AMPA receptors are cation-permeable ionotropic glutamate receptors of the non-NMDA receptor subfamily. To date four subunits, GluA1-4, have been identified which are of similar size (approx. 900 kDa) and share 68-73% amino acid sequence identity. The human GluA2 subunit is encoded by the GRIA2 gene located on the 4q32-33 chromosome. Each of the 4 subunits has four distinct domains: an extracellular amino acid terminal domain (ATD); the extracellular ligand binding domain (LBD); the transmembrane domain (TMD) with 3 transmembrane segments (M1, M3 and M4) and 1 cytoplasmic facing re-entrant loop (M2); and an intracellular carboxy-terminal domain. The functional receptor exists as a tetramer, either as homomers or heteromers (GluA1 and GluA4). The vast majority of excitatory fast synaptic transmission in the mammalian central nervous system is mediated by AMPA receptors of differing subunit combinations. It is well known that glutamate is a neurotoxin and it is proposed that overactivation of ionotropic glutamate receptors may underlie many neurodegenerative disorders such as ischemic stroke, epilepsy, Parkinson’s and dementia, amongst others. Enhancement of AMPA receptor activation by, for example, BDNF, has been proposed to have beneficial effects on learning and memory and has potential therapeutic value in the treatment of depression, Huntington’s and Parkinson’s diseases.

Here we present data collected on the SyncroPatch 384PE showing recordings of GluA2-mediated currents. Glutamate activated GluA2 receptors with an EC₅₀ similar to those reported in the literature. CNQX inhibited and LY404187 enhanced GluA2-mediated responses.

**Results**

hGluA2 expressed in HEK293 cells was activated using increasing concentrations of glutamate (Fig. 1). hGluA2 started to activate at a glutamate concentration of 10 μM. At the highest concentration tested (1 mM) a rise time of 34 ms was estimated (Fig. 1 inset). The EC₅₀ for glutamate was estimated to be 71.1 ± 2.1 μM (n = 222) in good agreement with the range found in the literature for GluA2 and with the value obtained for the Patchliner (see App Note).

**Summary**

AMPARs are cation-permeable ionotropic glutamate receptors of the non-NMDA receptor subfamily. To date, four subunits, GluA1-4, have been identified which are of similar size (approx. 900 kDa) and share 68-73% amino acid sequence identity. The human GluA2 subunit is encoded by the GRIA2 gene located on the 4q32-33 chromosome. Each of the 4 subunits has four distinct domains: an extracellular amino acid terminal domain (ATD); the extracellular ligand binding domain (LBD); the transmembrane domain (TMD) with 3 transmembrane segments (M1, M3 and M4) and 1 cytoplasmic facing re-entrant loop (M2); and an intracellular carboxy-terminal domain. The functional receptor exists as a tetramer, either as homomers or heteromers (GluA1 and GluA4). The vast majority of excitatory fast synaptic transmission in the mammalian central nervous system is mediated by AMPA receptors of differing subunit combinations. It is well known that glutamate is a neurotoxin and it is proposed that overactivation of ionotropic glutamate receptors may underlie many neurodegenerative disorders such as ischemic stroke, epilepsy, Parkinson’s and dementia, amongst others. Enhancement of AMPA receptor activation by, for example, BDNF, has been proposed to have beneficial effects on learning and memory and has potential therapeutic value in the treatment of depression, Huntington’s and Parkinson’s diseases.
Currents mediated by GluA2 could be reliably recorded on the SyncroPatch 384PE. Figure 2 shows the current traces and online analysis for a selection of 16 wells.

The GluA2 response was blocked by CNQX in a concentration-dependent manner with an IC\(_{50}\) within the range found in the literature for GluA2-containing receptors\(^6\). The positive allosteric modulator, LY404187, potentiated the glutamate response in a concentration-dependent manner with an EC\(_{50}\) within the range found in the literature\(^7\). LY404187 was pre-incubated prior to co-application with glutamate.
Figure 6: Timeline of an experiment on the SyncroPatch 384PE.

The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a 1 - 5 point concentration response curve on GluA2-mediated currents took approximately 22 - 27 min.

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot of depiction of raw data traces of GluA2-expressing HEK cells as recorded on one NPC-384 patch clamp chip. A single concentration for 5 different compounds was applied across the plate, with 4 columns receiving each compound and 4 columns receiving no compound as control. Compounds and concentrations are indicated. Compounds were pre-incubated and then co-applied with 100 µM glutamate. Multi-hole chips were used where 8 holes were present per well. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green (Rmemb > 50MΩ) or blue (Rmemb = 5 – 50 MΩ). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show raw data traces of GluA2 channels following activation first by glutamate (blue trace) followed by co-application with compound (black trace).
Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, raw traces are chosen and the graphs show control responses to glutamate followed by potentiation or inhibition of the response by co-application with the compound indicated. The experiment shows that a range of different compounds can be tested within 1 plate. An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, GluA2 expressed in HEK cells can be reliably and reproducibly recorded on the SyncroPatch 384PE. The timeline of each experiment was about 22 - 27 minutes (start – end) and included 1 - 3 control applications with glutamate followed by co-application with 1 - 5 concentrations of a modulator or a cumulative concentration response of glutamate. Finally, a max concentration of glutamate (2 mM) was typically applied. If required, compounds were pre-incubated and then co-applied with glutamate. The GluA2-mediated response was inhibited by CNQX in a concentration-dependent manner as expected and potentiated by LY404187 in a concentration-dependent manner with an EC50 within the range found in the literature.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording GluA2 receptor-mediated currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

Methods
Cells
HEK293 cells expressing GluA2 kindly provided by SB Drug Discovery.

Cell culture
Cells were cultured and harvested according to Nanion’s standard cell culture protocol. Cells were induced to express hGluA2 by incubation in 1 µg/ml tetracycline 18 - 24 hours prior to experiments.

Electrophysiology
Whole cell patch clamp recordings were conducted according to Nanion’s standard procedure for the SyncroPatch 384PE using multi-hole (8 or 9 holes per well) chips. Cells were held at -80 mV for the duration of the experiment. For pharmacology experiments, cells were pre-incubated in compound and then compound was co-applied with glutamate. The peak amplitude or area under the curve in the presence of the PAM or inhibitor was normalized to the current amplitude in glutamate alone. Single point concentration response curves were performed and the concentration response curves calculated across the whole plate. For glutamate concentration response curves, cumulative concentrations were added to each well and the data normalized to the response elicited with the highest concentration.