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Supporting Information

Graphene Oxide Nanosheets Hamper Glutamate Mediated Excitotoxicity and Protect Neuronal Survival In An *In vitro* Stroke Model

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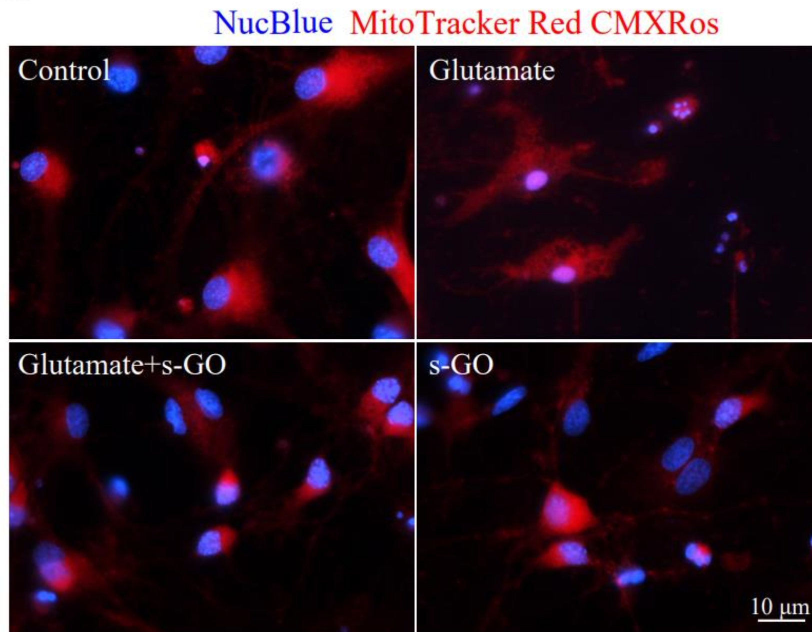
A



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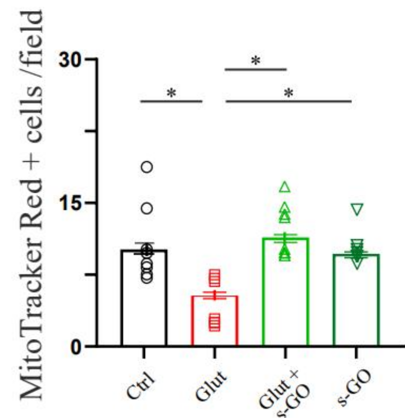
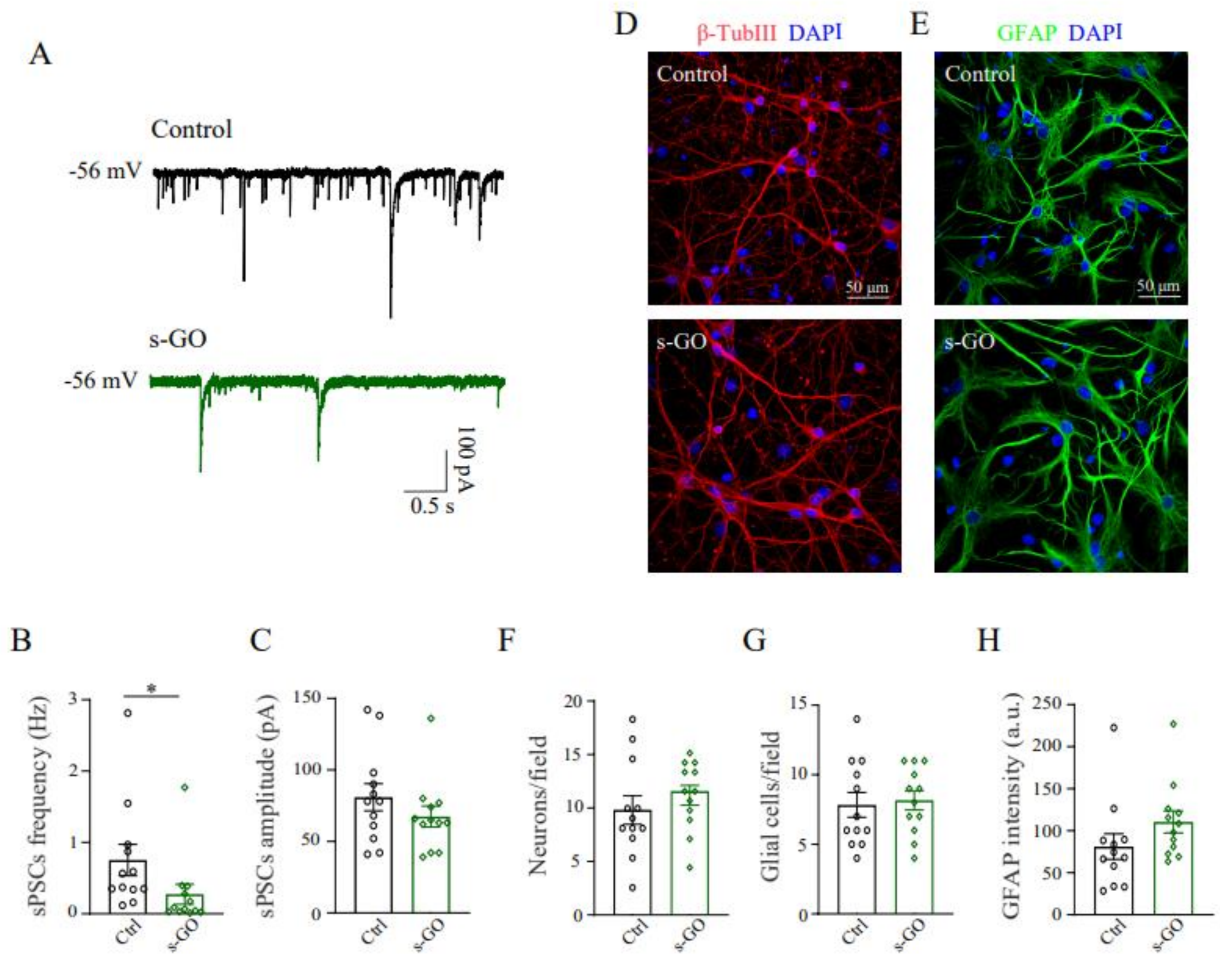


Supplementary Figure 1: In a model of primary excitotoxicity, s-GO exerts a protective effect against mitochondria damage. (A) Representative magnifications of confocal microscopy images in control, glutamate-treated, glutamate+s-GO and s-GO treated cultures, co-stained for NucBlue (in blue) and for the mitochondrial marker MitoTracker Red CM-XRos (in red). Live stainings were performed 24 hours after the excitotoxic insult, in which glutamate (40 µM) was applied for 30 minutes, with or without s-GO (10 µg/mL). As additional control, sister cultures were exposed to s-GO (10 µg/mL) only. Note that glutamate treatment induced a decrease in the number of MitoTracker Red CM-XRos positive cells and, in those marked, the typical reticular network structure of mitochondria[54]] appeared damaged. Both these aspects were reverted by the co-application of s-GO, while cultures treated with s-GO only were similar to controls. Scale bar 10 µm. (B) Bar plots showing the number of cells labelled by MitoTracker Red CM-XRos/field in the different conditions. These were 10.75 ± 0.06 cells in control, 5 ± 0.04 cells in glutamate, 12.57 ± 0.05 cells in glutamate+s-GO and 10.50 ± 0.05 cells in s-GO treated samples. Data are reported as mean ± SEM, while dots superimposed to the bars correspond to the single field values. N=8 fields for control and for glutamate, N=7 fields for glutamate+s-GO and N=6 fields for s-GO treated samples, *P<0.05.



Supplementary Figure 2: Application of s-GO in control cultures induces a decrease in synaptic activity, without modifying cell survival and reactivity. (A) Representative traces from control (in black) and s-GO treated cultures (in dark green). Bar plots of sPSCs frequency (B) and amplitude (C). N=12 each condition. Data are reported as mean ± SEM, while dots superimposed to the bars correspond to single cell values. Magnifications of representative confocal microscopy images obtained from cultures in control condition and exposed to s-GO (10 μg/mL), immunostained for the neuronal and glial markers β-tubulin III (in red, D) or GFAP (in green, E). In blue cell nuclei staining for DAPI. No changes were observed in neuronal density (F, 10 ± 1 cells/field in control and 12 ± 1 cells/field in s-GO treated samples), glial density (G, 8 ± 1 cells/field in control and 8 ± 1 cells/field in s-GO treated samples) or glial reactivity, measured as GFAP intensity (H, 81 ± 15 a.u. in control and 111 ± 13 a.u in s-GO treated samples). Data are reported as mean ± SEM, while dots superimposed to the bars correspond to the single field values. N=12 fields in each condition. *$P<0.05$