

Functional investigation of GABA and Glutamate re-uptake transporters EAAT3 and GAT1 using SSM-based electrophysiology

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Rocco Zerlotti^{1,2}, Alison Obergrussberger¹, Maria Barthmes¹, Cecilia George¹, Niels Fertig¹, Andre Bazzone¹

¹Nanon Technologies GmbH, Munich, Germany; ²Regensburg University, Germany

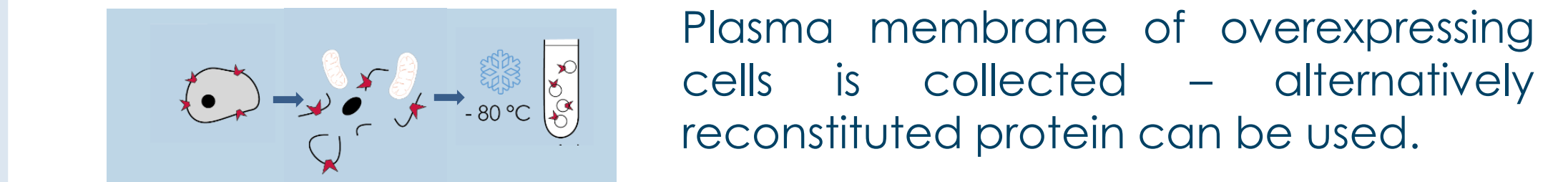
Introduction

GAT1 (SLC6A1) and EAAT3 (SLC1A1) are secondary active neurotransmitter transporters coupled to the ion gradients established by the NaK-ATPase. Both are expressed in neurons and play a major role in the depletion of the neurotransmitters GABA and glutamate from the synaptic cleft. Therefore, they play a major role in termination of synaptic transmission, maintaining the ambient extracellular neurotransmitter concentration and neurotransmitter recycling through reuptake. GAT1 is linked to epilepsy, schizophrenia, anxiety and ADHD. Subtype specific inhibition of EAAT3 is assumed to be beneficial during phases of insufficient energy supply by preventing reversal glutamate transport. Therefore, screening compounds to find inhibitors of these transporters is of high pharmacological interest. We used solid-supported membrane (SSM)-based electrophysiology using the SURFE²R N1 and SURFE²R 96SE to record GAT1 or EAAT3 purified from the plasma membrane of CHO or HEK cells

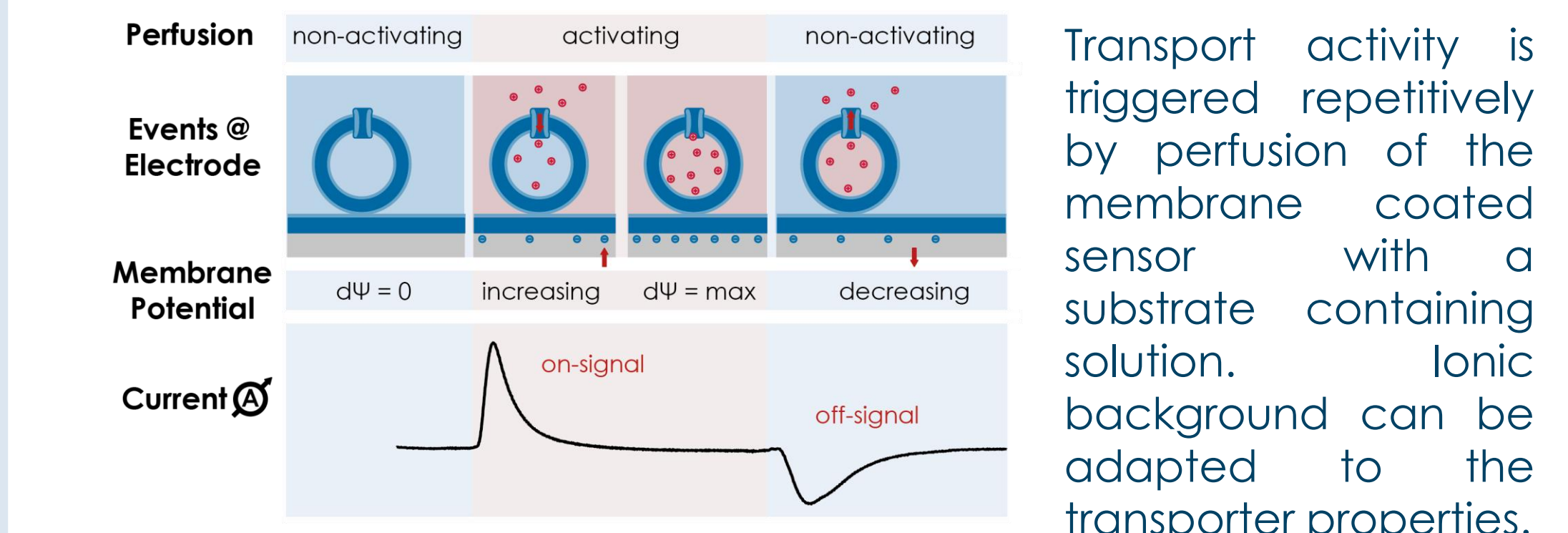


Solid supported membrane electrophysiology explained

SSM (solid supported membrane) based electrophysiology can resolve small currents as they are generated by SLC transporters like EAAT3 and GAT1.



A sensor is coated with the membrane or cells. Changes in the membrane potential caused by electrogenic transport events can be detected on the sensor.



Conclusions

- The SSM-based electrophysiology technique using the SURFE²R N1 or SURFE²R 96SE can be utilized to record GAT1 and EAAT3 transporters
- Small transport currents can be measured which might not be detectable in whole cell patch clamp electrophysiology
- EAAT3 activity was measured on the SURFE²R N1 and the assay was scaled-up to higher throughput using the SURFE²R 96SE. Different transport and binding reaction can be observed, and inhibition by published tool compounds was reproduced
- GAT1 currents generated by GABA jumps show two distinct components: a fast pre-steady state (PSS) current and a slow steady state
- Application of negative voltages affects the PSS component of GAT1 currents, enhancing it 2-folds. Na⁺ gradient affects V_{max} and the PSS component.

GAT1 transport in CHO membrane vesicles

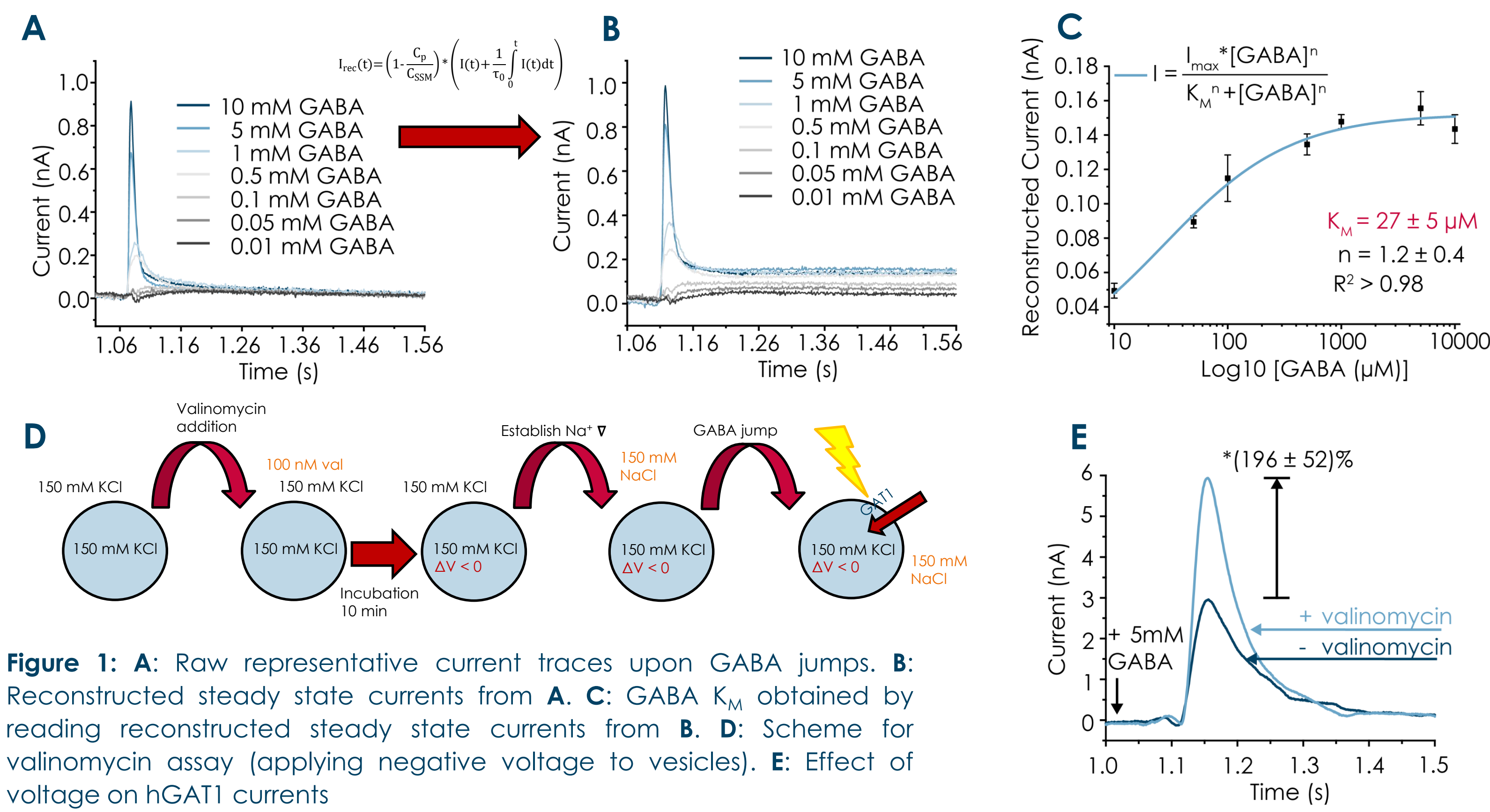


Figure 1: **A:** Raw representative current traces upon GABA jumps. **B:** Reconstructed steady state currents from **A**. **C:** GABA K_M obtained by reading reconstructed steady state currents from **B**. **D:** Scheme for valinomycin assay (applying negative voltage to vesicles). **E:** Effect of voltage on hGAT1 currents

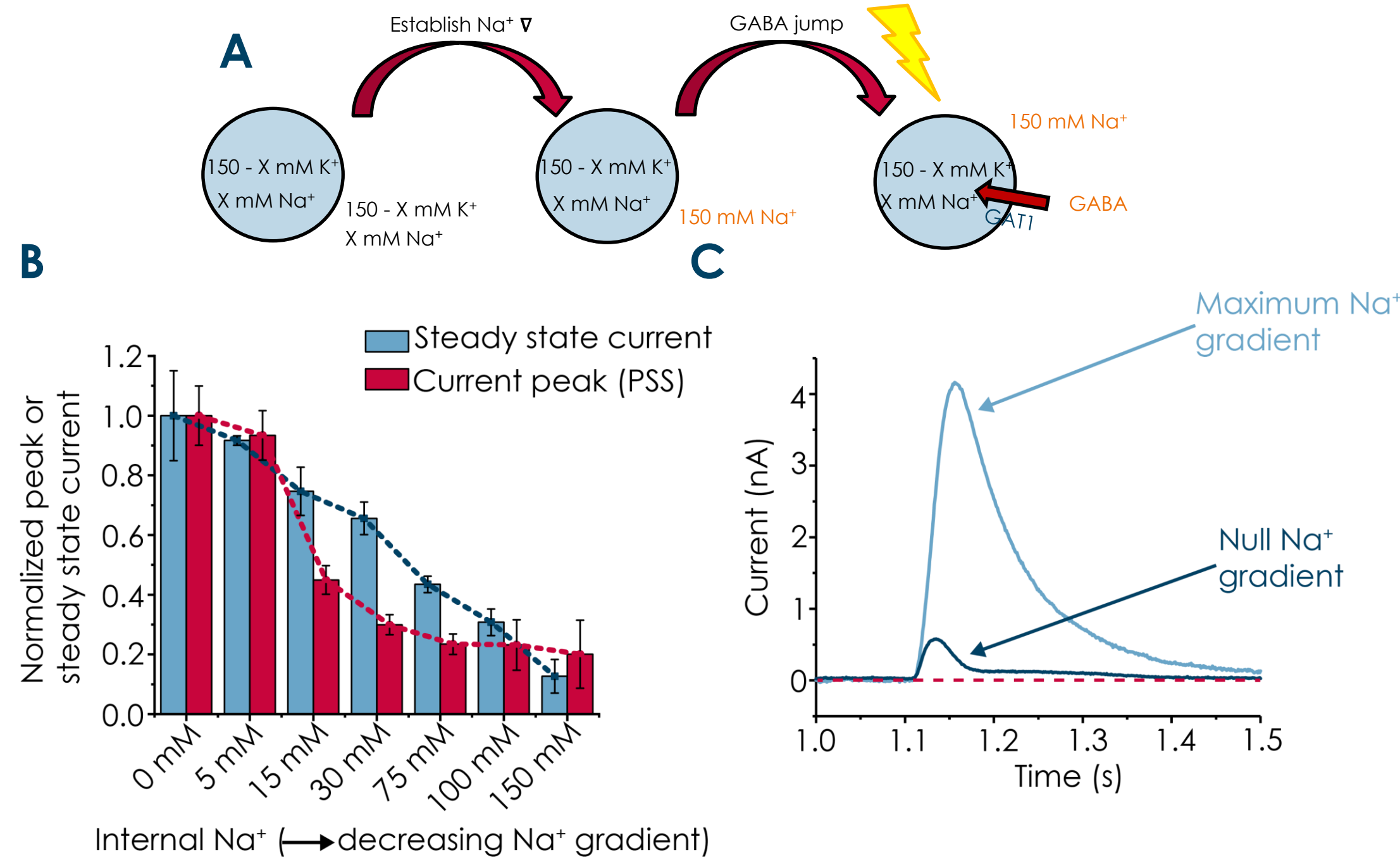


Figure 2: **A:** Scheme for GABA jump experiments in different Na⁺ gradients (lesser gradient are obtained incrementing internal Na⁺ concentrations, X). **B:** Steady state current (V_{max}) and peaks (pre-steady state, PSS) as a function of Na⁺ gradient. **C:** Representative traces obtained in maximum and null Na⁺ gradient.

EAAT3 activity measurement in overexpressing HEK cells

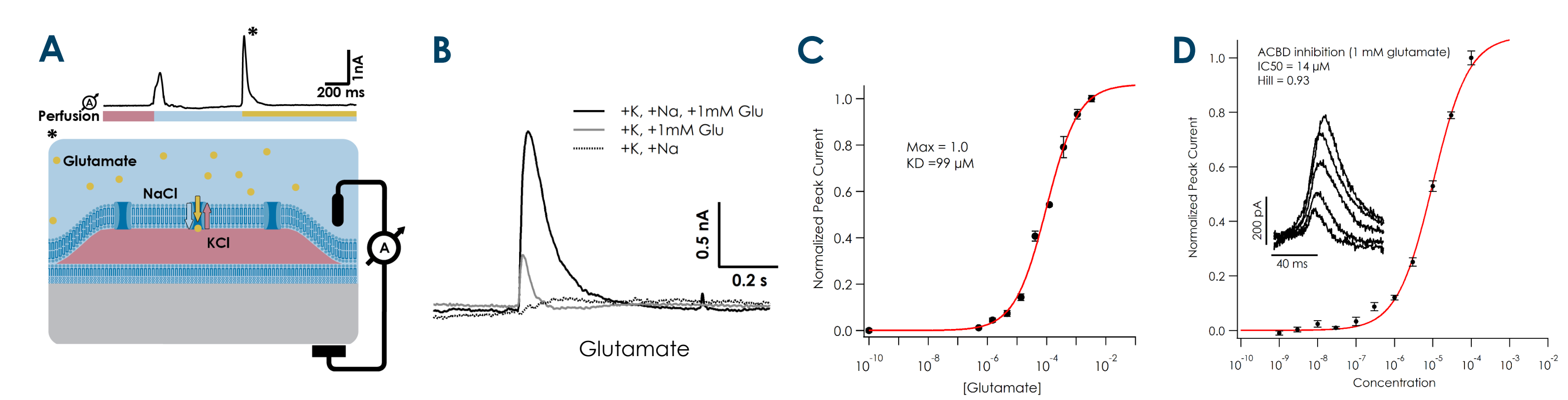


Figure 3: Purified plasma membrane of a HEK-EAAT3 cell line shows stable activity induced by glutamate and inhibited by ACBD. **A:** Activation scheme: Application of glutamate after establishing a Na/K ion gradient. **B:** EAAT3 activity is dependent on ionic background. **C:** Apparent glutamate affinity. **D:** Tool compound ACBD inhibits EAAT3 currents.

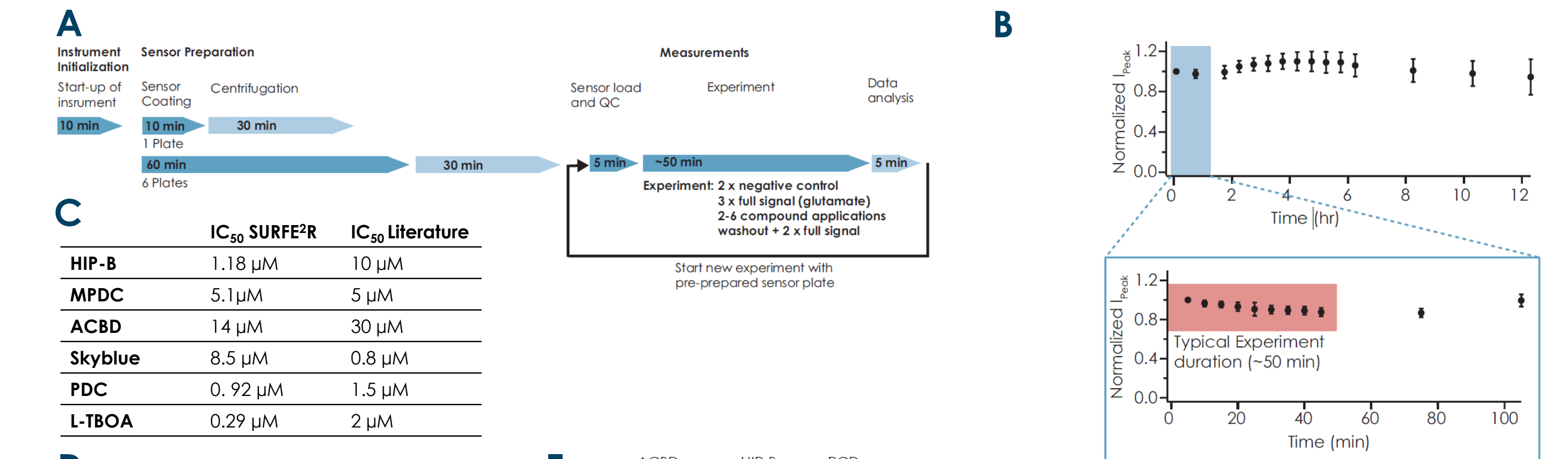


Figure 4: The assay was scaled up to an HTS format and validated using 6 known inhibitors. **A:** Workflow for the HTS experiment. Alternative workflows for one or 6 plates are displayed. **B:** Stability of the current signal during repetitive activation of a 96-well sensor plate **C:** Comparison of IC₅₀ values measured using SSM based HTS electrophysiology and published values. **D & E:** Two different assay modes: Single dose experiment using multiple compounds vs. full dose-response measurement.