APPLICATION NOTE

VALA SCIENCES
Unlocking the Cellular Secrets of Disease

Measuring voltage activity in hiPSC-dopaminergic neurons using Kinetic Image Cytometry®

INTRODUCTION

Neurons communicate with target cells through action potentials, which are transient electrical depolarizations (≤ 10 msec) resulting from the flow of sodium, potassium, and calcium through voltage- and ligand-gated ion channels in the plasma membrane (Figure 1). Each neuron type fires action potentials with different patterns and frequencies, which propagate along axons to trigger neurotransmitter release at synapses and modulate target cell activity.[1] Depending on excitatory synaptic input frequency, neurons can also exhibit other forms of electrical activity, including slow or long-lasting depolarizations. One hypothesized cause of neurodegenerative diseases is excitotoxicity: increased membrane depolarization frequency that can cause calcium overload, oxidative stress, inflammation, mitochondrial dysfunction, and neuronal cell death.[2] Measuring neuronal voltage at high temporal and spatial resolution is thus key to assessing neuronal health and function.

In Parkinson's Disease, excitotoxicity may play a role in the degeneration of dopaminergic neurons the substantia nigra pars compacta, a brain region responsible for voluntary movement.[3,4] In the brains of Parkinson's patients, the presynaptic protein α-synuclein misfolds and aggregates to form Lewy body inclusions and neurotoxic oligomers.[5] Mutations in α-synuclein (e.g., A53T) cause early-onset forms of Parkinson's, potentially by promoting formation of α-synuclein aggregates.[6,7] These aggregates increase neuronal activity and calcium transients in many model systems, and thus may promote excitiotoxicity in Parkinson's.[8]

Electrophysiologists use metal or glass electrodes to directly record neuronal electrical activity from single neurons, but applying this technique to high-througput screens is technologically difficult. Researchers have recently designed molecular voltage sensors that change fluorescence when cells depolarize, enabling simultaneous recording of voltage transients from all neurons in a field of view with high temporal and spatial resolution.[9] These sensors can be used in multiple cell types, including dopaminergic neurons differentiated from human induced pluripotent stem cells (hiPSC-DNs, Figure 2). hiPSC-DNs exhibit the pacemaker-like electrophysiological activity of substantia nigra dopaminergic neurons and form functional networks, synapses, and signaling pathways in vitro.[10]

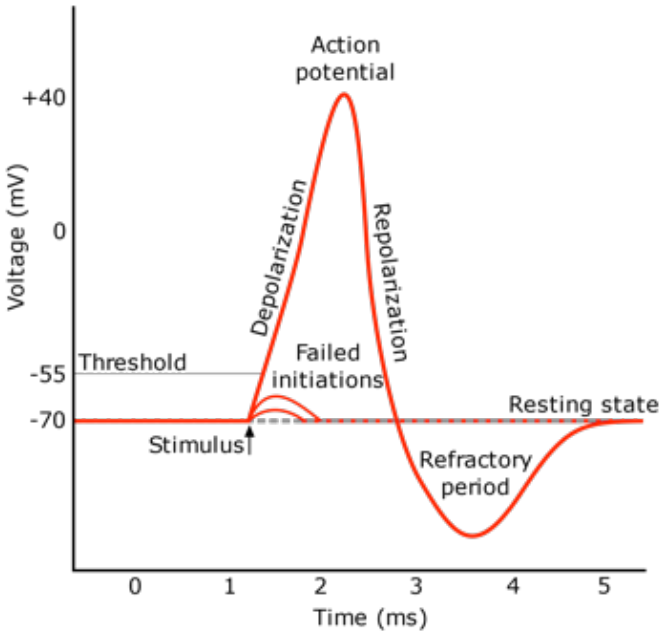

Figure 1. Typical neuronal action potential. *A stimulus can raise the negative resting potential above the threshold value to initiate action potential depolarization and repolarization, followed by an afterhyperpolarization, or refractory period, before a return to resting potential. The kinetics and voltage values for each step are determined by the sodium, potassium, and calcium channels expressed in each neuron. Figure details: original by en:User:Chris 73, updated by en:User:Diberri, converted to SVG by tiZom - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2241513*

In this pilot study, we tested if Vala's high content imaging technology can quantify voltage activity in normal hiPSC-DNs and an isogenic line with the A53T α-synuclein mutation introduced by genomic editing. We loaded both cell types with FluoVolt™, a molecular wire voltage sensor that has a high quantum yield and reports changes in membrane voltage on submicrosecond timescales.[11] We used Vala's automated digital microscopy workstation (the IC200 Kinetic Image Cytometer®) to record FluoVolt™ signal in hiPSC-DNs and detect either spontaneous voltage activity or activity following stimulation with electrodes built-in to the KIC®. We used algorithms encoded in Vala's cell image analysis software (CyteSeer®) to quantify the electrical activity of neurons on a cell-by-cell basis. Our results demonstrate that Vala's technology can simultaneously analyze neuronal activity from hundreds of hiPSC-dopaminergic neurons, enabling faster, higher throughput quantification of neuronal activity at the single cell level than possible with electrophysiological techniques.

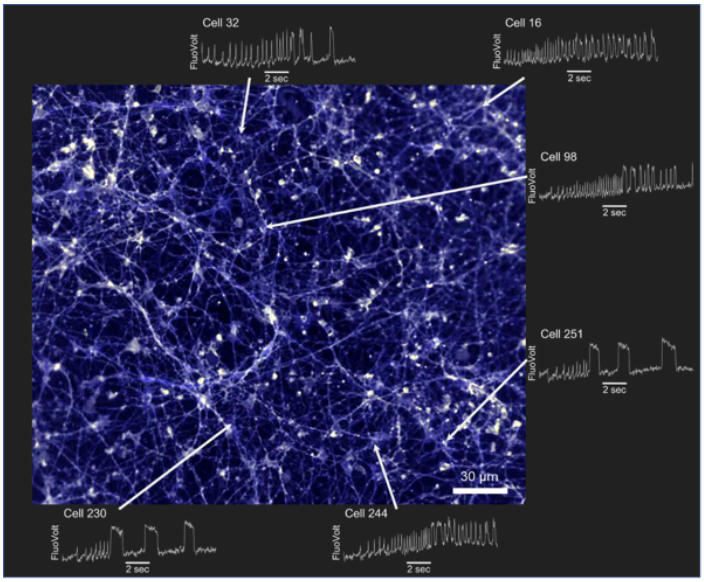

Figure 2. Voltage transients recorded from hiPSC-DNs with Vala's IC200-KIC®. *FluoVoltTM-loaded normal hiPSC-DNs were imaged at 60 frames per second for 10 seconds and the digital movies analyzed via CyteSeer®. Traces show FluoVolt™ intensity vs. time for the soma of each cell. Video at valasciences.com/application-notes.*

METHODS

hiPSC-DNs (iCell® DopaNeurons from Cellular Dynamics International, including a wild type line and an A53T α-synuclein (α-SNCA) isogenic line), were thawed and seeded in 96-well plates (Greiner Bio-One) coated with poly-L-ornithine/laminin at 200K cells per cm^2. Neurons were maintained in the media suggested by the manufacturer at 37°C and 5% CO_2 for 13 days prior to imaging. Before imaging, the neurons were loaded with FluoVolt™ according to the manufacturer's instructions, along with Hoechst 33342 as a live cell nuclear stain. FluoVolt™ signal was detected with Kinetic Image Cytometry® at 60 or 400 frames per second, with or without electrical stimulation from the IC200-KIC® electrodes. Voltage time series were analyzed by CyteSeer® using custom algorithms to identify live neuronal nuclei and to determine their associated soma.

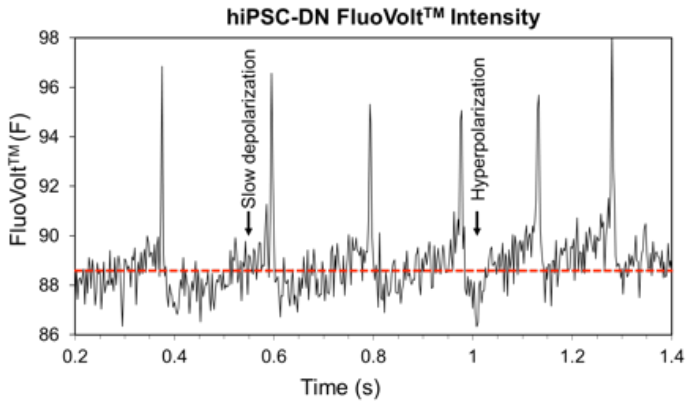

Figure 3. Action potentials recorded from a single hiPSC-DN by Kinetic Image Cytometry®. *CyteSeer®- analyzed trace of FluoVolt™ intensity vs. time for a neuron imaged at 400 frames per second. Arrows denote a slow depolarization and a hyperpolarization that precede and follow each action potential. Red line represents the approximate resting potential.*

RESULTS

Because FluoVolt™ intensity increases when cells depolarize, neuronal voltage activity causes increases in fluorescence of varying durations. Kinetic Image Cytometry® can detect FluoVolt™ signal to report hiPSC-DN action potentials from each cell in a field of view (Figure 2). A trace from a single hiPSC-DN soma (Figure 3) displays slow pacemaker depolarizations before each action potential and afterhyperpolarizations typical of dopaminergic neurons in substantia nigra slices.[1]

The temporal resolution of KIC® imaging at 400 frames per second (2.5 msec) is therefore sufficient to simultaneously capture detailed voltage transient information from hundreds of neurons in a field of view. Decreasing the field of view enables frame rates of up to 1500 per second to capture more voltage transient details. CyteSeer®, Vala's cell image analysis software, derives 30 different data parameters from each voltage transient from each neuron (e.g., rise time, decay time, and peak width at various points, see Figure 4). CyteSeer® thus provides detailed, high-throughput information on intra- and interneuron variablity in electrical activity.

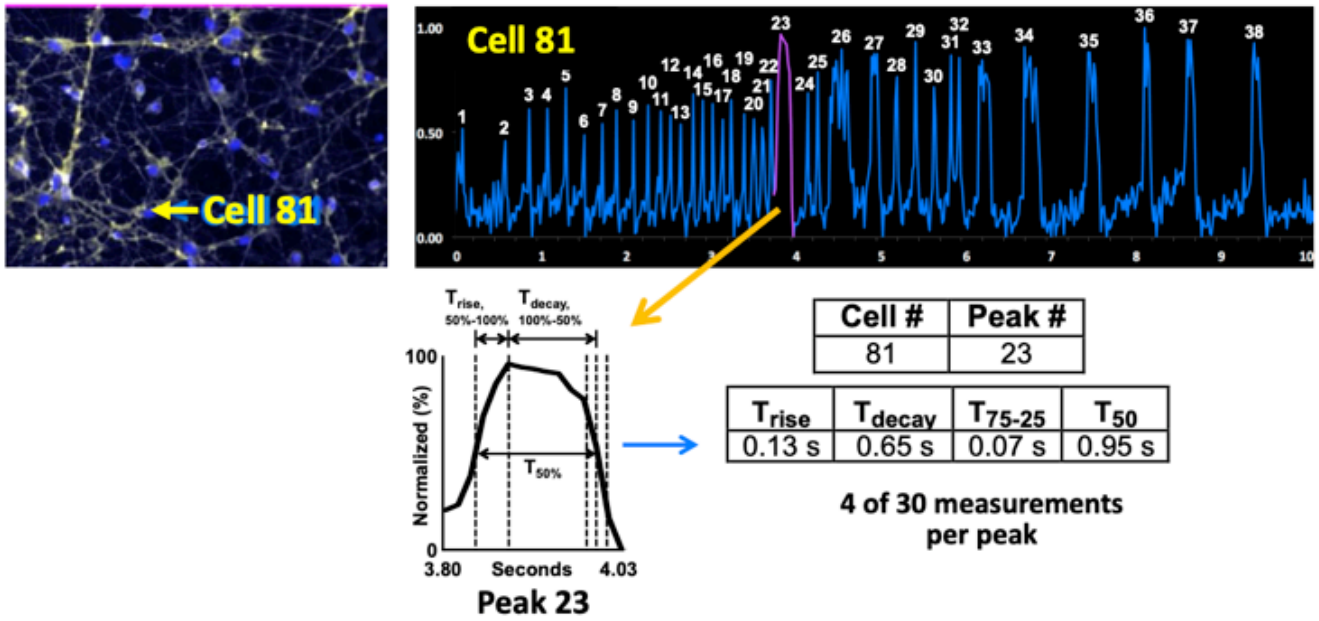

Figure 4. Analysis of the kinetics of the voltage transients. *Every transient in every cell can be quantified for a variety of kinetic parameters, such as rise time, decay time, and duration. In the example shown, cell 81 in the field of view displayed 38 separate voltage transients, and each of which was analyzed for 30 different kinetic parameters.*

FluoVolt™ traces recorded at 400 frames per second from A53T hiPSC-DNs exhibit spontaneous voltage activity in the absence of stimuli (Figure 5, left), consistent with the pacemaker activity of substania nigra dopaminergic neurons. These voltage transients are either brief (< 10 msec) depolarization/repolarizations that correspond to action potential spikes or prolonged (up to 1 sec) square wave depolarizations. Many hiPSC-DNs exhibited regular voltage transients in response to 5 Hz electrical pulses from built-in IC200-KIC® electrodes (Figure 5, right).

We next used Kinetic Image Cytometry® of FluoVolt™ signal to compare voltage transients in normal hiPSC-DNs and an isogenic line with the A53T α-synuclein mutation introduced by genomic editing (Figure 6). The IC200-KIC® dwell time per well is about 15 seconds (to autofocus, image for 10 seconds, and travel between wells). This acquisition speed enabled imaging of thousands of neurons across multiple wells in less than 10 minutes. hiPSC-DNs of both genotypes displayed a mixture of action potential spikes and longer depolarizations, but A53T α-synuclein neurons exhibited voltage transients at a frequency about two-fold greater than the controls. These data suggest that the A53T mutation in α-synuclein increases spontaneous activity in dopaminergic neurons, which may lead to excitotoxicity.

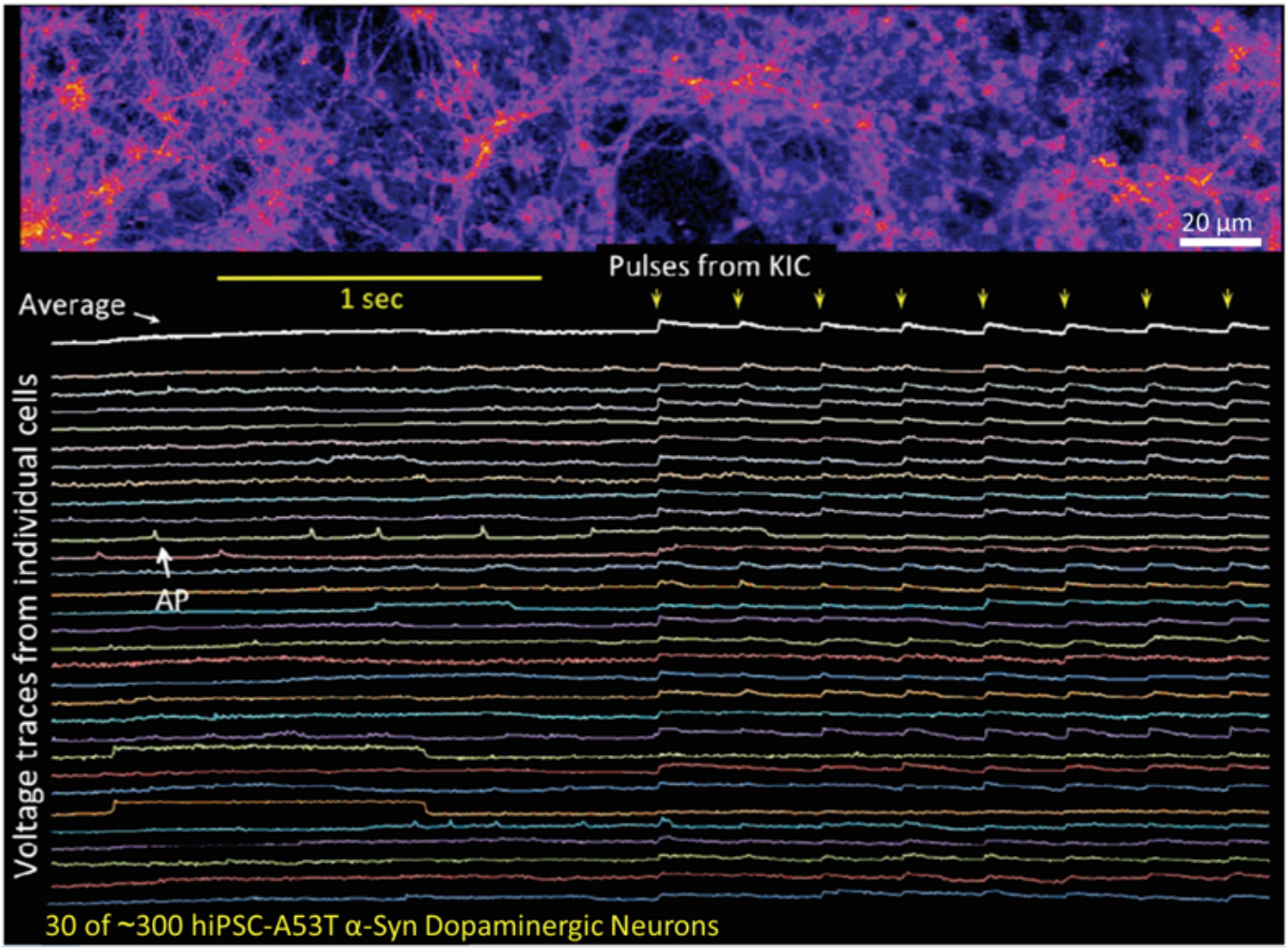

Figure 5. Voltage transients in hiPSC-DNs by Kinetic Image Cytometry®, recorded at 400 frames per second.
Upper, field of view of neurons labeled with FluoVoltTM. Lower, traces for 30 individual neurons (out of ~300 in the field of view); an average trace is also shown. FluoVoltTM signal was acquired at 400 frames per second. Electrical pulses were delivered by the KIC at 5 Hz at the midpoint of the recording. An arrow denotes an action potential (AP) displayed by individual neurons. Prolonged depolarizations are also common. Video at valasciences.com/application-notes.

DISCUSSION

Neurodegenerative diseases such as Parkinson's currently have no cures, affect tens of millions of people worldwide, and cost hundreds of billions of dollars in annual patient care.[12] Current Parkinson's treatment, which involves administration of the dopamine precursor L-dopa, is effective in the short term but eventually loses efficacy and causes L-dopa-induced dyskinesia.[13] Therapeutics that specifically reduce dopaminergic neuron excitotoxicity and cell death could slow Parkinson's progression and reduce the need for L-dopa treatment. The data above demonstrate that Vala's instrumentation (the IC200 KIC®) and image analysis software (CyteSeer®) can quantify voltage transients in dopaminergic neurons derived from hiPSCs and labeled with the molecular wire voltage sensor FluoVolt™. High-throughput assays on hiPSC-DNs using Vala's technology can increase the likelihood of discovering new treatments for Parkinson's by increasing the efficiency and accuracy of voltage-based screens for neuroprotective or neurotoxic compounds.

CONCLUSIONS

Vala's high content KIC® methods can simultaneously measure voltage activity in hundreds of hiPSC-neurons, representing a technological advance over traditional electrophysiological recording techniques. This assay can be expanded to include measurements of hiPSC-DN calcium transients using fluorescent calcium sensors such as Fluo-4. CyteSeer® can report many voltage and calcium parameters that can be combined with multidimensional analysis methods, machine learning, and artificial intelligence to detect changes relevant to neuronal electrophysiology. The ability of Vala's technology to automatically record and analyze voltage and calcium activity in neurons plated in 96- or 384-well dishes brings the opportunity for true high-throughput screening to search for cures of Parkinson's, Alzheimer's, and related neurodegenerative diseases. Vala's IC200 KIC® can also quantify action potentials and/or calcium transients in other disease-relevant cell types, including cardiomyocytes, skeletal muscle, and pancreatic beta cells. Thus, the IC200 KIC® is a versatile automated microscopy workstation with broad applications across biomedical and cell biology research.

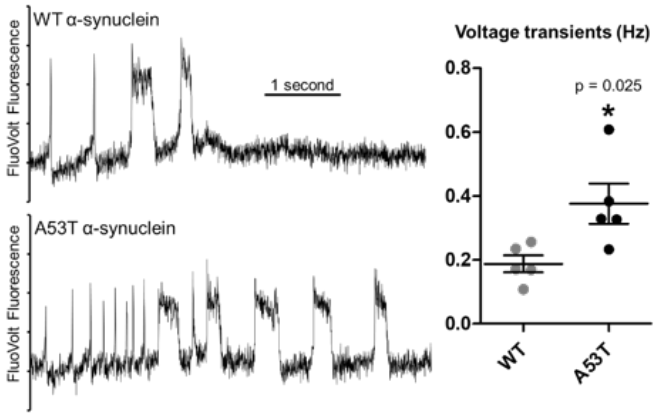

Figure 6. Effects of A53T α-synuclein on voltage activity in hiPSC-DNs. *Left, representative traces from FluoVoltTM-loaded hiPSC-DNs imaged at 400 frames per second (spontaneous activity). Right, Quantification of voltage activity. Each dot represents one of n = 5 wells per cell type, with each well representing 200 to 500 neurons (p = 0.025 by Student's t-test).*

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