**INTRODUCTION**

In vitro models for drug discovery or safety screening need to be relevant for particular pathological or physiological contexts and be highly reproducible in order to repeatedly obtain valuable results. Cancer cell lines, which are widely used in neuroscience for simple screening, are handled with standard procedures. A major drawback, however, is that these cells lack many of the key neuronal features, such as spontaneous neurite outgrowth and the ability to form functional neuronal networks that respond to neuronal pharmacology. Rodent primary neurons are a tool of choice to study most neuronal biology in vitro, including the measurement of neurotoxicity and pathophysiological modeling, however, obtaining these cells is labor intensive and time consuming, requiring the sacrifice of a number of embryos. Additionally a specific subpopulation of interest may be very poorly represented. iPSC derived neurons are a recent alternative which were compared to rodent neurons to determine their suitability for compound testing, in an array of basic neuronal functions.

**MATERIAL & METHODS**

- **Rat primary mesencephalic neurons** The ventral midbrain of E14 Sprague-Dawley rats were harvested and dissociated enzymatically and mechanically before plating in 96 well plates at 300000 per cm². Neurotoxins were applied after 24 hours for a duration of 72 hours before fixation and labeling with a Tyrosine Hydroxylase primary antibody and Alkaline Phosphatase secondary antibody and permanent red amplification of the signal. Images were acquired and neurite parameters obtained using Metamorph software.
- **Rat primary cortical and hippocampal neurons** The cortices or hippocampi of E19 Sprague-Dawley rat embryos were harvested and dissociated enzymatically and mechanically before plating at 60000 per cm² in 96 or 384 well plates. For Toxic treatments, hippocampal neurons were treated after 24 hours of culture and neurite outgrowth was followed kinetically using an Incucyte Zoom, acquiring phase contrast images every 4 hours. Neurite outgrowth kinetics were obtained after image analysis. For calcium oscillations and glutamate responses, cortical neurons were evaluated after 12 days in vitro, using a Calcium 5 probe in a FlirP Tetra platform.
- **Human iPSC-derived CNS.4U** These cultures contain a mix of neuronal cells representative of the Human cortex (Elastase/NEAT, GABAergic, Dopaminergic) and astrocytes. These cultures mature over time and display spontaneous synchronized oscillations after 33 days. These oscillations can be modulated. Cyclothiazide, an allosteric positive modulator of AMPA receptors increases the amplitude of oscillations, while the antiepileptic Valproic acid highly decreases the oscillation frequency. The iPSC-derived CNS.4U cultures contain a mix of neuronal cells representative of the Human cortex (Elastase/NEAT, GABAergic, Dopaminergic) and astrocytes. These cultures mature over time and display spontaneous synchronized oscillations after 33 days. These oscillations can be modulated. Cyclothiazide, an allosteric positive modulator of AMPA receptors increases the amplitude of oscillations, while the antiepileptic Valproic acid highly decreases the oscillation frequency.
- **iCell DOPA neurons** iCell DOPA neurons (Cellular Dynamics International) were thawed as per instructions and plated in 96 well plates. Neurotoxins were applied after 24 hours in the presence of cycloheximide or Caspase 3/7 probes and phase contrast and fluorescent time lapse images were acquired using an Incucyte Zoom platform.
- **CNS.4U neurons** CNS.4U cells (Ncella) were thawed and plated on PEl coated 384 well plates at 30000 per cm². After 33 days in vitro, calcium oscillations were monitored using Calcium 5 fluorescent calcium probe, in a FlirP Tetra platform.
- **Peri.4U neurons** Peri.4U neurons (Ncella) were thawed following the manufacturer’s instructions and plated at 30000 per cm² of a 96 well plate. 24 hours after plating, cells were treated with Taxol or Vincristine in dose response and time lapse images were acquired using an Incucyte Zoom platform. Neurite parameters were calculated kinetically.

**RESULTS**

**Neuronal models for Parkinson’s disease**

- Rat primary mesencephalic neurons display spontaneous synchronized calcium oscillations detected as calcium oscillations in a plate reader. Pentylenetetrazol (PTZ) increases the amplitude of oscillations, which is well reduced by the concomitant application of the antiepileptic Valproic acid.
- Neurites also display an aberrant curved morphology.

**Neuronal models for Epilepsy studies**

- Rat primary cortical neurons display spontaneous synchronized calcium oscillations detected as calcium oscillations in a plate reader. Pentylenetetrazol (PTZ) increases the amplitude of oscillations, which is well reduced by the concomitant application of the antiepileptic Valproic acid.

**Neuronal models for Chemotherapy-induced Peripheral neuropathy**

- Human iPSC-derived dopaminergic neurons are highly enriched in dopaminergic neurons (> 80%) and allow the use of labelling-free content kinetic analyzes. These Human neurons respond well to neurotoxins reproducing features of Parkinson’s disease neurodegeneration.
- The use of primary neuropeptide neurons implies an endpoint immunofluorescent labeling and a specific signal amplification to detect neurites. Neurotoxins affected neuronal health detected by neurite outgrowth analysis.

**CONCLUSION**

The iPSC-derived neuronal models respond to particular needs in terms of neuronal types, that may otherwise not be obtained as Human neurons for reasonable throughput experiments. Moreover, while models exist derived from rodent embryos, the Human iPSC alternative allows for purer populations and more practicality for screening campaigns, and avoids the sacrifice of numerous animals. These models respond well to different experimental paradigms, and with optimized experimental conditions to perform live content neurite outgrowth assays and high throughput intracellular calcium measurements, they allow for the measurement of neurotoxicity and neuronal network functionality in different physiological and pathological contexts such as in epilepsy (CNS.4U), Parkinson’s disease (iCell DOPA) and peripheral neuropathy (Peri.4U). Therefore, while reducing the effort and ethical concerns involved in using primary neurons obtained from rodents, iPSC-derived neurons, used in adapted experimental paradigms also have good validity as models for neurotoxicity and drug discovery studies.