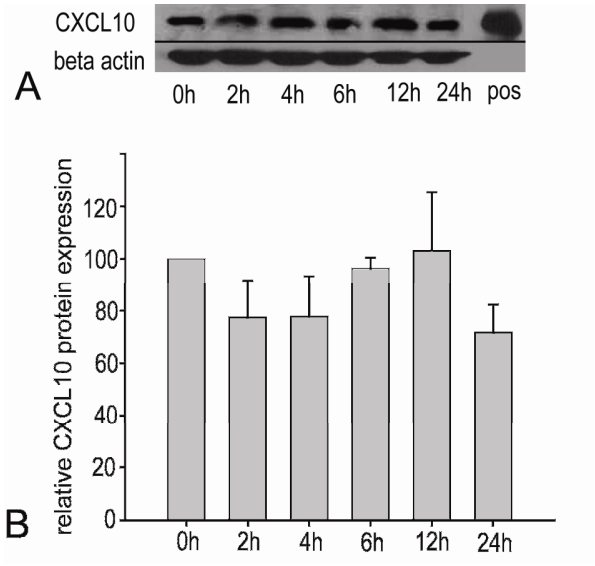
Expression of CXCL10 in cultured cortical neurons

Vinet, Jonathan; de Jong, Eiko K.; Boddeke, Hendrikus W. G. M.; Stanulovic, Vesna; Brouwer, Nieske; Granic, Ivica; Eisel, Ulrich L. M.; Liem, Robert S. B.; Biber, Knut

Published in:
Journal of Neurochemistry

DOI:
10.1111/j.1471-4159.2009.06495.x

Publication date:
2010



Supplementary figure 1. Western blot analysis corroborated the presence of CXCL10 in cultured neurons. (A) Comparably to the mRNA expression, CXCL10 protein levels remained at similar levels at every time point after glutamate stimulation. 100pg recombinant CXCL10 was used as positive control. (**B**) Quantification of the western blots revealed no significance difference in CXCL10 protein levels at any time points (0-24h) after glutamate treatment. Similar results have been obtained in 3 independent experiments and data are represented as mean +/- SEM (n=3).

Method

Neuronal cultures were solubilized in sample buffer and equal amounts of protein were loaded onto 12.5% SDS-polyacrylamide gels and transferred to Hybond-ECL nitrocellulose membrane (Amersham Biosciences). Membranes were blocked with 5% non fat milk in PBS with 0,1% Tween-20. and incubated O/N with rabbit anti-CXCL10 (PeproTech, 1:1000) or rabbit anti-β-Actin (Cell signaling Technology, 1:2000) primary antibodies in PBS with 1% nonfat milk, 0,1% Tween-20. The next day, membranes were incubated with the appropriate horseradish peroxidase conjugated antibody (Amersham

Biosciences). Bands were visualized by ECL. Digital images of the Western blots were made and quantification was done with ScionImage (Scion Corporation). Results were normalized against beta-actin data. Recombinant CXCL10 (PeproTech) was used as a positive control.. Blotting of recombinant CXCL10 resulted in 2 bands: one had a molecular weight of 10kDa and the other less prominent band was approximately 20kDa, which most likely consist of CXCL10 dimers. The dimerized form of CXCL10 was detected in neuronal cultures only.