Effect of inflammatory stress on neural stem cells behaviour: a rescue effect of phosphatidylcholine by modulating neuronal plasticity.

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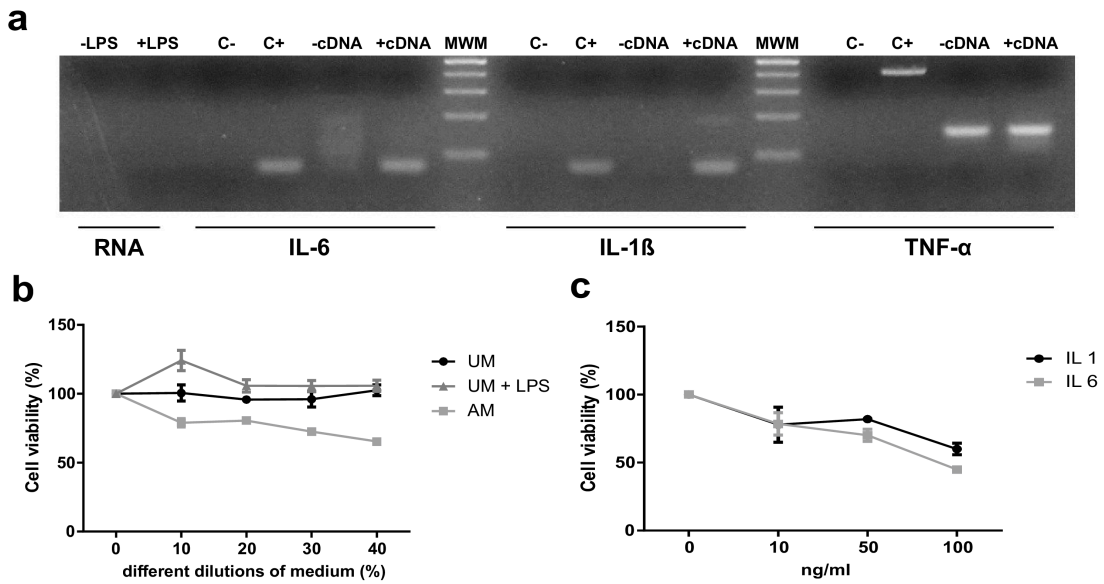
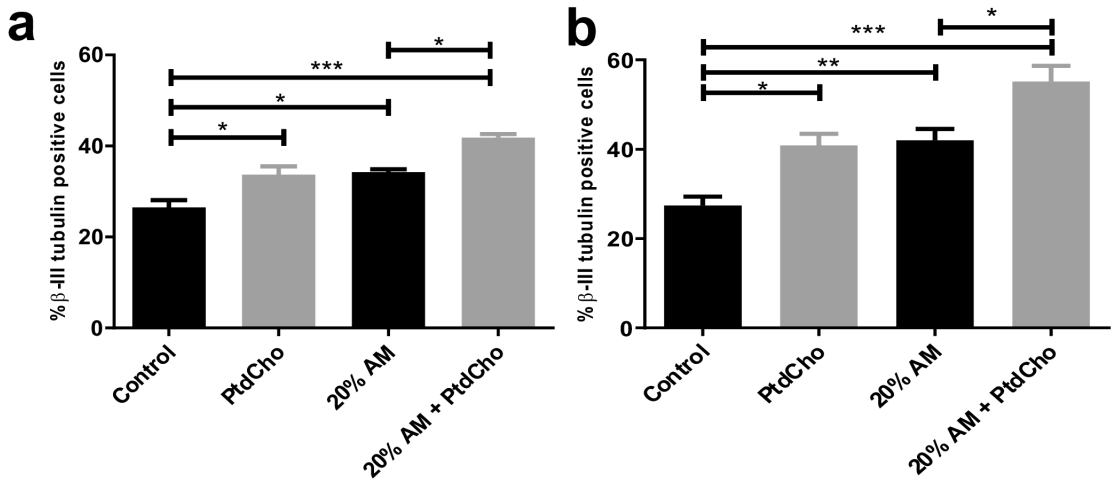
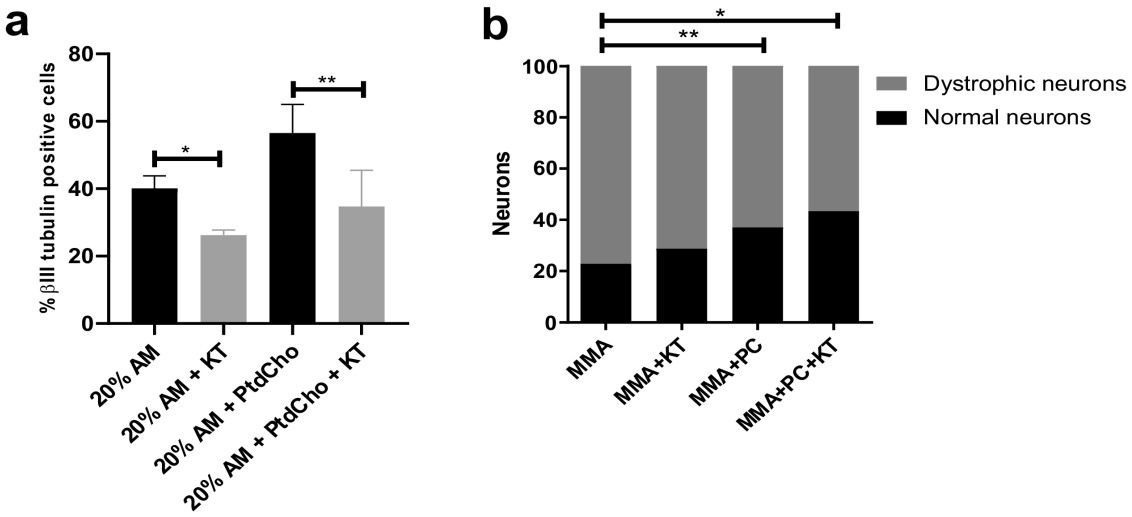


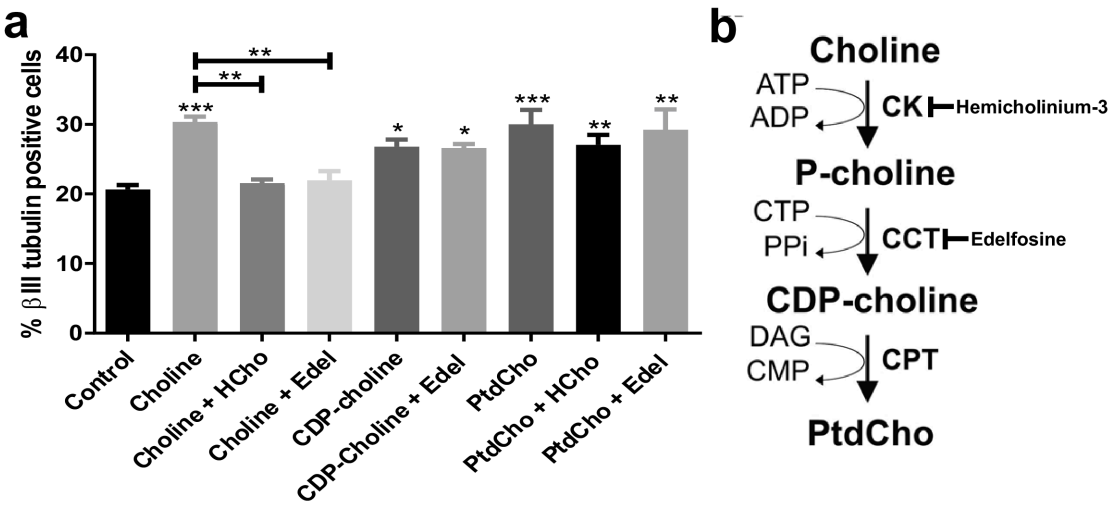
Supplementary figure 1 - Characterization of macrophages-activated media. a) Representative image of the amplification products obtained after the RT-PCR made from total RNA of Raw 264.7 cells grown in DMEM 10% SFB in the presence of 1 µg/ml LPS for 18 hours (n = 2). -LPS: RNA isolated from unstimulated Raw 264.7 cells and treated with RQ1 DNase was used as a template of the PCR reaction to control for the presence of traces of genomic DNA. +LPS: RNA isolated from LPS-stimulated Raw 264.7 cells and treated with RQ1 DNase was used as a template of the PCR reaction to control for the presence of traces of genomic DNA. C-: PCR water control (negative control). C+: PCR positive control (mouse genomic DNA). -cDNA: PCR product from total cDNA generated from unstimulated Raw 264.7 cells. +cDNA: PCR product from total cDNA generated from LPS-stimulated Raw 264.7 cells. MWM: Molecular weight marker, 100 bp. **b)** Cells were incubated with different dilutions of medium obtained from LPS-stimulated macrophages (AM), medium obtained from macrophages without activation as control medium (UM) or control medium with LPS (UM + LPS) for a period of 72 hours, the viability of the NSCs was analysed by MTT assay. **c)** After incubating the cells with the indicated concentrations of interleukins for a period of 72 hours, the viability of the NSCs was analysed by MTT assay.



Supplementary figure 2- NSCs differentiation is affected by inflammation and restored by PtdCho. Percentage of β-III Tubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs exposed to 20% V/V of AM and UM in the presence or in the absence of PC 50 µM during 24 **(a)** and 48 **(b)** hours. Graph is representative of three independent experiments. Data were presented as mean ± SEM. ***p < 0.001; **p < 0.01; *p < 0.05.



Supplementary figure 3 - PtdCho effects and the PKA/CREB signalling pathway under inflammatory conditions (AM). a) Percentage of β-III Tubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs exposed to 20% V/V of AM in the presence or in the absence of PC 50 µM and PKA inhibitor (KT5720 (10 µM)) after 72 hours. Cells were incubated during 30 min with the PKA inhibitor prior to liposomes addition. Graph is representative of three independent experiments. Data were presented as mean ± SEM. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. **b)** Number of normal and dystrophic neurons under inflammatory conditions in the presence or in the absence of PtdCho or KT5720, **$p < 0.05$ ***$p < 0.001$ (Student's T-test).



Supplementary figure 4 – Effect of choline, CDP-choline and PtdCho on neuronal differentiation. a) Percentage of β-IIITubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs treated during 24 hours with choline (50 µM), CDP-choline (50 µM), PtdCho (50 µM) in the presence or in the absence of the indicated inhibitors hemicholineum-3 (HCho, 50 µM) for choline kinase and Edelfosine (Edel, 5 µM) for phosphocholine cytidylyltransferase α. Cells were incubated during 30 min with the inhibitors prior to liposomes addition. Graph represents the percentage of neuronal differentiation measured in three independent experiments. Data were presented as mean ± SEM. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. **b)** Schematic representation of the Kennedy pathway for PtdCho biosynthesis.