

High throughput SSM recordings of EAAT3 (SLC1A1) activity on the SURFE²R 96SE

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Summary

The excitatory amino acid transporter 3 (EAAT3; also known as EAAC1) is a sodium-dependent neuronal uptake transporter encoded by the SLC1A1 gene¹. It plays a major role in the reuptake of glutamate from the synaptic cleft, thereby maintaining a low extracellular concentration of glutamate and regulating the excitatory neurotransmission. EAAT3 is also involved in the uptake of aspartate and cysteine into the cells^{1,2}. The transporter is highly expressed in mature neurons, where it is distributed in somata and dendrites^{1,2}.

EAAT3 functions as a co-transporter, coupling the uphill substrate transport into the cells to the electrochemical gradients of sodium and potassium. The stoichiometry of transport is 1 glutamate with 3 Na⁺ and 1 H⁺ moving into the cell to 1 K⁺ moving out of the cell^{1,2}. Therefore EAAT3 is an electrogenic transporter, generating a net charge flow.

Dysfunction of glutamate transporters leads to increased extracellular glutamate levels, thereby causing neurotoxicity and neurodegeneration². Regulatory mechanisms facilitating EAAT3 function are, therefore, interesting as targets for the treatment of neurodegenerative diseases².

Here we present EAAT3 activity measurements on the SURFE²R 96SE instrument using purified plasma membrane of CHO cells expressing EAAT3. The transporter was activated by glutamate and blocked by six different known inhibitors with IC₅₀ values similar to those found in the literature.

Results

To activate transport on the SURFE²R 96SE, the sensor plate containing 96 individual sensors with attached EAAT3-containing membrane fragments was 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 EAAT3 activation (Figure 1).

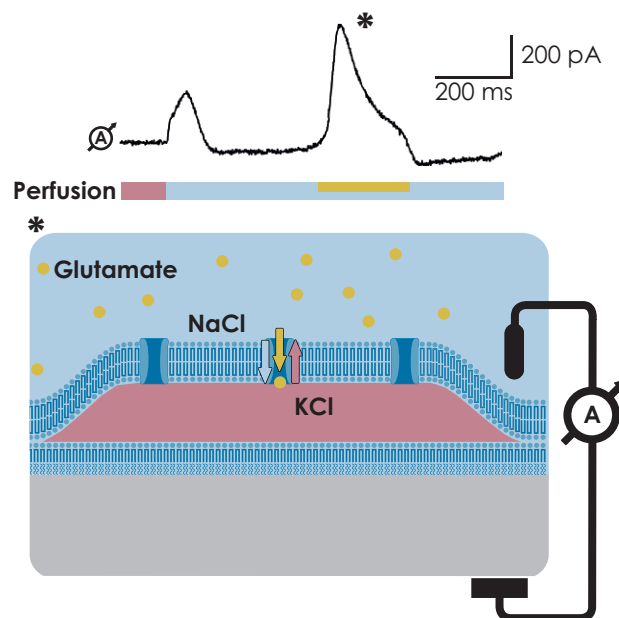


Figure 1: Typical EAAT3 current response on an individual sensor of the SURFE²R 96SE. Glutamate was used as the substrate for EAAT3. When applying glutamate to the sensor, a transient current occurs (top; actual recording trace from an experiment on the SURFE²R 96SE).

Application Note

Success rate and signal stability

An investigation into reproducibility revealed that in a typical experiment $97 \pm 3\%$ of the sensors in a 96 well plate (average of 10 plates) met the quality criteria and generated current signals >150 pA. A screenshot showing a typical plate with color-coding for quality of the SSM is shown in Figure 2.

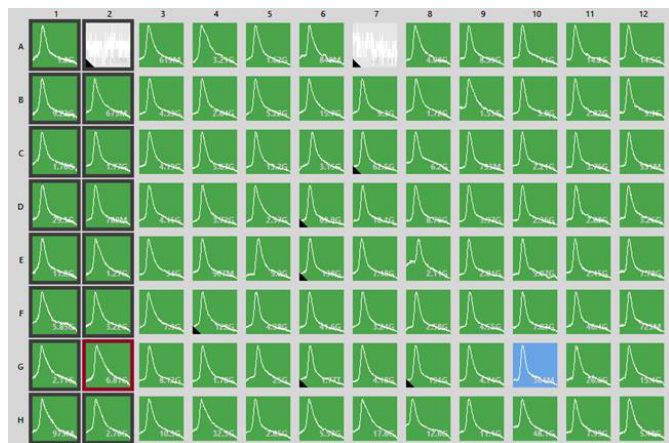


Figure 2: Success rate of experiments on the SURFE²R 96SE. Shown is a screenshot of one experiment on the SURFE²R 96SE. Wells are color-coded based sensor quality (green: high quality; blue: intermediate quality or grey: Failed well). Shown is signal generated upon perfusion with activating buffer (Na⁺ and glutamate).

Once prepared, sensors are very stable and signals are highly reproducible. Figure 3 shows normalized currents of EAAT3 activated by glutamate in the presence of sodium. EAAT3 can be repetitively activated for over 12 hours with similar amplitudes. A typical experiment duration is approximately 50 minutes (shown in the inset). In this time, EAAT3 was activated 9 times with similar peak amplitudes proving the suitability of the technique for pharmacological investigation.

Glutamate was applied to the sensors, a single concentration of glutamate was applied to each well and the concentration response curve (CRC) for glutamate was calculated across the whole plate with $n = 8$ wells per concentration. In each experiment, negative control (baseline) and maximum current are also recorded in each well and data was normalized to the maximum current in each well. The CRC was fit with a Hill Equation revealing an EC_{50} for glutamate of $99 \mu\text{M}$, similar to that recorded using the SURFE²R N1 (see Application Note).

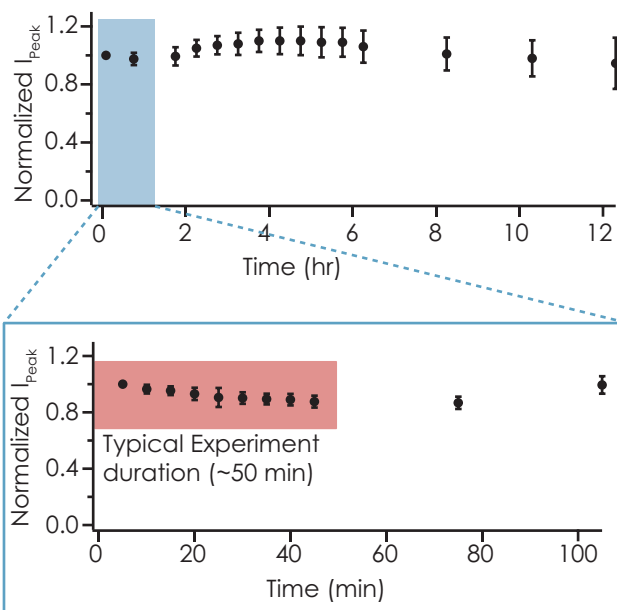


Figure 3: Stability of EAAT3 signals on the SURFE²R 96SE. EAAT3 can be reproducibly and repetitively activated for 12 hours. Over the course of a typical experiment (approx 50 mins; bottom panel), EAAT3 can be repetitively stimulated and reproducible currents are recorded. Data shown is average normalized peak current for a 96 well plate ($n = 94$ wells) $\pm$ standard deviation.

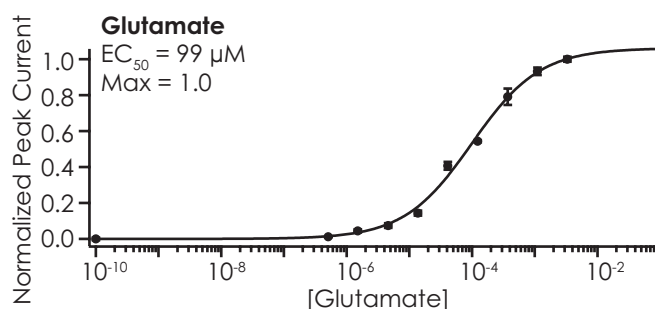


Figure 4: Concentration response curve for activation of EAAT3 by glutamate. Shown are average $\pm$ S.E.M of 8 wells per concentration of glutamate. The curve was fit using a Hill equation revealing an EC_{50} for glutamate of $99 \mu\text{M}$.

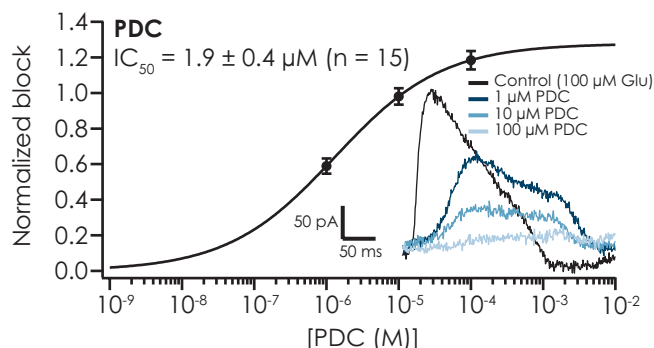
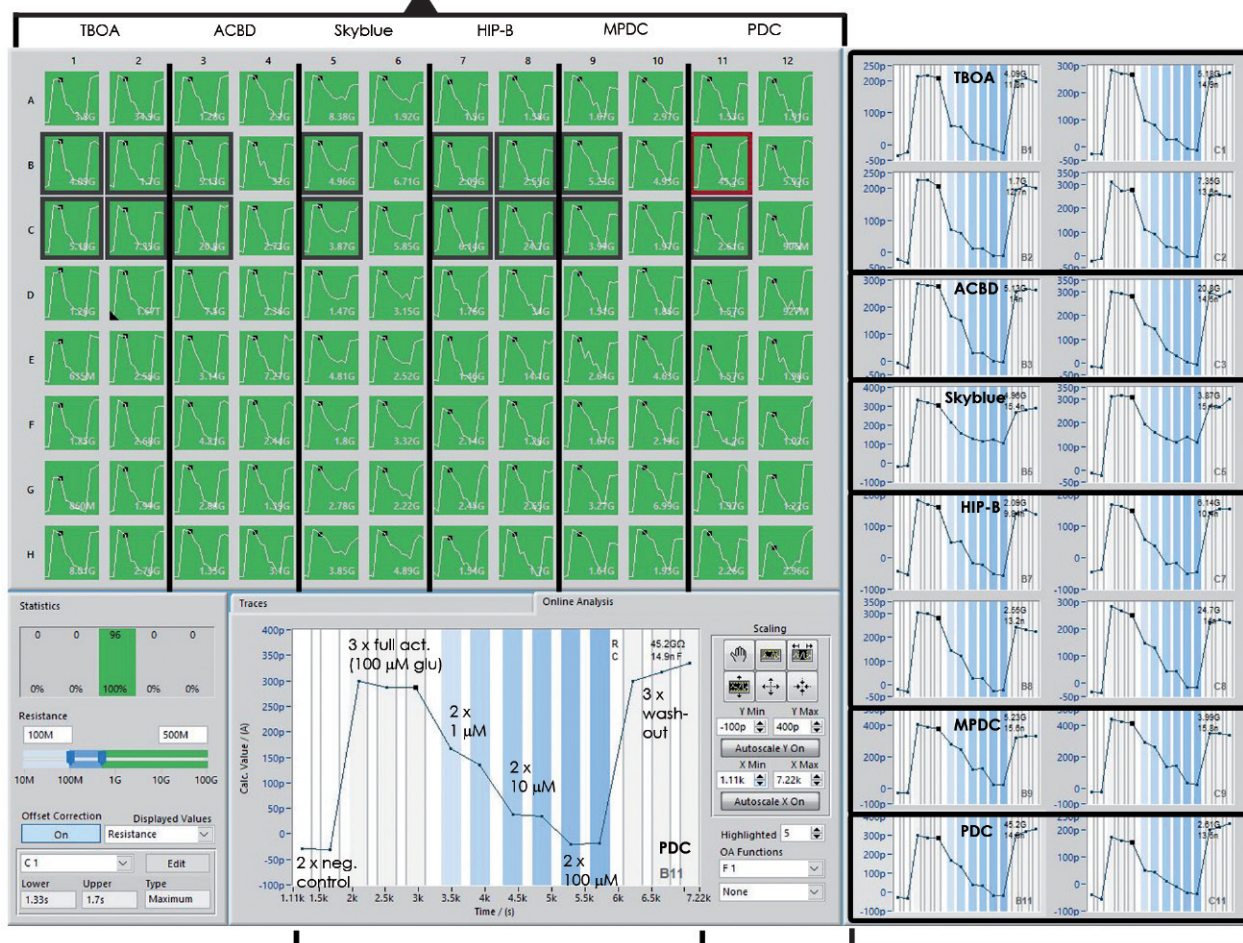


Figure 5: Concentration response curve for PDC. Shown is the concentration response curve for PDC for an average of 15 wells. Traces for an exemplar well are shown on the right.

Application Note

96 color coded depictions of data traces eases judgement of success rate



Highlighted OA plot of 1 well showing effect of HIP-B on EAAT3-mediated currents

Highlighted OA plots 16 wells showing block of EAAT3 by different compounds

Figure 6: Graphical user interface of the screening and data analysis software used on the SURFE²R 96SE. Screenshot of depiction of online analysis data of EAAT3 activated by glutamate and blocked by different compounds. Six different compounds were used on one plate, cumulative concentrations were added to each well and full concentration response curves in each well were calculated. Two columns of the plate were exposed to each compound giving $n = 16$ wells per compound. All wells were successful in terms of quality of sensor (green wells). Average IC_{50} values for each of the 6 compounds are given in Table 1, an example of a concentration curve for one of the compounds (PDC) is shown in Figure 5.

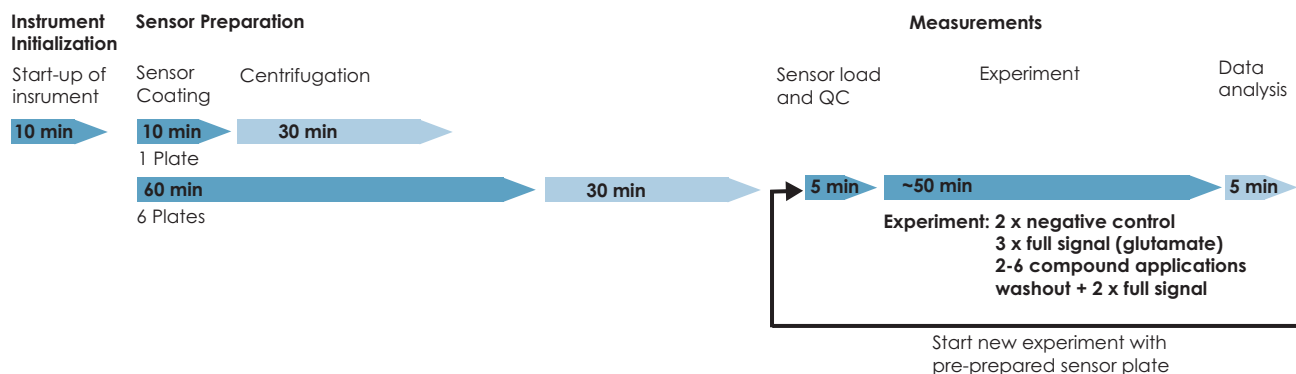


Figure 7: Workflow for experiments on the SURFE²R 96SE. Sensors (96 well plate format) are prepared on the robot and subsequently centrifuged. If multiple sensors are used on one day, they are prepared sequentially and centrifuged together. One sensor plate containing 96 sensors is then loaded onto the SURFE²R 96SE and experiments conducted. In these experiments, total time was approximately 50 mins. Once the experiment is finished, a new sensor plate containing 96 sensors is loaded onto the SURFE²R 96SE and a new experiment started.

Application Note

Compound	IC ₅₀ (μM)	Literature value (μM)
HIP-B	3.3 ± 1.4 (16)	17-18 ³ (IC ₅₀) U
MPDC	7.2 ± 1.5 (15)	5 ⁴ - 7 ⁵ (K _i) U
ACBD	1.5 ± 0.2 (16)	30 ⁵ (K _i) U
SkyBlue	0.6 ± 0.1 (16)	0.8 ⁶ (IC ₅₀) B
PDC	1.9 ± 0.4 (15)	1.5 ⁵ (K _i) U
L-TBOA	0.4 ± 0.05 (15)	7 - 15 ^{7,8} (IC ₅₀) U

Table 1: IC₅₀ values for 6 different blockers of EAAT3 calculated using the SURFE²R 96SE. Shown are average IC₅₀ values ± S.E.M obtained on the SURFE²R 96SE (number of wells shown in brackets) and the expected literature IC₅₀ or K_i values. B = binding assay; U = uptake assay.

Pharmacology experiments on the SURFE²R 96SE can be performed in different ways. Single point CRCs can be performed calculated across the whole plate or cumulative CRCs can be performed on individual wells, as shown in Figure 6. This increases the number of data points that can be obtained from 1 sensor plate. In the experiment shown in Figure 6, six different compounds were investigated in one experiment. The experiment started with 2 applications of resting buffer (negative control) followed by 3 applications of glutamate (100 μM). Once a stable signal was established, increasing

concentrations of the compound were added in duplicate (1, 10 and 100 μM) via the activating buffer. At the end of the experiment, the compounds were washed out and control activating buffer was added. Two columns per compound were used giving n = 16 wells per compound. An example of a concentration curve for one of the drugs (PDC) with the corresponding traces from one example well is shown in Figure 5. The IC₅₀ values calculated on the SURFE²R 96SE are summarized in Table 1. Shown are average IC₅₀s calculated from the individual IC₅₀ values from each well ± S.E.M. The IC₅₀ values calculated on the SURFE²R 96SE agreed well with the literature³⁻⁸, some being a little lower, e.g. HIP-B, ACBD and L-TBOA presumably due to differences in the technique used (binding versus electrophysiology).

In conclusion, EAAT3 can be reliably measured on the SURFE²R 96SE with expected EC₅₀ values for glutamate and IC₅₀ values for a number of known blockers. The SURFE²R 96SE is a highly sensitive SSM-based electrophysiology device for higher throughput recordings of transporters and ion pumps.

References

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Methods

Plasma membrane preparation

According to the Nanion's standard procedure ("Quickguide Membrane Preparation from CHO cells"). Total protein concentration was between 5 - 10 μg/μl of the undiluted protein preparation which is about 0.1 μg per well.

Buffers

EAAT3 experiments were performed by the exchange of

a sodium and glutamate free ("resting") buffer for a sodium containing ("control") buffer and afterwards a substrate and sodium containing ("activating") buffer. Resting buffer contained: 140 mM KCl, 2 mM MgCl₂, 30 mM HEPES, pH 7.4 with NMG.

Control buffer contained: 140 mM NaCl, 2 mM MgCl₂, 30 mM HEPES, pH 7.4 with NMG, activating buffer identical to control plus x mM glutamate.

SURFE²R sensor preparation

According to the Nanion standard procedure "SURFE²R Sensor Preparation". Sensors are prepared in resting buffer, membrane is diluted 1:10 with resting buffer.

SURFE²R 96SE measurement workflow

EAAT3 can be activated by providing glutamate, aspartate or cysteine as a substrate. A sodium gradient needs to be established in advance of substrate addition. Therefore, any double solution exchange protocol is suitable. All sensors are prepared at the start of the day and then used sequentially.