Validation of Ligand-gated Ion Channel Applications on QPatch HTX

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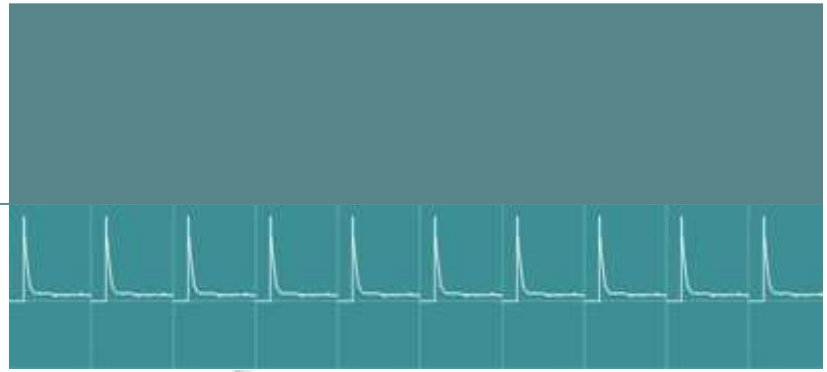
Outline

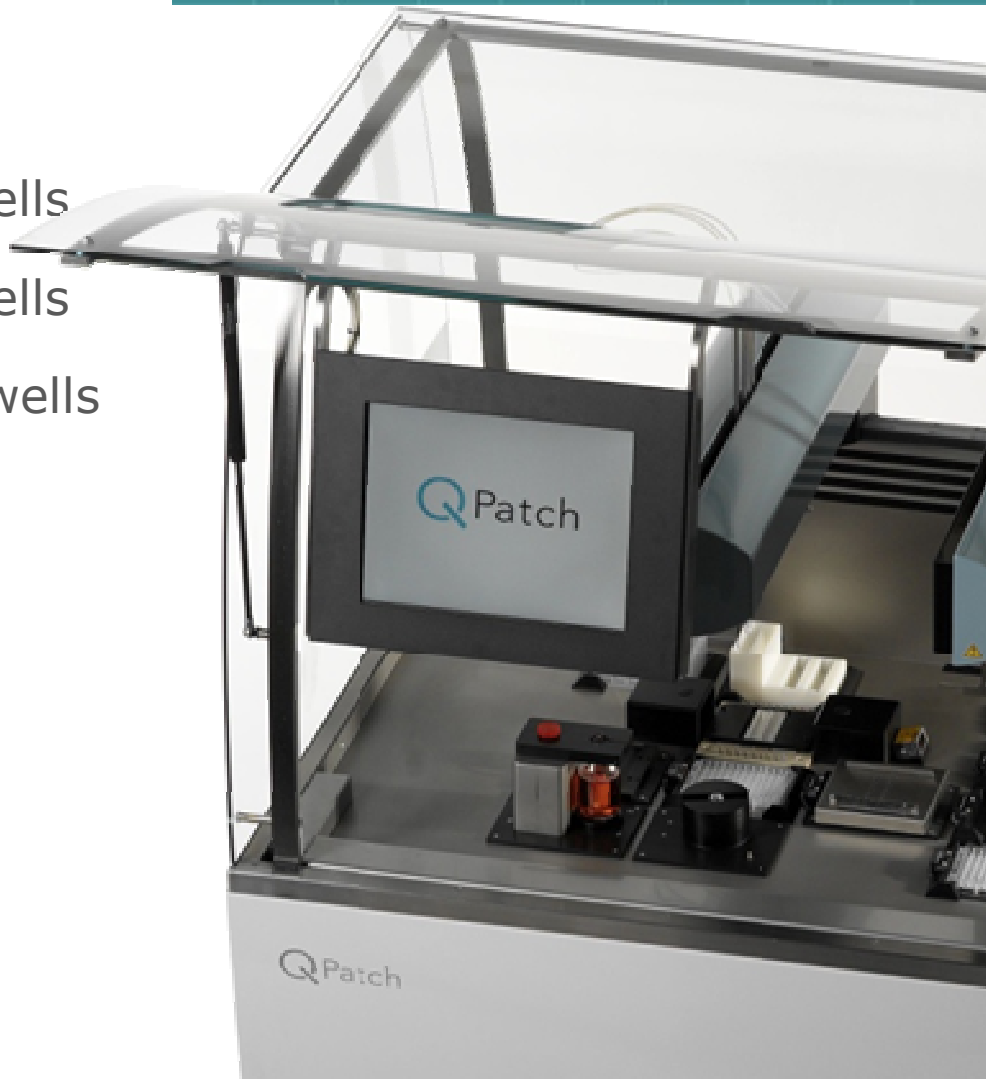
- Ligand gated applications on QPatchHT
- Introduction to QPatch HTX
- Challenges
- Experimental data
  - RBL 2H3
  - GABA a
  - P2X3
  - α1
  - GluR5
  - α7
- Summary and conclusions

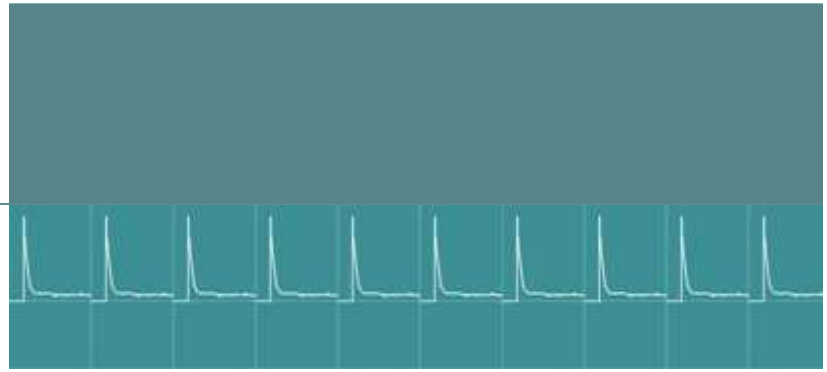
sophion



The QPatch Family

- QPatch 16 16 cells in 16 wells
- QPatch HT 48 cells in 48 wells
- QPatch HTX 480 cells in 48 wells





Ligand gated applications on QPatchHT

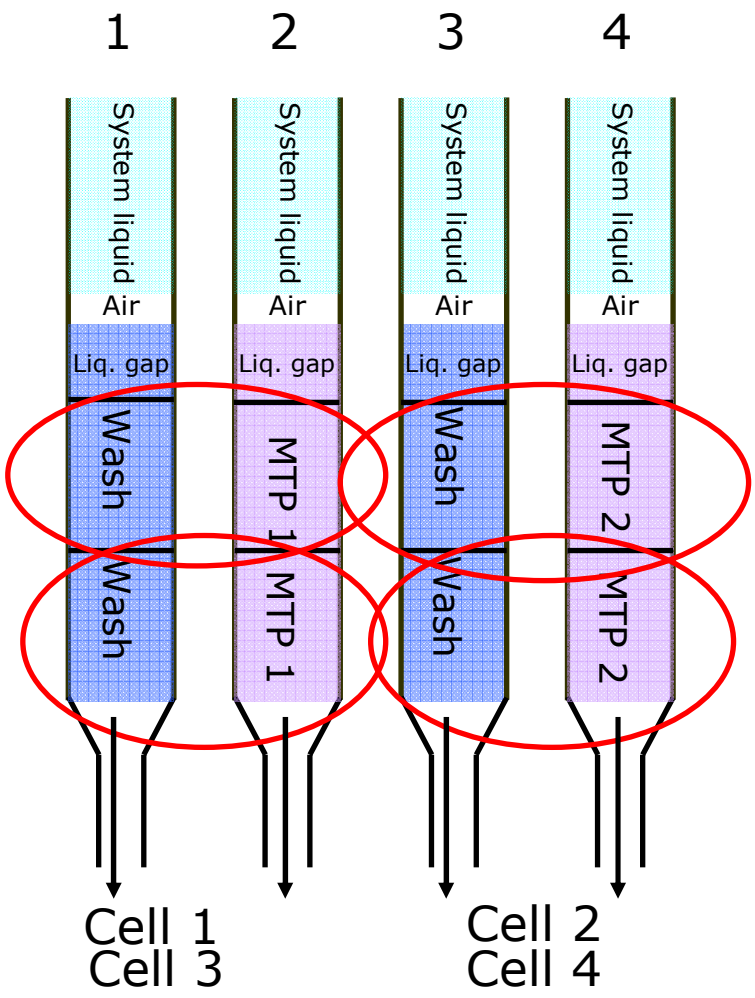
- 48 simultaneous and individually controlled patch clamp recordings
- Eight pipettes for liquid-handling for faster and complex ligand-gated applications
- Flexible software enabling e.g. preincubation of compound
- Flow channels integrated in the QPlate
- 12 application cycles pr. Cell -> 576 pt. pr. plate
- 12 pt agonist dose response takes app. 60 min pr. plate.

Typical Application Protocol for 5 pt antagonist dose response

Liquid periods

	Liquid	Volume [µl]	Wash	Data acquisition	Details
1	Res: Saline	5	☐	☐	
2	MTP: agonist alone	5	☑	☑	Res: Reference (4)
3	MTP: agonist alone	5	☑	☑	Res: Reference (4)
4	MTP: agonist alone	5	☑	☑	Res: Reference (4)
5	MTP: agonist alone	5	☑	☑	MTP: blocker_conc_1 (4)
6	MTP: blocker_conc_1 plus agonist	5	☑	☑	MTP: blocker_conc_2 (4)
7	MTP: blocker_conc_2 plus agonist	5	☑	☑	MTP: blocker_conc_3 (4)
8	MTP: blocker_conc_3 plus agonist	5	☑	☑	MTP: blocker_conc_4 (4)
9	MTP: blocker_conc_4 plus agonist	5	☑	☑	MTP: blocker_conc_5 (4)
10	MTP: blocker_conc_5 plus agonist	5	☑	☑	Res: Reference (4)
11	MTP: agonist alone	5	☐	☑	

Ligand gated applications on QPatchHT
- Advanced pipetting strategy



4 tips; n=2 as an example

Sequence:

Wait for start -> MTP 1 + 2 -> wash -> next 2 cells

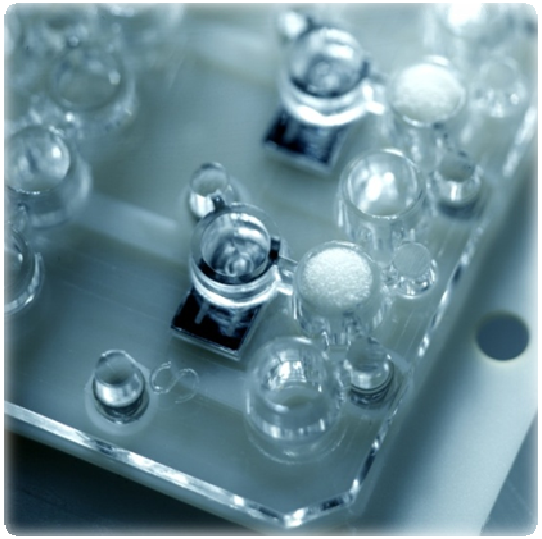
MTP 1 + 2 -> wash ->

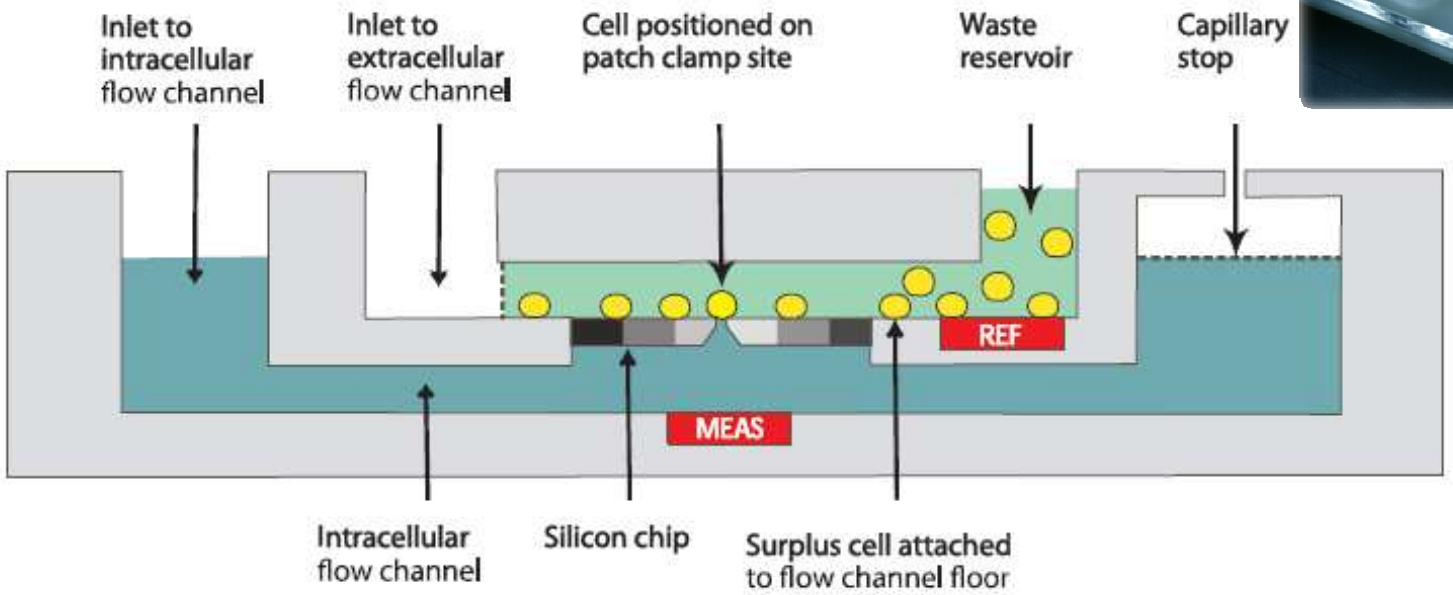
Wash pippetes -> pick up compound

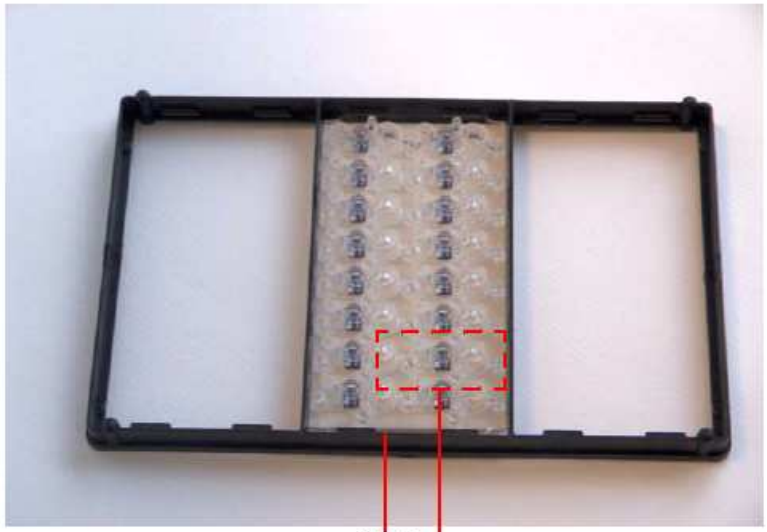
Etc…….

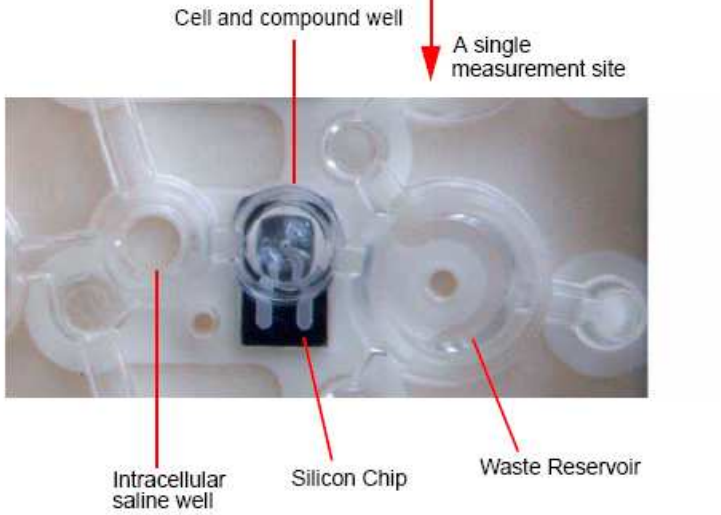
Ligand gated applications on QPatchHT - Chip intergrated flow channels

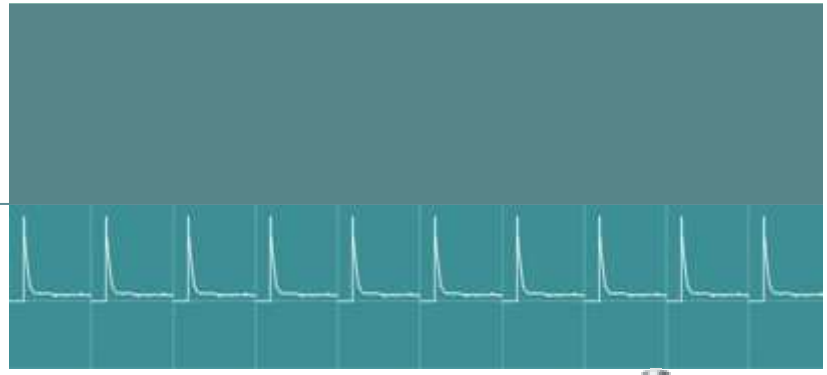
- Laminar flow
- Sequential addition of compounds
- Limited waste











70µl and 250µl independent waste volume

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- **Introduction to QPatch HTX**
- Challenges and questions
- Experimental data
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  - GABA a
  - P2X3
  - α1
  - GluR5
  - α7
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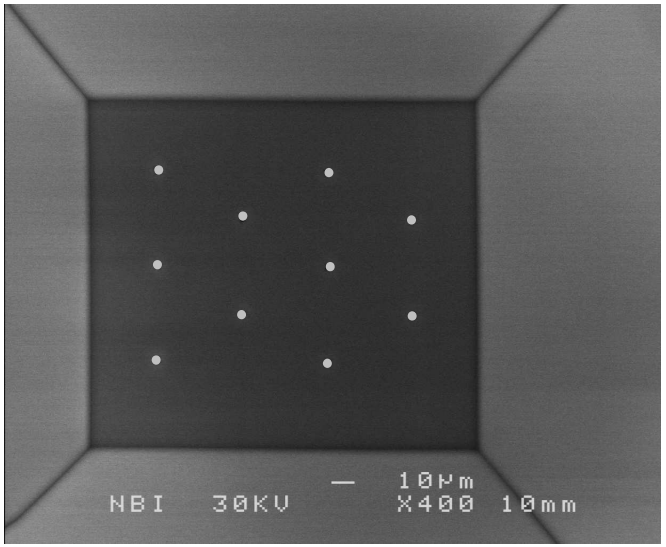
Introduction to QPatch HTX

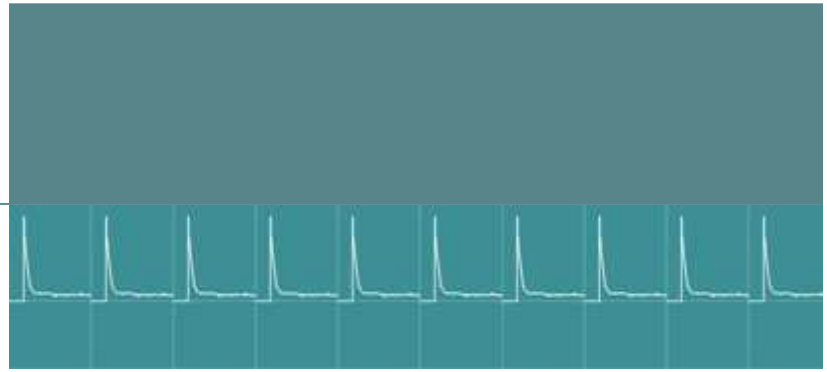


- The QPlate has been modified - ten patch holes pr. chip
- Substrate is the same –> true gigaseals
- Flow channels are the same –> laminar flow
- The gain on the amplifiers have been reduced
- Software has been optimized to make experiments run more syncronized

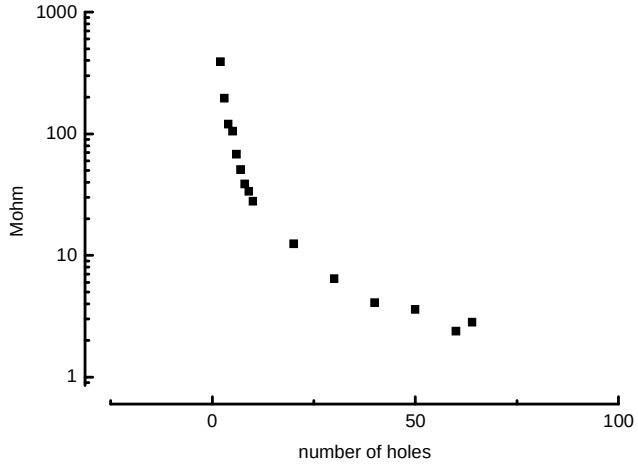
Resulting in

- Increased success rates
- Higher throughput

Why 10 holes?



- There seems to be a lower seal limit
- Likelyhood of getting a bad cell/seal increases as the number of holes increases
- Easier to modify amplifier and chip



Estimated site resistance as a function of holes in parallel from 300 single hole CHO hERG DUO

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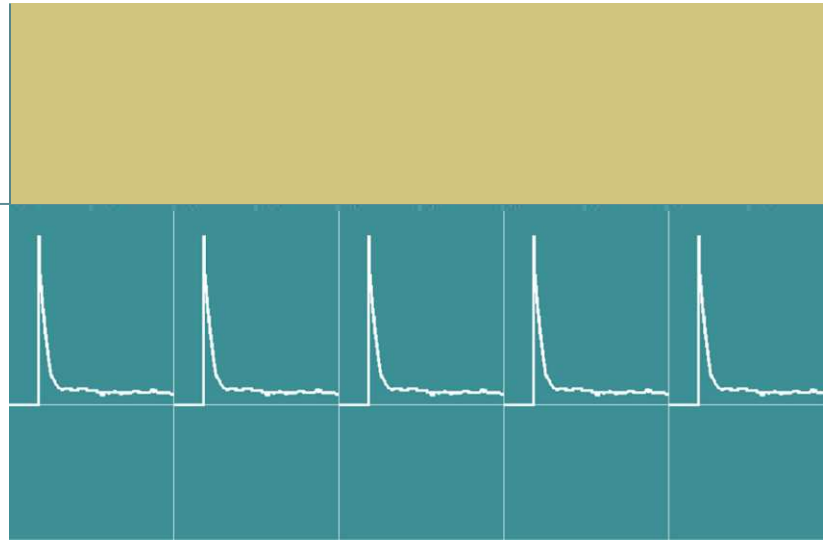
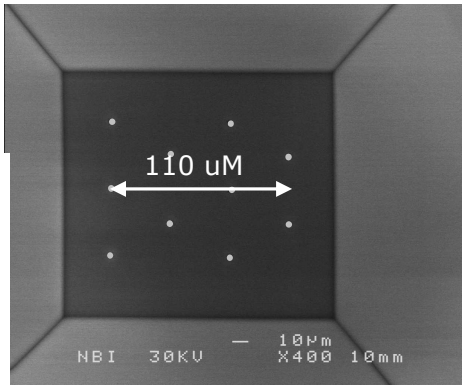
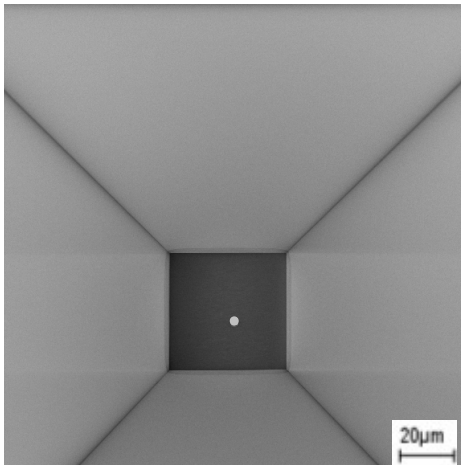
Challenges and questions



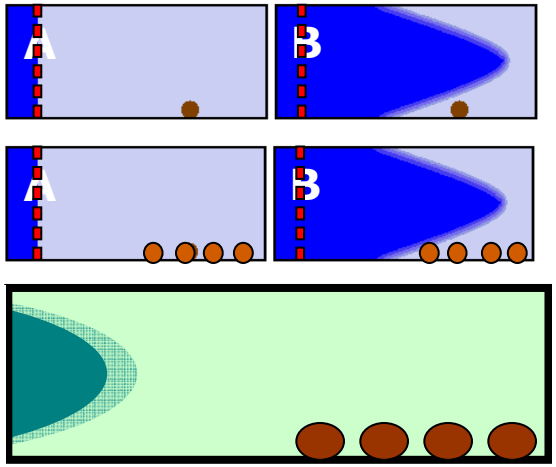


HTX: 110 uM
+ 1 cell diameter



HT: 1 cell diameter

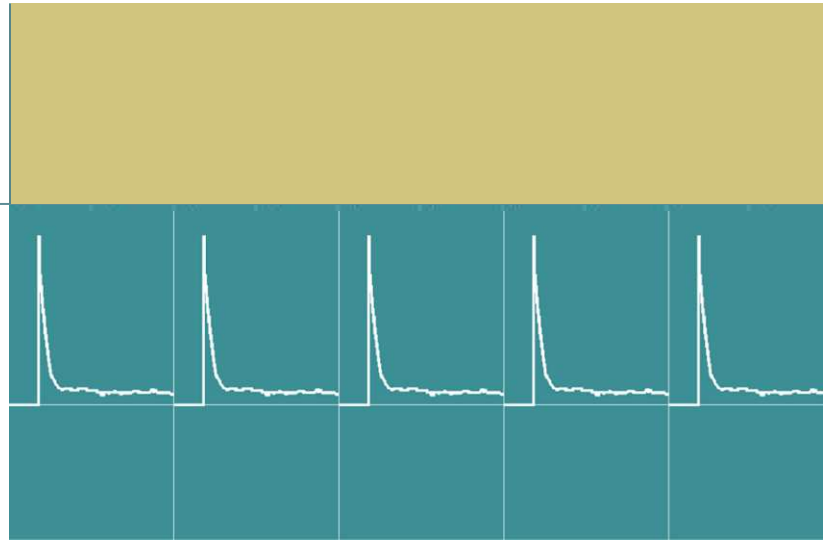
What is the effect of increased flow distance on risetime signals from ligand gated ion channels?



Larger area → Slower signal onset?

PatchHTX

Challenges and questions



Additional questions

- How are success rates with HTX?
- How does pharmacology look?

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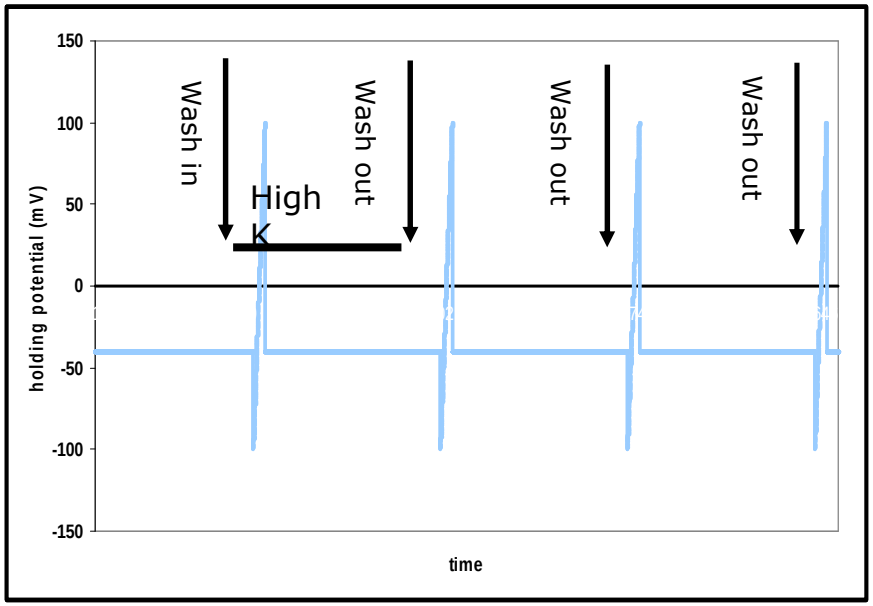
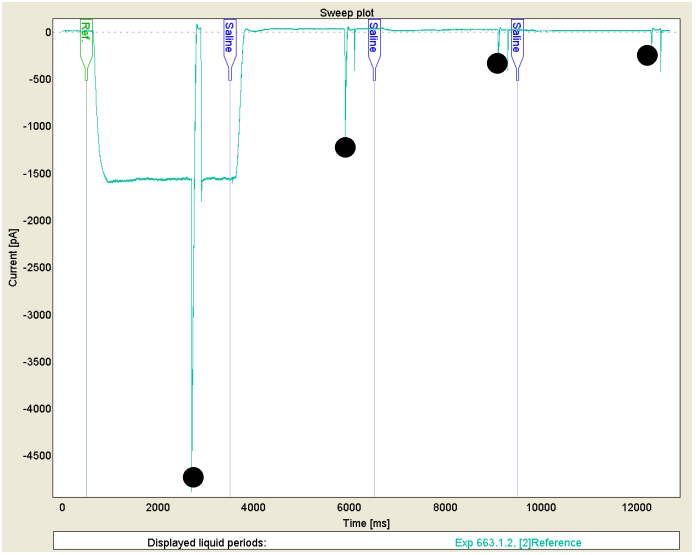
Evaluation of exchange rate with endogenous Kir in RBL 2H3

- RBL 2H3 is an excellent test cell line
- Very good patchability
- Endogenously expressed Kir in all cells
- RBL 2H3 expresses Kir in large amounts in all cells
- Exchanging extracellular sodium with potassium to change driving force is a widely used assay to evaluate exchange rates.

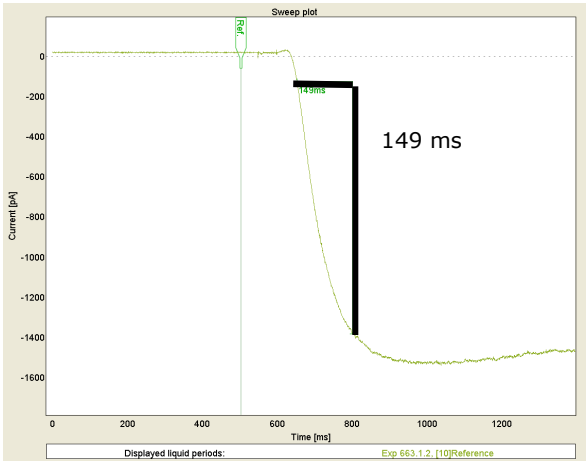


Typical voltage/application protocol High K added for 3 seconds and washed out.

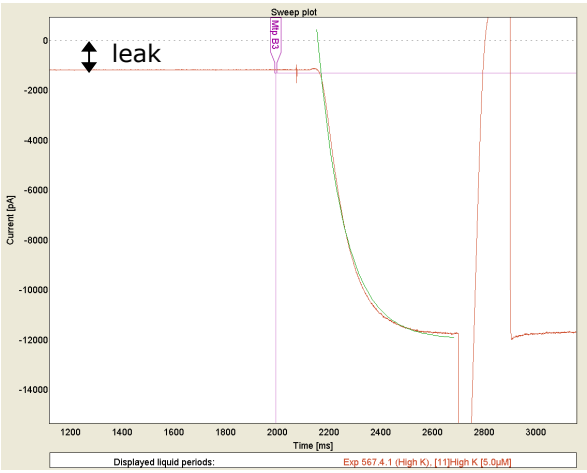
Typical response single hole



Evaluation of exchange rate using RBL 2H3



10 – 90 % Rise time cursor
single hole data

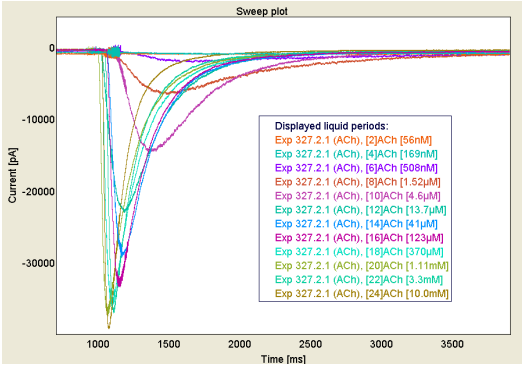


HTX data fitted to
A + Bexp(-Ct)

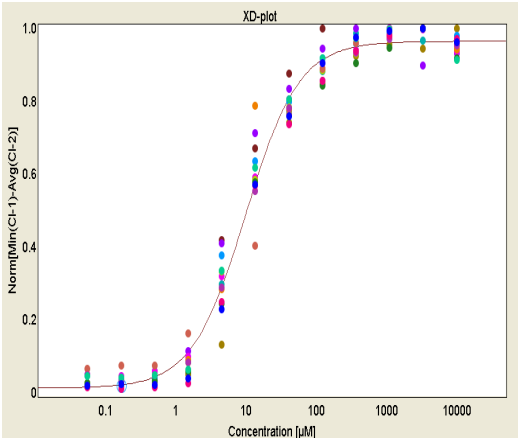
	Single hole	HTX
Current response (nA)	0,7 +/- 0,3	5,05 +/- 2,0
Rise time (10 – 90 %) (ms)	170 +/- 49	183 +/- 27
”Activation” 1/C (ms)	89 +/- 18	65 +/- 14

Testing rise times on nicotinic α1 currents in TE671 cells

- Fast currents widely endogenously expressed



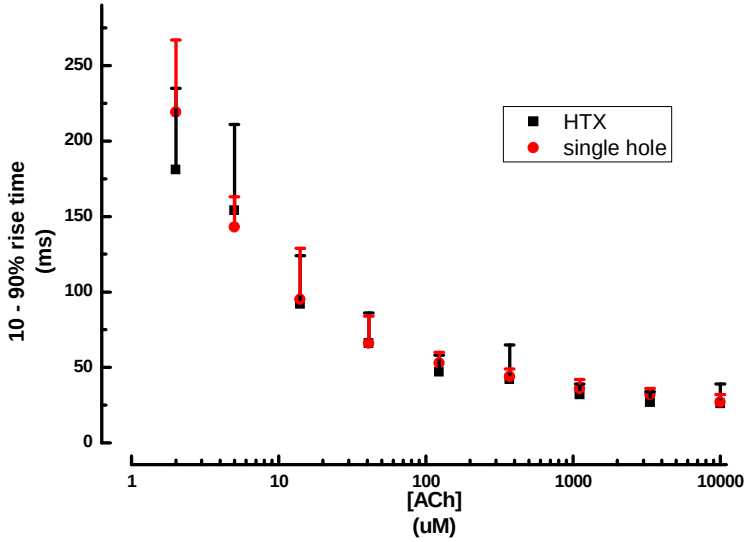
Rawdata plots of nAchR α1 currents in TE671 cells recorded as response to increasing concentrations of acetyl choline.



Resulting concentration response curve from 14 seperate measurement sites.
EC50 = 8 µM.

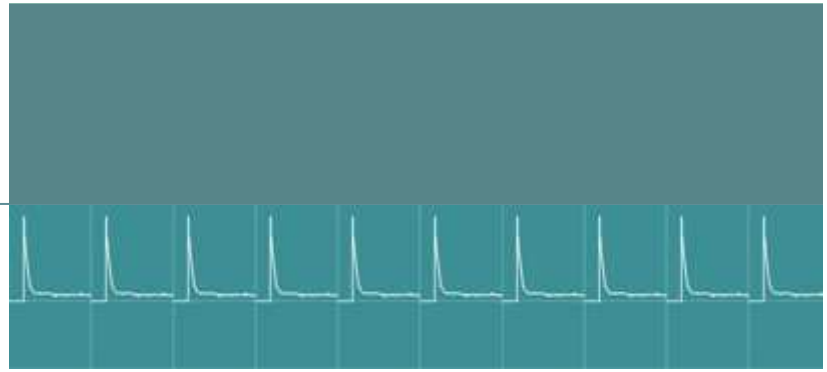
Signal rise time analysis TE671

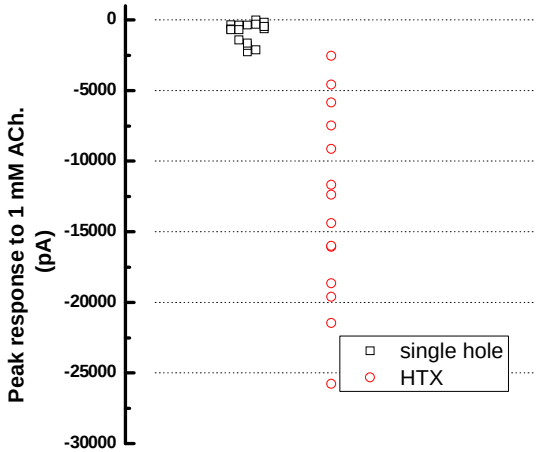
- Rise times are identical between HT and HTX.



Comparison of 10 – 90 % rise time between HTX and single hole as a function of [ACh]

α1 TE671 current amplitudes single hole vs. HTX





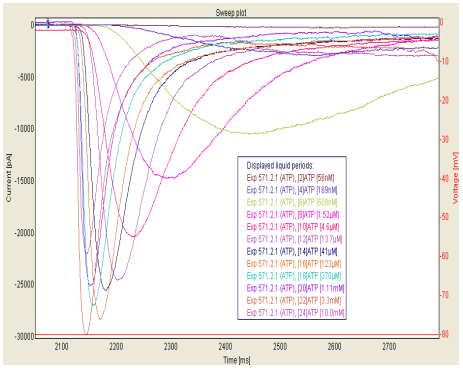
α1 peak responses from 16 sites in HTX and single hole mode

	Single hole	HTX
% Sites with α1 current	**90 %**	**93 %**
Current amplitude@ 1 mM ACh (nA)	**-1,2 +/- 0,9**	**-16,1 +/- 7,4**

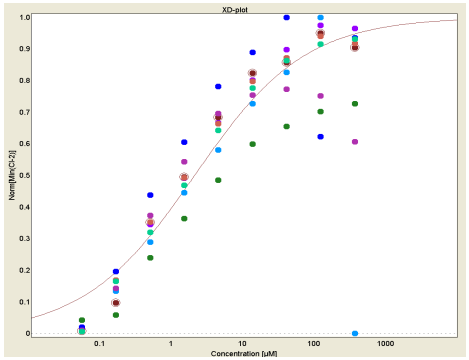
Testing rise times on rP2X3 with ATP

- rP2X3 has fast activating currents
- interesting history…

ATP dose response



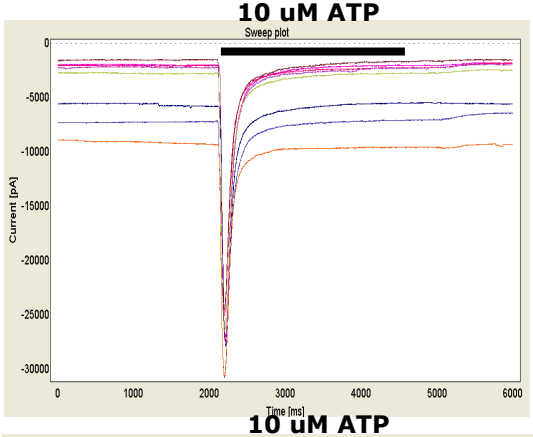
Leak subtracted sweeps from a 12 point dose response with ATP



EC50: 1.5 uM

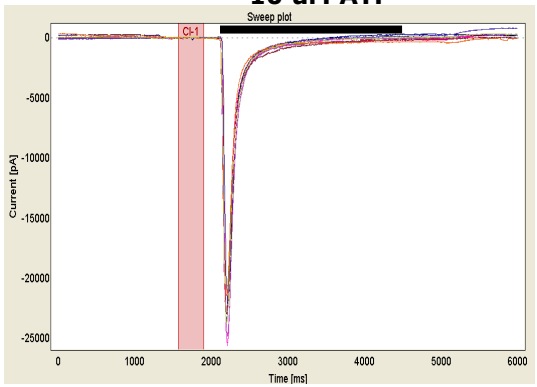
Handling leak in ligand gated assays

10 uM ATP



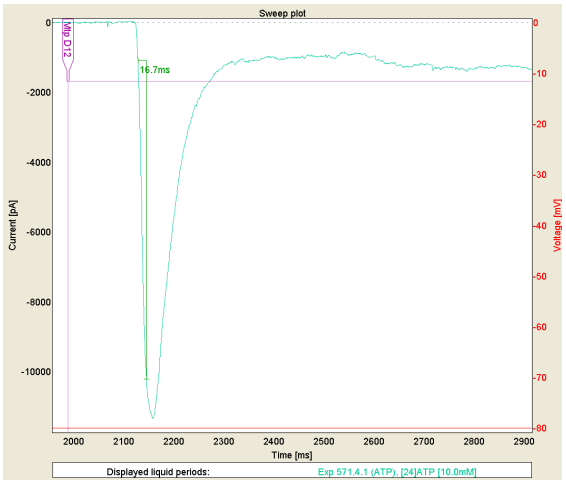
8 repeated additions of ATP (10 uM) the leak is improving over time. Difficult to evaluate raw data.

10 uM ATP

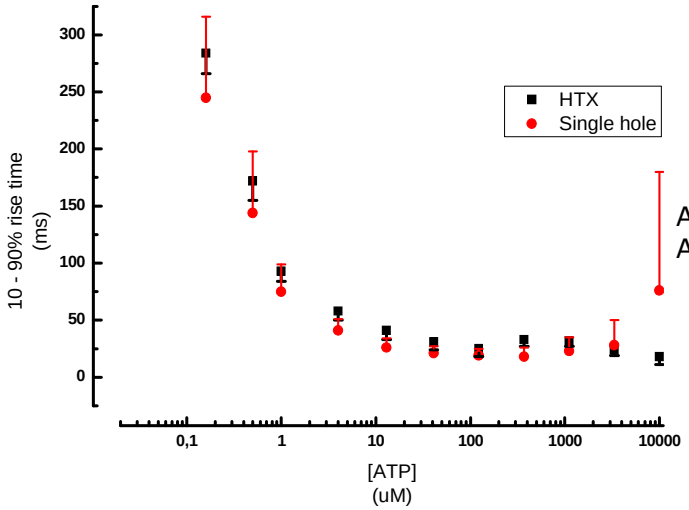


Same data but with zero subtraction.

Testing rise times on rP2X3 with ATP



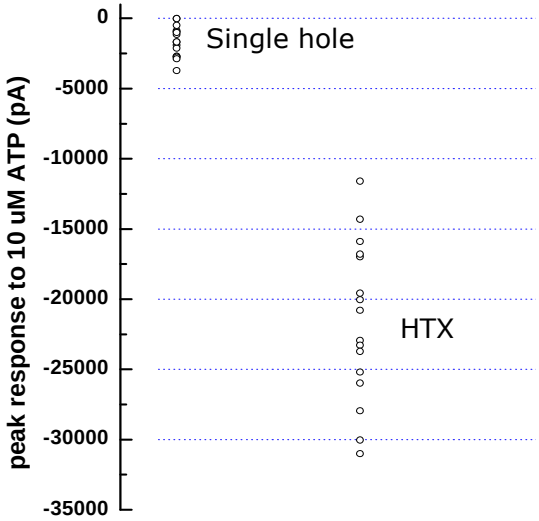
Leak subtracted 10 mM ATP induced P2X3 response with 10 – 90 % rise time cursor. Rise time is 16 ms.



Average rise time +/- SD vs ATP concetration

Rise time data is found to be identical Between HT and HTX

P2X3 current amplitudes single hole vs. HTX

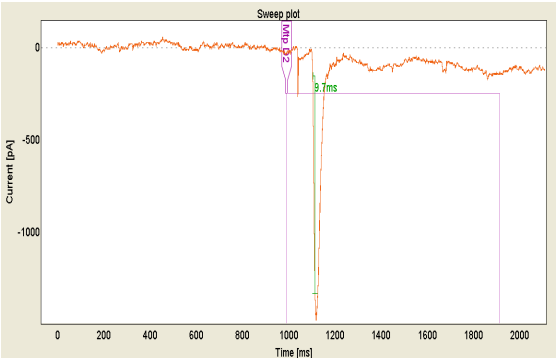


P2X3 peak responses from 16 sites in HTX and single hole mode

	Single hole	HTX
% Sites with P2X3 current	**81 %**	**100 %**
Current amplitude (nA)	**-2,0 +/- 0,8**	**-21,6 +/- 5,7**

Nicotinic α7 in GH4 C1 cells

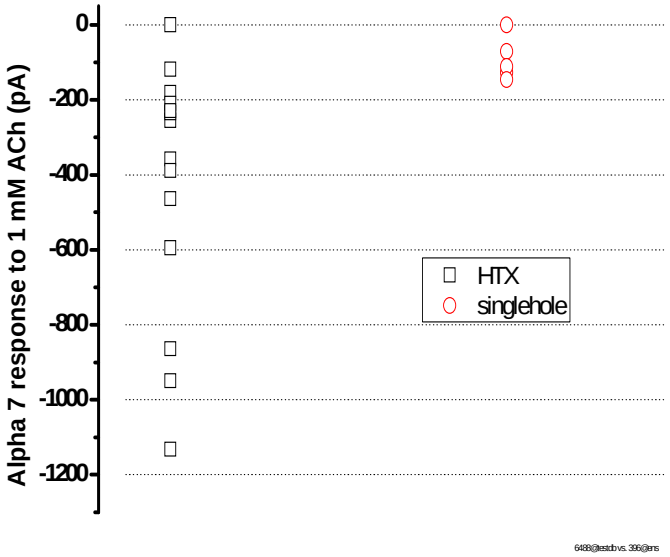
- Fast kinetics
- Hard to express in amounts high enough to be usefull



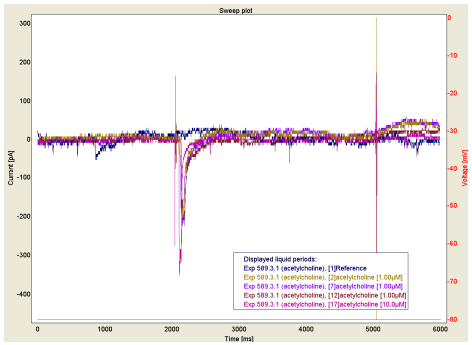
Leak subtracted sweep of α7 currents
stimulated with 10 mM ACh.

Rise time: 9,6 +/- 2,5 ms @ 10 mM ACh on HTX, not different from single hole

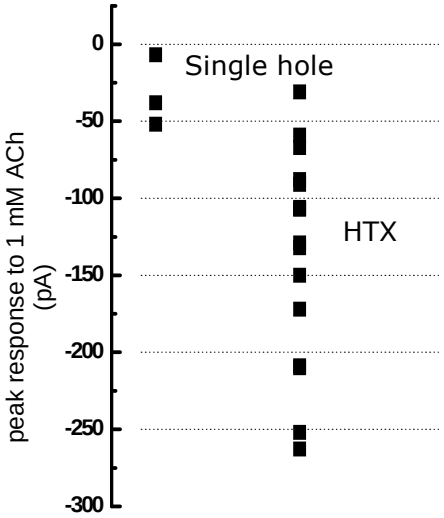
α7 signal from frozen cell batch



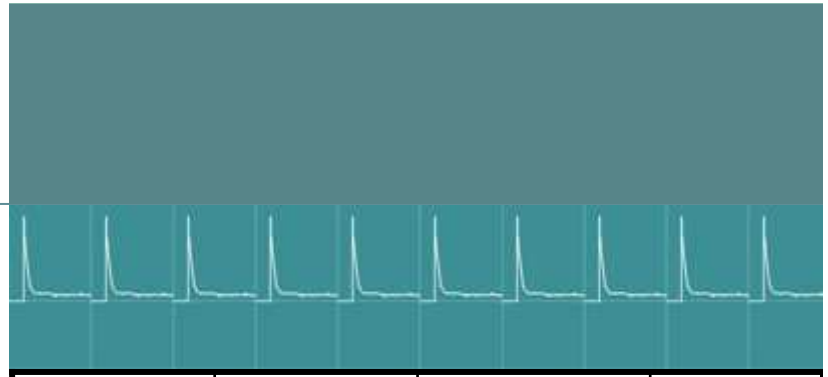
Nicotinic α7 in GH4 C1 cells



Raw data sweeps from non optimized batch. Cells stimulated with 3 x 1 And 10 mM ACh.

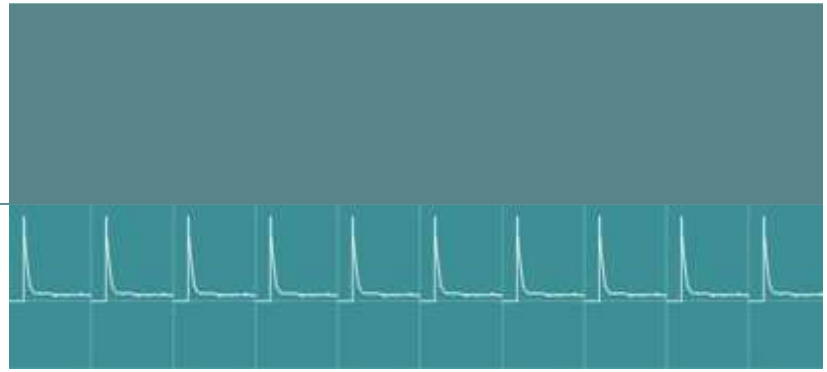


α7 peak responses from 16 sites in HTX and single hole mode

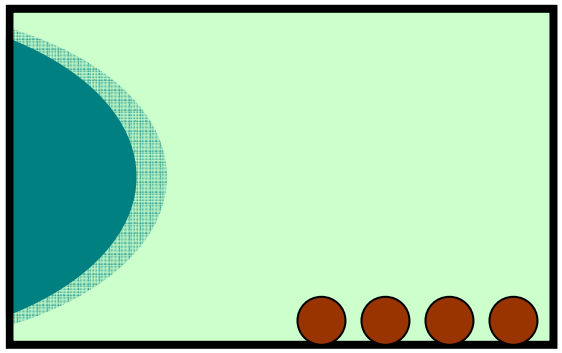


	Single hole	HTX	
Peak current @ 1 mM ACh (pA)	-114 +/- 32	-460 +/- 328	Frozen cell batch
% sites with alpha 7 current	25%	81%	Frozen cell batch
Peak current @ 1 mM ACh (pA)	-33 +/- 23	-136 +/- 69	Non optimised batch
% sites with alpha 7 current	6%	28%	Non optimised batch

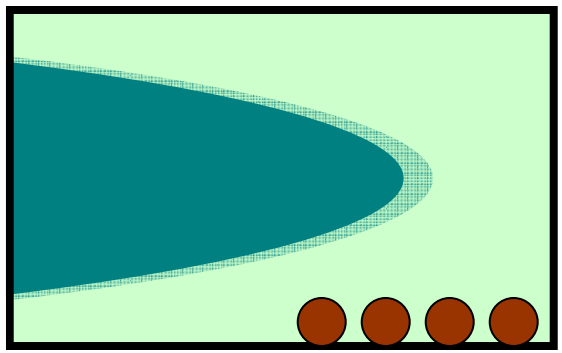
Summary - rise time experiments



Angle of front is high - cells are hit from the side



Angle of front is low - cells are hit from above



Question:

What is the effect of increased flow distance on risetime signals from ligand gated ion channels?

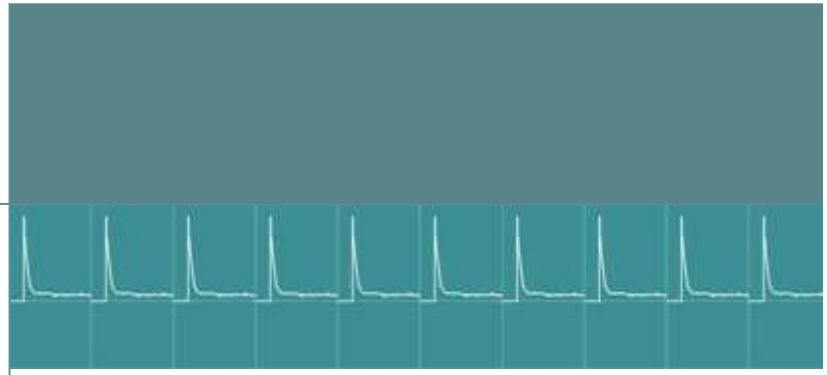
Answer:

We have looked at the onset of the signal in a number of assays and do not see any difference in rise times in any of our assays so far.

This could be explained by the angle of the liquid front in the flow channel – the liquid hits the cells ”from above” rather than from the side.

Pharmacology

- Test of known compounds on ligand targets



Typical protocol

- 4 - 5 baseline recordings with agonist @ EC50
- Wash and preinkubate with xx uM compound for 2 minuttes
- Record effect of compound plus agonist @ EC50
- Wash and preinkubate with yy uM compound for 2 minuttes
- Repeat 5 – 6 rounds to generate 5 or 6 points pr. well.

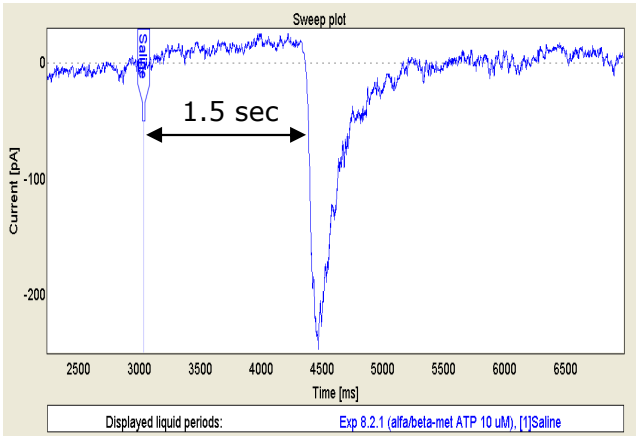
Typical protocol setup in software

Liquid periods

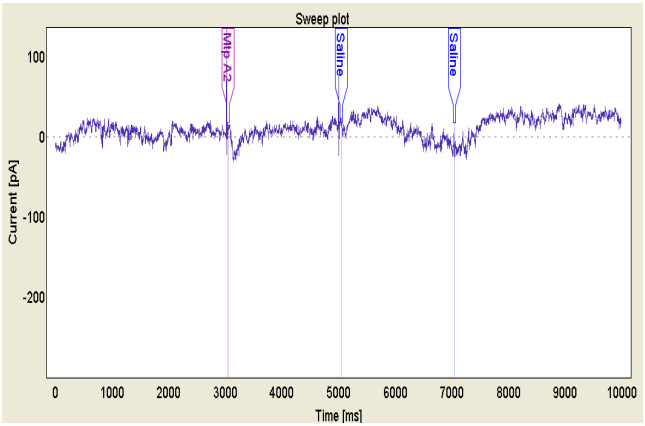
	Liquid	Volume [µl]	Wash	Data acquisition	Details
1	Res: Saline	5	☐	☐	
2	MTP: agonist alone	5	☑	☑	Res: Reference (4)
3	MTP: agonist alone	5	☑	☑	Res: Reference (4)
4	MTP: agonist alone	5	☑	☑	Res: Reference (4)
5	MTP: agonist alone	5	☑	☑	MTP: blocker_conc_1 (4)
6	MTP: blocker_conc_1 plus agonist	5	☑	☑	MTP: blocker_conc_2 (4)
7	MTP: blocker_conc_2 plus agonist	5	☑	☑	MTP: blocker_conc_3 (4)
8	MTP: blocker_conc_3 plus agonist	5	☑	☑	MTP: blocker_conc_4 (4)
9	MTP: blocker_conc_4 plus agonist	5	☑	☑	MTP: blocker_conc_5 (4)
10	MTP: blocker_conc_5 plus agonist	5	☑	☑	Res: Reference (4)
11	MTP: agonist alone	5	☐	☑	

Baseline stability rP2X3 – hexokinase

Historical data:



Delayed P2X3 response when adding saline was seen initially…

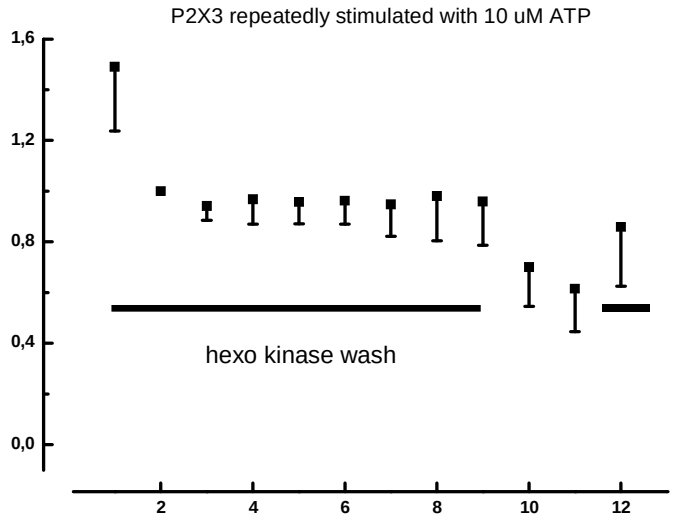


Followed by no response when adding agonist.

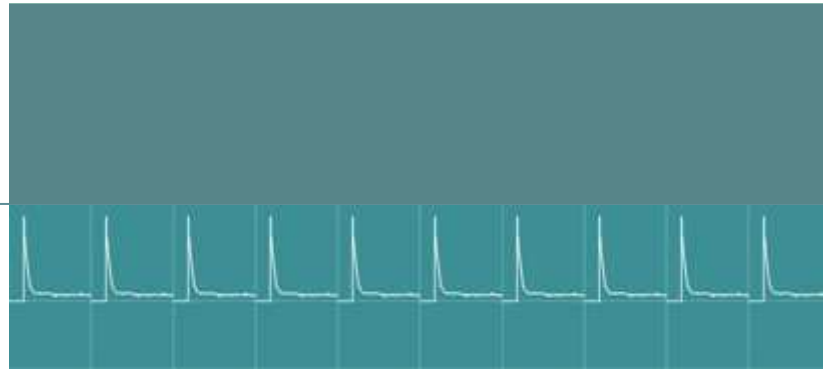
- Solution was found to be to add hexokinase

Hexo kinase + glucose + ATP ⟶ ADP + glucose-6-phosphate

- Hypothesis is that cells remaining in the inlet well leaks ATP desensitising channels further down in the flow channel
- Hexokinase also stabilised responses

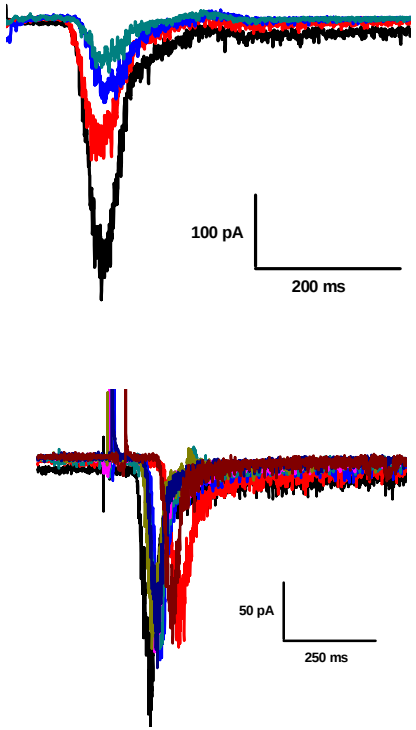


Baseline stability alpha7 – acetyl choline esterase

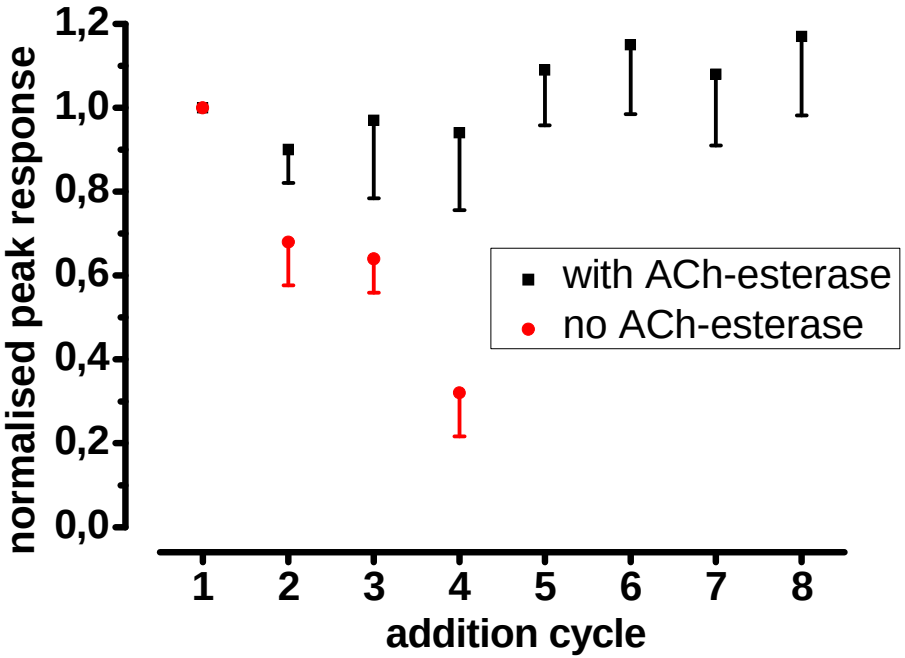


Single hole alpha7 raw data continuosly stimulated with 1 mM ACh

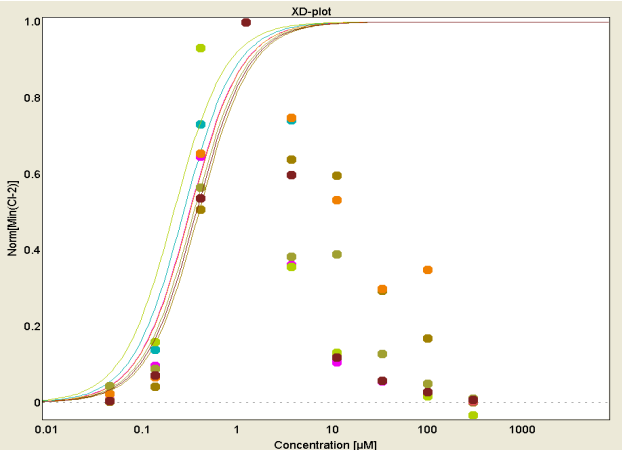
In absence and presence of acetyl choline esterase



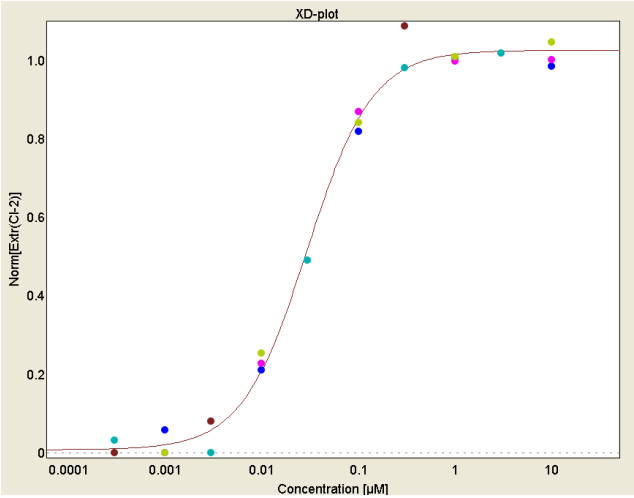
Acetyl choline esterase stabilizes responses by hindering acumulated desensitization.



rP2X3 pharmacology



Normalised αβ- met-ATP rP2X3 peak responses vs. concentration

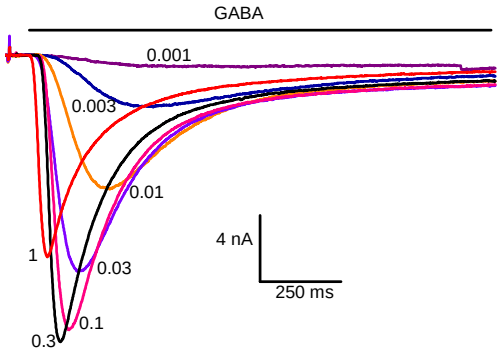


Normalised TNP-ATP induced block
Cells stimulated with 1 uM αβ- met-ATP
IC50: 0.029 uM.

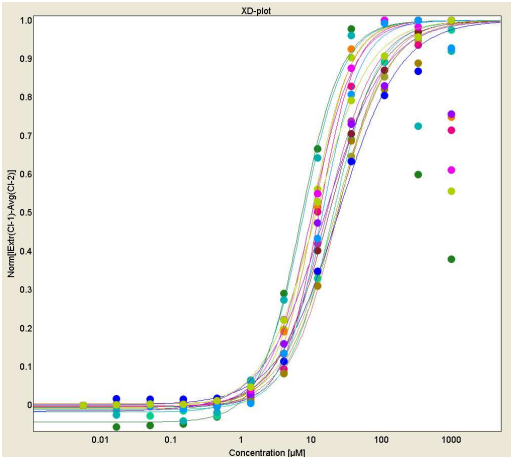
XC50 for known agonists/antagonists (uM)

	Single hole	HTX	Lit values
ATP	0.7	1.5	1.8 Wildman et al, 1999
αβ-met-ATP	1.5	0.3	2.7 Pratt et al, 2005
TNP-ATP @ 1 uM αβ- met-ATP	0.021	0.029	0.009 Virginio et al, 1998

GABAA pharmacology



Leak subtracted GABA α1β2γ2 currents recorded as response to increasing concentrations of GABA (mM).

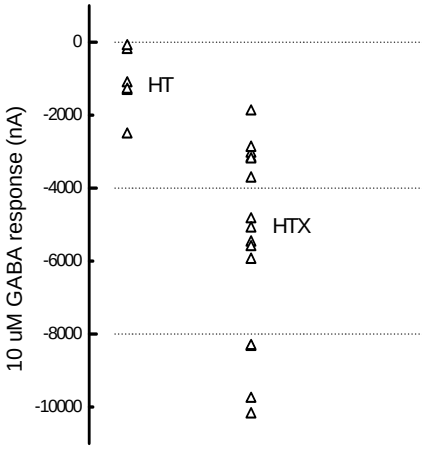


Normalised GABA responses from 16 individual sites fitted to the Hill equation.
Each cell was exposed to 12 different concentrations of GABA.

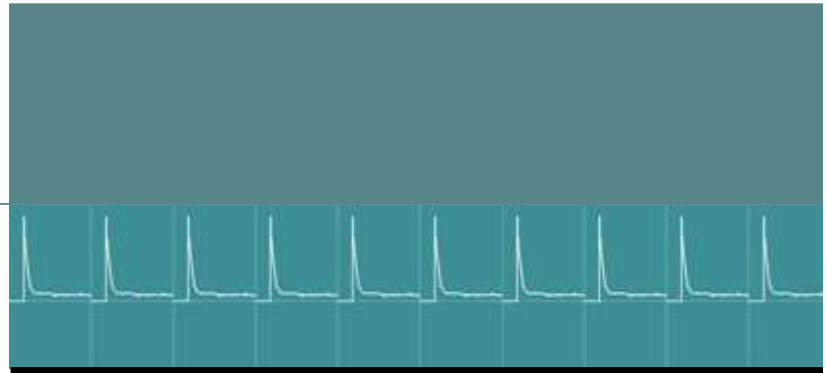
XC50 for known agonists/antagonists (uM)

	Single hole	HTX	Lit values
GABA	5.6	12	9-18 Curtis et al, 1970
Bicuculline	1.2	1.5	1-3 Curtis et al, 1970
Diazepam @ 1 uM GABA	ND	0.14	0.15 Kapur et al. 1996
% sites with current	37%	93%	

GABA$_A$ pharmacology



GABA current response on QPatch HT vs. HTX evaluated on 16 sites.
Sites with current were 37 vs. 93 % respectively.



XC50 for known agonists/antagonists (uM)			
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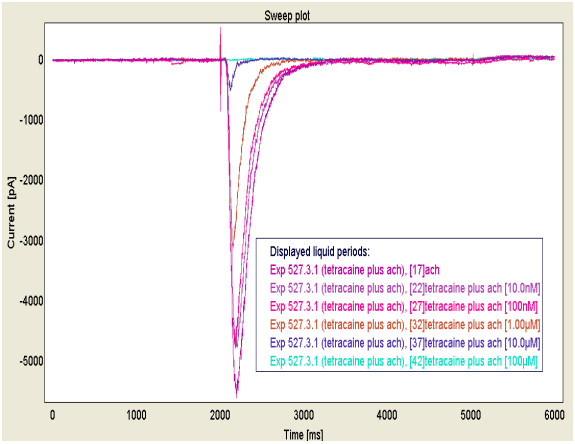
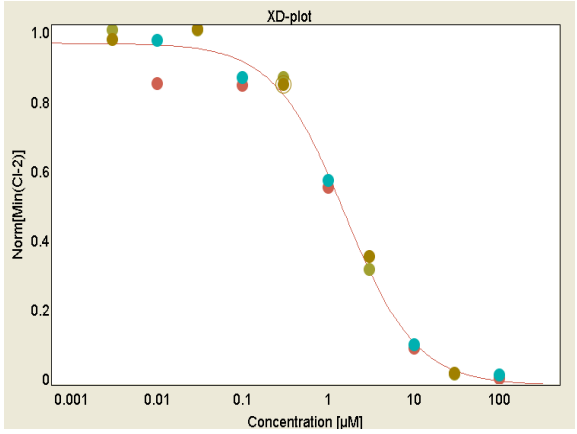
TE671 alpha1 pharmacology



Alpha1 current stimulated with 1 uM ACh in the presence of increasing concentrations of tetracaine (0.01 – 100 uM).

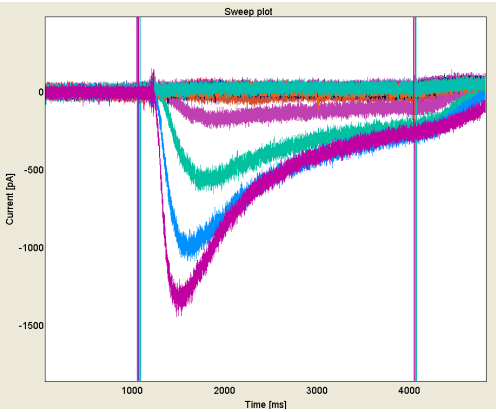


Resulting concentration respose plot from 4 sites.
IC50 = 1.6 uM for tetracaine and >100 uM for lidocaine

XC50 for known agonists/antagonists (uM)

	Single hole	HTX	Lit values
Acetyl choline	5.6	12	8.5 Shao et al, 1998
Tetracaine	ND	1.6	13 Gentry & Lukas, 2001
Lidocaine	ND	<100	52 Gentry & Lukas, 2001
Gallamine	5.3	2.2	0.9 Poul et al, 2002
Rocuronium	ND	0.13	0.33 Poul et al, 2002
Sites with current	90%	93%	NA

GluR5 pharmacology



Leak subtracted GluR5 currents recorded as response to increasing concentrations of kainate. EC50 = 157 uM.

XC50 for known agonists/antagonists (uM)

	Single hole	HTX	Lit values
Glutamate	236	344	630 Lerma et al, 2001
Kainate	119	299	33-177 Lerma et al, 2001
CNQX	1.9	0.8	
% sites with Kainate current	65	91	

Summary

- We have found excellent agreement between single hole and HTX rise time data.
- We have tested a number of known agonists and antagonists and have found good agreement between HTX, single hole and litterature reference values.
- We have increased the success rates in all assays on QPatch HTX.

We therefore conclude that QPatch HTX is a very well suited platform for ligand gated assays just as flexible as QPatch HT but with an increased success rate and throughput.

Acknowledgements

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