Biolin Scientific
[Progress Together]

QPatch applications on iPSC-derived cardiomyocytes and neurons

Precision Medicin & Ion Channel Retreat 2016
Guangzhou, China
November 11, 2016

Kazuya Tsurudome Ph.D.
Senior Application Scientist, Biolin Scientific K.K.

Attension
[Together with Biolin Scientific]

Biolin Scientific
[Progress Together]

1. Introduction – Automated patch clamp and iPS-derived cells
2. iPS-derived cardiomyocytes
3. iPS-derived neurons
4. Conclusion

Attension
[Together with Biolin Scientific]

History of Automated Patch Clamp

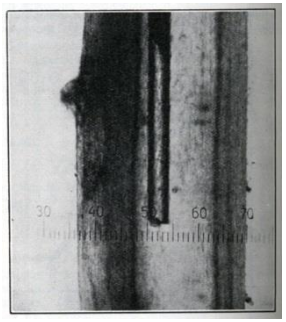
Development

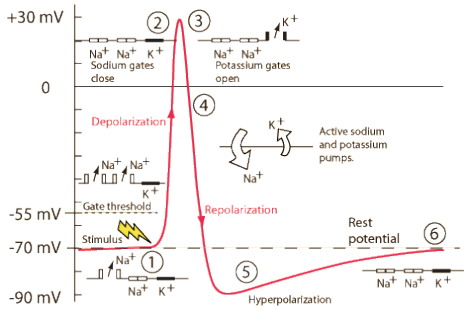
The Nobel Prize in Physiology or Medicine 1963

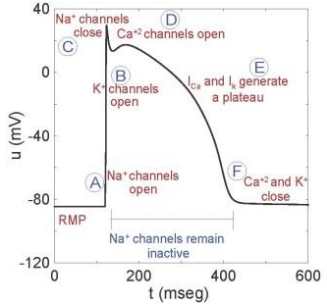

Sir John Carew Eccles
Prize share: 1/3


Alan Lloyd Hodgkin
Prize share: 1/3


Andrew Fielding Huxley
Prize share: 1/3



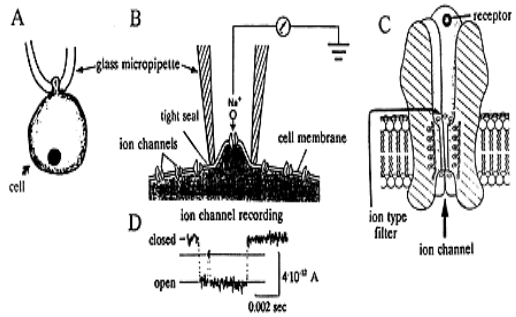




The Nobel Prize in Physiology or Medicine 1991

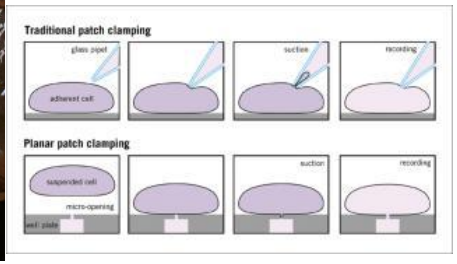

Erwin Neher
Prize share: 1/2


Bert Sakmann
Prize share: 1/2



Applications

Planar patch clamp yr2001



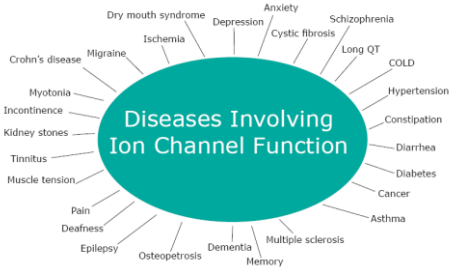
Qpatch (16/48 channel)

yr2004

Qube (384 chennels)

yr2014

Examples of channelopathies



LONDON--(BUSINESS WIRE)--The global ion channel modulators market is forecast to reach a value of USD 21.4bn by end-2018, driven largely by deeper understanding of channelopathies and ongoing advances in electrophysiology, particularly automated electrophysiology.

Attension
[Together with Biolin Scientific]

Whole cell patch clamp system

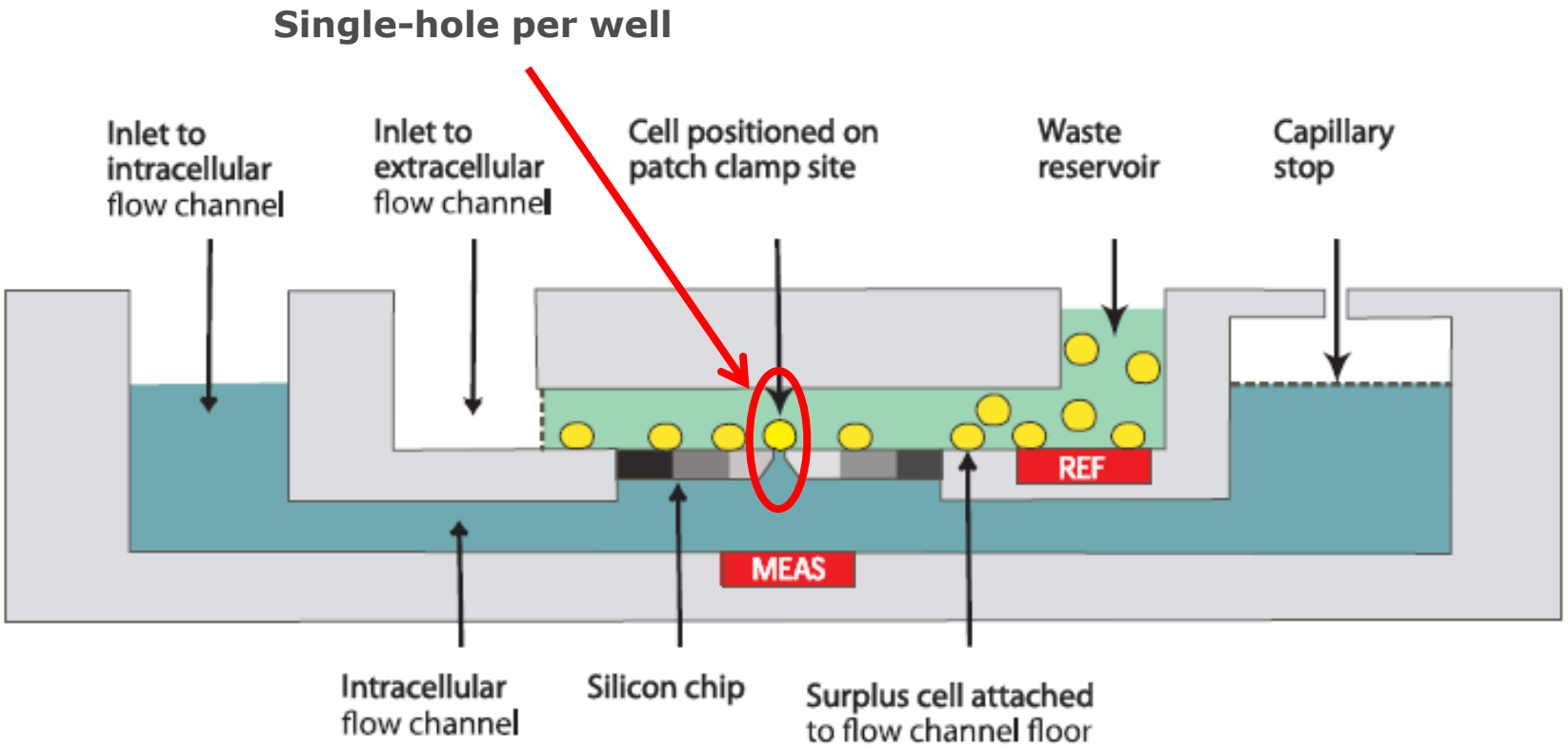
- Need to keep cells/animals alive in good condition
- Whole cell patch clamp configuration by manipulator and syringes
- Run voltage protocols and apply drugs manually
- Every step performed by skillful researcher

Time consuming experiments

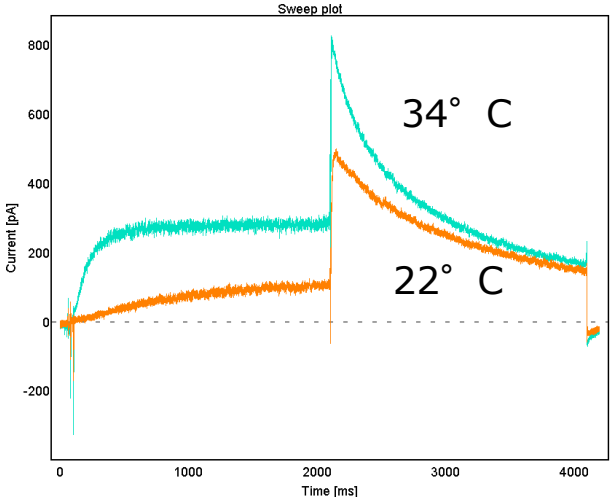
QPatch 16/48 channel automated patch clamp system

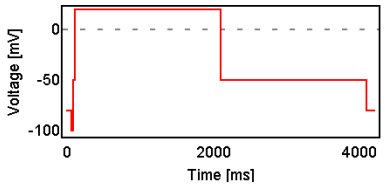
- Automated cell preparation
- Whole cell patch clamp configuration by pressure system
- Programmed assays are conducted by robot and amplifiers
- Precise auto drug application
- 100% Unattended system after ‘start’

QPlate; single-hole technology

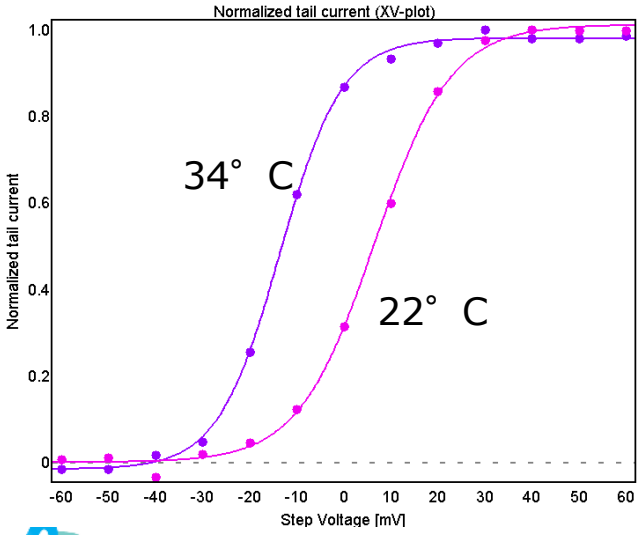


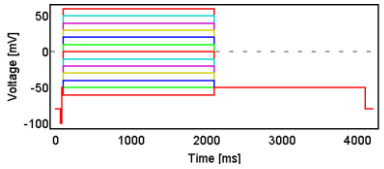
Other features: Temperature control with voltage clamp





- Currents were recroded from hERG expressing CHO cells. The experiments were performed at 22℃(orange) or 34℃(green).

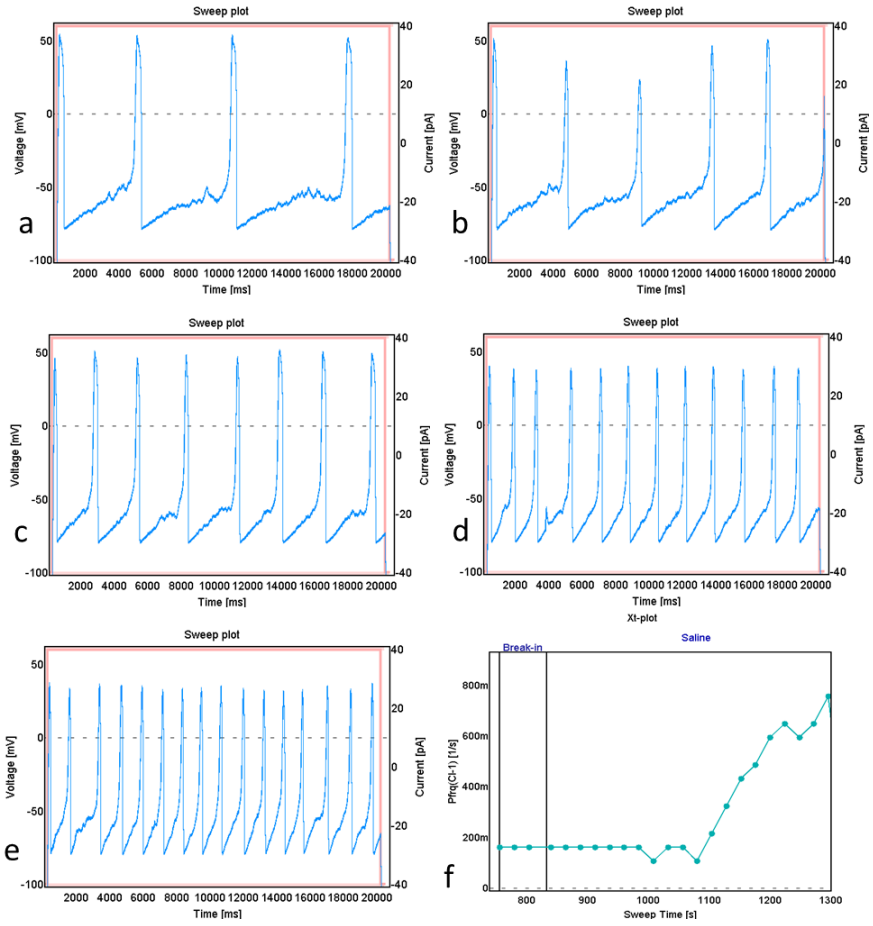




- I-V plots showed a shift of activation curve. There was about -15mV shift of $V_{0.5}$ at 34℃(purple) compared to 22 ℃(pink)

Attension
[Together with Biolin Scientific]

Other features: Current clamp



- Membrane potentials were recroded from hERG expressing CHO cells. The experiments were performed at 22℃ or higher temperature up to 34℃.
- The pulse frequency was 0.16pulse/sec at 22 ℃ and it increased up to 0.76pulse/sec at 34℃

Automated systems with cultured cells

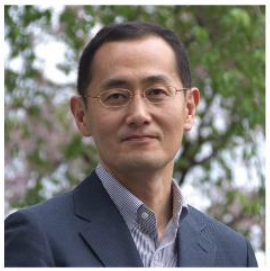
- Scalable
- Stable cell condition
- Cell lines stably expressing specific ion channels
- Suitable cells (such as CHO or HEK293 cells) for planar patch

Nowadays, we have a demand of testing iPSC-derived cells such as cardiomyocytes or neurons on automated patch clamp system

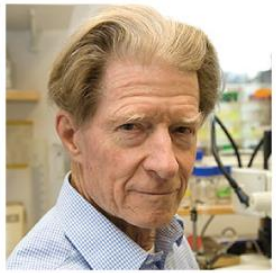
Induced Pluripotent Stem Cell (iPSC)

Discoveries

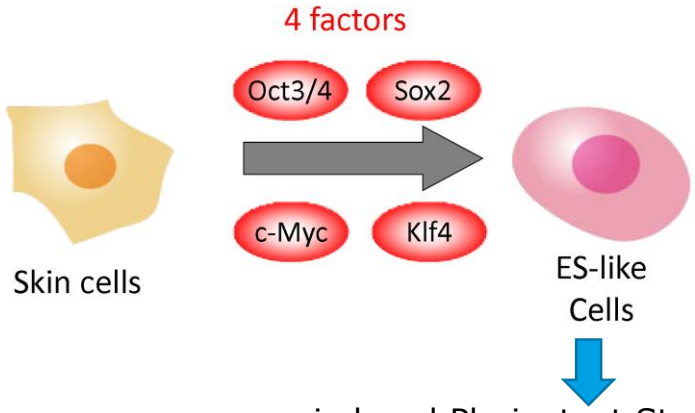
2012 Nobel Prize in Physiology or Medicine



Shinya Yamanaka
University of Kyoto, Japan
Photo Credit:
Center for iPS cell Research and Application, Kyoto University



John B. Gurdon
Gurdon Institute in Cambridge, UK



induced Pluripotent Stem (iPS) Cell

Mouse 2006 Human 2007

From Dr. Yamanaka's Novel prize lecture

Commercially available

iPSC-derived cells
(cardiomyocytes/neurons etc.)

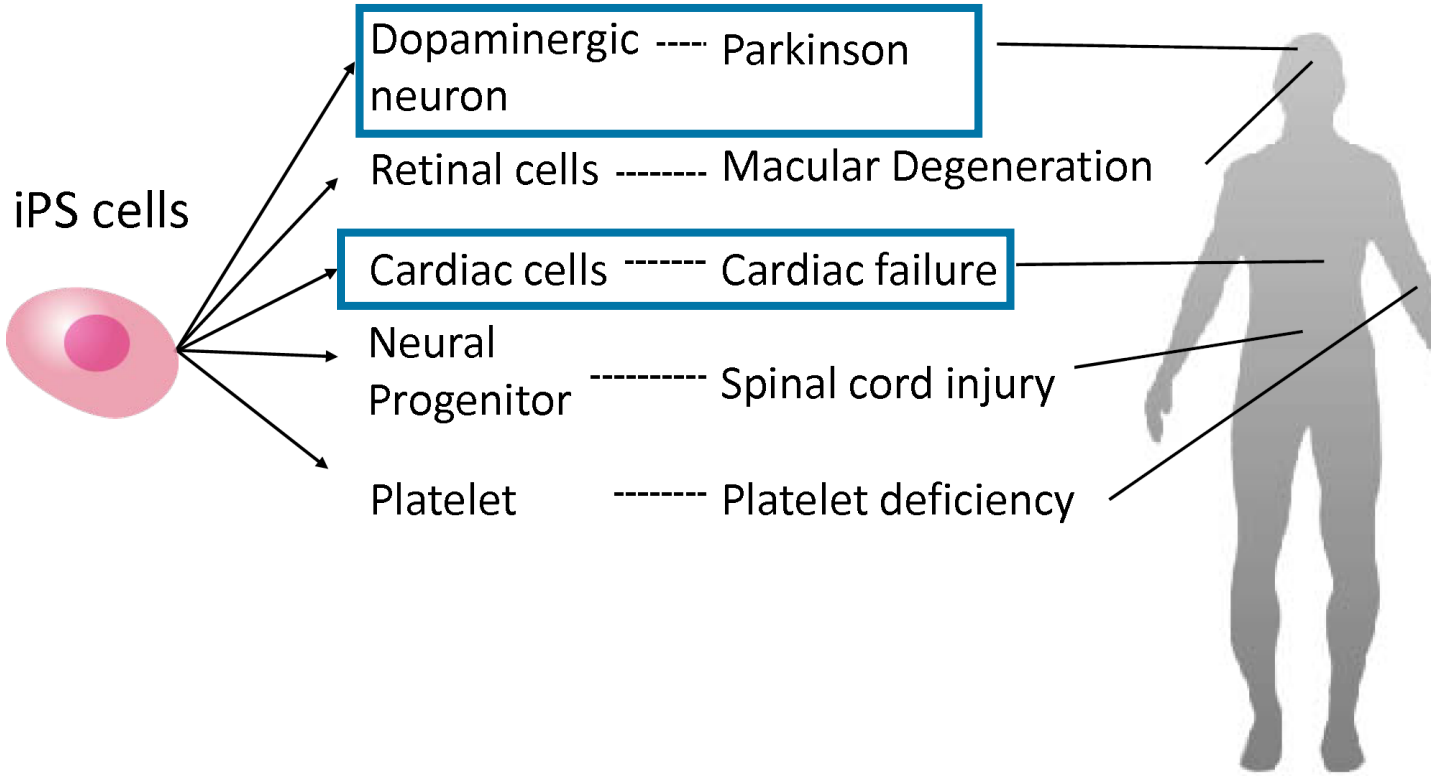
TaKaRa

Cellular Dynamics international
a FUJIFILM company

axioGENESIS

REPROCELL
INTEGRATED TOOLS FOR TRANSLATIONAL RESEARCH

iPSCs for research and application



From Dr. Yamanaka's Novel prize lecture

Cardiomyocytes

- iCell Cardiomyocytes by Cellular Dynamics

- Cor.At, Cor.4U by Axiogenesis AG

Neurons

- Dopa.4U by Axiogenesis AG
- ReproNeuro by ReproCELL

Cardiomyocytes

- iCell Cardiomyocytes by Cellular Dynamics

- Cor.At, Cor.4U by Axiogenesis AG

iCell from CDI

- Seals were obtained from 53% of cells. Whole cell configuration rate was 22%
- The currents observed: $Na^+ > K^+ > Ca^{2+}$

Detachment: Trypsin
Cell suspension media: EX-Cell ACF CHO media

	Single-hole
Seal	53 % (±12, n=9)
Whole-cell	22 % (±13, n=9)

	Single-hole	Multi-hole
Cell size	29 pF (±15, n=41)	N.D
Cells expressing I_{Na}	91 % (±16, n=40)*	N.D
Cells expressing I_{Ca}	41 % (±39, n=58)*	N.D
Cells expressing I_K	80 % (±35, n=17)*	N.D
Usable I_{Na} data/QPlate	16 % (±10, n=224)	58 % (±22, n=80)
I_{Nav} amplitude	-3.5 nA (±2.2, n=24)	-6.4 nA (±42., n=46)
IC_{50} TTX for I_{Na}	10.3 µM (±0.5, n=6)	6.3 µM (±4.5, n=12)
Tau for I_{Na} inactivation* **	0.85 ms (±0.29, n=22)	0.99 ms (±0.16, n=20)

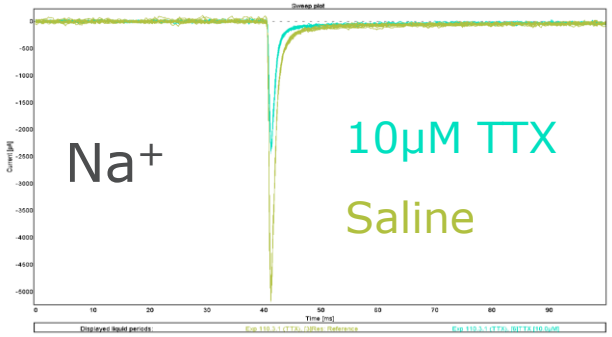
Ringer solution used for current recording

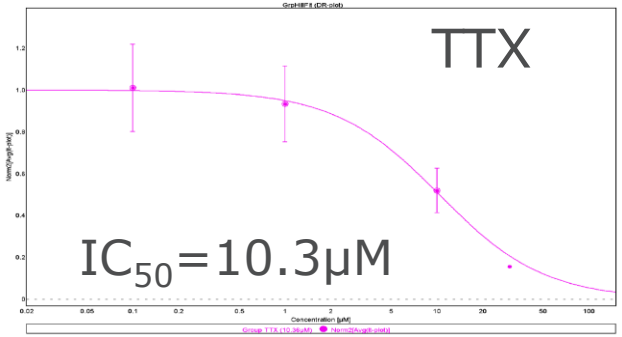
- Extracellular solution for I_{Na} and I_{Ca} (in mM)
  - 120 NaCl, 5 KCl, 3.6 CaCl2, 1 MgCl2, 20 TEA-Cl, 10 HEPES. pH7.4
- Intracellular solution for I_{Na} and I_{Ca} (in mM)
  - 120 CsCl, 3 MgCl2, 10 EGTA, 5 HEPES, 5 MgATP. pH7.3

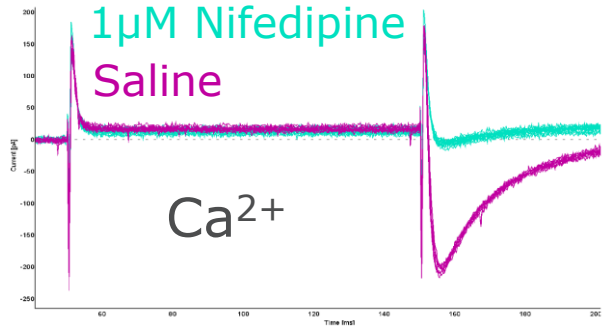
- Extracellular solution for I_K (in mM)
  - 15 NaCl, 140 KCl, 1.2 CaCl2, 1 MgCl2, 10 HEPES. pH7.4
- Intracellular solution for I_K (in mM)
  - 5.374 CaCl2, 1.75 MgCl2, 3.125/10 KOH/EGTA, 120 KCl. pH7.2

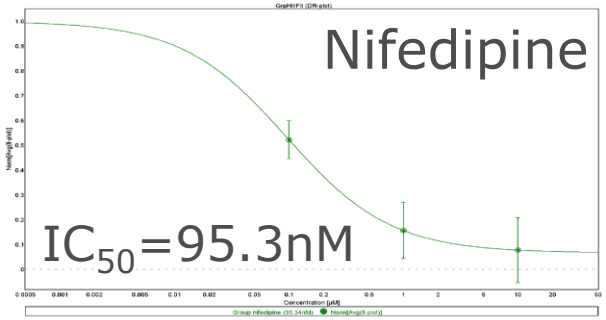
iCell from CDI

- IC_{50} values of TTX, Nifedipine were in range of those values for $Na_V1.5$, $Ca_V1.2$ channels

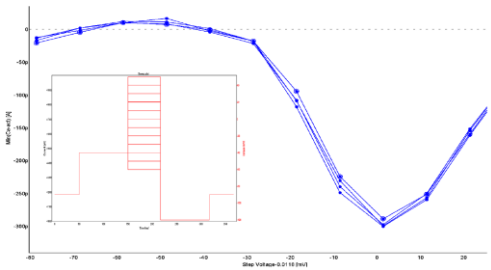








$V(I_{max})$=0 to 10mV



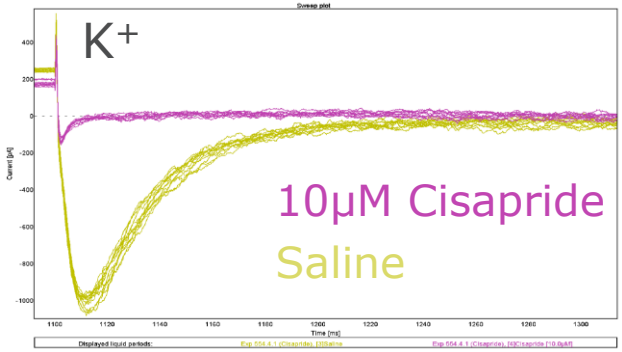
Current sweeps

Dose response curve (Hill fit)

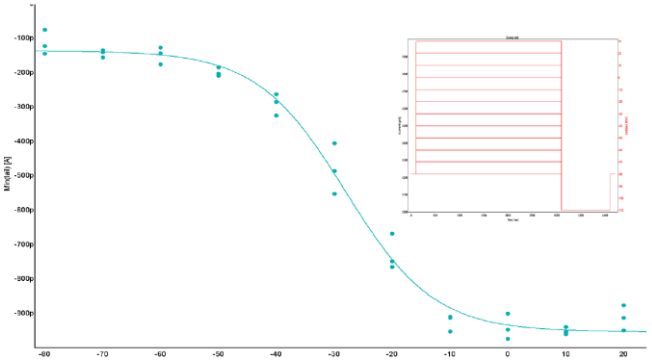
I-V plot

iCell from CDI

- Half activated voltage step ($V_{0.5}$) of tail K^+ current was similar to literature value (-23.1mV vs -21.5mV*)



Current sweeps



I-V plot
(Boltzmann fit)

$V_{0.5}$=-23.1mV
(literature value: -21.5mV*)

$$\frac{[K^+]o}{[K^+]i} = \frac{140}{123}\,[\text{mM}]$$

E_K=3.3mV

* Sanginetti and Jurkiewicz 1990

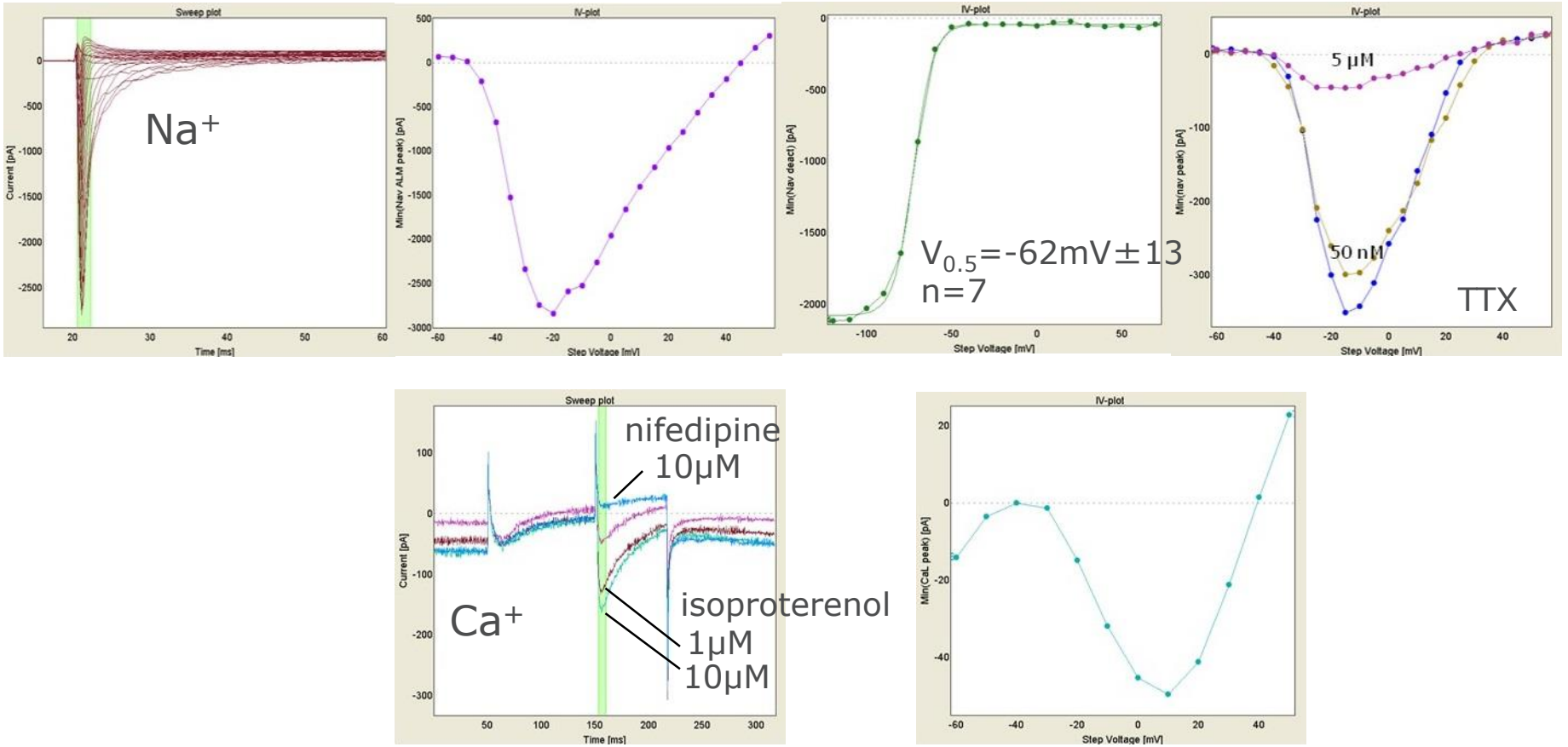
Cor.At (ESC derived mouse cardiomyocyte) from Axiogenesis

Detachment: Trypsin
Cell suspension media: EX-Cell ACF CHO media

	Single-hole
Seal	60.9 % (±17, n=4)
Whole-cell	50 % (±11, n=4)

Subject	Result
Viability after harvest procedure (%)	87± 3, n= 4
Size of cell (pF)	17 ± 7, n= 112
Peak I_{Na} (pA) (at -30 mV)	1842 ± 2521, n= 52
Current density I_{Na} (pA/pF)	104 ± 129, n= 52
Cells with recordable I_{Na} amplitude (%)	68 (15/22)
Peak I_{Ca} (pA) (at +10 mV)	35 ± 27, n= 32
Current density I_{Ca} (pA/pF)	2 ± 1.5, n= 32
Cells with recordable I_{Ca} amplitude (%)	55 (12/22)

Cor.At (ESC or iPSC derived mouse cardiomyocyte) from Axiogenesis



Cor.4U from Axiogenesis

Detachment: TrypLE Express

Cell suspension: manually suspended with EC

Solution	Whole cell method	R seal [MΩ]	R whole cell [MΩ]	Success rate (%)
Physiological	Suction	712	485	18.7
Physiological	Perforated	1255	561	18.8
KF	Suction	511	613	27.3
KF	Perforated	469	886	57.1

Extracellular solution:

EC000: 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose (mM), pH7.4

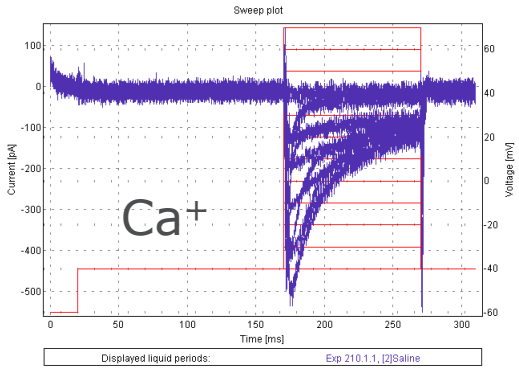
Physiological intracellular solution:

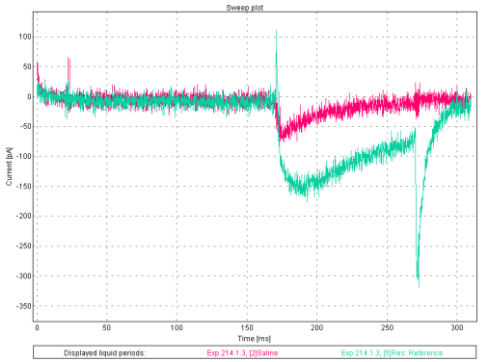
IC000: 120 KCl, 1.75 $MgCl_2$, 5.374 $CaCl_2$, 31.25/10 KOH/EGTA, 10 HEPES, 4 Na_2-ATP (mM), pH7.2

KF containing intracellular solution:

IC700: 120 KF, 20 KCl, 10 HEPES, 10 EGTA (mM), pH7.2

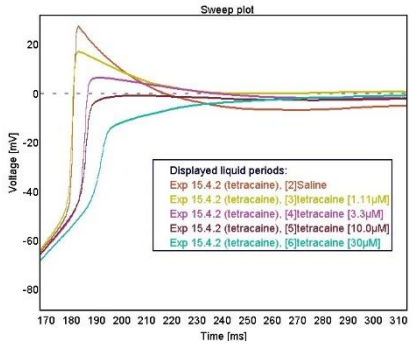
Cor.4U from Axiogenesis

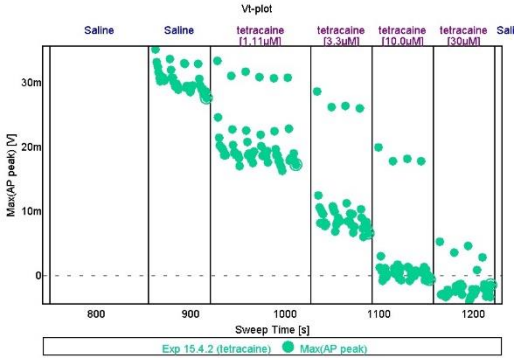


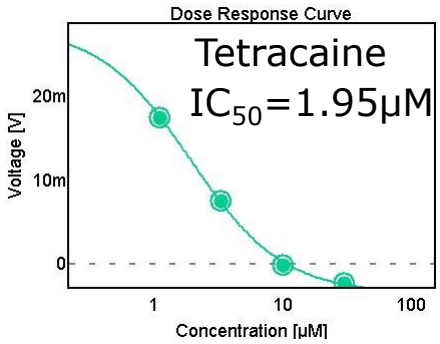


Reference: 1µM FPL 64176

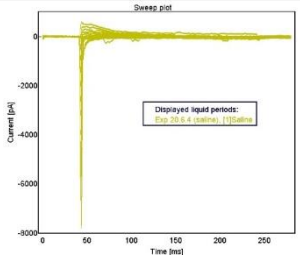
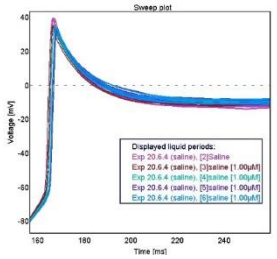
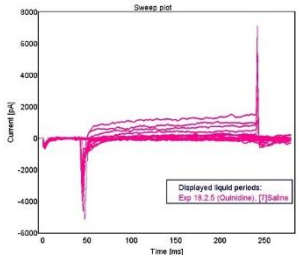
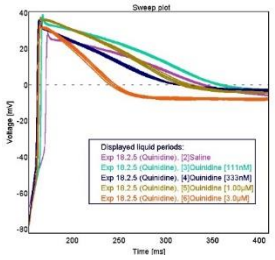
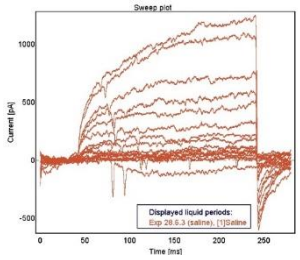
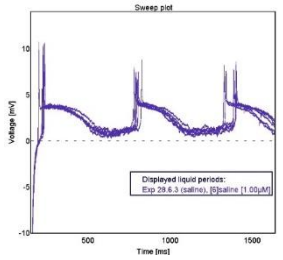
Membrane potentials (Current clamp recording)







Cor.4U from Axiogenesis

Current types	Current	Membrane potential
Transient inward/outward		
Transient inward Sustained outward		
Inward tail Sustained outward		

Neurons

- Dopa.4U by Axiogenesis AG

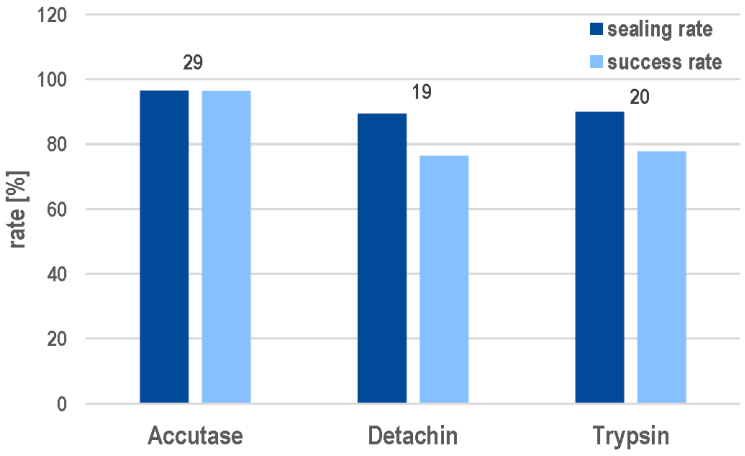
- ReproNeuro by ReproCELL

Dopa.4U from Axiogenesis

	attached cells	compl. experiment	seal	whole cell with good Rs
number of cells	380	209	217	125
Success rate	67%	55%	57%	33%

Detachment: Accutase, Detachin or Trypsin

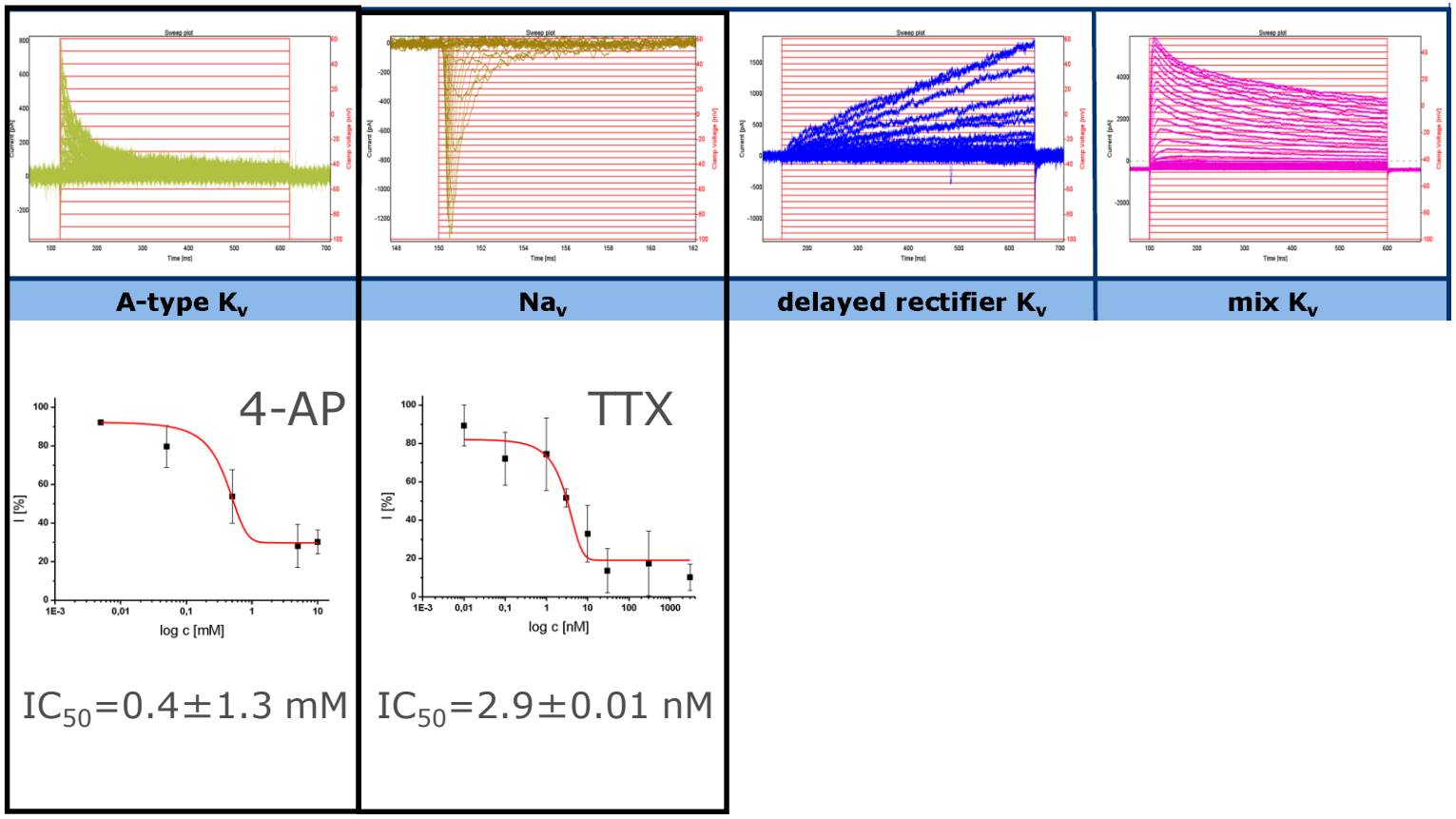
Cell suspension: manually suspended with EC



Ringer solution used for current recording from neurons

- Extracellular solution (in mM)
  - 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 Glucose, pH7.4
- Intracellular solution (in mM)
  - 120 KF, 20 KCl, 10 HEPES, 10 EGTA, pH7.2

Dopa.4U from Axiogenesis



ReproNeuro from ReproCELL

Detachment: TrypLE
Cell suspension media: EX-Cell ACF CHO media

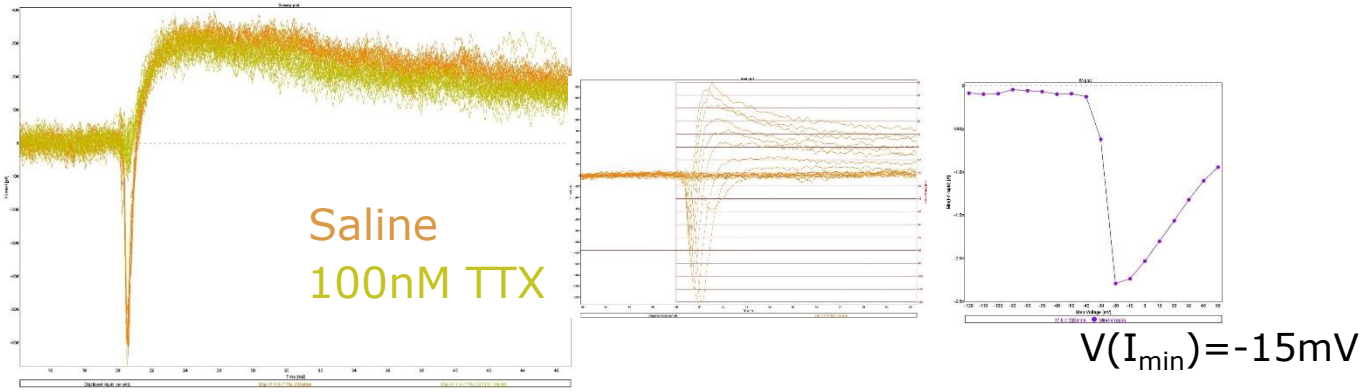
	Single-hole
Cell attached	45% (±12, n=19)
Whole-cell	26% (±11, n=19)
WC per cell attach	57% (±19, n=19)

Currents	%
Inward	39
Outward	61

	R seal	R whole cell
>100MΩ	117 (98.3 %)	119 (100%)
>500MΩ	52 (43.7 %)	88 (73.9%)
>1000MΩ	36 (30.3 %)	69 (58.0%)
Average [MΩ]	1049.6	1285.0

ReproNeuro from ReproCELL

Inward current

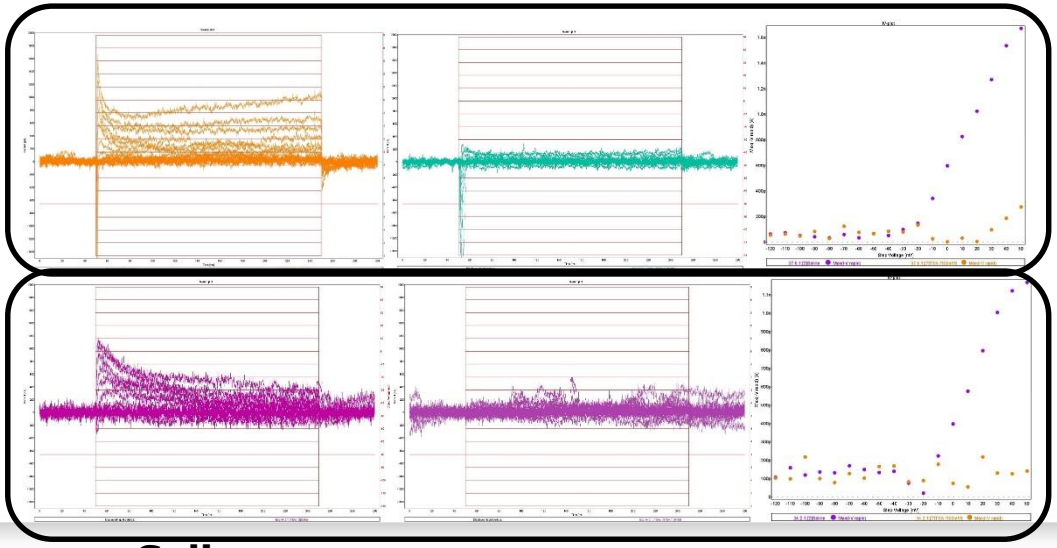


$V(I_{min})$=-15mV

Outward current

A-type Kv
+
delayed rectifier Kv

A-type Kv



Saline **100 mM TEA**

Summary

- **Success rates** were 50-60% at sealing state and then dropped to 20-30% at W.C. Those success rates were strongly affected by the harvesting method and whole cell method.

- **Low cell density:** The cell attached success rate did not achieve near 100%, which is probably because of the low cell density. (the whole cell configurations were successfully obtained over 50% of the attached cells)

- **Excitability:** The recorded membrane currents and membrane potentials were consistent with the channels expressed in each cell types. However, the channel expression was not even and the excitability strongly depends on the current expression pattern.

Acknowledgement

Sophion A/S
- Rikke S Perrier
- Denise Franz
- Hervør L Olsen

NMI TT Pharmaservices
- Timm Danker

Thank you!!