency was calculated by averaging the firing rate on all electrodes in each microchannel type in hippocampal control cultures (n = 19 in µ-ch and n = 12 in µ-ch_ws). The normalization procedure is explained in the methods section of the main manuscript and Table S1.

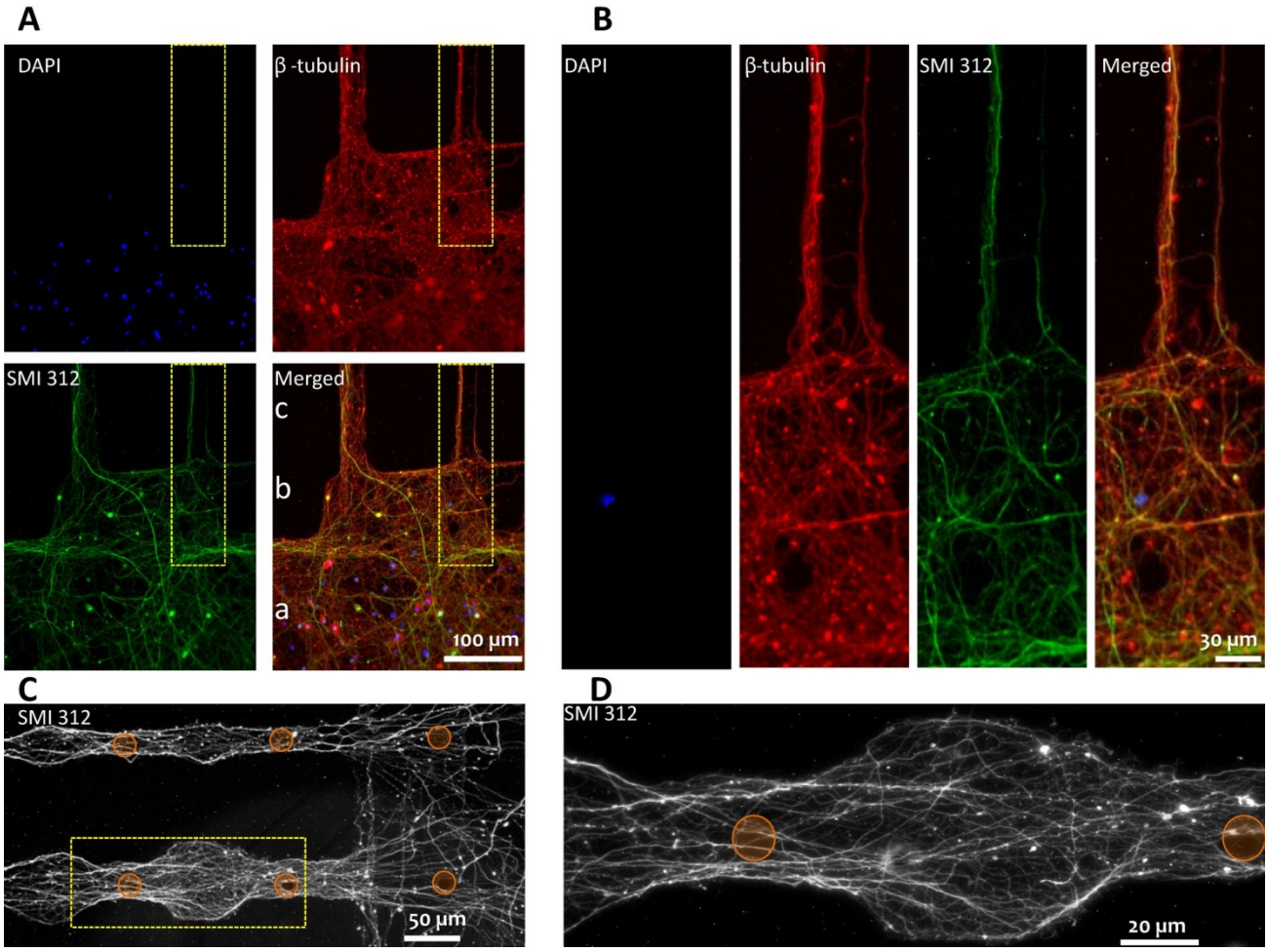

Fig. S2. Immunofluorescence images prepared from cultures on coverslips (A and B) and MEAs (C and D). A) Nuclei (DAPI), neurons (β-tubulin III), axons (SMI 312) and merged images. Reservoir (a), neurite cavity (b) and microchannels (c) of a PDMS device. **B**) Magnified view of a selected area (yellow rectangles in A) including the neurite cavity and the proximal region of a microchannel. **C**) SMI 312-labeled axons growing in a µ-ch (top) and µ-ch_ws (bottom). Orange circles indicate the recording electrodes. **D**) Magnified view on the axonal branching in a working station (yellow rectangle in C).

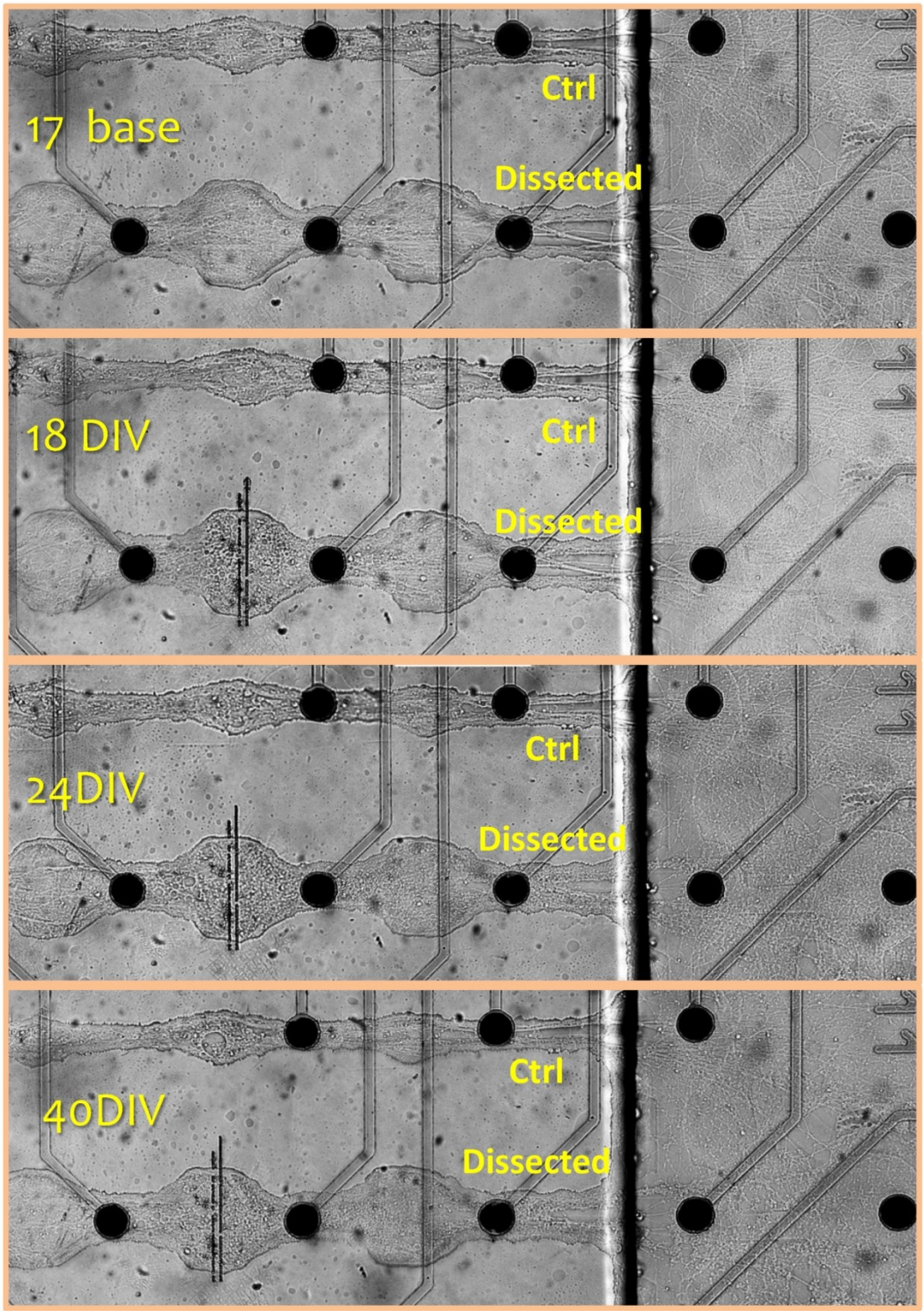

Fig. S3. Long-term evolution of axonal morphology in control *vs.* dissection microchannels. Axonal morphology in two adjacent microchannels, the lower one with dissected axons and the upper one with intact axons at different days. Axons in the dissection microchannel experienced a complete bundle transection at 17 DIV along the two vertical black lines. Electrode pitch: 200 µm.

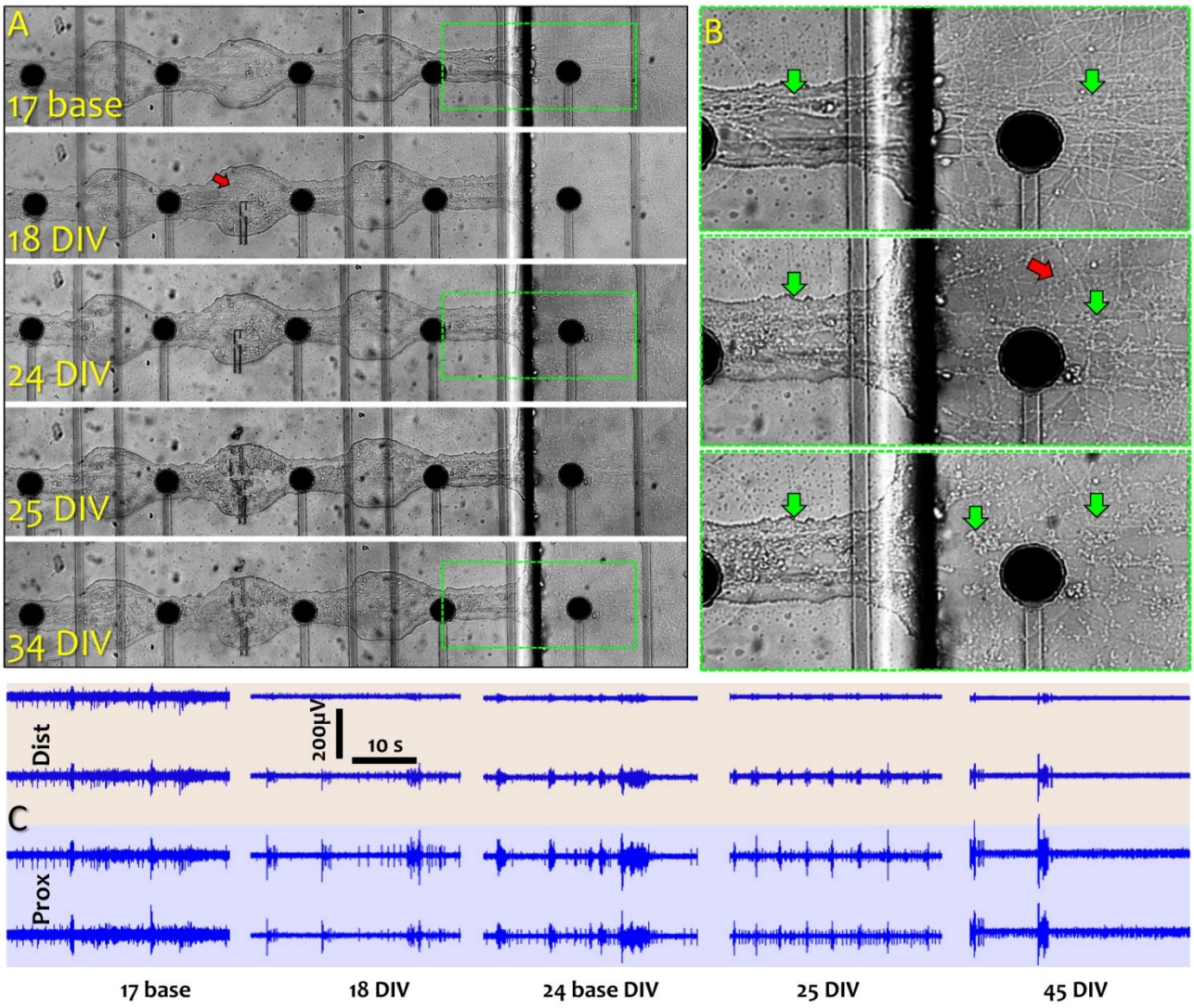

Fig. S4 Evolution of axonal morphology and activity after subsequent partial dissections. A) Axonal morphology after the first partial dissection at 17 DIV and a second partial dissection at 24 DIV. **B**) Magnified view of the green areas outlined in A showing the morphology of distal axons before the dissection (upper panel), one week after the first partial dissection (middle panel) and 10 days after the second partial dissection (lower panel). The deteriorative effect of the second partial dissection was more dramatic than that of the first partial dissection. Green arrows indicate degenerating axons, red arrows intact or regenerating axons. **C**) Recorded activity profile (30 s) on two proximal (bottom) and two distal electrodes (top) at different days.

Part II. Legends for the Supplementary Movies S1-S4

Movie S1. 3D animation of the assembled MEA-microchannel device placed on a motorized microscopy stage with the laser microdissection setup situated underneath. https://youtu.be/9aVunIJvhzY

Movie S2. Dissection example in a microchannel without working station. Axonal bundles are growing on two sides. The dissection of one axonal bundle not only cut all axonal fibers in that bundle, but also mechanically affected the axons on the opposite edge of that microchannel. Chosen picosecond UV laser dissection settings: average power of 10:25 µW at the sample, pulse repetition rate of 100 Hz. https://youtu.be/dKO8THPQHRU

Movie S3. To induce a complete axonal dissection in a working station, the motorized microscopy stage with the culture was moved back and forth with respect to the fixed focused laser beam. To ensure that all axons were dissected, the procedure was repeated twice at 17 DIV. Chosen picosecond UV laser dissection settings: average power of 10:25 µW at the sample, pulse repetition rate of 100 Hz. https://youtu.be/omwS-wYml6Y

Movie S4. A partial axonal dissection was induced by cutting the axons only in one half of the working station at 17 DIV. Chosen picosecond UV laser dissection settings: average power of 10:25 µW at the sample, pulse repetition rate of 100 Hz. https://youtu.be/2mZljzSm5Z0

Movie S5. A local axonal dissection was performed by cutting only a few axons in one corner of the working station at 17 DIV. Chosen picosecond UV laser dissection settings: average power of 10:25 µW at the sample, pulse repetition rate of 100 Hz. https://youtu.be/atP9cyAGjpU

Part III. Supplementary Tables

Table S1 Data treatment and averaging in four steps to calculate normalized or relative activity

Method A (Normalized activity), Fig. 6	Method B (Relative activity), Fig. 7
1 **Spike frequency (SF_i)** SF_i was extracted for each individual electrode *i* of each MEA	***1*** **Spike frequency (SF_i)** SF_i was extracted for each individual electrode *i* of each MEA
2 **Normalization (NSF_i)** For each day, the SF_i on each electrode *i* was divided by the maximum spike frequency (SF_{max}) recorded by one of the MEA electrodes during a 15 min recording session (Table 1). $NSF_i = SF_i / SF_{max}$	***2*** **Percentage of activity change (ΔSF_i)** For each electrode *i*, the spike frequency in any recording session (SF_{DIVi}) was expressed as the percentage change with respect to the spike frequency at 17 DIV (SF_{17i}) $\Delta SF_i = (SF_{DIVi} - SF_{17i}) / SF_{17i}$
3 **Baseline subtraction ($NSF_{DIVi\text{-}17i}$)** The normalized spike frequency for each electrode *i* preceding a dissection at 17 DIV (NSF_{17i}) was subtracted from the normalized spike frequency of the same electrode *i* in every other recording session. $NSF_{DIVi\text{-}17i} = NSF_{DIVi} - NSF_{17i}$	***3*** **Compartment average per session (ΔSF_{avS})** For each recording session S (*e.g.,* 15 min recording at18 DIV,Table 1), ΔSF_i of all electrodes belonging to the same compartment category *c* were averaged across all *n* MEAs that had been subjected to the same dissection type. $\Delta SF_{avS} = \frac{1}{n \cdot \#c} \sum_{j=1,\ i \in c}^{n} (\Delta SF_i)_j$
4 **Compartment average per session (NSF_{av})** The normalized and baseline-subtracted spike frequencies $NSF_{DIVi\text{-}17i}$ for electrodes belonging to the same compartment category *c* were averaged across all *n* MEAs that had been subjected to the same dissection type for each recording session (Table 1). $NSF_{av} = \frac{1}{n \cdot \#c} \sum_{j=1,\ i \in c}^{n} (NSF_{DIVi-17i})_j$	***4*** **Compartment average per time span (ΔSF_{avTS})** For each time span TS (*e.g.,* 18 DIV to 24 DIV, which corresponds to the first week after the first dissection;), ΔSF_{avS} of each compartment was averaged across all *m* recording sessions belonging to that time span. $\Delta SF_{avTS} = \frac{1}{m} \sum_{j=1}^{m} (\Delta SF_{avS})_j$

Table S2 Activity trends for different dissection types and time spans

DIV (time spans)				5 h after dissection		1 week recovery			5 h after dissection		3 weeks recovery	
				17d_D1		18d-24d			24d_D2		25d-32d	34d -45d
Cortex	Res			∨ -20.46		∧ 135.84			∧ 14.07		∧ 38.02	∧ 40.55
	Ctrl	Prox		∨ -23.17		∧ 51.48			∨ -16.92		∨ -1.88	∨ -11.09
		Dist		∨ -30.61		∧ 20.77			∨ -25.37		∨ -6.24	∨ -12.89
	Local	Prox	D1	∨ -19.01		∧ 92.21		D2	∨ -2.79		∧ 33.89	∧ 5.55
		Dist	D1	∨ -68.17		∨ -4.92		D2	∨ -45.27		∨ -2.28	∨ -12.03
	Partial	Prox	D1	∨ -28.36		∧ 28.10		D2	∨ -10.28		∧ 19.06	∧ 18.03
		Dist	D1	∨ -57.64		∨ -26.50		D2	∨ -31.91		∨ -25.52	∨ -14.08
	Complete	Prox	D1	∨ -41.96		∧ 40.36			∨ -22.87		∨ -5.27	∧ 14.88
		Dist	D1	∨ -76.41		∨ -49.00			∨ -25.99		∨ -6.44	∧ 61.61
Hippocampus	Res			∧ 3.50		∧ 34.02			∧ 1.76		∨ -37.06	∨ -13.63
	Ctrl	Prox		∧ 25.16		∧ 109.97			∧ 16.14		∨ -0.93	∨ -22.38
		Dist		∧ 12.14		∧ 104.71			∧ 13.37		∨ -0.70	∨ -26.79
	Local	Prox	D1	∨ -4.66		∧ 59.99		D2	∨ -11.58		∨ -36.41	∨ -63.76
		Dist	D1	∨ -27.13		∧ 40.12		D2	∨ -62.08		∨ -46.98	∨ -68.74
	Partial	Prox	D1	∨ -9.83		∧ 85.65		D2	∨ -0.26		∨ -23.62	∨ -48.45
		Dist	D1	∨ -44.64		∧ 66.27		D2	∨ -58.00		∨ -61.74	∨ -76.22
	Complete	Prox	D1	∨ -12.19		∧ 70.00			∧ 13.03		∧ 0.84	∨ -12.33
		Dist	D1	∨ -45.62		∧ 7.99			∨ -3.12		∧ 0.65	∨ -0.52

The yellow and green bars represent the first and the second dissection, respectively. Details on daily recording times, imaging and cell culture medium exchange are found in Table 1 in the main manuscript. Spike frequencies at different days were averaged over different time spans (Table 1 and Fig. 7). All data are given as the percentage of increase or decrease in mean spike frequency (SF) with respect to the pre-dissection values. For 18 to 24 DIVs, the pre-dissection activity at 17 DIV (17 base) was considered as the baseline; for 25 to 45 DIVs, the pre-dissection activity during the time span between 18 and 24 DIVs (18-24 base) was considered as the baseline. Res: reservoir, Ctrl: control axons, Prox: proximal axonal segment, Dist: distal axonal segment, D1: first dissection, D2: second dissection. An activity decrease or increase with respect to the previous time span is represented by a ∨ or ∧, respectively. The effect of the first dissection on the activity decrease was more profound in cortical cultures, whereas the effect of the second dissection was more profound in hippocampal cultures.