Case Study: Bringing Human Neuronal Biology to HTS

Abstract

The development of pharmacological compounds requires highly predictive in vitro test systems. Assays based on primary neuronal cultures obtained from animals are commonly used, although correlation between animal and human data might be weak in some cases. As a solution to this problem, the drug discovery field is strongly moving towards human induced pluripotent stem-cell derived (hiPSC) neurons. Here we developed several live-cell Ca^{2+} imaging screening assays on the Hamamatsu µCell instrument based on Cellular Dynamics' iCell Glutaneurons. Intracellular Ca^{2+} dynamics as indicator of neuronal network activity was recorded and modulated using different pharmacological tool compounds with known neurophysiological effects. We focussed on compounds with seizurogenic potential from the current NeuTox panel of HESI and mGluR modulators. As astrocytes are highly important for the neuronal network activity, we were also interested in a comparison between data obtained from a pure iCell GlutaNeuron culture and a coculture of iCell GlutaNeuron and iCell Astrocytes. For a subset of experiments the results were benchmarked against data obtained from rat cortex primary neuron culture. iCell GlutaNeurons were responsive to the tested compounds concentration-dependently and with, in parts, higher sensitivity compared to the primary cell system. Furthermore, the cocultivation with iCell Astrocytes had a substantial impact on the network activity after compound treatment, indicating a possibly even more physiological reaction than iCell GlutaNeuron cultivation alone.

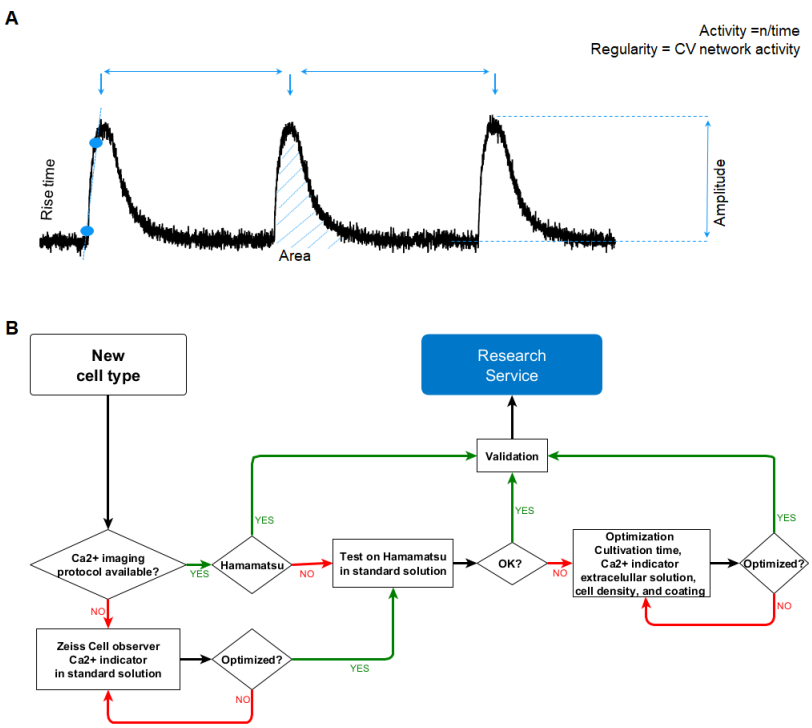

Figure 1. Definition of the analyzed parameters and the flow diagram for assay optimization. The analysis of Ca^{2+} transients is widely used to measure neuronal network activity. **A)** Parameters that are analysed from the intracellular Ca^{2+} recordings: 1) Ca^{2+}-transients magnitude is measured as the peak amplitude and area under the curve, 2) Rise time is measured as time interval between 10 and 90 percent of the peak amplitude, 3) Network activity is defined as the number of peaks over time, and 4) The regularity is measured as the coefficient of variation of the network activity. **B)** The flow diagram describes the steps that are followed during the assay optimization phase, which is extremely important to obtain stable, sensitive, and reproducible recordings in a time- and cost-efficiently manner.

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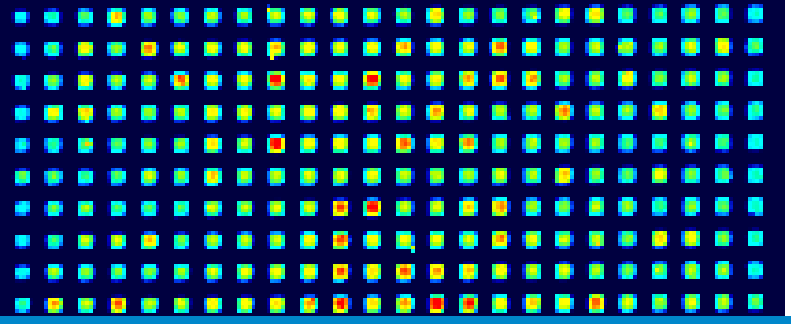

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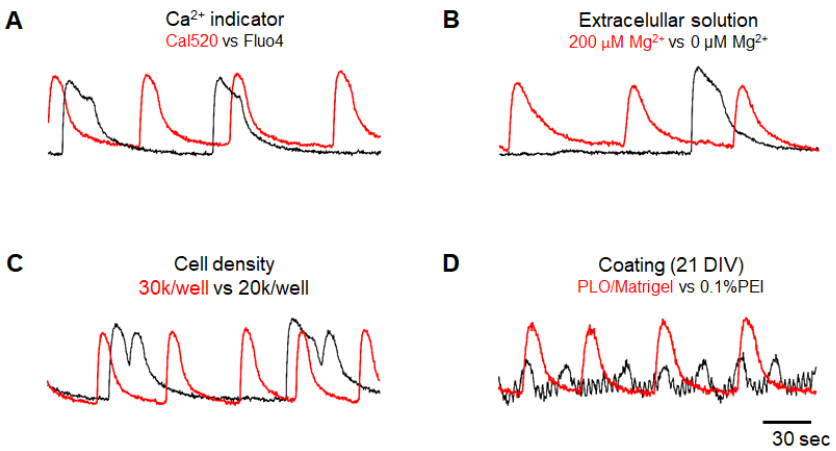

Figure 2. Examples that highlight the importance of assay optimization. A) The choice of the correct Ca^{2+} indicator has a significant impact on the network activity. **B)** The concentration of divalent cations has a major influence on the occurrence of network bursts. **C)** Finding the optimal cell density is key to a robust experiment. **D)** The coating of the cultivation wells can largely influence the establishment of the neuronal network activity. Please note that the assay optimization presented here is specific for iCell GlutaNeurons.

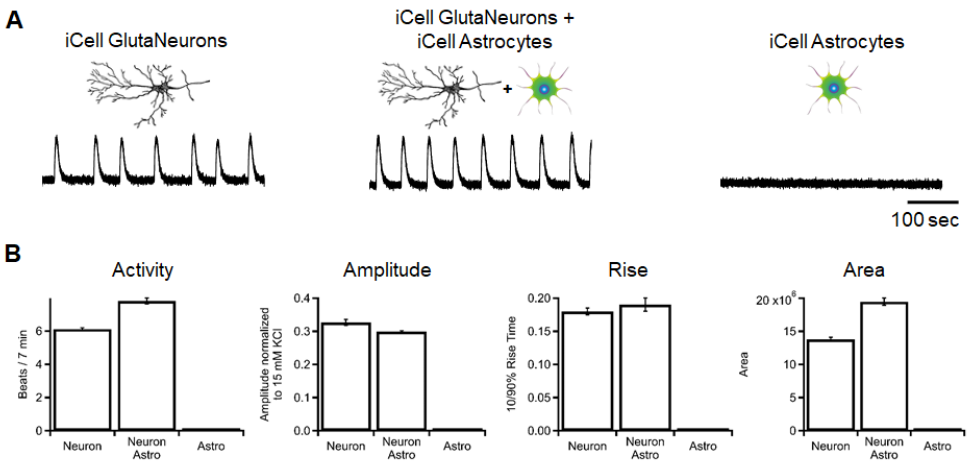

Figure 3. Overall GlutaNeurons network activity is qualitatively independent of the presence of Astrocytes. A) Previous studies indicate that astrocytes are highly important for neuronal network activity. Therefore, we were interested in comparing the network activity from iCell GlutaNeuron cultures with and without iCell Astrocytes. **B)** Our results suggest that the standard parameter including network activity, time course and Ca^{2+}-transients magnitude (peak amplitude and area under the curve) are similar between iCell GlutaNeuron and iCell GlutaNeuron+Astrocytes cultures. As expected, no network activity was detectable in pure iCell Astrocytes cultures.

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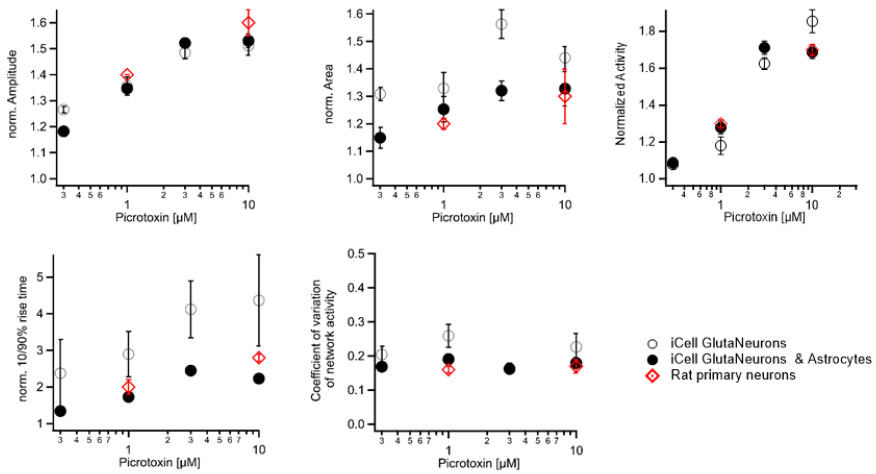

Figure 4. Effects of Picrotoxin on the network activity . The seizurogenic compound Picrotoxin is a GABAA antagonist, which is known to increase neuronal activity by disinhibiting the network. This is reflected as by our results showing that picrotoxin triggers an increase in the Ca^{2+}-transients magnitude and the neuronal activity in a dose-dependent manner, without altering the activation regularity. Sensitivity of iCell Glutaneurons was comparable to the gold standard rat primary neurons (indicated in red). Note that the robustness of the data is significantly increased in co-cultivated cultures compared to iCell Glutaneurons alone.

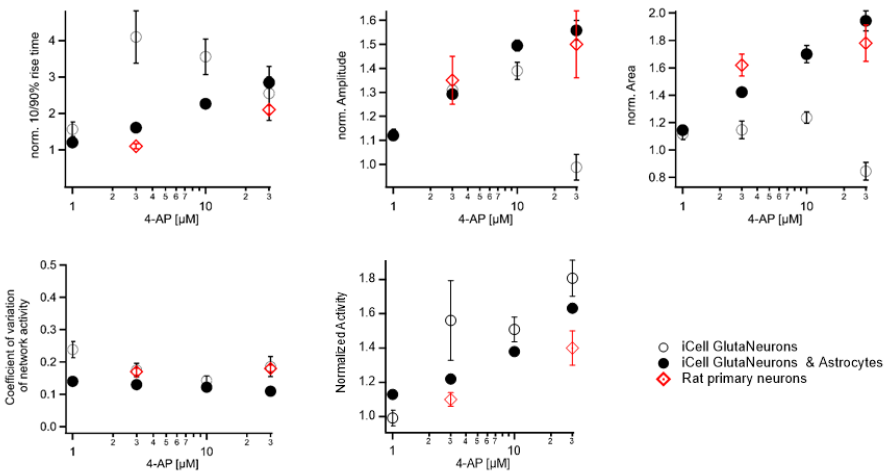

Figure 5. Effects of 4-Aminopyridine on the network activity. The IKA antagonist 4-Aminopyridine is known to activate neuronal activity. As anticipated, our results show that the presence of 4-Aminopyridine induces an increase in the Ca^{2+}-transients magnitude and the neuronal activity in a dose-dependent manner, without altering the activation regularity.

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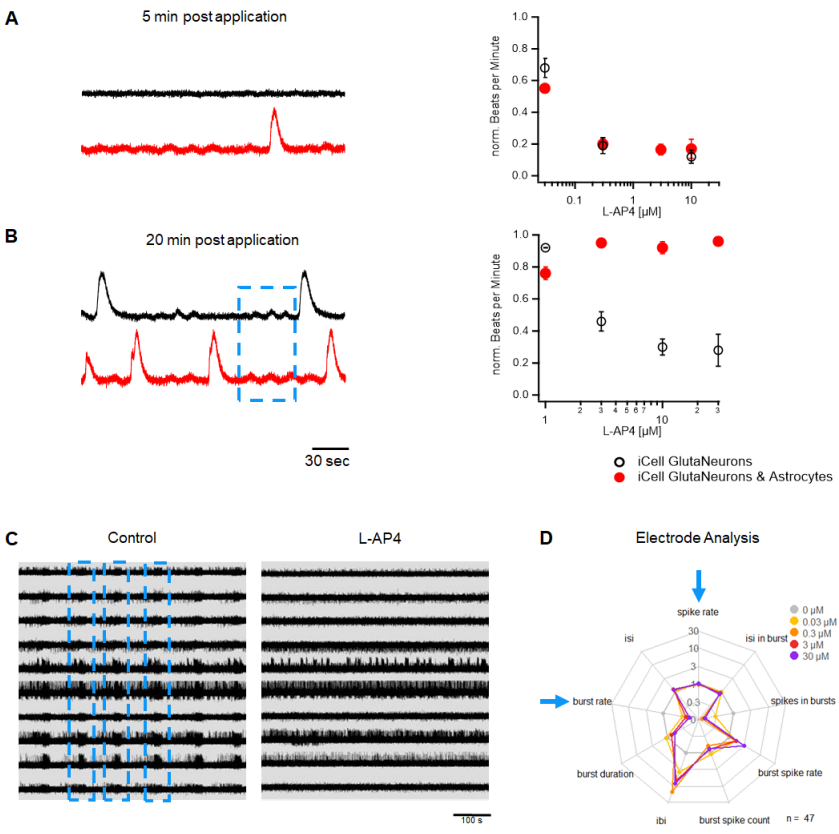

Figure 6. L-2-amino-4-phosphonobutyric acid (L-AP4) reduces the number of Ca^{2+} transients and disturbs the network synchronicity without altering the spike rate. The amino-4-phosphonobutyric acid (L-AP4) is a mGluR III agonist that has been reported to reduce the network activity. **A)** Our results showed that the number of Ca^{2+}-transients was concentration-dependently reduced by the same amount in both iCell Glutaneurons and cocultivated with iCell Astrocytes when recorded within 5 min post application. **B)** Interestingly, 20 min post application of L-AP4, the reduction was immanent only in the pure iCell Glutaneuron cultures while the activity recovered fully in the co-cultivated wells, indicating that the co-cultures have a higher robustness. Please note the Ca^{2+}-basal level oscillations in the cells that were treated with L-AP4 in both cultures (highlighted with a blue box), suggesting that the treatment might either reduce the neurons firing or desynchronize the burst activity. Since the intracellular Ca^{2+} recordings performed in the Hamamatsu FDSS/µCell instrument integrates cell activity over the entire well, we further dissected the mode of action using MEA technology, which allows a higher spatio-temporal resolution. **C)** Ten traces from a single MEA well with electrical activity on all electrodes. Deflections in the trace indicate single action potentials. Left: control; please note the highly synchroneous burst activity, highlighted with the blue boxes. Right: after L-AP4 application burst activity was gone while the cells on the electrodes were still active. **D)** Radar plot of 9 (out of 20) parameters obtained for analysis, clearly indicates that L-AP4 does not decrease spike rate but has a major impact on the burst rate, demonstrating that synaptic transmission and by this network synchronicity is disturbed..

Conclusion

Using the FDSS/µCell (Hamamatsu) instrument and the iCell Glutaneurons (Cellular Dynamics), we successfully developed a robust live-cell Ca^{2+} imaging assay. Our results show that iCell GlutaNeurons are an excellent model to test potential neuronal modulators or neurotoxicity effects, since the observed effects were in line with previous reports and comparable to primary cells. Furthermore, the cocultivation with iCell Astrocytes increased the assay robustness and simulates a condition that is more physiological. Finally, using a Ca^{2+} imaging assay, we detected L-AP4-mediated abnormalities, which could only be characterized using the MEA platform, highlighting the importance of combining different methodologies to answer a scientific question.

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