

## Supplementary Material

### Neonicotinoid pesticides affect developing neurons in experimental mouse models and in human induced pluripotent stem cells (iPSC)-derived cells and organoids

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#### 1. Supplementary Materials & Methods

##### 1.1 Media composition

###### CCM

Neurobasal medium, 2% B27 Supplement (Gibco™), 2% horse serum (Life Technologies), 0.5 mM L-glutamine, 25 µM 2-mercaptoethanol 1% penicillin/streptomycin, 25 µM Glutamate (Sigma Aldrich Inch.), 10 ng/ml BDNF (Miltenyi Biotec)

###### DAM

DMEM/F-12, 1% penicillin/streptomycin (Sigma Aldrich Inch.), 10% heat-inactivated fetal bovine serum (FBS, Euroclone).

###### NDM

Neurobasal medium, 2% B27 Supplement, 1% GlutaMAX™-I Supplement and CultureOne™ Supplement (Gibco™), 200 uM Ascorbic Acid (Sigma Aldrich), 20 ng/mL human recombinant GDNF and BDNF (Miltenyi Biotec)

###### ADM

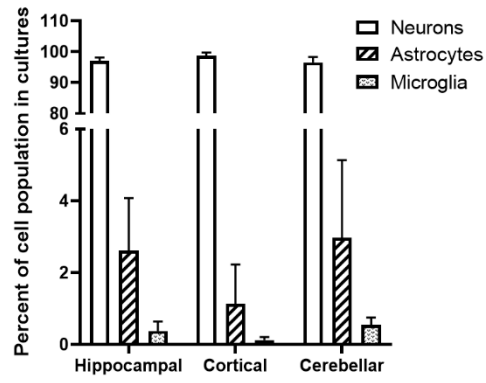
DMEM, 1% N2 Supplement, GlutaMAX™-I Supplement (Gibco™) and FBS (Euroclone)

###### hCS medium

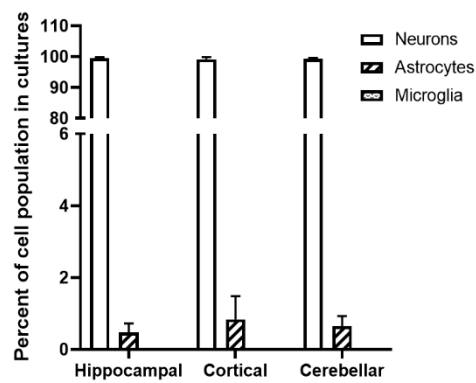
Neurobasal Plus Medium, 2% B-27 Plus, GlutaMAX™-I Supplement, 20 ng/mL human recombinant GDNF and BDNF

1.2 Supplementary Figure 1 (Fig S1)

A



B



**Figure S1. Mouse primary cell culture characterization**

Primary neuron cultures were obtained from different areas of mouse embryo brain, and analyzed for the abundance of neurons or glial cells fraction by immunocytochemistry at 6 DIV (**A**) or 14 DIV (**B**) with specific markers for neurons, astrocytes and microglia (NF200, GFAP and IBA-1, respectively).