

# A Human iPSC-Based Platform for Functional Toxicity Screening of CNS-Targeted RNA Therapeutics



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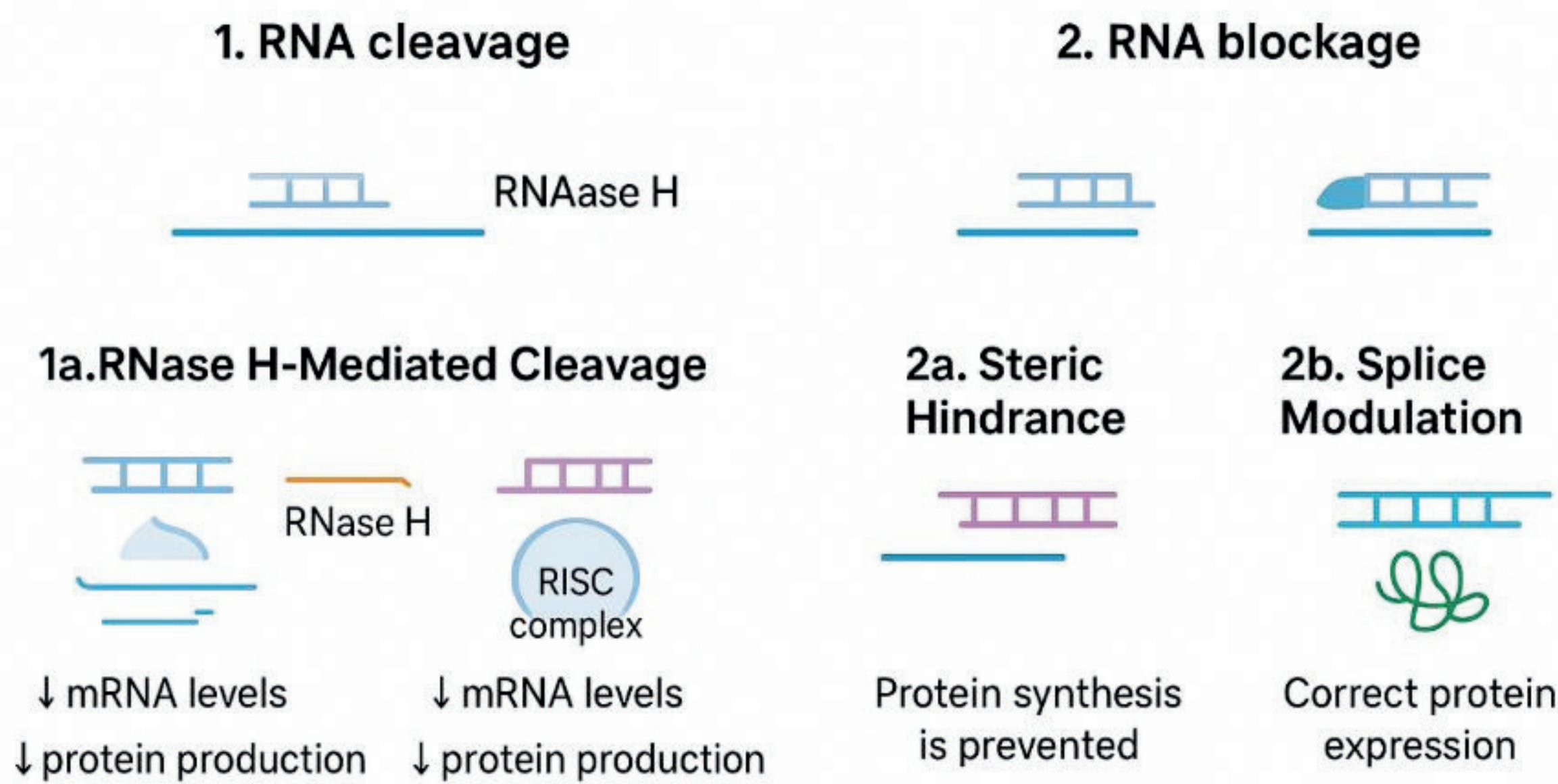
## Background and Purpose

Human induced pluripotent stem cell (iPSC)-derived neural co-cultures recapitulate key structural and functional features of the human central nervous system (CNS) and provide a physiologically relevant approach for evaluating potential CNS safety liabilities. Antisense oligonucleotides (ASOs) are an emerging therapeutic modality; however, some ASOs have been associated with adverse CNS effects, including seizures, convulsions, and altered neuronal function. The objective of this study was to develop and evaluate human iPSC-derived functional assays capable of detecting and characterizing ASO-induced neurotoxicity relevant to CNS safety pharmacology.

## Methods

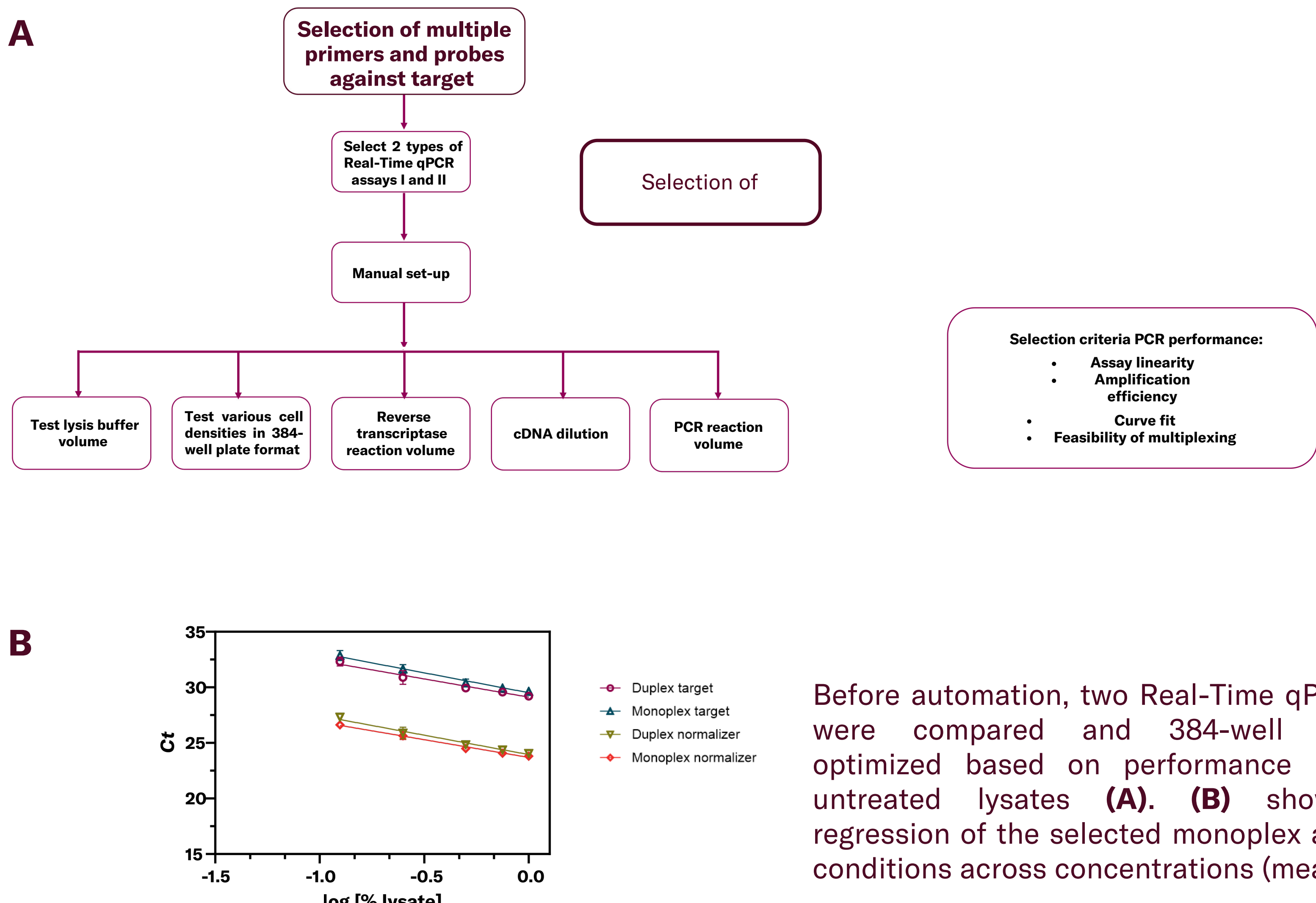
Human iPSC-derived neurons and astrocytes were used to establish high-throughput calcium imaging and multi-electrode array (MEA) assays. Calcium imaging was used to quantify spontaneous calcium transients and assess neuronal network excitability, while MEA recordings were used to evaluate neuronal network activity through measurements of spike amplitude, network burst frequency, and network burst interval. Functional responses were assessed across a panel of 70 preclinical ASO candidates and compared with available in vivo CNS toxicity observations

## Mechanisms of Action: Antisense Oligonucleotides (ASOs)

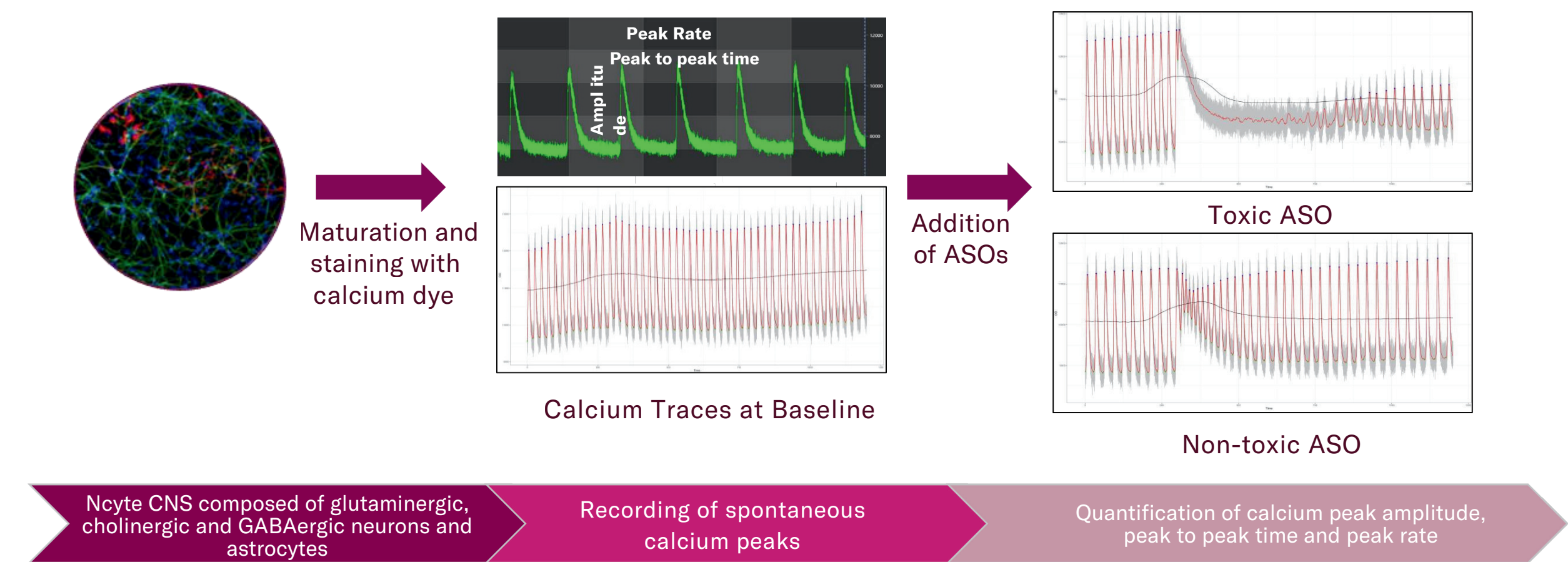


Designed to bind complementary RNA targets, ASOs can modulate RNA processing to reduce, restore, or modify protein expression through several distinct mechanisms. These include promoting RNA degradation via RNase H, altering splicing patterns, or blocking translation, thereby enabling precise and targeted regulation of gene expression at the RNA level.

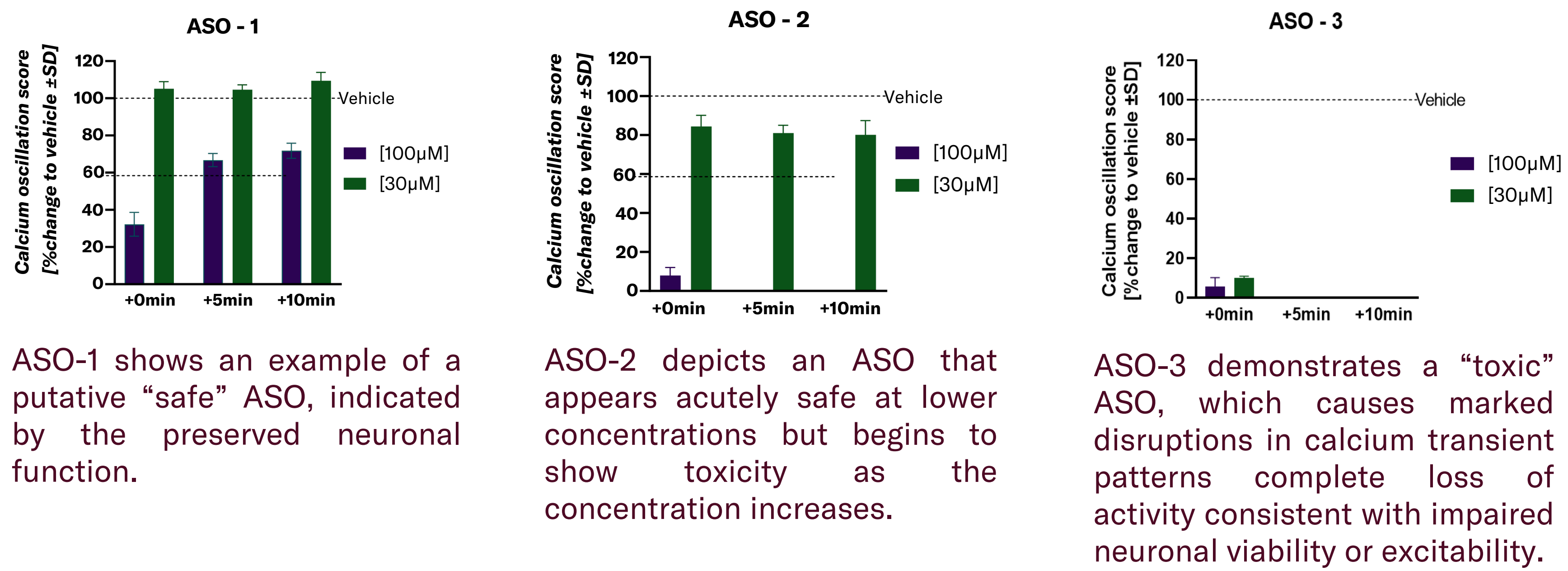
## Automated platform for ASO screening



## In vitro safety assessment of ASOs: Ca<sup>2+</sup> traces



Exemplar calcium traces from Ncyte CNS cultures treated with toxic (upper trace) and non-toxic ASOs (lower trace). Toxic ASOs cause the disruption of spontaneous calcium activity.



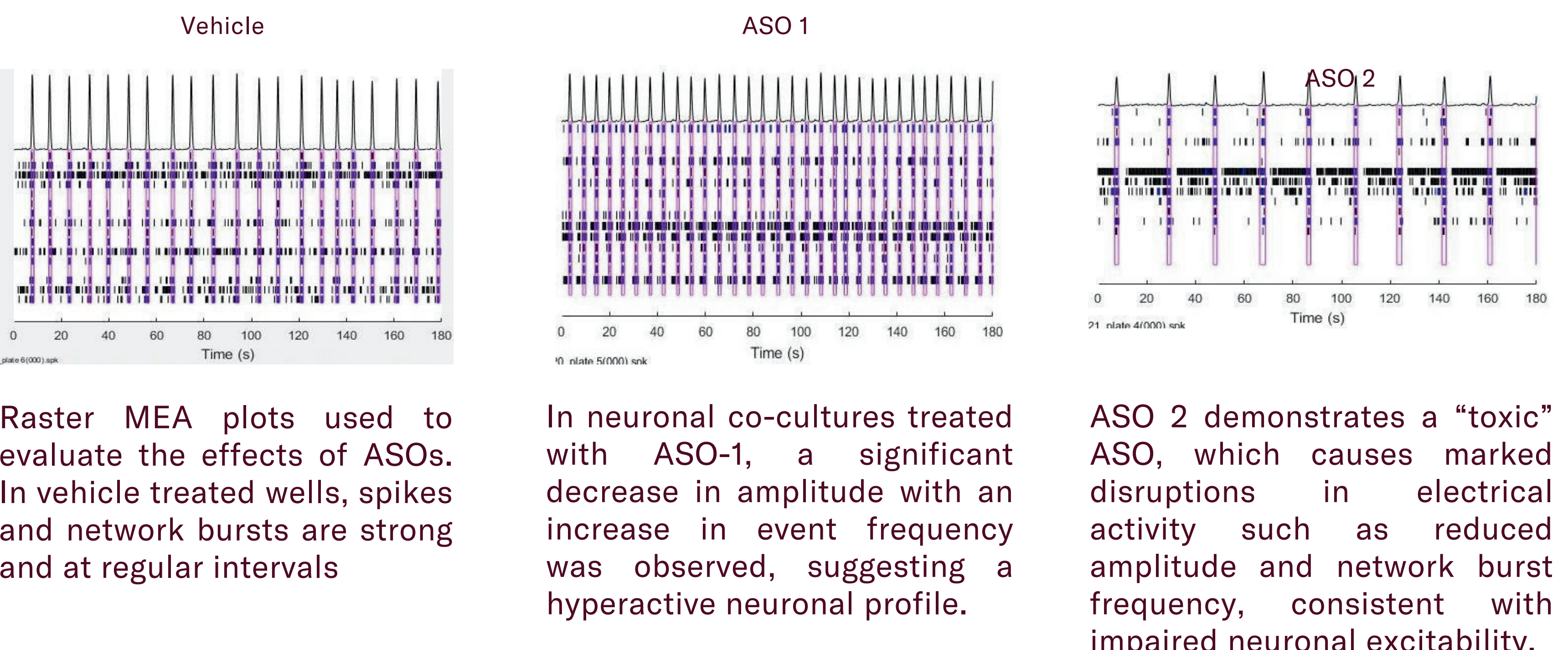
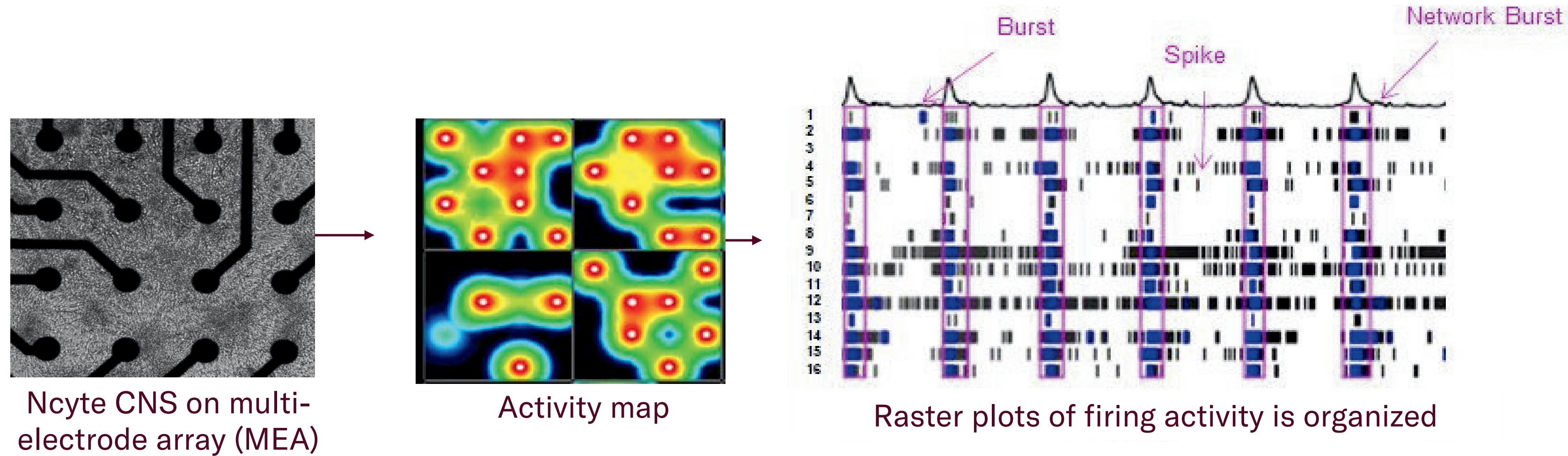
ASO-1 shows an example of a putative “safe” ASO, indicated by the preserved neuronal function.

ASO-2 depicts an ASO that appears acutely safe at lower concentrations but begins to show toxicity as the concentration increases.

ASO-3 demonstrates a “toxic” ASO, which causes marked disruptions in calcium transient patterns complete loss of activity consistent with impaired neuronal viability or excitability.

These examples show how the Calcium assay can be used to distinguish between safe and toxic ASOs

## In vitro safety assessment of ASOs: MEA traces



Collectively, these examples highlight how MEA can be applied as a sensitive, functional screen to discriminate between safe and neurotoxic ASOs.

## Conclusion

These findings demonstrate that human iPSC-derived functional assays can detect and differentiate ASO-induced alterations in neuronal network activity and may provide a translational approach for identifying potential CNS safety liabilities during nonclinical developme:

- Fully automated RT-qPCR assay established to assess ASO efficacy in human iPSC-derived neurons, delivering highly robust data (<5% inter-plate variability) and applicable across multiple iPSC-derived cell types.
- High-throughput calcium transient assay using Ca<sup>2+</sup> imaging in hiPSC-derived CNS cultures to evaluate dose-dependent acute neurotoxicity of ASOs.
- MEA (microelectrode array) assay developed to assess ASO-induced effects on neuronal network activity, capturing changes in firing rate, burst frequency, and synchrony.

Ncardia’s hiPSC-derived neuronal cell models enable early screening for ASO efficacy and neurotoxicity, providing a powerful platform for improving drug development efficiency.

Questions? Contact us at: [support@ncardia.com](mailto:support@ncardia.com), [www.ncardia.com](http://www.ncardia.com)

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