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AUTOMATING IMAGING CALCIUM IN NEURONS USING MICROFLUIDICS WITH FLUIGENT'S ARIA.

INTRODUCTION TO IMAGING CALCIUM IN NEURONS TO STUDY NEURONAL COMMUNICATION IN VITRO

Measuring neuronal activities in vitro is critical in assessing the functionality, health, intrinsic properties and/or reaction to different stimulus of the culture. As calcium plays a crucial role in cell signaling, tracking calcium influx within neurons is an indirect readout of the electrical activities of neurons (1). This method, called imaging calcium in neurons, emerges as a wonderful technique, now able to detect single action potentials in individual neurons (2). This method employs fluorescent "dyes" that react to the binding of Ca+ ions, resulting in alterations in their fluorescence properties, which enables researchers to optically measure shifts in calcium concentration within neurons and neuronal tissue.

Analysis tools associated with calcium imaging facilitate the analysis of neuronal events, synchrony of the network as well as amplitude of the response and number of events to characterize the functionality of the culture.

MICROFLUIDICS FOR CALCIUM IMAGING

Most imaging calcium in neuron experiments with chemical stimulation are carried out using microfluidic devices to temporally control the environment while recording neuronal activity (2). Recent advancements in microfluidic technology have revolutionized the way we study neural cells by enabling automated recordings of calcium activity. Microfluidic platforms offer a controlled environment for cell cultures, allowing researchers to observe neuronal behavior with minimal disturbance. This automation not only reduces experimental time but also simplifies data analysis, paving the way for more efficient and insightful research outcomes.

By integrating microfluidics into neuroscience experiments, researchers can track the calcium activity of individual neurons or neuronal networks over extended periods. This approach offers a deeper understanding of how neuronal function evolves in response to various stimuli, providing valuable insights into neurodegenerative diseases, drug effects, and neural plasticity.

AIM OF THE STUDY

After publishing a first application note covering neuron cells immunolabeling using Aria, the aim of this study was to maintain the initial field of interest of neurons throughout all recordings to track them individually while performing imaging calcium in neurons. This greatly facilitates the analysis process and significantly reduces thetime required for experimentation. Indeed, manually changing the media poses a risk of losing the field, emphasizing the importance of maintaining consistency in the experimental setup.

In this application note, we investigate the significance of measuring calcium activity in neural cells and explore the benefits of automating this process using Fluigent's Aria.

This application note has been produced by Maxime Poinsot, PhD student in Institut de Neuro-sciences de la Timone & Fluigent.

Figure 1 shows an overview of the study.

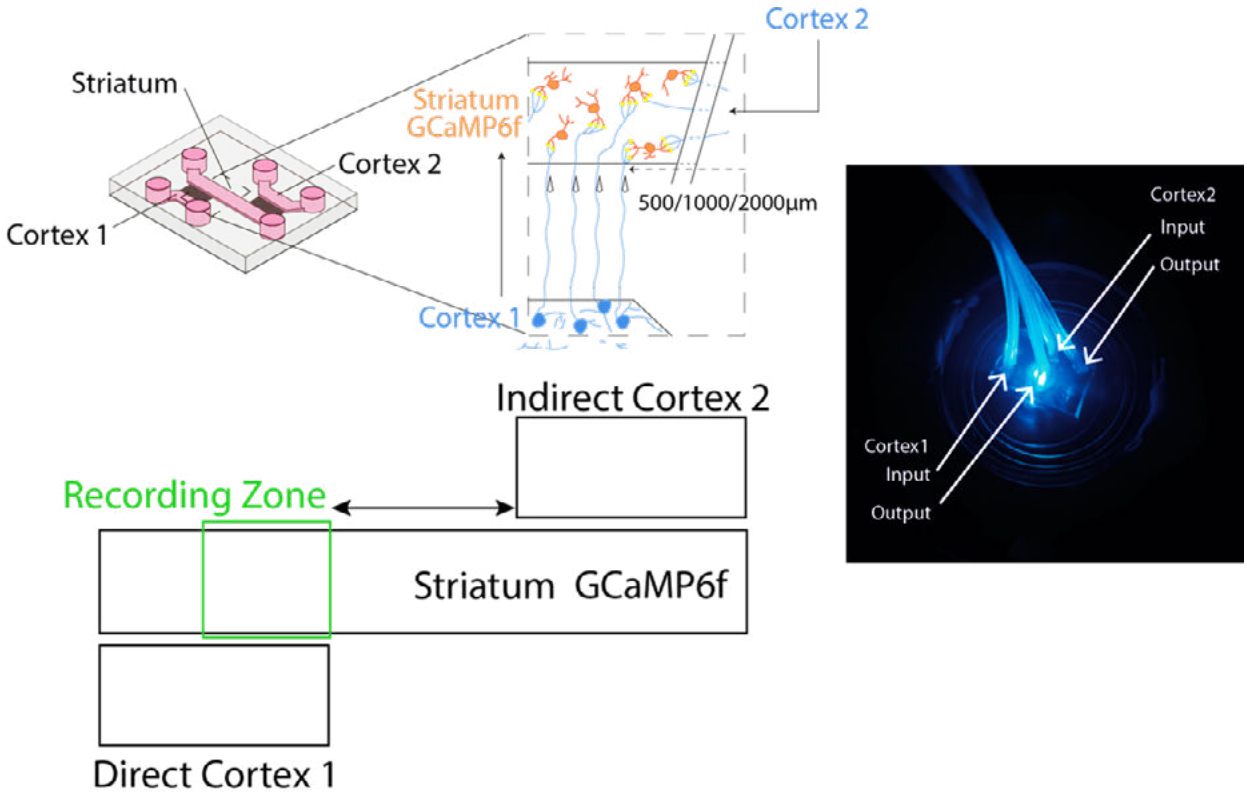

Figure 1: Overview of calcium imaging in neuronal cells.

HOW TO PERFORM MICROFLUIDIC IMAGING CALCIUM IN NEURONS

Materials

Cells & reagents

Progenitor neuron cells from rat embryos at 17.5 days of gestation were used in this application note to validate the abilities of Aria in studying such delicate cells while preserving axons and dendrites.

The Striatum was transduced with AAV5.SYN.GCaMP6f after one day in vitro (DIV). GCaMP6f with a genetically ultra-sensitive fluorescent calcium sensor utilized to accurately detect individual action potentials.

Tetrodotoxin (TTX) at a concentration of 5µM was prepared in water for cellular cultures. Tetrodotoxin is a potent neurotoxin that functions as a sodium channel blocker, effectively inhibiting the firing of action potentials in neurons.

Microfluidic Brain on Chip

The microfluidic chip (Figure 2) used for this application note is a homemade chip, fabricated using PDMS (Polydimethylsiloxane) with a curing agent ratio of 1 part curing agent to 10 parts of the base silicone, specifically utilizing Sylgard 184 as the base material. This PDMS-based chip is designed to replicate two distinct cortex zones and study their projection onto remote areas of the striatum, enabling research on axonal rewiring and cortical axon distribution within the striatum. It provides a valuable platform for investigating the effects of altering axonal architectures in neuron cells.

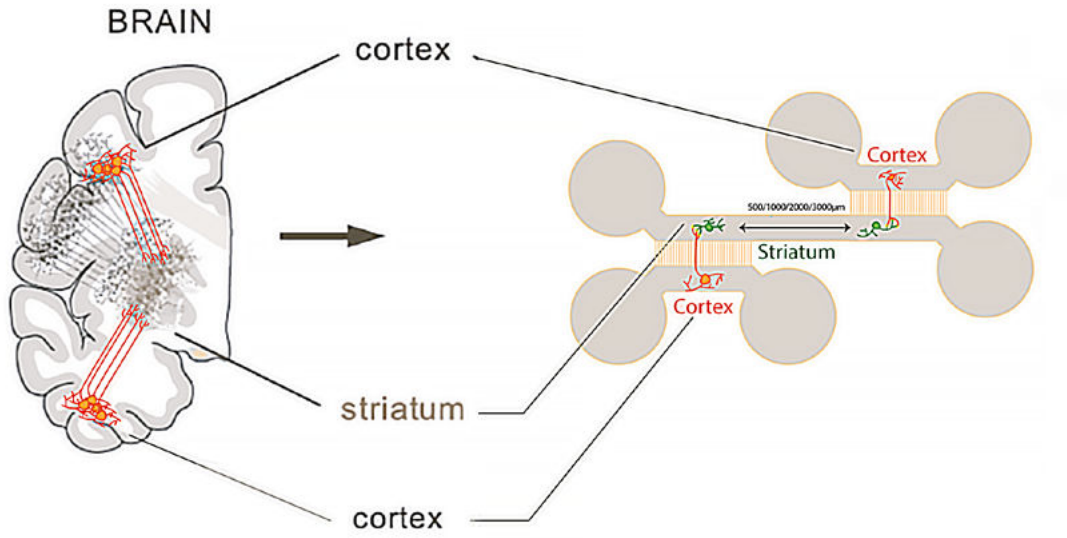

Figure 2: Illustration of the microfluidic chip used to perform calcium imaging in progenitor neuron cells.

Aria serial output

Aria Serial Output (Figure 3) is a performing microfluidic control device enabling the precise and controlled delivery of fluids for microfluidic applications. It facilitates the regulation of the flow rates and temporal delivery of up to 10 different fluids or solutions while using user-friendly protocols. With Aria Serial Output, researchers can automate complex protocols, reducing human error and ensuring consistent results in microfluidic experiments. This system is a suitable tool for imaging calcium in neurons, requiring precise and gentle fluid perfusion, to maintain the integrity of the cells and define the field of interest under the microscope.

Figure 3: Photography of experimental setup showing Aria, M-switch, Aria software.

Live imaging Super Resolution Microscopy: ZEISS Elyra 7

The confocal microscope used is an inverted ZEISS microscope. The microscope stage is maintained at 37°C and 5% CO2, providing high-resolution imaging for neuron cell visualization. Recordings were made using a 20x oil-immersion objective and a 488 nm laser at 70% intensity with a 200 ms exposure time every 200ms for 1 minute allowing to capture precise and detailed calcium events in recorded neurons.

Protocol: calcium imaging using Aria

- Neuron cells are cultured for 21 days inside the microfluidic chip to have a mature and synchronous neuronal circuit before starting the imaging calcium in the neurons process.

- Once the cells are ready and mature enough, the chip is placed under the microscope and the preferred field of interest is localized, making sure the chip will no longer move. Connecting the chip to Aria will allow for a smooth and automated delivery of the various reagents without the user touching the chip, and thus ensuring a steady localized field of view under the microscope.

- The basal calcium activity of the striatum is measured every 200 ms during 1 minute of time. This is considered the t0 of the experiment.

- Using Aria, the direct cortex (Cortex 1) culture medium is replaced by the medium containing TTX, which will inhibit cellular activity monitored with the microscope.

- A waiting time of 5 minutes allows the TTX to act, this is t1 of the experiment.

- The calcium activity of the striatum is recorded every 200 ms for 1 min. This is t2.

- If there is still neuronal activity, the recorded striatum receives input from the indirect cortex (Cortex 2). Therefore, changing the indirect cortex culture medium and replacing it with a medium containing TTX will inhibit the activity.

- A waiting time of 5 minutes allows the TTX to act, this is t3 of the experiment.

- The calcium activity of the striatum is recorded every 200 ms for 1 min. This is t4.

- The protocol used to perform imaging calcium in neurons is summarized below (Figure 4).

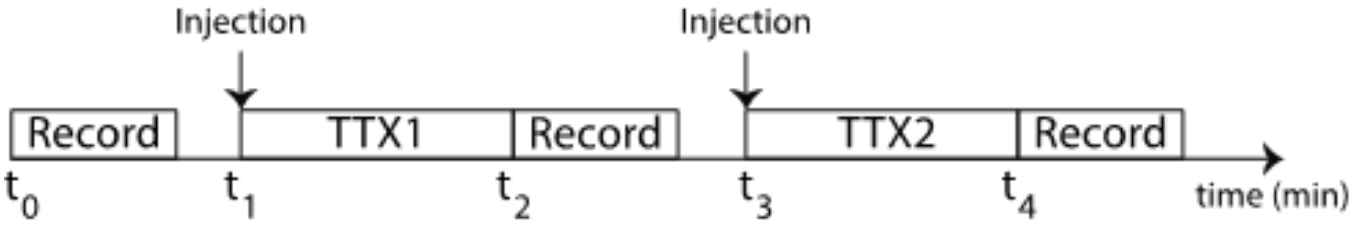

Figure 4: Protocol used to perform imaging calcium in neurons.

RESULTS

Figure 5 represents progenitor neuron cells inside a microfluidic chip after experiencing different types of stimulation. The images show the recording of striatum neurons alongside their normalized amplitude trace. Following the administration of TTX into the direct Cortex 1, persistent activity is observed within the striatum, suggesting functional connectivity from the indirect cortex 2 to the recorded striatum. When the TTX is administered into both direct Cortex 1 and 2, no neuronal activity is recorded.

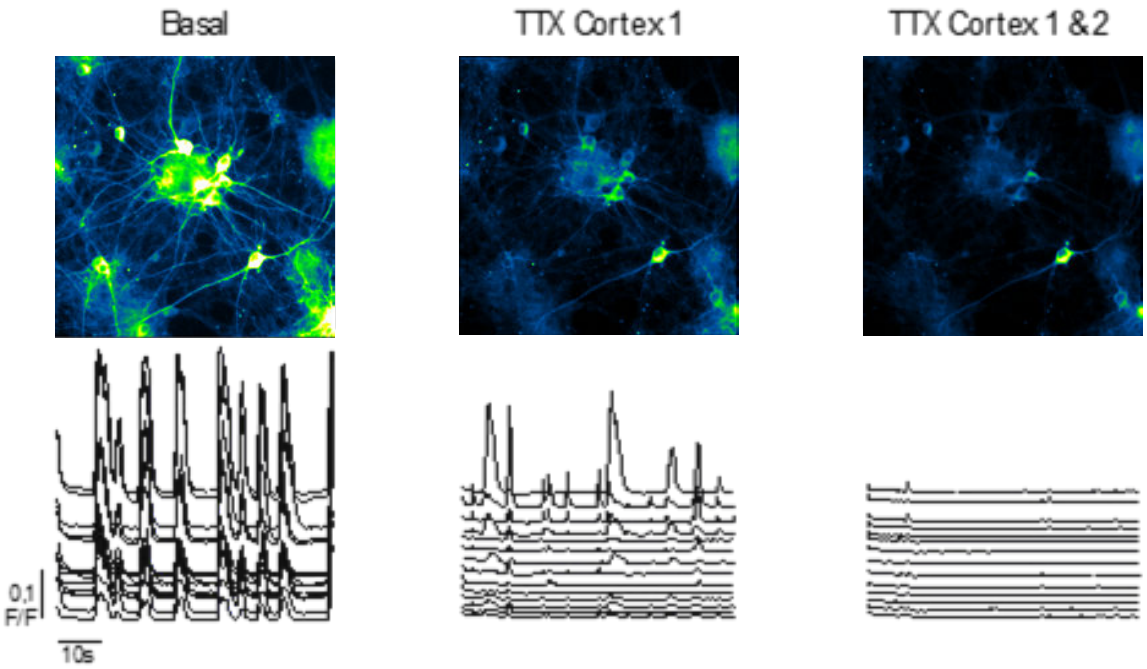

Figure 5: Functional activity of neuron cells after stimulation.microfluidic chip used for neuron cells.

CONCLUSION

With Aria's capabilities, we achieved full automation of this calcium imaging in neurons protocol, facilitating the rapid execution of many microfluidic experiments within a short timeframe. Furthermore, using Aria ensured the sustained stability of the recorded field, laying a solid foundation for subsequent in-depth analyses. This approach enhances experimental throughput and the reliability and reproducibility of experimental outcomes, thereby advancing our understanding of neuronal circuit dynamics. Aria, in combination with the M-Switch, offers a transformative solution for microfluidic calcium imaging challenges.

REFERENCES

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