Electromodulated Delivery of BACE1 siRNA to Primary Neurons in Culture Restores Electrical Function in Alzheimer's Disease Models

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GENE therapy is an exciting and emerging approach being considered for a wide variety of neuropathologies such as Alzheimer's disease (AD), immune deficiency disorders etc. There are significant challenges in delivery of specific gene constructs in safe and efficient ways to targeted groups of neurons and also in the assessment of treatment efficacy (via parsimonious functional assays). In this study, we report a novel technique using voltage pre-conditioning for electromodulated delivery of siRNA for silencing BACE1 protein (a key enzyme implicated in AD pathology) into primary hippocampal neurons in culture. The technique reported here also simultaneously allows the careful determination of functional changes in neurons. The scalable nature of the technique reported here allows for the realization of high-throughput drug discovery tools readily.

II. METHODS

Voltage preconditioning is used for tunable delivery of BACE1 siRNA in targeted neurons using a rapid, low voltage pulse train (+3V to -3V range) [1] in a microelectrode array (MEA). Primary rat hippocampal neurons were seeded on customized MEAs and subjected to a previously established AD-based neurotoxicity model. Cells at individual electrodes were selectively transfected at different dose levels by tuning the electrical parameters (-1 V for high siRNA uptake, +1 V for moderate siRNA uptake, and +3 V for low siRNA uptake) with BACE1 siRNA and negative control siRNA on DIV18-21.

III.RESULTS

Neurons displayed dose-dependent changes in firing rate (an order of magnitude change in firing rate at the highest siRNA loading levels compared to negligible changes at lowest loading levels). Surprisingly, significant changes in spontaneous activity and waveform shapes are observed within 1 hour after transfection indicating more rapid functional changes than the 24 hour duration that is generally used in current molecular assays.

IV.CONCLUSIONS

In this study, we used an electromodulated delivery mechanism (that does not use electroporation) to controllably deliver siRNAs to primary neurons cultured (DIV18-21 i.e. 18-21 days in vitro) on a customized MEA platform. Calibrated dosing in neuronal populations is achieved at different voltage amplitudes with maximum siRNA loading occurring at -1V compared to negligible uptake at +3V. We quantitatively assessed the functional effect of simultaneous, modulated uptake of BACE1 siRNA and fluorescently tagged scrambled siRNAs. Changes in spike rate activity and spike waveform shapes at different levels of BACE1 silencing are likely due to the regulatory role played by BACE1 in Nav1.1, Nav1.2, Nav1.4 voltage-gated ion channels. Since a significant proportion of AD therapeutics is directed towards BACE1 inhibitors, optimal knockdown of BACE1 needs to be carefully investigated for maximum drug efficacy and restoration of synaptic function. The above reported method will enable rapid, high-throughput drug discovery *in vitro* and also controlled and targeted non-viral delivery of RNAi therapeutics *in vivo*.

REFERENCES

[1] A. Sridharan, C.Patel, and J.Muthuswamy "Voltage preconditioning allows modulated gene expression in neurons using PEI complexed siRNA" *Mol Ther Nucleic Acids.* vol.26(2), pp:e82. doi: 10.1038/mtna.2013.10, 2013.

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