

Electrophysiological recordings of LGIC and AA transporters in iCell® GlutaNeurons

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iCell® GlutaNeurons kindly provided by FUJIFILM Cellular Dynamics, Inc.



Summary

Human neurons derived from induced pluripotent stem cells (hiPSCs) are becoming increasingly important for studying basic neuronal physiology and can provide good models for studying neurological disorders. hiPSC-derived neurons provide a viable alternative to primary cells and animal models in the drug discovery industry for finding novel therapeutics to treat seizure-related and neurodegenerative disorders. iCell® GlutaNeurons are glutamatergic-enriched cortical neurons derived from hiPSCs. Single cell gene transcription analysis has shown the presence of glutamate receptors: AMPA, kainate and NMDA, as well as glutamate and GABA transporters¹. Ionotropic glutamate receptors mediate the majority of excitatory neurotransmission in the mammalian CNS and removal of glutamate from the synaptic cleft by reuptake via glutamate transporters plays a role in regulating neuronal excitability. GABA is the major inhibitory neurotransmitter in the brain and is important in controlling excitability. After release, GABA is removed from the extracellular space by GABA transporters (GATs), thus terminating inhibitory synaptic transmission. Both GABA and glutamate transporters may provide novel therapeutic targets for, e.g. Parkinson's disease², Alzheimer's disease³, and epilepsy⁴.

We recorded ligand-gated ion channel currents mediated by GABA_A and AMPA receptors from iCell® GlutaNeurons on the Patchliner and SyncroPatch 384PE. Furthermore, we could measure GABA and glutamate transporters in these neurons using the SURFE²R N1 device.

Results

To activate transport on the SURFE²R N1, a sensor with attached iCell® GlutaNeurons was inserted into the device and perfused with a buffer containing NaCl and glutamate. When the substrate is present, Na⁺ and K⁺ movement across the membrane can be observed until an electrochemical equilibrium is reached. To generate Na⁺ and K⁺ gradients, necessary as a driving force, the sensor was flushed with KCl before and after the glutamate transporter activation. The traces and concentration response curve for glutamate for an example sensor is shown in Figure 1, confirming the presence of a glutamate transporter in iCell® GlutaNeurons, although the exact identity was not investigated further.

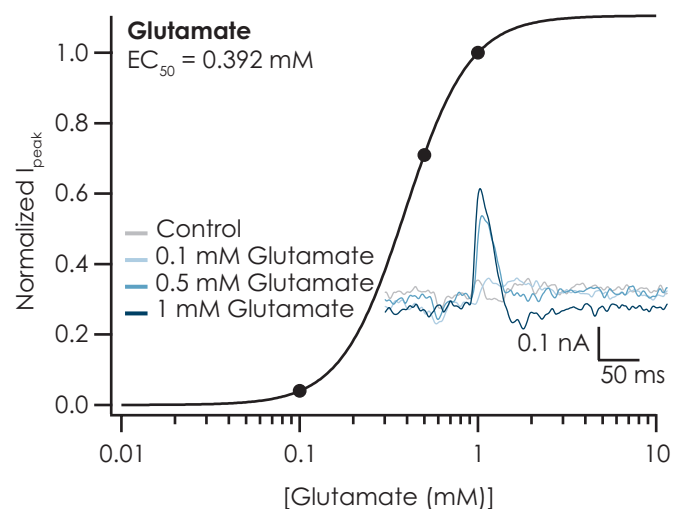


Figure 1: Glutamate transport was activated in iCell® GlutaNeurons upon addition of glutamate. Peak amplitude increased with increasing glutamate concentration with an EC₅₀ of 0.391 mM. Traces recorded from the cells on the sensor of the SURFE²R N1 are also shown.

Application Note

GATs transport GABA in exchange for Na⁺ and Cl⁻ with the proposed stoichiometry 2 Na⁺: 1Cl⁻: 1 GABA². Using the SURFE²R N1, GABA transport could be observed in the presence of Na⁺ and Cl⁻ upon addition of GABA (Figure 2), confirming the presence of a GABA transporter in iCell[®] GlutaNeurons although the exact identity of the GAT was not further investigated.

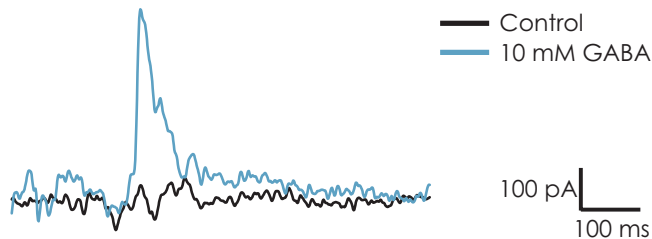


Figure 2: A Addition of 10 mM GABA to sensors with attached iCell[®] GlutaNeurons resulted in the activation of a current mediated by a GABA transporter.

iCell[®] GlutaNeurons were also used on the medium and high throughput patch clamp instruments, the Patchliner and SyncroPatch 384PE. Figure 3A shows the current response of an iCell[®] GlutaNeuron to glutamate in the absence and presence of the AMPA specific positive allosteric modulator (PAM) LY404187 on the Patchliner.

References

1. Chen, Y. 2018. In: Neurotoxins. DOI: 10.5772/intechopen.77043
2. Jin, X-T. *et al* 2011. *Frontiers in Systems Neuroscience*. 5:Article 63
3. Fuhrer, T.E., *et al*. 2017. *Neuroscience*. 351: 108-118
4. Björn-Yoshimoto, W. & Underhill, S.M. 2016. *Neurochem Int*. 98: 4-18

Methods

Cell Culture

iCell[®] GlutaNeurons were kindly provided by FUJIFILM Cellular Dynamics, Inc. (Catalog # R1061 or R1034) and were cultured and harvested according to Nanion's standard procedures for hiPSCs.

Electrophysiology

iCell[®] GlutaNeurons were used on the Patchliner and SyncroPatch 384PE using Nanion's standard protocols.

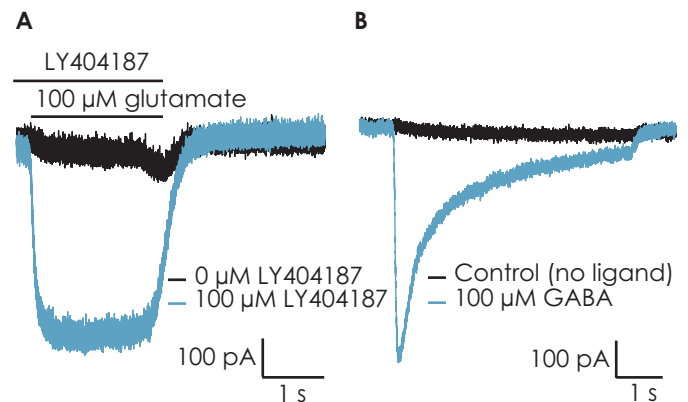


Figure 3: A In the presence of the AMPA-specific PAM, LY404187, a response to glutamate in iCell[®] GlutaNeurons recorded on the Patchliner was seen. B GABA_A receptor mediated response was recorded on the SyncroPatch 384PE

GABA induced an inward current when applied to iCell[®] GlutaNeurons on the SyncroPatch 384PE mediated by the GABA_A receptor (Figure 3B).

In conclusion, iCell[®] GlutaNeurons can be successfully used on the SURFE²R N1 to measure transporter activity, and on the Patchliner and SyncroPatch 384PE to measure whole cell currents using the patch clamp technique for drug discovery and ion channel/transporter research.

For glutamate experiments, cells were held at -80 mV, pre-incubated in LY404187 and this was then co-applied with glutamate. For GABA experiments, cells were held at -80 mV and GABA was applied for ~5 s.

SURFE²R sensor preparation

According to the Nanion standard procedure "SURFE²R Sensor Preparation". Whole cells were used.

SURFE²R N1 measurement workflow

Glutamate and GABA transport are activated by providing the respective substrate. In both cases a sodium gradient is established prior to substrate addition, and for GAT experiments, an additional chloride gradient is established. Therefore, any 3-buffer Nanion standard protocol is suitable. Buffers are HEPES based including NaCl with KCl for EAAT and K-Gluconate for GAT as the main salt.

Please ask us for further experimental details and for more information about buffers and recording solutions.