Using Micro-electrode Array (MEA) to test the impact of patient autoantibodies on sensory neuron excitability.

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Background:

- **Autoantibodies**, which target self-antigens within the nervous system, play a crucial role in the development of **neuropathic pain**, a long-lasting pain condition typically caused by a lesion or disease of the somatosensory nervous system.
- Increased **excitability and spontaneous activity** of primary sensory neurons are vital drivers of neuropathic pain
- **Autoantibody-mediated mechanisms** may be a prevalent phenomenon across different diseases and contribute to the manifestation of pain. Further investigation of these autoantibody-mediated signalling properties and their target, and their contributions to pain could be essential for developing **novel and targeted analgesic therapies**.
- *In vitro* data regarding primary sensory neuron excitability have been mainly derived from cell patch-clamp recordings, However, patch-clamp methods are invasive, incompatible with long-term measurements, and low-throughput***. Maestro Edge Micro-electrode array system (Axion BioSystems) (MEAs)*** have recently emerged as a new technology to measure electrical signals from cells.

MEA wells provide a cell-culture surface with integrated electrodes used for assaying electrically excitable cells, thus allowing the measurement of cell activity devoid of cell disruption (Figure 1).

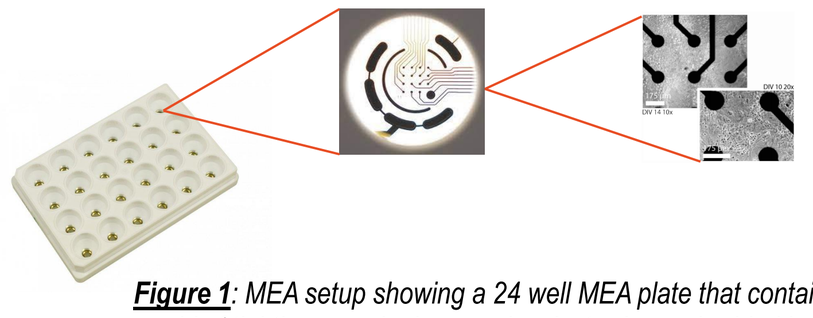

Figure 1*: MEA setup showing a 24 well MEA plate that contains a grid of tightly spaced microscopic electrodes embedded in the bottom of each well. Cells were cultured above the electrodes and their electrical activity was assessed.*

Aim:

Use of MEA as a platform to perform comprehensive assessment and analysis of neuronal excitability and spontaneous activity using sera samples from neuropathic pain patients. Repeated testing of excitability changes was performed and monitored over time, which is essential for understanding linked pathological mechanisms.

Assay Overview and benefits:

1. MEAs enable **long-term measurements of action potentials from large populations of cells.** Unlike conventional electrophysiology techniques, **several excitability parameters**, such as number of action potential spikes, firing rate, number of network bursts and duration at different timepoints can be **recorded and analysed.**
2. The technique provides **more insightful and comprehensive data and is a high-throughput** approach to allow testing of many samples in one plate.
3. The approach is important to **validate assays for identifying pathogenic antibodies and their possible targets.**
4. It will support a more complete understanding of the **cellular targeting of autoantibodies** in different autoantibody-mediated conditions such as fibromyalgia and complex regional pain syndrome.
5. The assay is being adapted for **human induced pluripotent stem cell-derived neurons** due to their similarity to human DRG, which makes it more **clinically relevant.**

Experimental workflow:

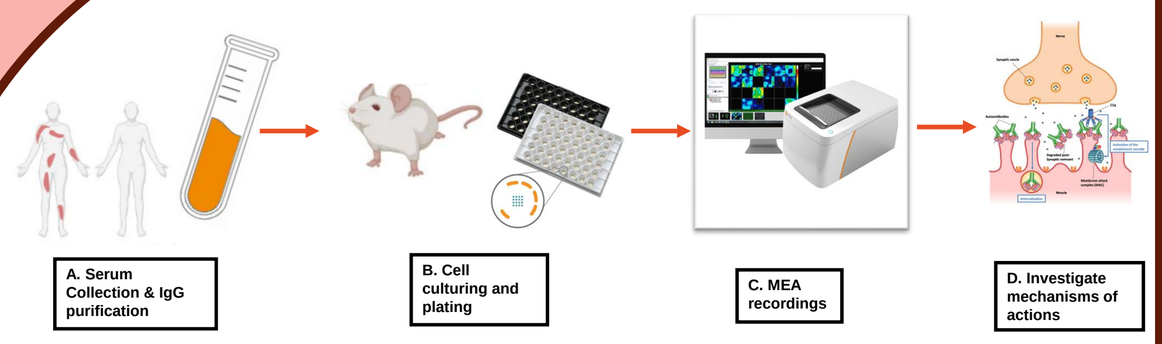

Figure 2: *Experimental workflow. (A) Sera samples have been collected from pain patients as well as healthy controls. IgG purification will also be performed. Culturing of mouse primary neurons onto 24 MEA well plates (B). Neurons will be treated with sera or purified IgG from pain and healthy control patients, and then MEA recordings will be performed to assess neuronal excitability(C). Different assays will be utilized as an approach to detect potential mechanisms of autoantibody pathogenicity (D- Ludwig et al., 2017).*

Assay Application:

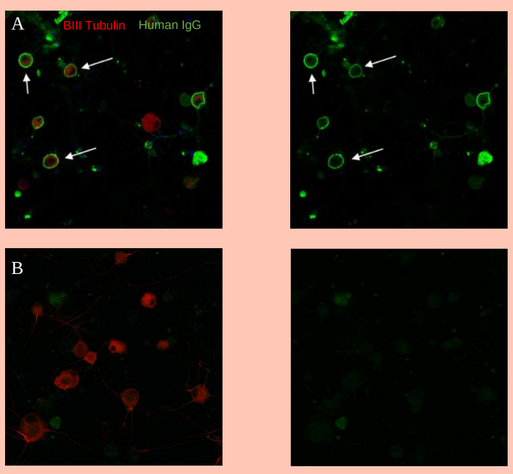

Figure 3: *IgG binding in mouse primary sensory neurons treated with sera from pain patient (A) or healthy control (B) at 1:100 dilution. Neurons marked with BIII-tubulin (red). Human IgG binding is shown in green.*

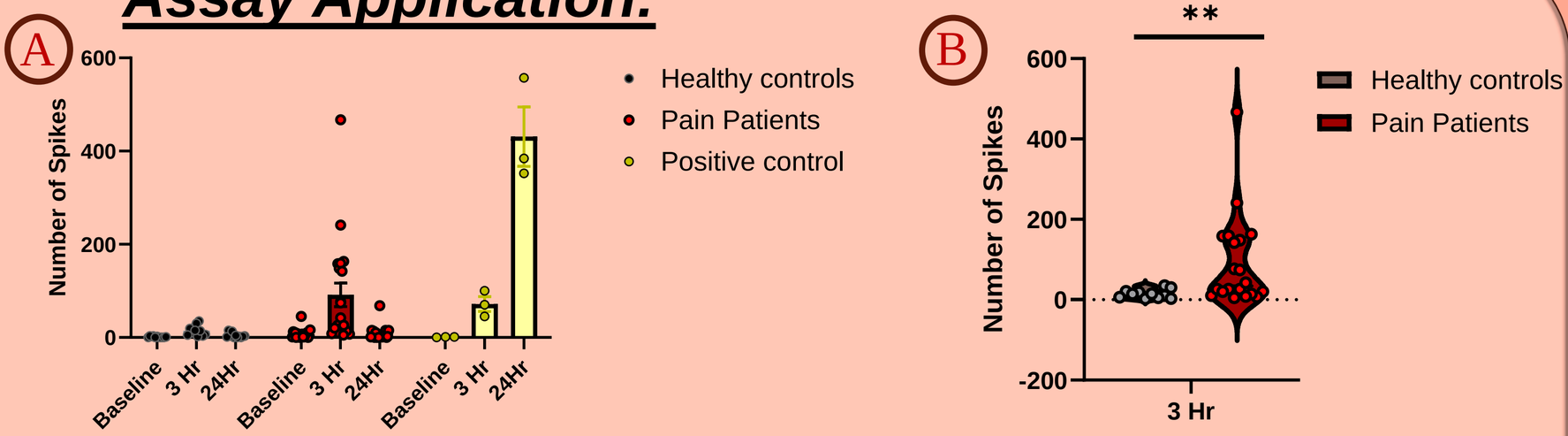

Figure 4: ***(A)****Sera from 20 pain patients and 11 healthy controls were added to DRG neuron cell cultures. Recordings were made at different timepoints, and number of spikes were recorded and analyzed.* A selected pain patient serum with known target (CASPR2) was used as a positive control since it showed significant levels of excitability changes and antibody internalization after 24Hrs of treatment.
(B) *Mouse sensory neurons treated with pain patients' sera show a significant increase in neuronal excitability after 3-hour treatment as indicated by the increase in number of action potential spikes.*

Conclusion & potential impact:

- This work may be of direct benefit to patients as it will confirm the pathogenicity of the autoantibodies involved, which could be essential for developing novel and targeted analgesic therapies potentially of interest to pharma companies.
- Interested in talking to companies that might wish to access the platform, please contact adham.farah@ndcn.ox.ac.uk

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