

Development and validation of ASIC1a ligand-gated ion channel drug discovery assays on automated patch clamp platforms.

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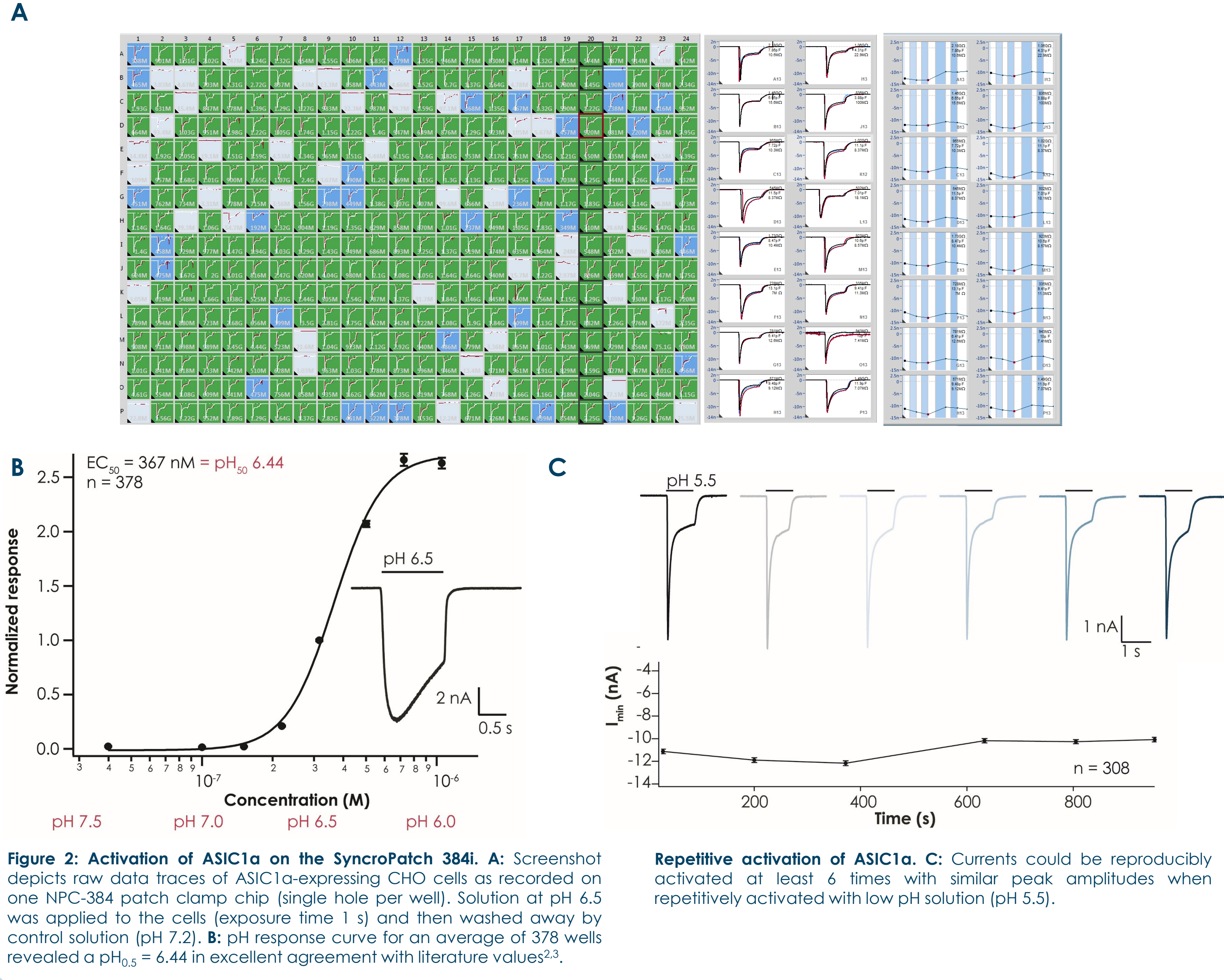
Introduction

Acid-sensing ion channels (ASICs) are proton-gated ion channels which are highly sensitive to extracellular acidosis and are permeable to cations¹, predominantly Na⁺. To date, six different ASIC subunits (1a, 1b, 2a, 2b, 3 and 4) encoded by four genes have been identified². Three subunits assemble to form homomeric or heteromeric channels which are expressed throughout the CNS and PNS. Excitatory ASIC channels have a proposed role in nociception and pain, and other neurological diseases such as ischaemia, inflammation and learning and memory².

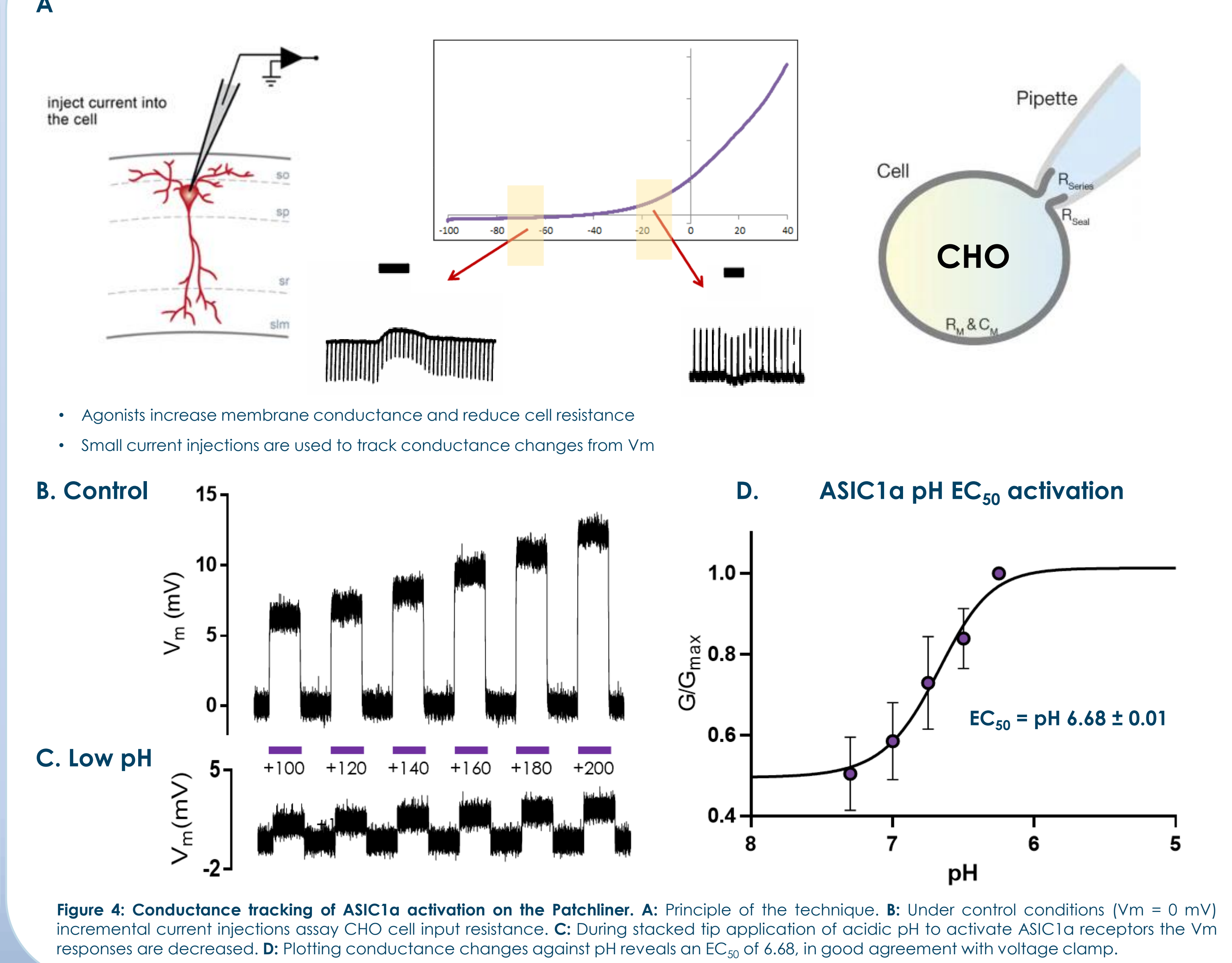
Ligand-gated ion channels with rapid activation and desensitization present specific challenges on automated patch clamp (APC) systems due to low channel expression, the need for rapid application and wash-off of ligands, and loss of responsiveness due to short- and long-term desensitization. In this study we used ASIC1a channels expressed in CHO cells as an exemplar rapid ligand-gated ion channel to develop reliable screening assays on medium (Patchliner) and high throughput (SyncroPatch 384i) APC devices. ASIC1a-mediated current responses were reproducible and could be repetitively activated with similar peak amplitudes at least 7 times in the same cell using techniques which minimize agonist exposure time. ASIC1a was activated by decreasing pH and blocked by benzamil and amiloride. Finally, we demonstrate successful implementation of the classic historical input resistance/conductance-tracking technique to measure ligand-gated current activation on an APC platform.



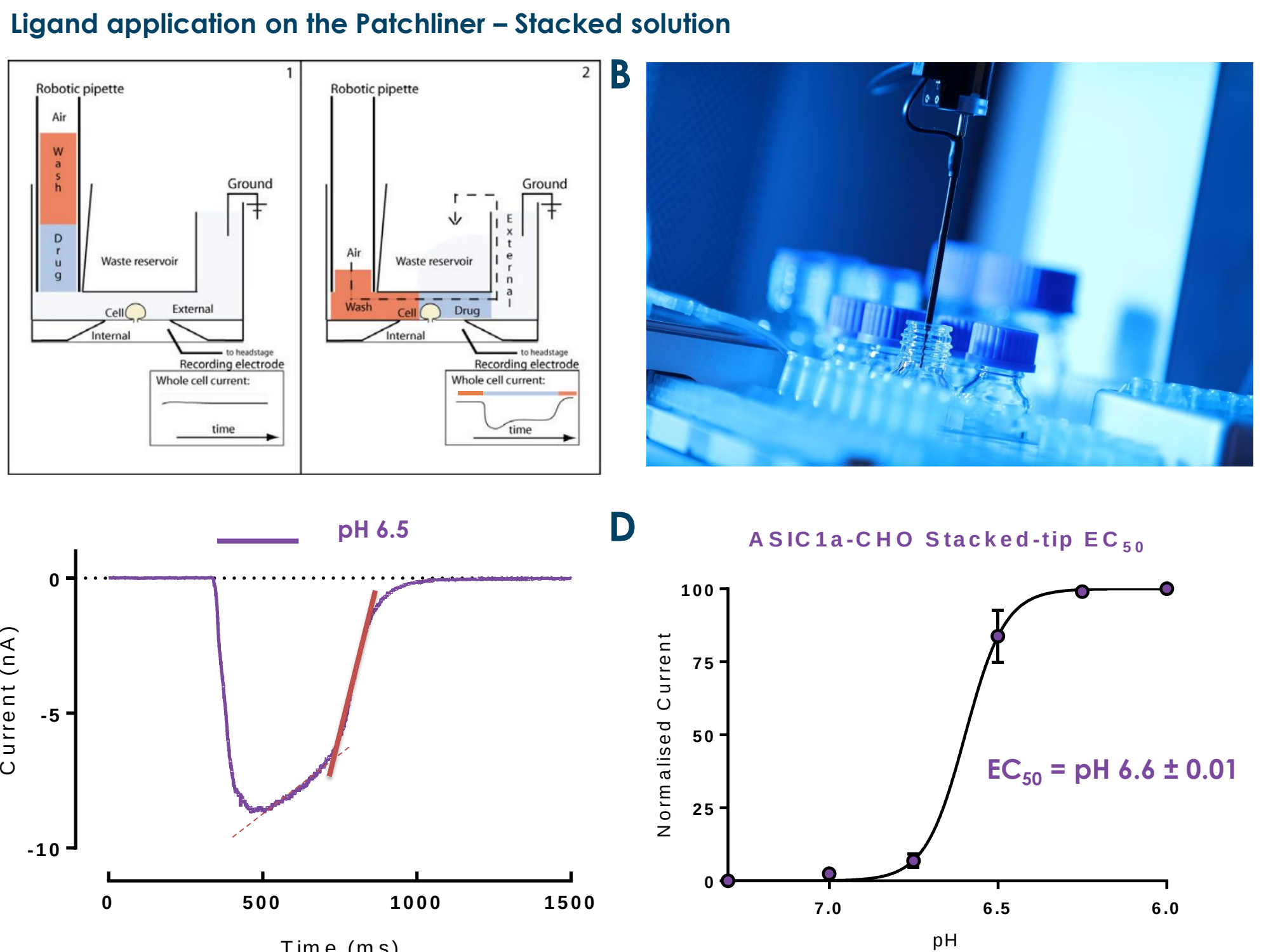
High throughput activation of ASIC1a



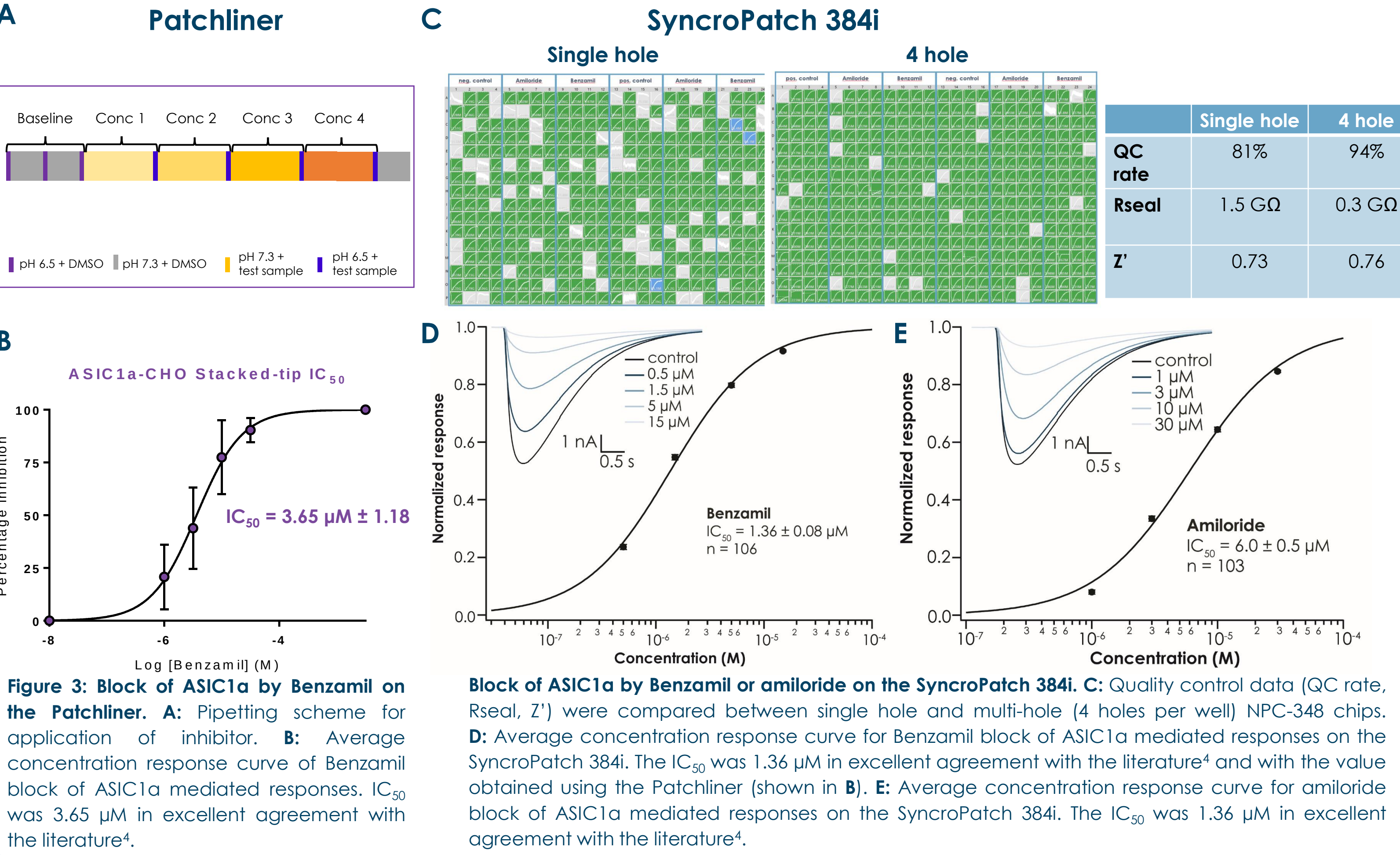
ASIC1a conductance tracking



Optimization of ligand addition



Pharmacology of ASIC1a on Patchliner and SyncroPatch 384i



Summary

- ASIC1a was reliably measured on **automated patch clamp** devices with medium (**Patchliner**) or high (**SyncroPatch 384i**) throughput using the stacked tip approach.
- ASIC1a was activated with $pH_{0.5}$ 6.4 on the **Patchliner** and $pH_{0.5}$ 6.6 on the **SyncroPatch 384i**, in excellent agreement with the literature^{2,3}.
- ASIC1a could be **repetitively activated** with similar peak amplitudes on the **Patchliner** and **SyncroPatch 384i** enabling pharmacological studies.
- ASIC1a was blocked by **benzamil** and **amiloride**. Concentration response curves were constructed with IC_{50} values in good agreement with literature values⁴.
- Either **single hole** or **multi-hole** chips can be used. The use of multi-hole chips results in higher success rates for completed experiments (**94% vs. 81%**) and higher Z' values (**0.76 vs 0.73**).
- **Conductance tracking** can also be used to study ASIC1a channels on the **Patchliner**.

References

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