Supplemental Digital Content

Paclitaxel-induced painful neuropathy is associated with changes in mitochondrial bioenergetics, glycolysis and an energy deficit in dorsal root ganglia neurons

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Supplementary Figure 1. Oxygen measurements of isolated DRG neurons from paclitaxel/vehicle-treated animals at day 7

In each well, 10 sequential oxygen measurements of the media surrounding the plated cells are taken each time the fluorophore probe is lowered and the recording microchamber formed. As the cells continue to respire during the measurement, oxygen levels decrease. The fluorophore probe then rises and the subsequent mixing cycle allows the media to re-oxygenate before another set of 10 sequential oxygen measurements. These raw data show that our timings of the mix-wait-measure XF24 cycles are of appropriate length. The straight diagonal lines, without plateau or horizontal drift, formed by the 10 sequential oxygen measurements show that the measurement period is not too long. The first oxygen measurement, prior to and following additions from each injection ports (A-D), is at a very similar oxygen level, i.e. returns to the same starting point as the previous measurement cycle, showing that the mix-wait period is sufficient to allow complete reoxygenation of the media.
Supplementary Figure 2. Titration of optimal FCCP concentration for DRG neurons

A shows oxygen consumption rates of naïve dissociated DRG neurons after sequential addition of FCCP from ports A-D, concentrations above dotted lines indicate final concentration of FCCP at cells. B shows a more detailed concentration response; with FCCP injected in smaller increments from ports A-D, concentrations above dotted lines indicate final concentration of FCCP at cells. A & B show that an FCCP concentration above 0.2μM is toxic to DRG neurons (at our plating density) causing damage to the integrity of the mitochondrial membrane, as evidenced through decreased oxygen consumption rates.
Supplementary Figure 3. Titration of optimal oligomycin concentration for DRG neurons

Figure shows oxygen consumption rates of naïve dissociated DRG neurons after sequential addition of 0.5μM oligomycin from ports A-C, leading to exposure to increasing oligomycin concentrations; 0.5μM, 1.0μM & 1.5μM. 0.2μM FCCP was injected from port D to verify that the neurons are still respiring.
Supplementary Figure 4. Neuronal and non-neuronal contributions to oxygen consumption rates in isolated DRG cells

A shows the effect of removing neurons on the bioenergetic profile, graph shows the mean ± SEM of OCR measurements per well (n=5). Dashed lines indicate injection of media + DMSO (A; control), oligomycin (B), FCCP (C), antimycin A and rotenone (D). B shows the effect of removing neurons on the glycolytic profile, graph shows mean ± SEM of ECAR measurements per well (n=5). Dashed lines indicate injection of media + DMSO (A; control), oligomycin (B). No washing refers to isolated, naive DRG cells that were plated and incubated overnight before the bioenergetic assessment; the method which was followed throughout these studies. Wash times refer to the length of time isolated, naive DRG cells were kept at 37°C before washing wells with media. These profiles were observed on three separate occasions (n=5 wells per condition, per experiment) spread over a number of months. Across three experimental replicates, five wells per condition, non-washed wells contained 8.8-10.2% neurons, whilst washed wells contained 0.2-1.2% neurons.