Application of organotypical slice cultures for investigating selective neurotoxicity of nanoparticles

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Nanoparticles and neurotoxicity

Nanomaterials have unique properties and applications, as in diagnostics and drug delivery in medicine. However, the properties that make nanoparticles (NPs) so useful could also be coupled to unintentional health effects. As particle size decreases the surface area increases, as do the reactivity. Thus NPs can generate distinct effect not seen with the same material in a larger form and access tissues normally protected by barriers.

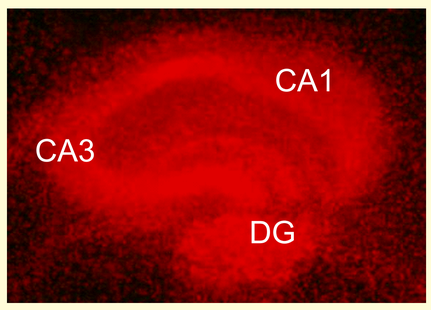
The fact that NPs can get access to the brain via olfactory neurons or by crossing the blood brain barrier, points towards the brain as a target organ. Inhaled NPs have been detected in the olfactory bulb and also in deeper brain regions as the hippocampus. So far there is scant knowledge about effects of NPs in the brain.

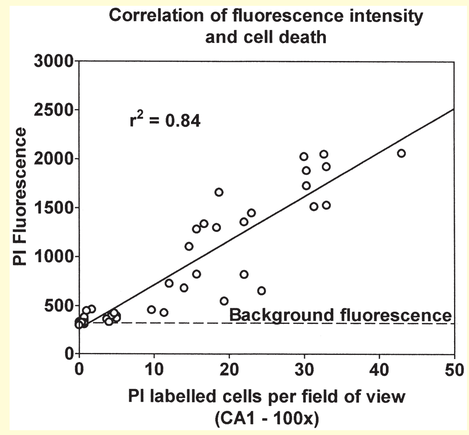
The aim of the project is to study toxicity of selected NPs towards different types of glial cells and neurons.

Easy and reliable quantification of cell death

Cell death can be easy and reliable quantificated by use of propidium iodide (PI), which is a fluorescent, membrane impermeable dye labelling dead or dying cells.

Shown here is a slice culture stained with PI after a toxic insult.

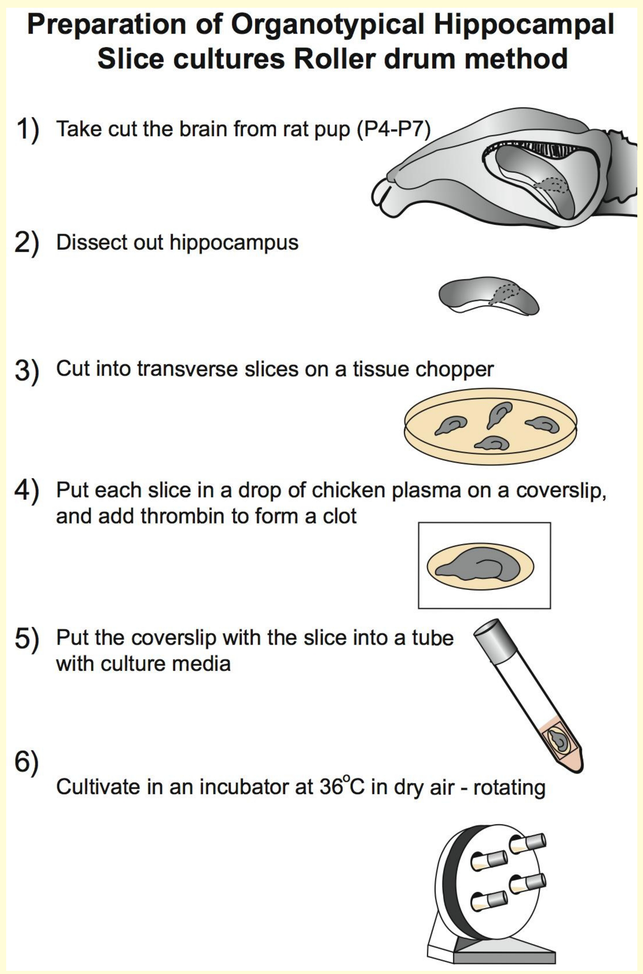




We have earlier demonstrated a good correlation between PI- fluorescence intensity and the number of dead cells.
(Laake *et al.*, Brain Res Protoc, 1998).

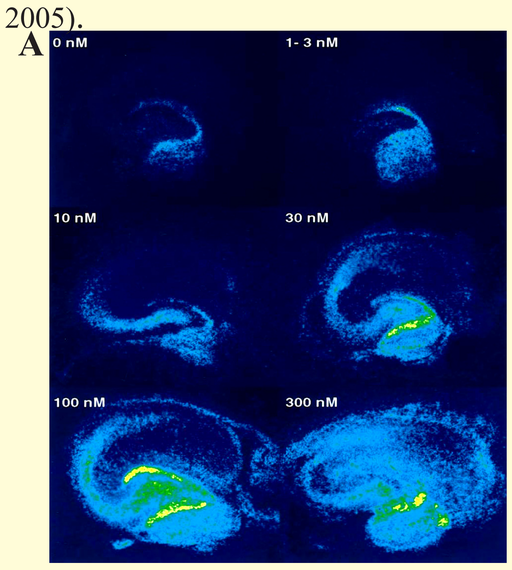
Organotypical slice cultures

We have in our laboratory established an *in vitro* model for studying selective toxicity based upon hippocampal organotypic slice cultures.



Validation of selectivity in the model

We have demonstrated selective vulnerability of the CA3 pyramidal cell after treatment with the algae toxin and protein phosphatase inhibitor Okadaic Acid (Rundén *et al.*, J Neurosci, 1998), in contrast to the selective vulnerability of the CA1 pyramidal cell to ischemic cell death (Rundén-Pran *et al.*, Neurosci 2002 and 2005).



Pseudo-color images of PI-labelled cultures

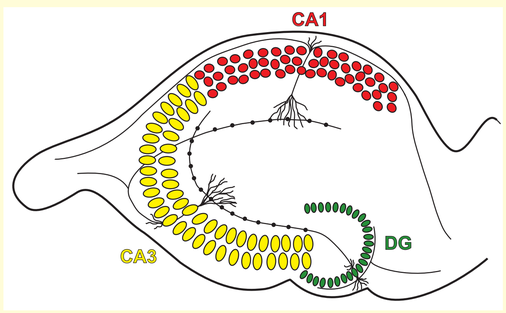
A) The algae toxin Okadaic Acid induced cell death selectively in the **CA3** pyramidal cell layer in a dose dependent manner (48 h after the insult).

B) Oxygen and glucose deprivation (OGD) induced cell death selectively in the **CA1** pyramidal cell layer (as also seen *in vivo*).

C) NS1619 (BK potassium channel opener) induced cell death mainly in **glial** cells (astrocytes).

Advantages of using the slice culture model

- no confounding effecs of circulation or systemic parameters, and thus the effect you see of a drug must reflect an action directly on neurons or glia
- cytoarchitecture is retained *in vitro*
- can be used for studying **selective vulnerability** of the hippocampal subregions (CA1, CA3, dentate gyrus (DG)) to different toxins and insults



Applications

- Nano-Ag and TiO_2 will be tested in the NorPol project (see separate poster).
- TiO_2, quantum dots and iron oxide will be tested in the NanoTEST (EU FP7) project (see separate poster).

- The NPs will be tested for cytotoxicity towards different hippocampal cell types (astrocytes, pyramidal cells, granule cells).

- Mechanism underlying cytotoxicity in the slice cultures can be investigated by assays for reactive oxygen species (ROS), changes in protein expression (Western blotting, immunofluorescence histochemistry and postembedding immunogold labelling) or changes in gene expression (Taqman, RT-PCR).

Why study selective vulnerability?

The mechanism underlying the selective vulnerability of the hippocampal pyramidal cells might hold the key to a better understanding of neurodegenerative disorders and development of medical treatment.

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