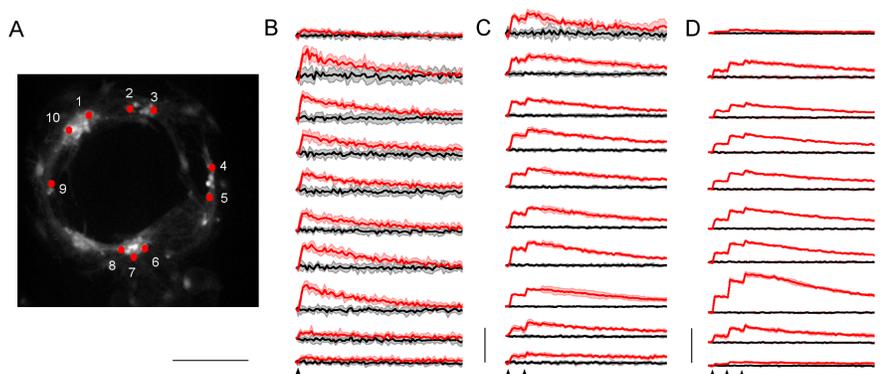


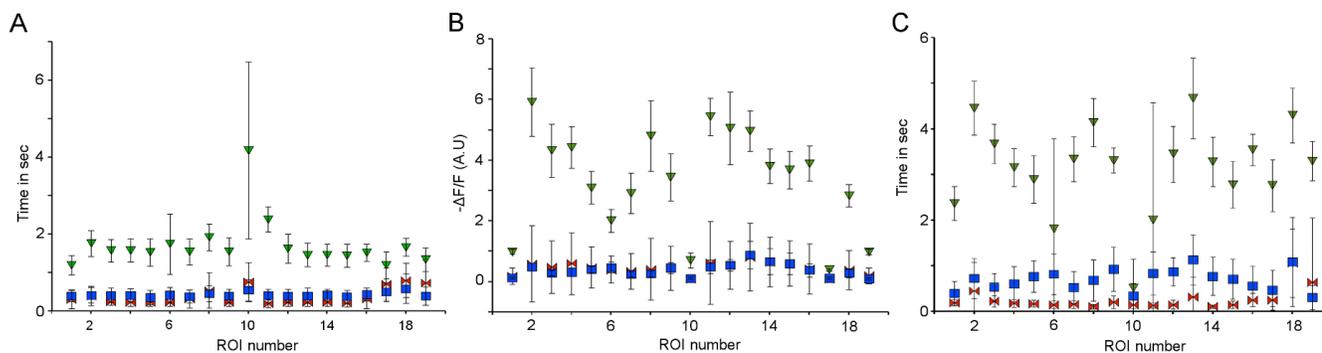
Ring-Shaped Neuronal Networks: A platform to study persistent activity

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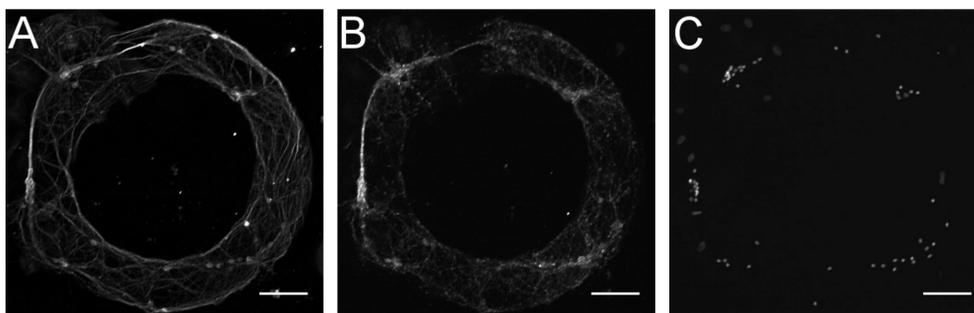
Electronic supplementary information



SFig 1: Correlated activity in neuronal ring cultures. (A) Fluorescent micrograph of neuronal cultures show a neuronal ring culture with 10 regions of interest (red dots). Regions of interest are chosen from brightfield images without knowledge of network connectivity or fluorescent loading intensity. The stimulating electrode position is shown in green prongs. (B) Red traces show an average response ($n=5$ trials) of each ROI following a single stimulation pulse (arrowhead). The background signal (black trace $n=5$ trials) is also shown. Average response for multiple stimulation pulses are shown for two (C) and three (D) stimulation pulses. Scale bars are: fluorescence image = $100\mu\text{m}$, X-scale is 1.6sec for all traces, Y-axis scales are $-0.5\Delta F/F$, $-1.0\Delta F/F$ and $-2.5\Delta F/F$ for B, C and D, respectively.



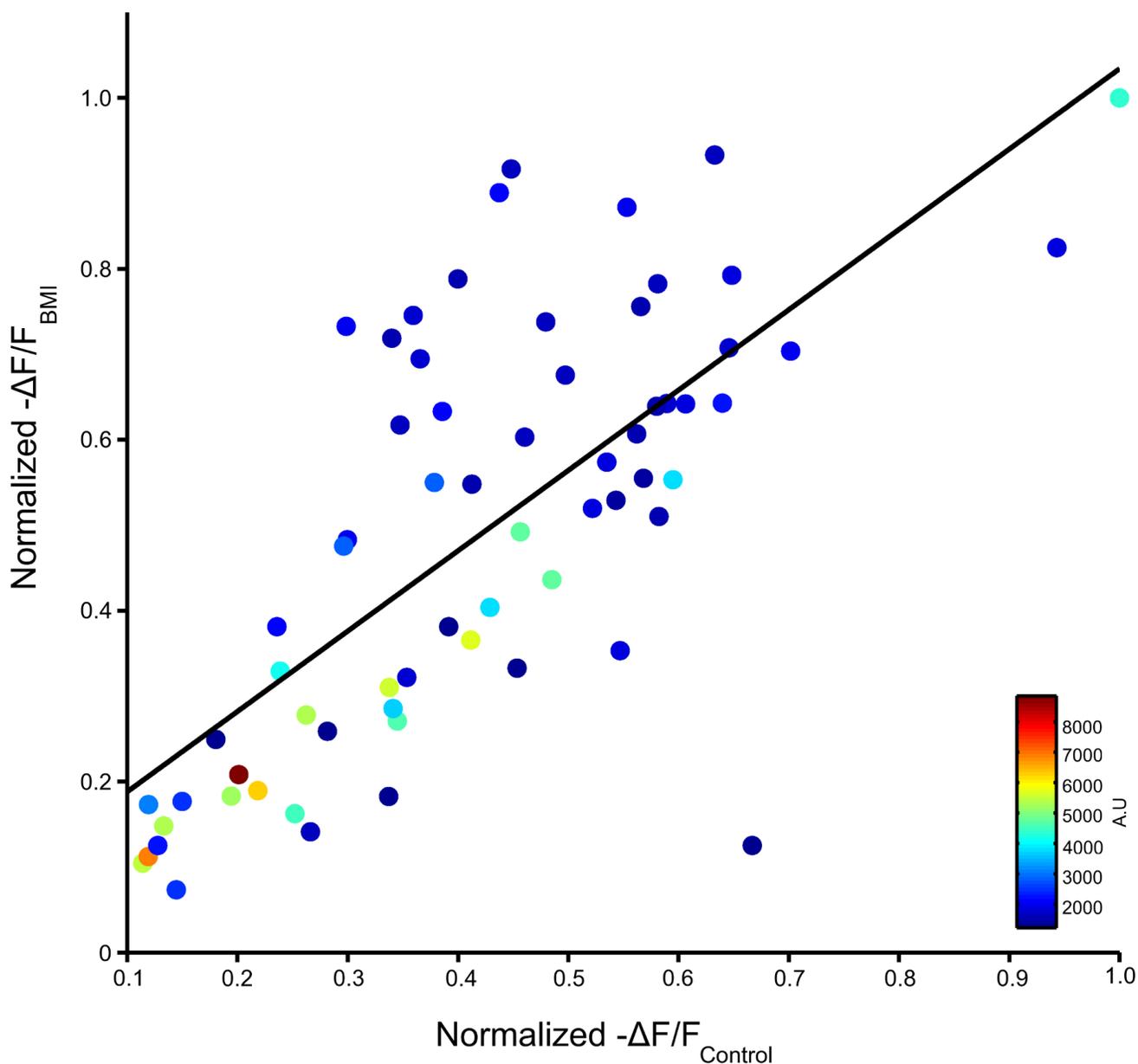
SFig 2: Response of networks perfused with medium+BMI are stronger and more sustained when compared with normal perfusion medium or media containing BMI+EGTA-AM. (A) Time of maximal response following stimulation for control medium (blue squares), +BMI (green diamonds), and +EGTA-AM (red bow-ties) of 10 ROIs from a single network (n=10 for each ROI). (B) Cumulative distribution of maximum response time from ROIs from multiple networks under control conditions (blue trace, n=66), BMI (green trace, n=80), and BMI+EGTA-AM (red trace, n=50). Maximal fluorescence response from a single culture (C) and cumulative distribution from multiple cultures (D). Duration of response at 50% of maximum from a single culture (E) and cumulative distribution from multiple cultures (F). Insets show average values ± SEM. Significance ($p < 0.05$) determined through Wilcoxon signed-rank test is shown (*).



SFig 3: Synaptic connectivity in the network. Fluorescent images from a neuronal network at DIV12 immunostained for neuronal processes (A, tubulin), pre-synaptic marker (B, synapsin1), and nuclear marker (C, Hoechst33342). Scale bar is 100µm

STable 1: Reverberation characteristics for ring networks at track-widths 150, 100 μm .

	Track width 150 μm		Track width 100 μm	
	Control	BMI	Control	BMI
Max $-\Delta F/F$ (A.U)	0.23 ± 0.01	1.41 ± 0.14	0.28 ± 0.05	1.25 ± 0.11
Time to $-\Delta F/F_{Max}$ (sec)	0.36 ± 0.01	0.83 ± 0.08	0.47 ± 0.02	0.94 ± 0.04
Half-width (sec)	0.43 ± 0.04	1.35 ± 0.13	0.28 ± 0.02	1.52 ± 0.12



SFig 4: Relationship of ROI activity strength between control and BMI conditions. A scatter plot is generated for the normalized maximal fluorescence change (relative to the other ROIs of the same network) at control conditions vs. the normalized maximal fluorescence change at BMI conditions. Data is plotted for all cultures. The solid line shows the linear trend of the data. Each data point is color-coded with the baseline fluorescence intensity of the ROI. The linear trend holds regardless of the baseline intensity. $R^2 = 0.5$