Graphene Oxide disrupts lipid composition, Ca^{2+} homeostasis and synaptic transmission in primary cortical neurons, without affecting neuronal survival and excitability

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Graphene (G) is considered one of the most promising nanomaterials for biomedical applications and it is already employed in many industrial sectors such as optics, electronics and mechanics [1]. Because of its two-dimensional shape and unique electrical and mechanical properties, its potential employments range from industrial applications to biomedical use, particularly in the field of neuro-interfaces and neuroprostheses [2]. Thus, a detailed exploration of the biocompatibility and functional consequences of the contact between graphene-based materials and neurons is of paramount importance for paving the way to future applications.

In particular, we studied the effects of the contact of thoroughly characterized nanosheets [3] of either pristine graphene (GR) or graphene oxide (GO) with primary cortical neurons from the rat brain. Parameters such as cell viability, morphology and functionality, as well as the intracellular route of internalized flakes, were first studied by employing a range of cell biology techniques, confocal and electron microscopy, and electrophysiology. One fundamental finding is that the interaction of neurons with GR and GO flakes does not affect neuronal cell viability (Figure 1). Furthermore, also the passive and active electrophysiological properties as well as the intrinsic excitability of neurons were fully preserved, demonstrating that G-exposed cultures are substantially healthy.

Large flake aggregates (in the micro-size order) mostly adhered to the cell membrane, while nano-flakes were up-taken and internalized by neurons (Figure 1). Imaging analysis revealed the intracellular location of nano-flakes and their trafficking from the early contact with the cell membrane to their final destination. Flakes that underwent endocytosis preferentially followed the endo-lysosomal pathway, with a significant percentage of G flakes ending up into lysosomes. To understand in more detail the neuro-physiological changes induced by flake exposure we investigated the protein and lipid contents of cells exposed to GO by undertaking proteomics and metabolomics approaches, and identified variations of key neuronal processes such as Ca^{2+} metabolism, membrane trafficking and autophagy. Results from the "omic" analysis were confirmed by live-cell Ca^{2+} imaging, electrophysiology and confocal microscopy analysis of treated cells. Altogether, while no strong effects on cell viability, excitability and network formation were observed, our results show a clear effect of GO on the lipid composition and Ca^{2+} metabolism of treated cells, which resulted in (i) a reduction of frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) (Figure 2), (ii) a decrease in the number of excitatory synaptic contacts, and (iii) a strongly upregulated autophagic reaction(Figure 2).

This work performs a comprehensive analysis of the effects of graphene flakes exposure to primary neuronal cells, under controlled experimental conditions. Overall, although G flake exposure does not impact on cell viability and network formation, it does nevertheless have important effects on neuronal transmission and network functionality, thus warranting caution when planning to employ this material for neurobiological applications such as drug delivery. As a final remark, while this work focuses on the biocompatibility of flakes, it leaves still open the issue of biocompatibility of planar G surfaces to be used as electrodes for *in vivo* applications. This is altogether a different challenge, which is currently being investigated. We believe the information given by this type of analysis will be crucial when attempting to design and engineer G-based devices for biomedical applications.

References

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[2] Kostarelos and Novoselov, Science, **344 (6181),** (2014) 261-3
[3] Leon et al., ACS nano, **14(2)**, (2013), 63-75

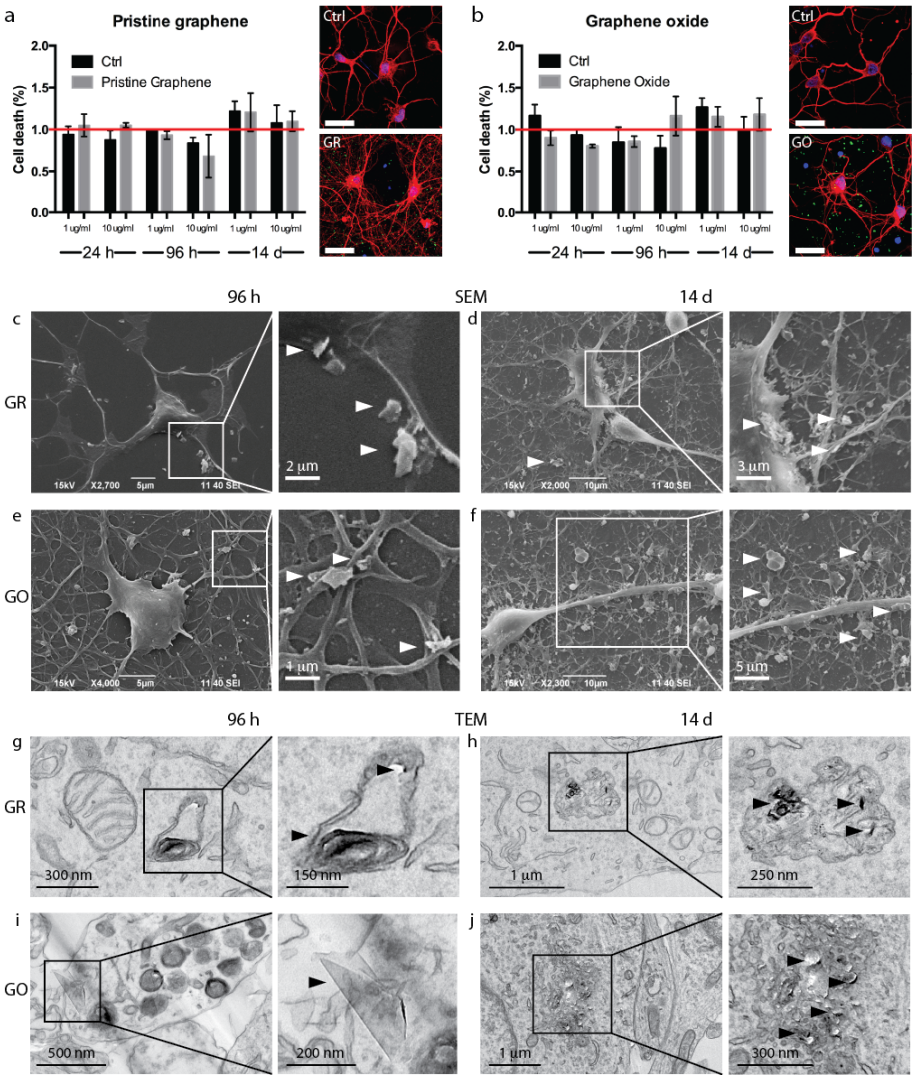

Figure 1. Viability and morphology of primary neurons exposed to GR and GO flakes.

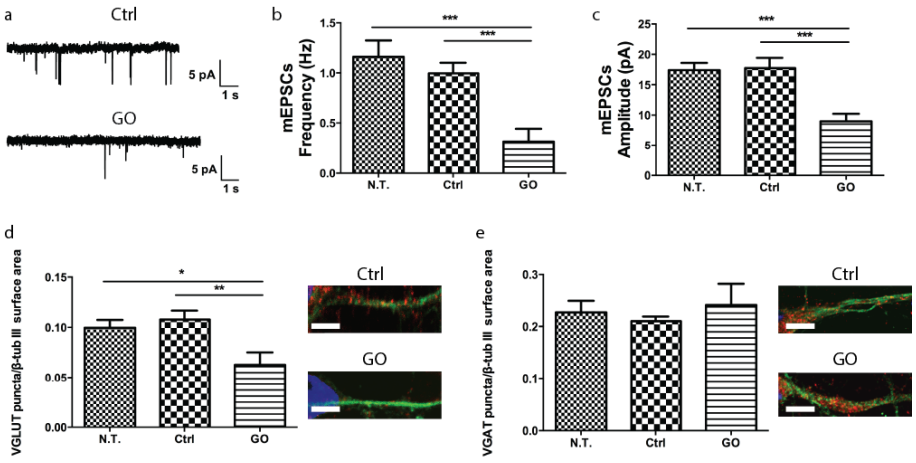

Figure 2. Synaptic density and activity in GO-treated primary cortical neurons.