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MOMENTUM Microplastics and Human Health Consortium

Micro- and nanoplastics: uptake in rodent brain and neurotoxicity measured *in vitro* using microelectrode array (MEA) recordings

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Introduction & Aim

- Micro- and nanoplastics (MNPs) are small plastic particles that vary in shape, composition and size
- Humans are increasingly exposed to MNPs through drinking water, dust, food and daily use products
- The possible human health effects of MNPs are largely unknown
- **The aim of this research is to study the uptake of MNPs in mice brain and the potential neurotoxic effects of MNPs *in vitro* using microelectrode array (MEA) recordings**

Methods

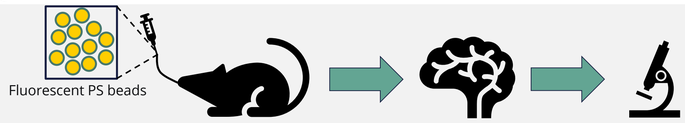

Figure 1. *In vivo* uptake of micro- and nanoplastics in mice brain
C57/BL6 mice (8-9 weeks old, ~25 gram) were exposed to 1 or 10 µm fluorescent polystyrene (PS) beads (Fluoresbrite® YG Microspheres) via oral gavage at 4 mg/day for 1 or 10 days. Brain slices were taken for immunohistochemistry.

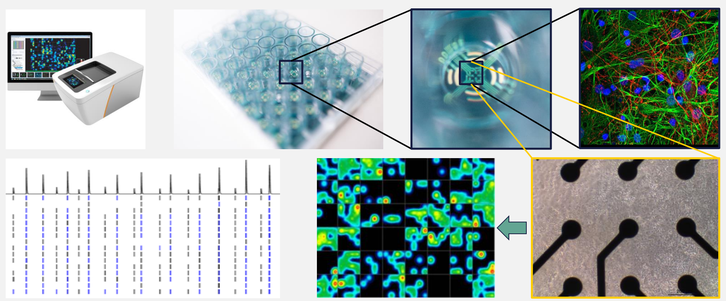

Figure 2. *In vitro* neurotoxicity testing using MEA recordings
Rat primary cortical cells were cultured on multi-well microelectrode arrays (MEA). Spontaneous neuronal activity was measured using a Maestro Pro MEA system (Axion BioSystems) after acute (on day *in vitro* [DIV] 10, 30 minutes) and chronic (DIV7-DIV28) exposure to different sizes and types of micro- and nanoplastics (10 µg/mL). The number of spikes was compared before and after exposure. Metabolic activity was determined using an alamarBlue assay after 28 days of exposure to distinguish neurotoxic effects from cytotoxic effects.[1]

Results

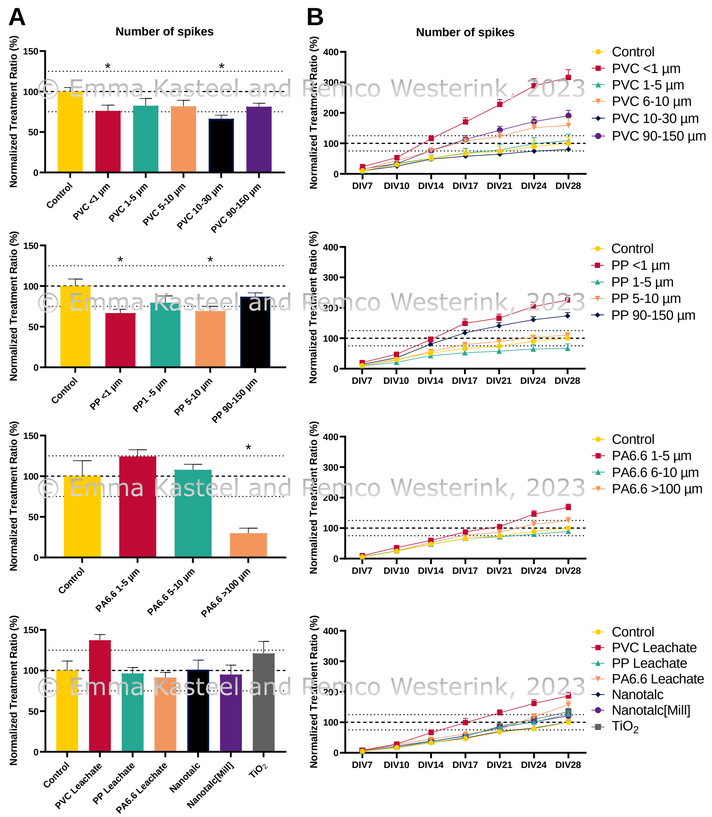

Figure 3. Effects on neuronal activity (number of spikes) after acute (A) and chronic (B) exposure to micro- and nanoplastics
Data (mean+SEM) are presented as the average % of control for different sizes of polyvinylchloride (PVC), polypropylene (PP), polyamide 6.6 (PA6.6), the leachates of these plastics and non-plastic nanoparticles (nanotalc and TiO_2) at 10 µg/mL from 14-16 individual wells, 2 independent experiments (acute) and 7-16 individual wells, 1-2 independent experiments (chronic). No cytotoxicity was observed at these exposures. *p<0.05 vs control

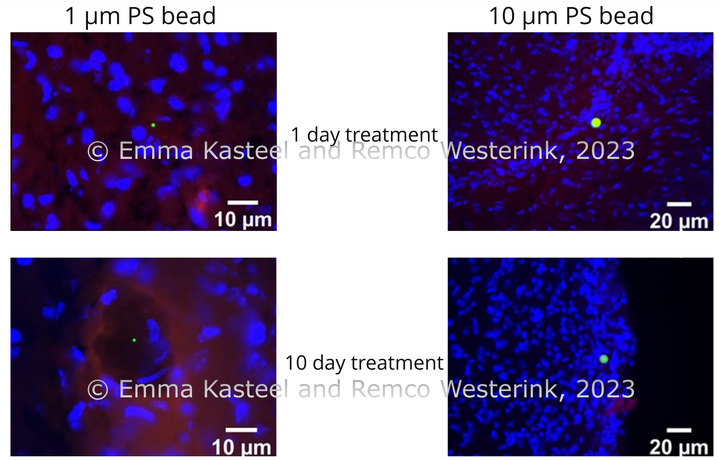

Figure 4. Presence of polystyrene microbeads in brain slices from mice
Pictures were taken at 100x objective magnification (left) from 10 µm slices or 40x objective magnification from 20 µm slices (right) in brain slices from mice exposed for 1 day (top) or 10 days (bottom) to 4 mg/day polystyrene (PS). Microbeads are visible in green; nuclei are stained with DAPI (blue); endothelial cells are stained with CD31 (red). % recovery of particles is indicated for each treatment.

Conclusions

- **Polystyrene microbeads are able to reach the brain in rodents after 1 and 10 days of exposure**

Preliminary data shows that:

- **PVC <1 µm and 10-30 µm, PP <1 and 5-10 µm and PA6.6 >100 µm are able to inhibit neuronal activity after 30 minutes of exposure**
- No inhibition of neuronal activity is seen after 30 minutes of exposure for other sizes of these plastics, their leachates and non-plastic nanoparticles
- **PVC <1 and 90-150 µm, PP <1 and 90-150 µm and PA6.6 1-5 µm show an increase in neuronal activity after 3-14 days of exposure**
- Leachates of PVC and PA6.6 also show an increase in neuronal activity after 3 and 21 days of exposure, respectively
- No inhibition of neuronal activity is seen after 21 days of exposure for other sizes of these plastics, PP leachate and non-plastic nanoparticles

Acknowledgements

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[1]Gerber et al. (2021). *Curr Protoc*

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