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Tuning Graphene Oxide nano-flakes reduction differently affects neuronal networks in the zebrafish

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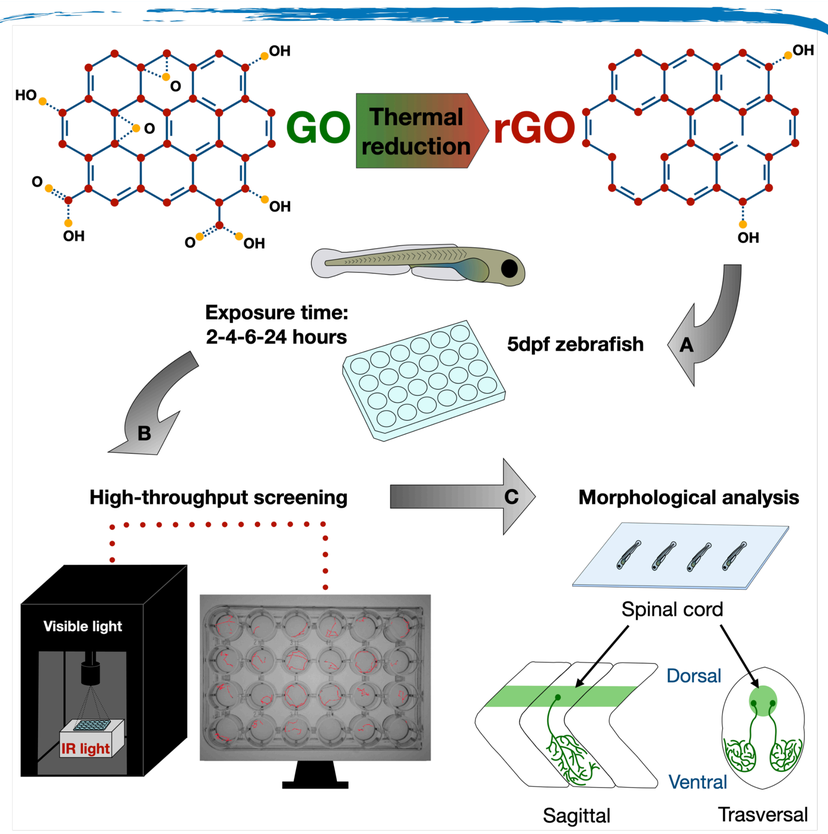
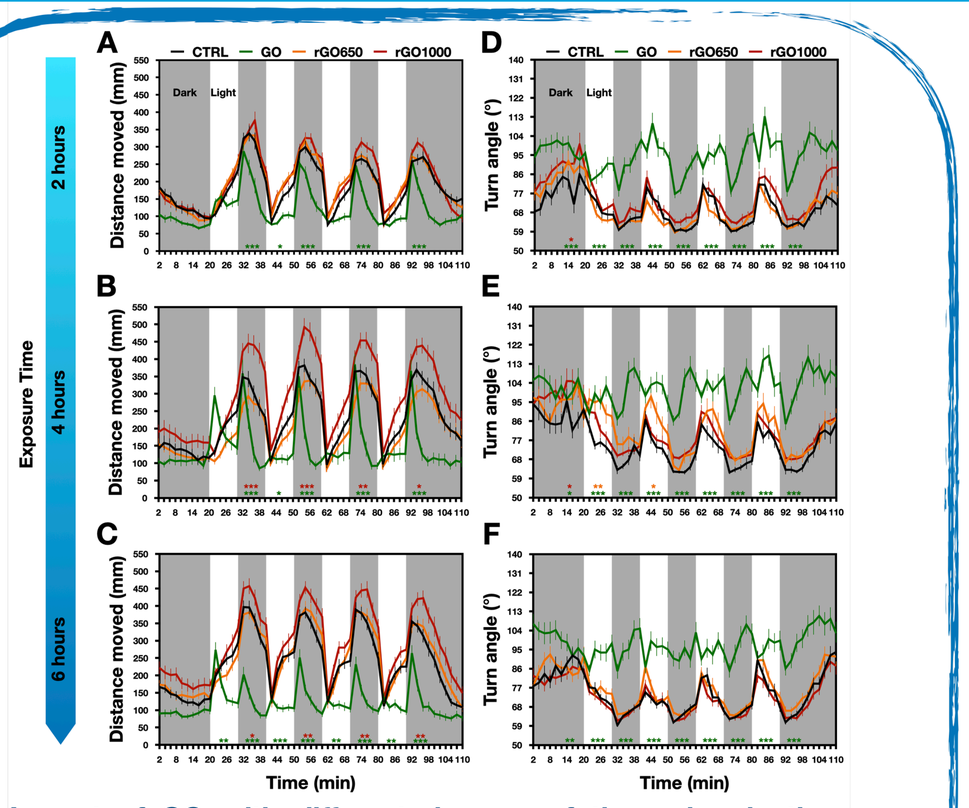


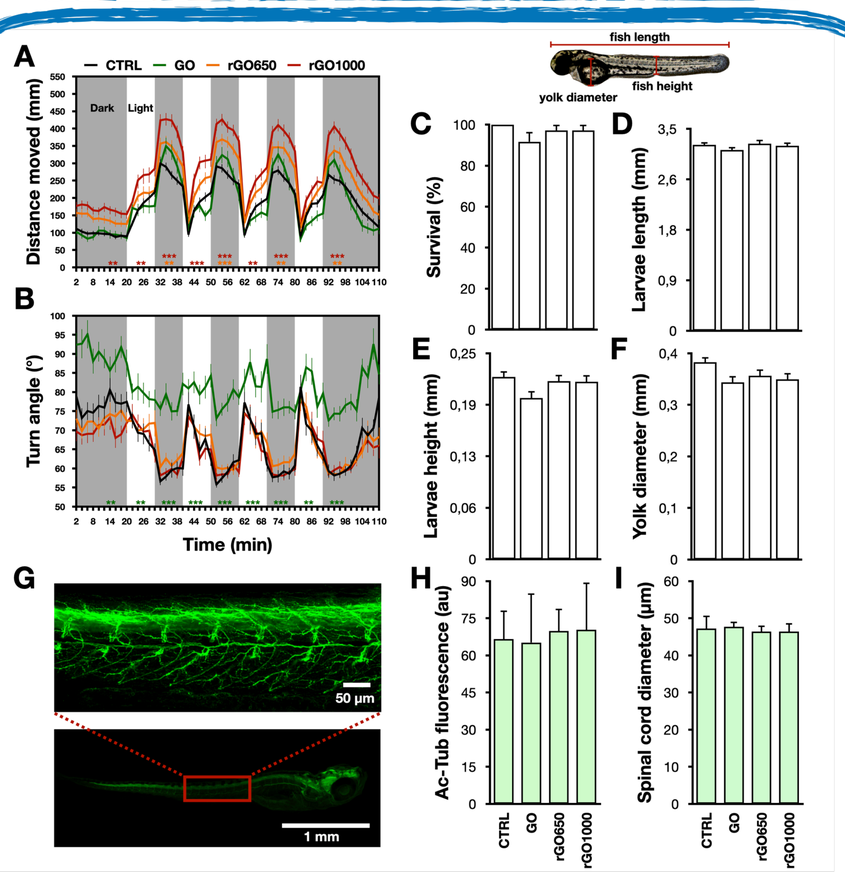
Experimental design of high throughput screening by using zebrafish larvae. rGO were obtained by thermal reduction of GO at 650 and 1000 °C **(A)**. Early-stage zebrafish (5 day post fertilization, dpf) were treated chronically **(B)** and used as behavioral model to investigate the effect on the sensory motor nervous system **(C)**. After behavioral experiments, animals were analyzed for anatomical traits and spinal cord characterization **(D)**.

1



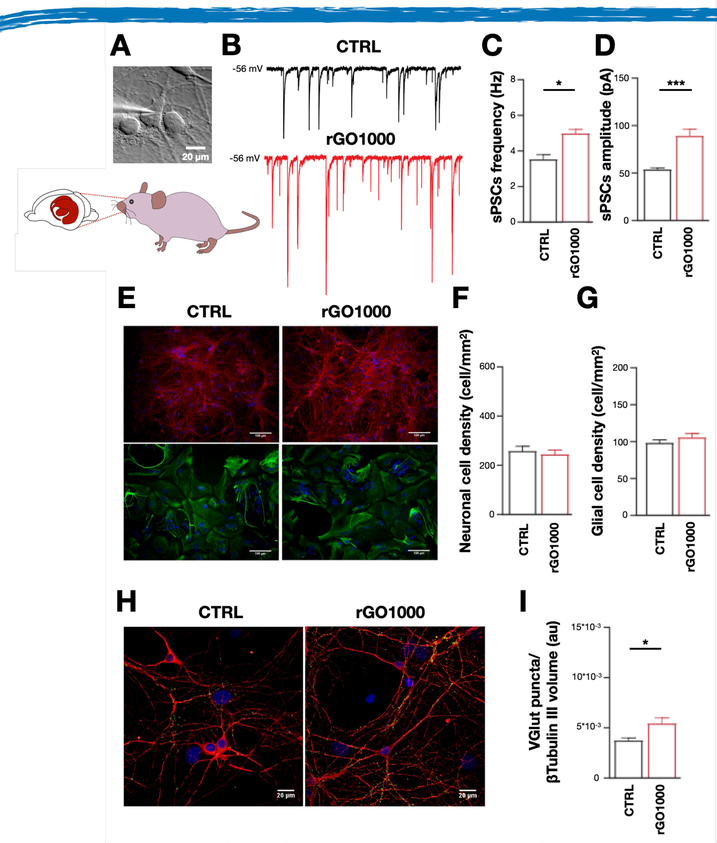
Impact of GO with different degrees of thermal reduction on locomotor behavior upon 2, 4 and 6 hours long lasting treatments. Locomotor activity was analyzed by measuring the distance moved and the turn angle (left and right columns, respectively). Animals were subjected to light (white bars) and dark (grey bars) alternating periods of 10 minutes each one. Line plots after 2 **(A-D)**, 4 **(B-E)** and 6 hours of treatment **(C-F)**. Statistical significance refers to the treatments respect to the control.

2



1 day chronic exposure of zebrafish larvae to rGO increase the locomotor performance without impair anatomical and neuronal traits. Locomotor activity of larvae was analyzed for distance moved **(A)** and turn angle **(B)**. Bar plots reported the anatomical analysis of larvae (in the inset on the top, the measured parameters), respectively the survival **(C)**, larvae length **(D)**, height **(E)** and yolk diameter **(F)**. On the bottom, a representative image of a whole mounted larvae labelled with the neuronal marker acetylated-tubulin (Ac-Tub), while on the top the spinal cord region in the red square is magnified **(G)**. Bar plots of the Ac-tub fluorescence intensity **(H)** and spinal cord diameter **(I)**.

3



6-8 days in vitro incubation of neuronal cultures with rGO1000 boosts synaptic activity. Dissociated neuronal cultures were obtained from rat brain and after exposure to rGO1000 were analyzed through patch clamp technique to monitor neuronal activity **(A)**. Exemplificative voltage clamp traces **(B)**. Bar plots represent pooled data of spontaneous postsynaptic currents (sPSCs) frequency **(C)** and amplitude **(D)**. Immunofluorescence images and plots are shown to visualize neurons (anti-β-tubulin III in red, **E-F**) and glial cells (anti-GFAP in green, **E-G**) in the two different conditions. **(H-I)** Confocal reconstructions and plot of control and rGO1000 treated neurons (in red) immunolabeled for the vesicular glutamate transporter 1 (VGLUT1 in green).

4

Conclusion: the increasing engineering of biomedical devices enriched by graphene-based components demand careful investigations of the impact on the nervous system. By exploiting the thermal reduction of GO to generate materials with different oxygen/carbon ratio, we reported that GO down regulated the zebrafish swimming performance. Conversely, rGO treatments boosted motor nervous system. Electrophysiological evidence suggested that such effects might depend on the interference of nanomaterials with synaptic communication. We concluded that the manipulation of a single chemical property, as the degree of GO reduction, is enough to induce differential effects of nanomaterials on nervous system function.

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