Supporting Information

Graphene oxide nanosheets modulate spinal glutamatergic transmission and modify locomotor behaviour in an *in vivo* zebrafish model

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Preparation of s-GO and physicochemical characterization

Preparation of s-GO. Graphite flakes (Graflake 9580) were purchased from Nacional de Grafite Ltda. (Brazil), whereas sodium nitrate, sulfuric acid 99.999% and potassium permanganate were acquired from Merck-Sigma Aldrich (UK). These reagents were mixed for 30 min, following a previously described modified Hummers method [10,29,30,33]. Dropwise addition of 37.5 mL of water for injections (Fresenius Kabi, UK) promoted a dramatic temperature rise, after which it was stabilised at 98 ^{o}C for another 30 min. The mixture was then diluted with 112.5 mL of water for injection and 12.5 mL of 30% hydrogen peroxide (Merck-Sigma Aldrich, UK), in order to reduce the residual potassium permanganate, manganese dioxide, and manganese heptoxide to soluble manganese sulfate salts. After the reaction terminated, the resulting mixture was purified after several centrifugation steps at 9000 rpm for 20 min until a viscous orange/brown gel-like layer of pure GO could be carefully extracted from the graphitic pellet using warm water.

The obtained GO dispersion was sonicated in a bath sonicator (VWR, UK) for 5 min at 80 W, and centrifuged at 13000 rpm for 5 min at room temperature to prepare the s-GO. *Transmission Electron Microscopy.* Structural properties such as lateral dimension and thickness of the GO materials have been studied by TEM and AFM. TEM was performed using a FEI Tecnai 12 BioTwin microscope (FEI, Netherlands), operating at an acceleration voltage of 100 kV, with spot size = 1 and aperture size = 3, and the electron beam exposure time set at 0.1 s. Images were taken with a Gatan Orius SC1000 CCD camera (GATAN, UK), with exposure time of 3 s. Twenty μL of s-GO (100 μg/mL) were placed on a Formvar/carbon-coated copper grid (CF400-Cu, Electron Microscopy Services, UK) and allowed to adsorb for 1 min. Filter paper was used to remove excess liquid.

Images were processed on ImageJ (version 1.49, NIH, USA) and lateral size distribution was obtained after manual segmentation of hundreds of sheets, obtaining their Feret diameter. *Atomic Force Microscopy.* A Multimode 8 AFM (Bruker, UK) was used on tapping-mode in air with a J-type scanner, Nanoscope V8 controller, and an OTESPA silicon probe (Bruker, UK). Images were acquired after depositing 20 μL of s-GO (100 μg/mL) on a freshly cleaved mica surface (Agar Scientific, Essex, UK) coated with poly-L-lysine 0.01% (Merck-Sigma Aldrich, UK) and allowed to adsorb for 5 min. Excess unbound material was removed by rinsing with Milli-Q water and then allowed to dry overnight at 37^{o}C.

Lateral dimension and thickness distributions of GO were carried out using NanoScope Analysis software (version 1.40 Bruker, UK).

Raman Spectroscopy. Samples were prepared for analysis by drop casting 20 μL of s-GO (100 μg/mL) onto a glass slide. After drying for at least 2 h at 37 °C, spectra were recorded using a DXR micro-Raman spectrometer (Thermo Scientific, UK) set with a 50× objective lens. Samples were exposed to a $\lambda = 633$ nm laser for 25 s at an intensity of 0.4 mW.

Spectra were averaged over five independent locations and considered between 250 and 3500 cm^{-1}. The average intensity ratio between the D and G bands (I_D/I_G) was then calculated, in order to evaluate the presence of sp^3 defects in the graphene lattice. *Zeta-Potential Measurements.* Electrophoretic mobility (μ) was measured at room temperature using a ZetaSizer Nano ZS (Malvern Instruments, UK), after dilution of s-GO in water (100 μg/mL). Default instrument settings and automatic analysis were used for all measurements, including a backscattering angle of 173°.

The equipment software converted automatically μ to zeta-potential (ζ) values following the Henry’s equation. Reported values correspond to the mean ± SD of triplicate measurements. *X-ray Photoelectron Spectroscopy.* Lyophilised s-GO was analysed at the National EPSRC

XPS Users' Service (NEXUS) facility, hosted by nanoLAB at Newcastle University. XPS measurements were achieved using a Thermo Theta Probe XPS spectrometer with a monochromatic Al K-α source of 1486.68 eV. The survey XPS spectra were acquired with pass energy (PE) of 200 eV, 1 eV step size, 50 ms dwell time and averaged over 5 scans. The etching was 90 s. The high resolution C1s XPS spectra were acquired with PE of 40 eV, 0.1 eV step size, 100 ms dwell time and averaged over 20 scans. Spectral analysis was carried out using CasaXPS software (Casa Software Ltd, UK), after charge calibration to the adventitious carbon component (284.6 eV). High-resolution C 1s spectra were deconvoluted after a Shirley background subtraction, using Gaussian-Lorentzian (70%:30%) models to fit the identified functional groups (π−π*: 290.0 – 292.0 eV; O−C=O: 288.6 – 290.0 eV; C=O: 286.8 − 287.8 eV; C−O: 285.5 − 286.6 eV). The asymmetric carbon-carbon peak, corresponding to sp^2 and sp^3 hybridisations, was fitted with an asymmetric Lorentzian function (284.5 – 284.6 eV). Apart from the $\pi - \pi^*$ contribution, the remaining deconvoluted peaks had their full width half maximum value constrained between 0.5 and 2 eV.

Preparation of dfG and physicochemical characterization

Preparation of dfG. The graphene ink is formulated from graphite *via* liquid phase exfoliation [31] in water [32,57]. In details, 1.5 g of graphite flakes (Sigma Aldrich, 100+ mesh) and 250 mg of 1-Pyrenesulfonic acid sodium salt (from Sigma Aldrich) were mixed into 500 mL of de-ionized (DI) water. The mixture was then sonicated at 600W using a Hilsonic bath sonicator for 7 days. Afterwards, unexfoliated graphite was removed by centrifugation (Sigma 1-14k refrigerated centrifuge) at 3500 rpm (903g) for 20 minutes. The supernatant containing graphene and PS1 in water was collected and then centrifuged again at 15000 rpm for 1 h to collect the sediment. After centrifugation, the supernatant containing excess amount of pyrene in water was discarded. The precipitate was re-dispersed in water. Structural properties such as lateral dimension and thickness of the dfG materials have been studied by AFM.

Transmission Electron Microscopy. TEM was performed using a FEI Magellan 400L XHR SEM at 20 keV in transmitted electrons mode using a high angle annular dark field (HAADF) STEM detector and a specially adapted holder was used for sample visualization. Samples dispersed in ultrapure water were deposited onto a copper grid coated with a lacey carbon film (Agar-Scientific).

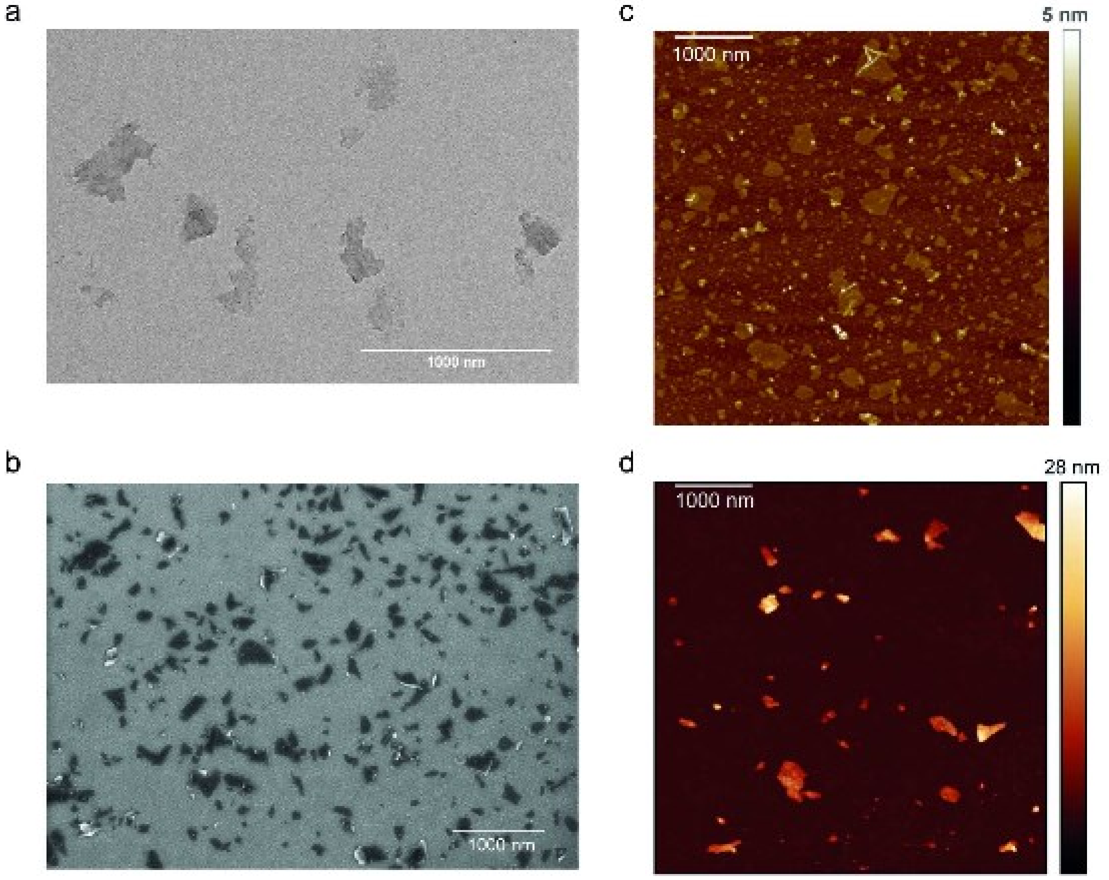
Atomic Force Microscopy. A Bruker Atomic Force Microscope (MultiMode 8) in Peak Force Tapping mode, equipped with ScanAsyst-Air tips is used to determine the lateral size distribution of the flakes. The sample was prepared by drop casting the solution on a clean silicon substrate; several areas of 100 μm^2 were scanned and about 130 flakes were selected for lateral size analysis. Lateral dimension and thickness distributions of dfG were carried out using NanoScope Analysis software (version 1.40 Bruker, UK).

Raman Spectroscopy. The sample was prepared by drop casting the solution on a clean silicon substrate. Raman measurements were performed using a Renishaw Invia Raman spectrometer equipped with 633 nm excitation line with 1.0 mW laser power. 100X NA0.85 objective lens and 2400 grooves/mm grating were used. Spectra were measured between 250 and 3500 cm^{-1}, enabling visualization of the D, G and 2D bands.

The average I_D/I_G ratio for each sample was then calculated.

Zeta-Potential Measurements. Electrophoretic mobility (μ) was measured using a ZetaSizer Nano ZS (Malvern Instruments, UK) after dilution of samples with water (100 μg/mL) in disposable cuvettes (Malvern Instruments, UK). Default instrument settings and automatic analysis were used for all measurements, performed at room temperature. The equipment software converted automatically the μ to zeta-potential (ζ) values by Henry's equation. All values for samples prepared are triplicate measurements, and values were mean ± SD.

X-ray Photoelectron Spectroscopy. XPS was performed using a SPECS Near-Ambient Pressure XPS instrument (operating under UHV conditions). X-Rays were produced by a monochromated Al Kα source (1486.6 eV). The analyser was a SPECS Phoibos 150 NAP analyser. Samples were fixed using double-sided carbon tape. Survey and high-resolution spectra were acquired using a spot size of 300 μm and pass energy of 60 eV and 30 eV, respectively. Analysis of the data was carried out with CasaXPS software. A Shirley background was used in curve-fitting along with a GL (30) line-shape (70% Gaussian, 30% Lorentzian using the Gaussian/Lorentzian product form) for the C 1s, N 1s and O 1s spectra. Samples were referenced to the C 1s emission by adventitious hydrocarbon contamination at 284.8 eV.



Supplemetal figure 1. Physicochemical characterization of s-GO (a, c) and dfG (b, d). (a, b) TEM micrographs of s-GO and dfG, respectively. (c, d) AFM images of s-GO and dfG, respectively. Note the similar dimensions for s-GO and dfG. Lateral dimension distributions can be found elsewhere [33,58].

References

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