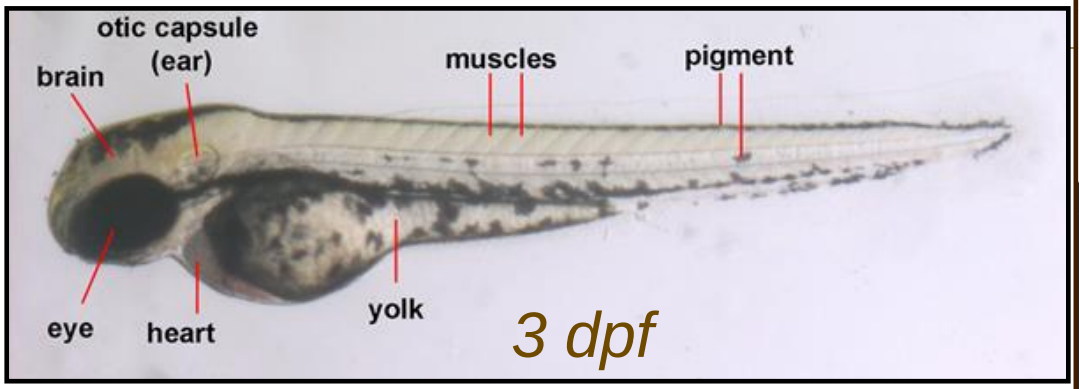
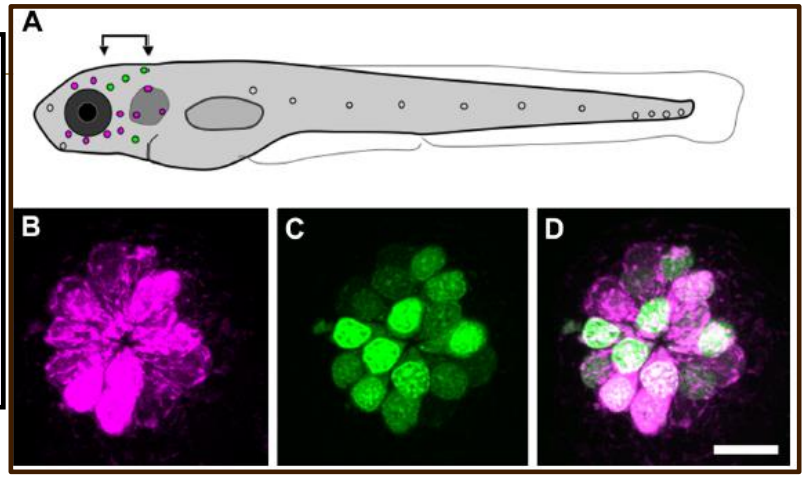
以斑馬魚為模式研究聽覺毛細胞以及耳毒性藥物
Using zebrafish as a model animal for hair cell and ototoxin studies





Owens, 2007

報告人：洪君琳
台北醫學大學 解剖暨細胞生物學科副教授
斑馬魚模式核心實驗室主任

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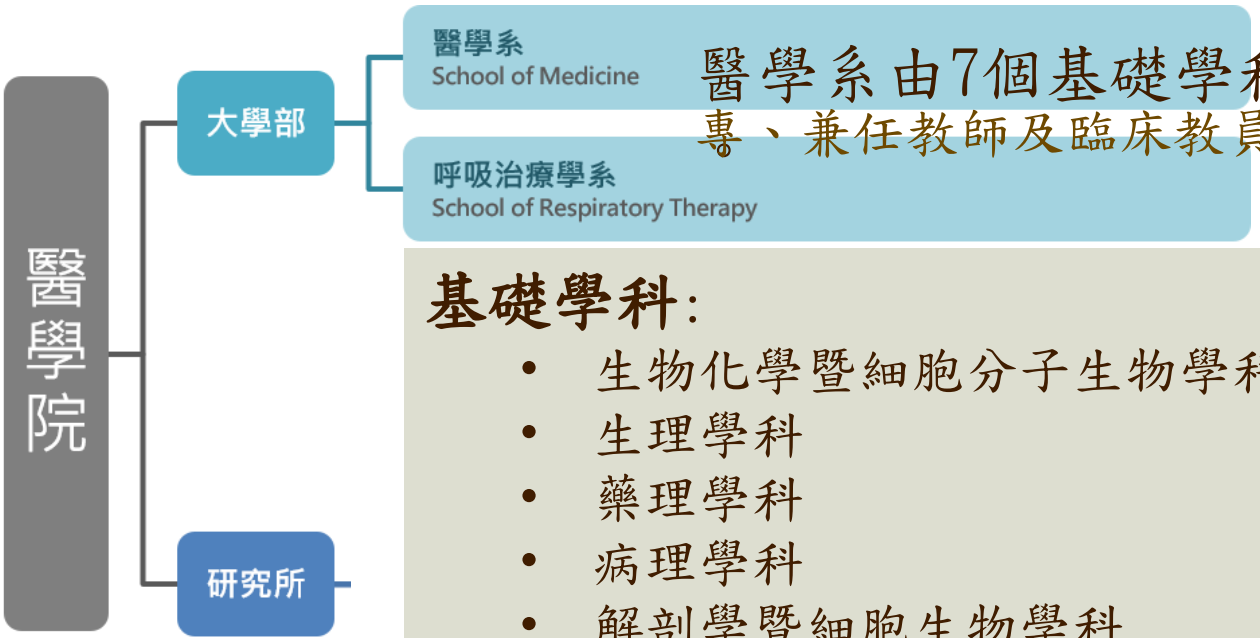
誠樸・關懷・卓越・創新

台灣醫學系排名

1.國立台灣大學
2.國立成功大學
3.國立陽明大學
4.私立長庚大學
5.私立台北醫學大學
6.私立中國醫藥大學
7.私立高雄醫學大學
8.私立馬偕醫學院
9.私立中山醫學大學
10.私立輔仁大學
11.私立慈濟醫學大學



醫學系由7個基礎學科及19個臨床學科組成
專、兼任教師及臨床教員多達600 餘人

基礎學科:

- 生物化學暨細胞分子生物學科
- 生理學科
- 藥理學科
- 病理學科
- 解剖學暨細胞生物學科
- 微生物免疫學科
- 分子寄生蟲暨熱帶疾病學科

臨床學科:

公共衛生學科；內科學科；外科學科；婦產學科；小兒學科；耳鼻喉學科；眼科學科；骨科學科；泌尿學科； 皮膚學科；神經學科；精神學科；麻醉學科；家庭醫學科；復健學科；急診學科；一般醫學學科；醫學教育暨人文學科；放射線學科；胸腔外科；神經外科；心臟血管外科；一般外科；感染科；胸腔內科；消化內科；腎臟內科；過敏免疫風濕科；心臟血管內科；血液腫瘤科

台北醫學大學　斑馬魚模式核心實驗室 設施

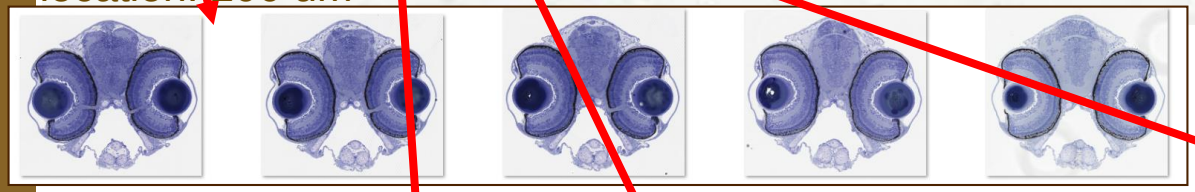
- 協助動物中心實驗動物管理
- 協助學校教師，醫師，研究人員斑馬魚動物模式建立
- 提供斑馬魚
- 支援教學課程

以樹酯包埋的方式建立斑馬魚胚胎之切片圖譜

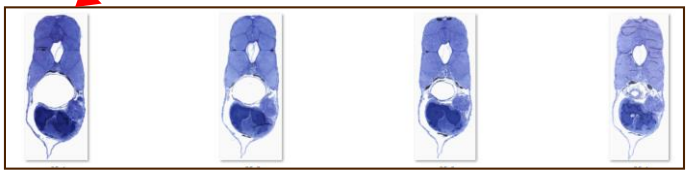
-建立斑馬魚胚胎器官組織之精準定位切片圖譜

每隔10 um 切片厚度1 um

location: 200 um



location: 1200 um

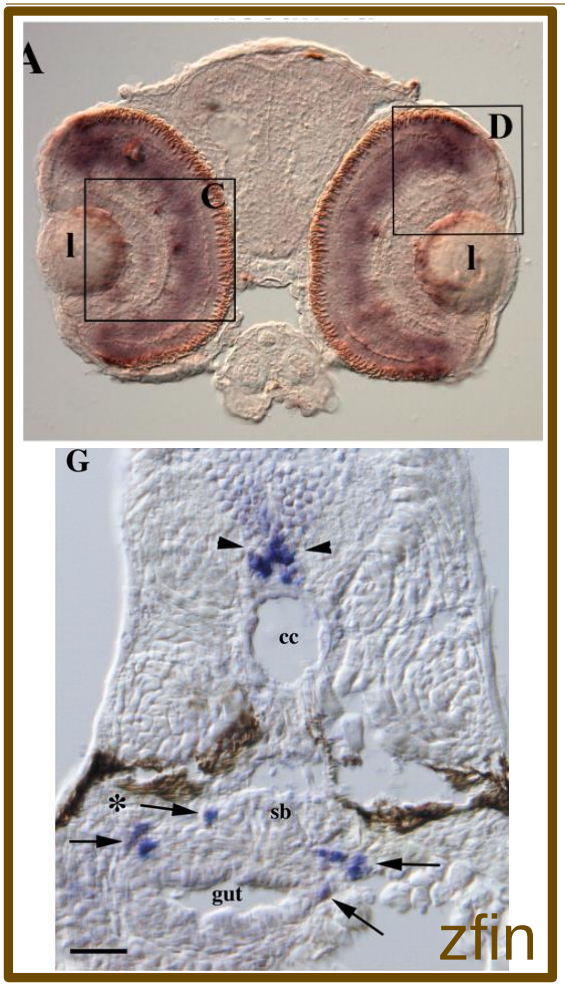


location: 600 um

location: 800 um

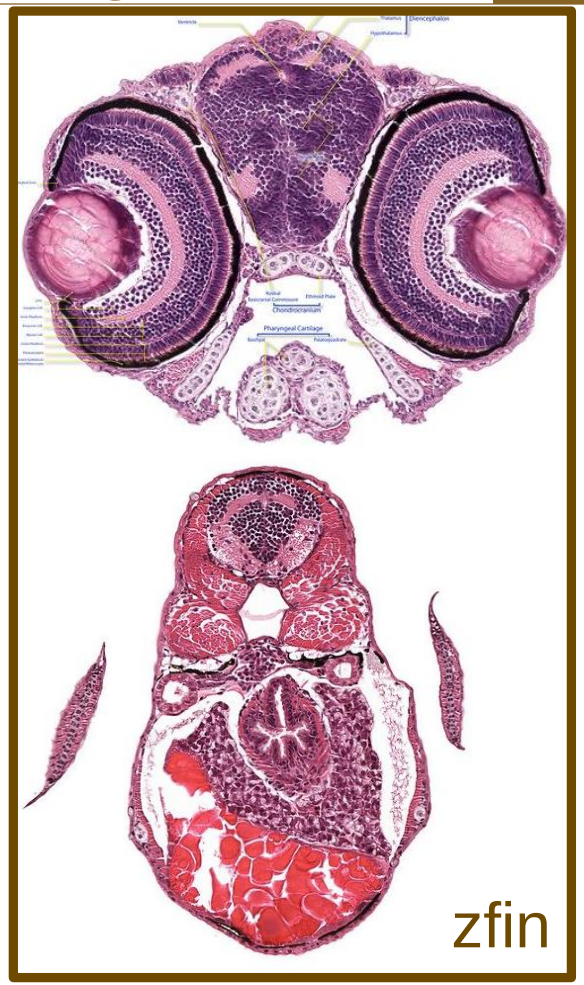
常用的切片方式

冷凍切片
快速、抗體染色



20 μm

石蠟切片
形態、HE stain

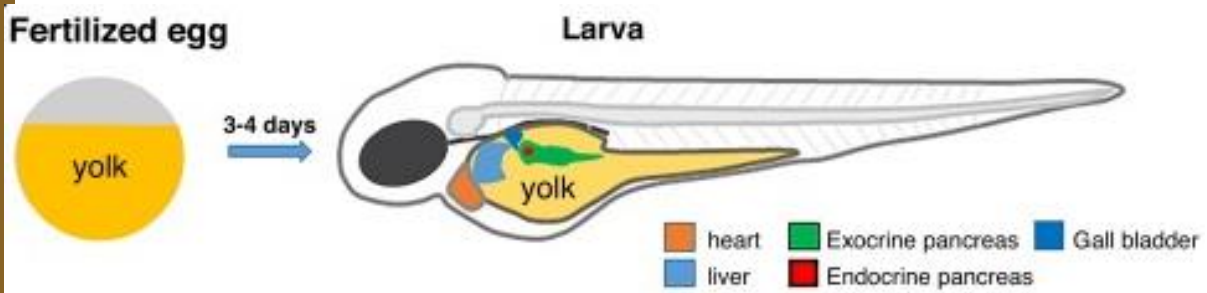


10 μm

樹酯包埋切片
形態完整、超薄

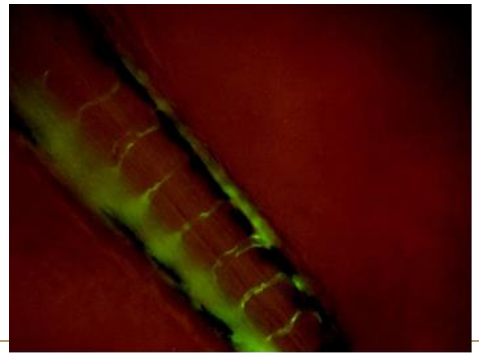
1 μm

斑馬魚胚胎器官短小
-切片厚度太大易錯過



Eye 300 um
Heart 120 um
Liver 100 um
Gall bladder 50um

內皮細胞

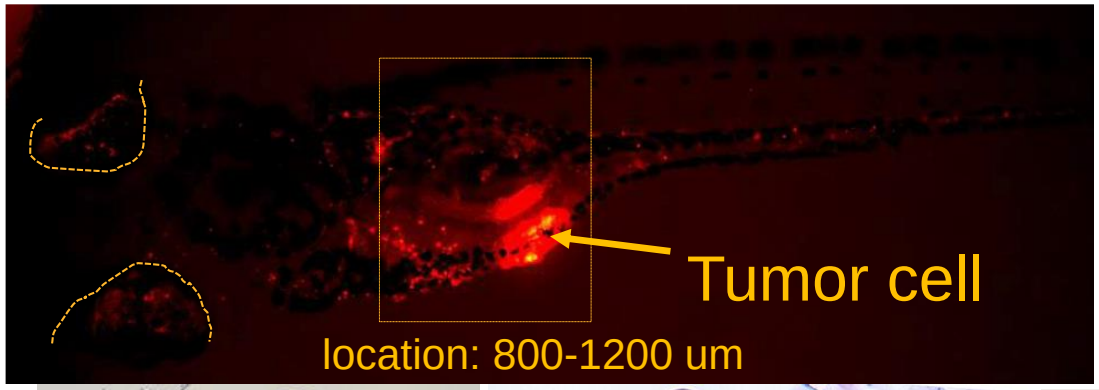


肝細胞

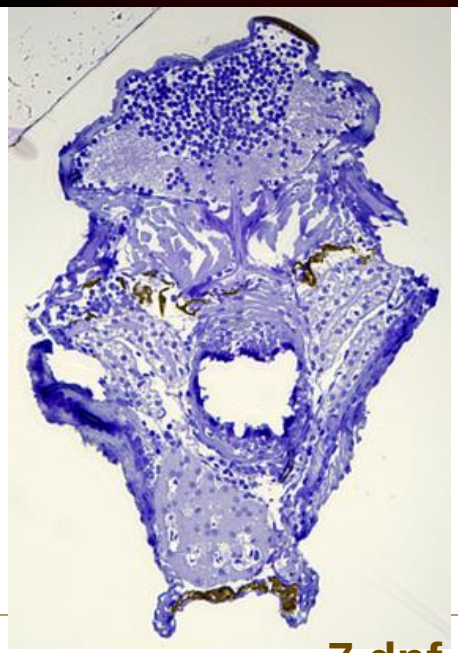
Kamel M, Ninov N. (2017) Curr Opin Pharmacol. 37:41-50

人類癌細胞異體移植模式

提供參考斑馬魚胚胎器官、組織在體內的位置與結構型態



1. 切片位置的定位
2. 組織形態的辨別


7 dpf


7 dpf


正常魚5 dpf

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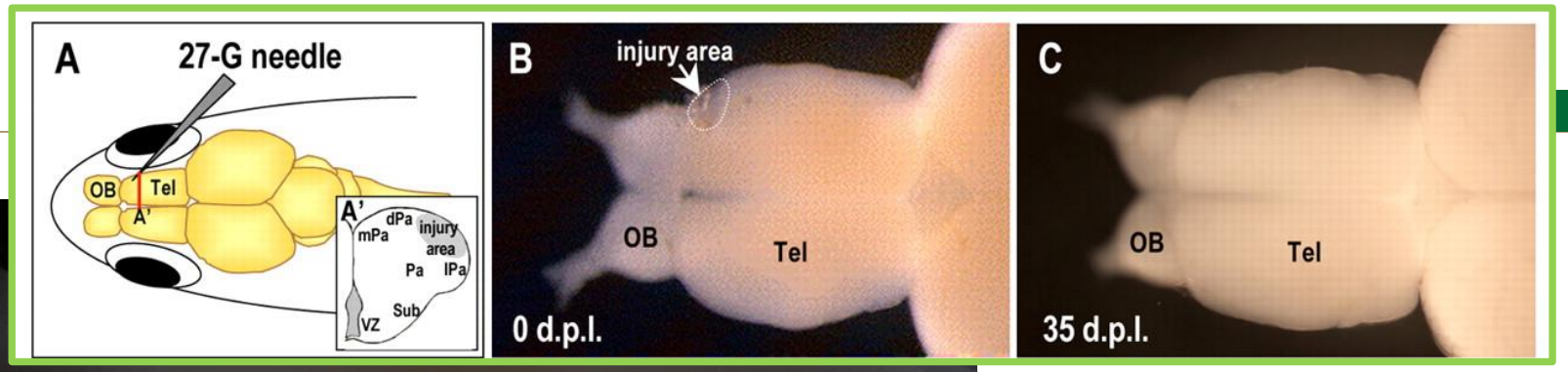
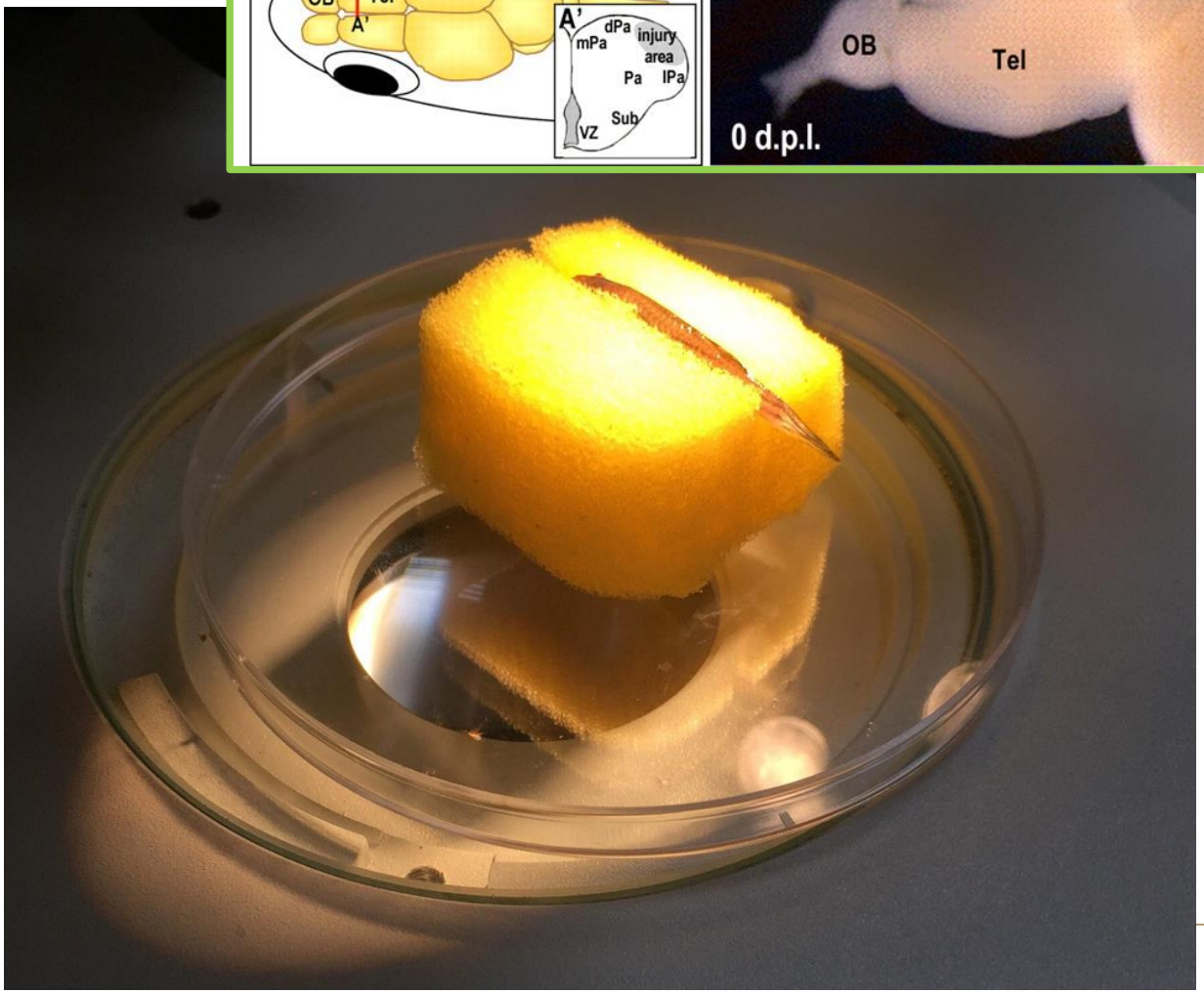
Traumatic brain injury (TBI)腦傷模式





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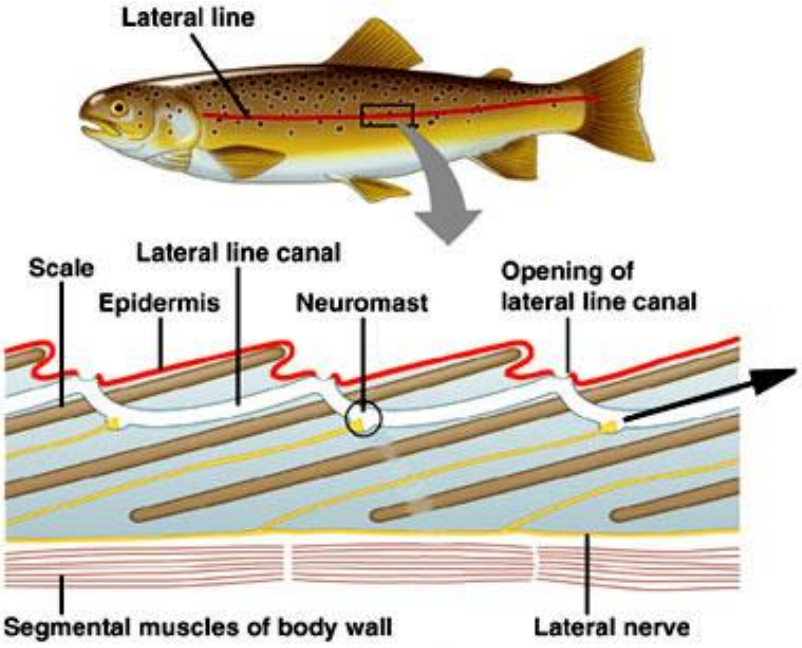
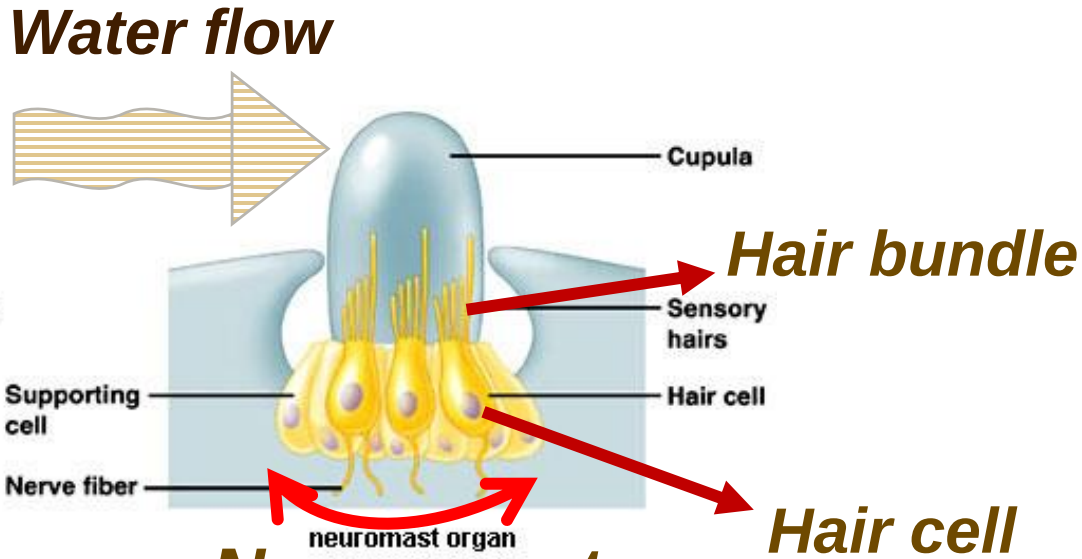
Lateral line in adult zebrafish魚類的側線

Sapede, 2002

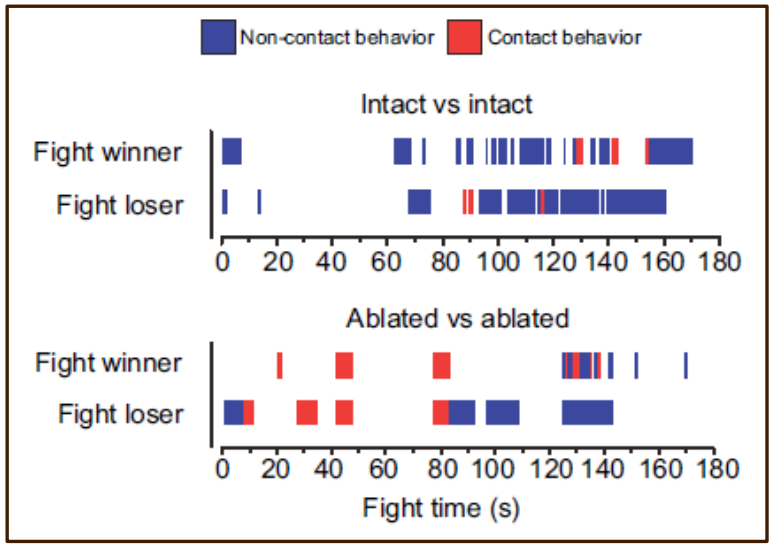


Biology 8th Cambell and Reece, 2008



Function of lateral line in social behavior

側線參與魚類的社會行為



Aggression

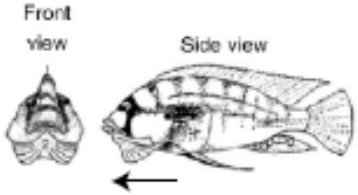
Border fight

Fish orient in front of each other and push foward and back to delineate the borders of their territory.

Lateral display

Fish orient parallel to each other, erect fins, distend jaws and shake their bodies.

Frontal threat



Fish distend jaws and flare opercula. Often accompanied by a lunge at another fish.

Mouth fight

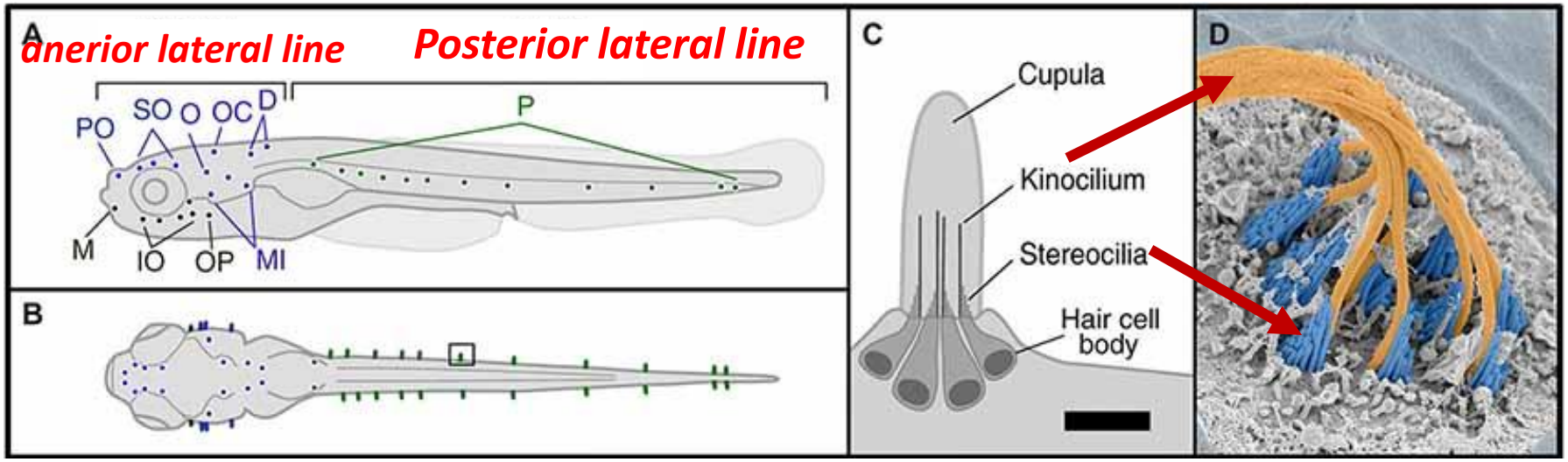
Fish grasp jaws and push/pull each other.

Bite/nudge

One fish rams opponent typically on the trunk with an open mouth (bite) or closed mouth (nudge).

JEB. 2016. 219: 2781-2789

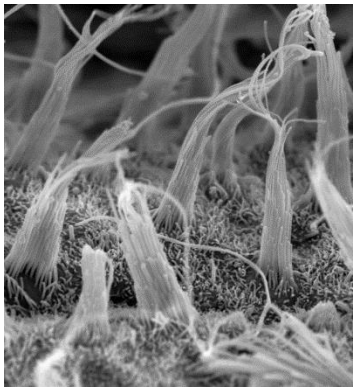
Neuromast hair cells of posterior lateral line of zebrafish larvae
斑馬魚仔魚側線神經丘



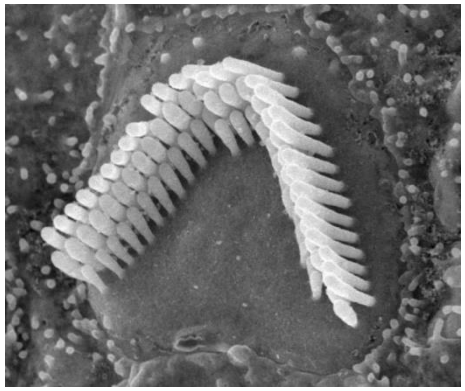
Monroe JD, et al. Front. Cell. Neurosci. 2015

- Hair cell morphology is similar between species毛細胞型態在物種間具保守性
- Zebrafish hair cells are sensitive to damage from ototoxic drugs 斑馬魚毛細胞對於人類耳毒性藥物具高敏感度性

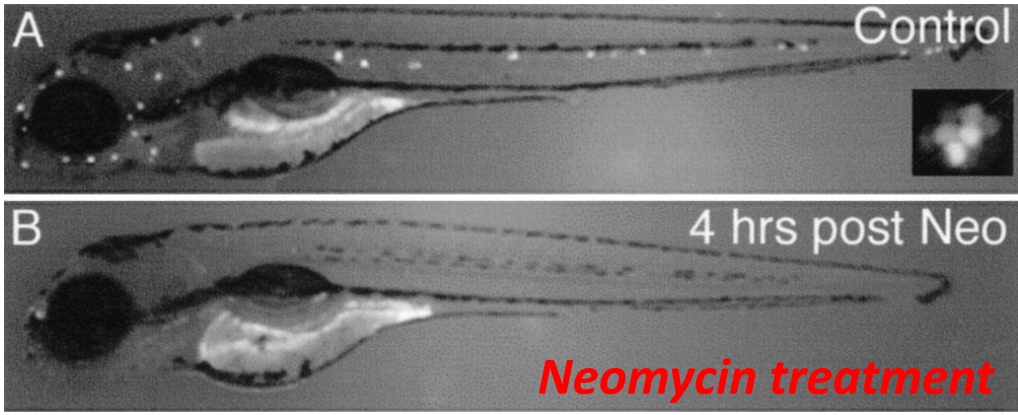
Ex. Gentamicin, quinine, aspirin



Mouse Vestibute hair cell

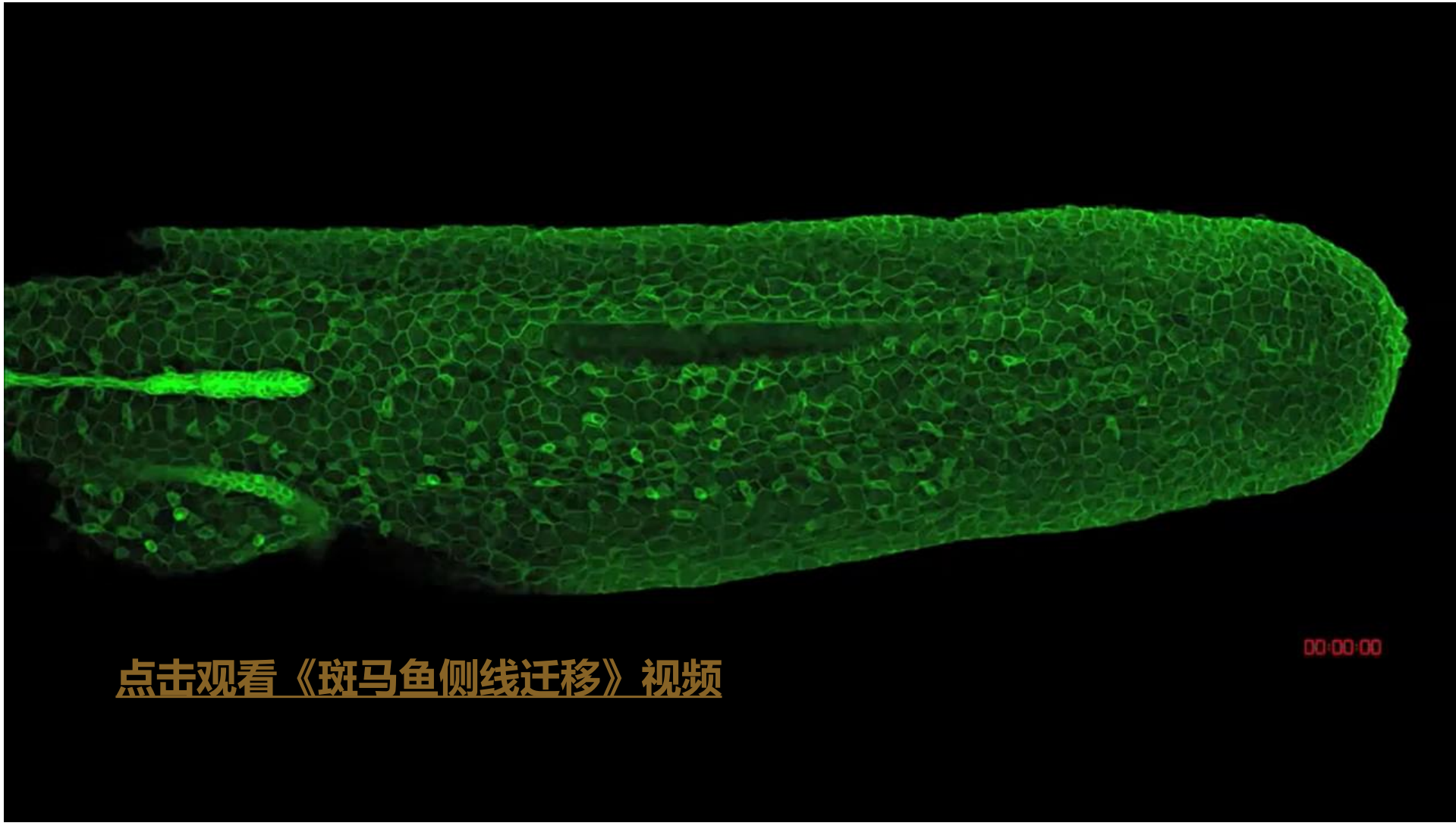


Mouse cochlear hair cell



Harris JA, JARO2003

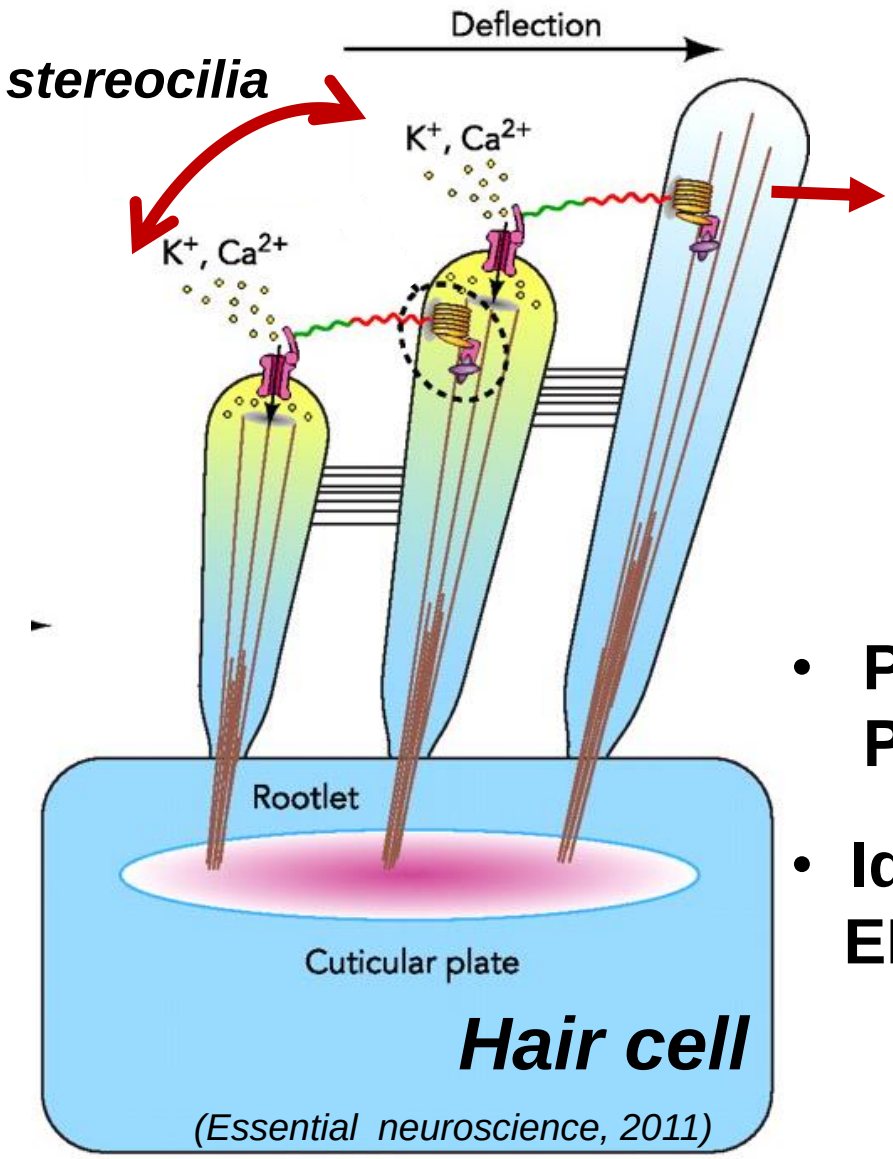
Zebrafish Lateral Line Migration



点击观看《斑马鱼侧线迁移》视频

Nipam H. Patel, 2012

Hair cell毛細胞



- **Hair bundle**
- **stereocilia** are lined up increasing height
- **Kinocilium**, the tallest

- **MET channel** on the tip of stereocilia

(mechanoelectrical transduction (MET) channels)機械性感測傳導通道

- **Permeability of MET channel: PCa > PLi > PNa ≈ PK >PRb**
- **Identity of MET channel? ENaC? TRP channel? TMC1/2?**

Article

Neuron

TMC1 Forms the Pore of Mechanosensory Transduction Channels in Vertebrate Inner Ear Hair Cells

Highlights

- TMC1 assembles as a dimer and resembles TMEM16 ion channels
- Cysteine modification with MTS reagents alters hair cell sensory transduction
- The data support a revised topology of TMC1 with 10 transmembrane domains

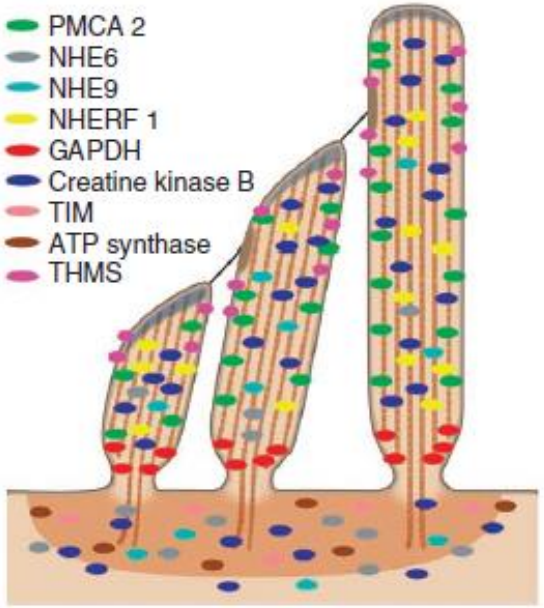
Authors

Bifeng Pan, Nurunisa Akyuz, Xiao-Ping Liu, ..., Marcos Sotomayor, David P. Corey, Jeffrey R. Holt

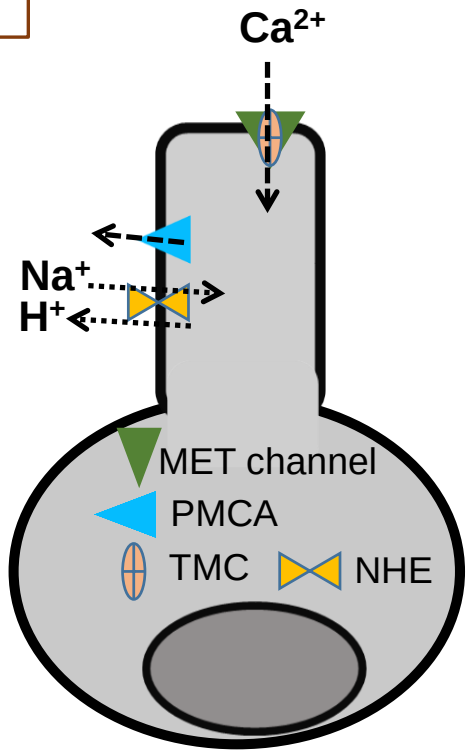
Correspondence

dcorey@hms.harvard.edu (D.P.C.), jeffrey.holt@childrens.harvard.edu (J.R.H.)

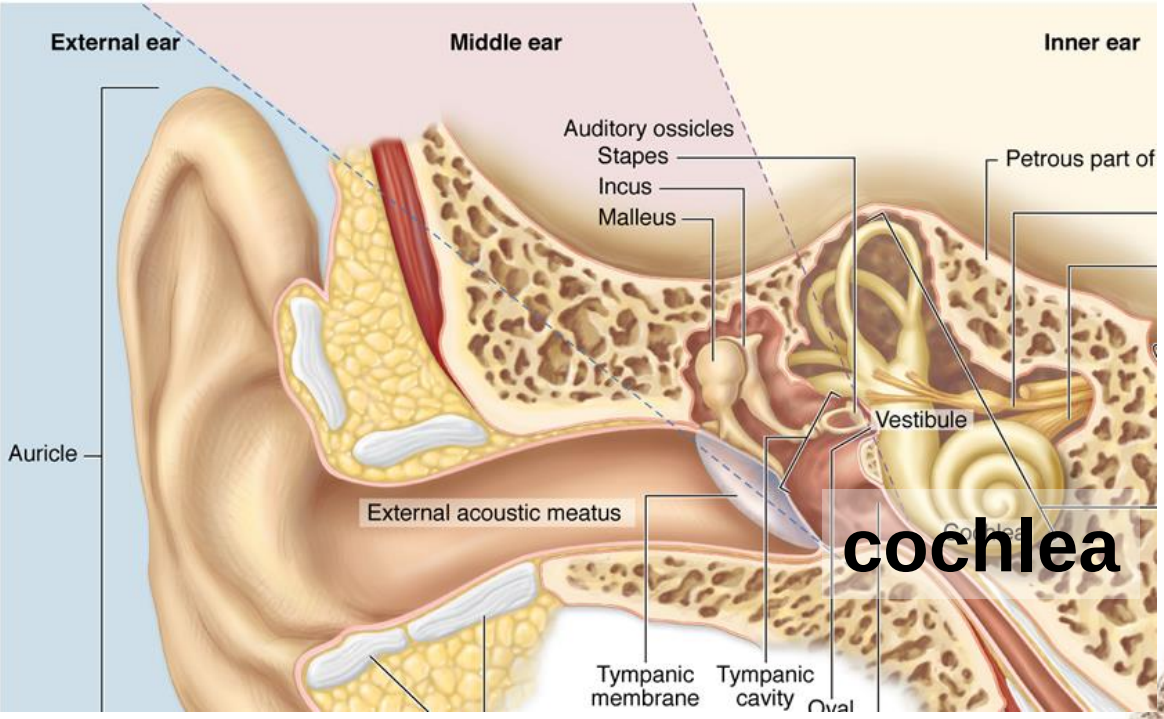
transmembrane channel-like protein (TMC)



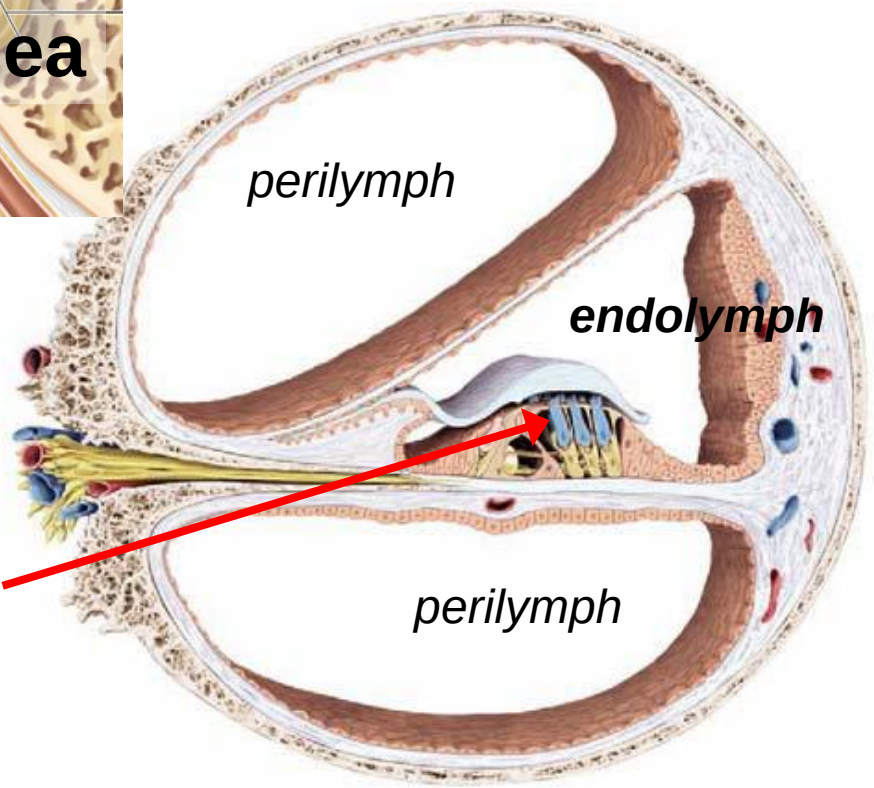
Nat Commun. 2011. 2:523.



Inner ear內耳



內耳毛細胞:
- 難以取得
- 難以控制給藥濃度
- 型態特殊



Hair cell

Ototoxin耳毒性藥物

症狀包含聽力喪失（10-20分貝為輕度聽力喪失，21-40分貝為中度聽力喪失，超過40分貝則為重度聽力喪失）、耳鳴、暈眩、運動失調、噁心、嘔吐等

List of Known Ototoxic Substances

Antibiotics
Aminoglycoside antibiotics
Streptomycin
Dihydrostreptomycin
Kanamycin
Gentamicin ← 建大黴素
Neomycin ← 新黴素
Tobramycin
Netilmicin
Amikacin
Macrolide antibiotics
Erythromycin
Clindamycin
Azithromycin

Miscellaneous Drugs
Salicylates
Acetylsalicylic acid (aspirin)
Nicotine
Quinine
Loop diuretics
Furosemide
Ethacrynic acid
Bumetanide
Platinum-based antineoplastic agents
Carboplatin
Cisplatin

Environmental Chemicals
Butyl nitrite
Nicotine
Mercury
Carbon disulfide
Styrene
Carbon monoxide
Tin
Hexane
Toluene
Lead
Trichloroethylene

藥品成分	耳毒性機率	血中波峰濃度	血中波谷濃度
Amikacin	0 - 28.5 %	> 35-40 mg/L	> 10 mg/L
Gentamicin	0 - 63 %	> 12 mg/L	> 2 mg/L
Kanamycin	5 - 26.9 %		
Neomycin	UP to 60 %		
Streptomycin	4 - 75 %		
Tobramycin	0 - 43 %	> 12 mg/L	> 2 mg/L

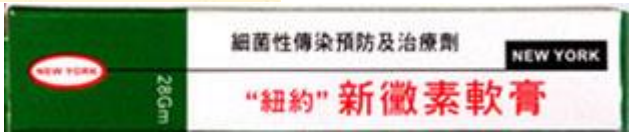
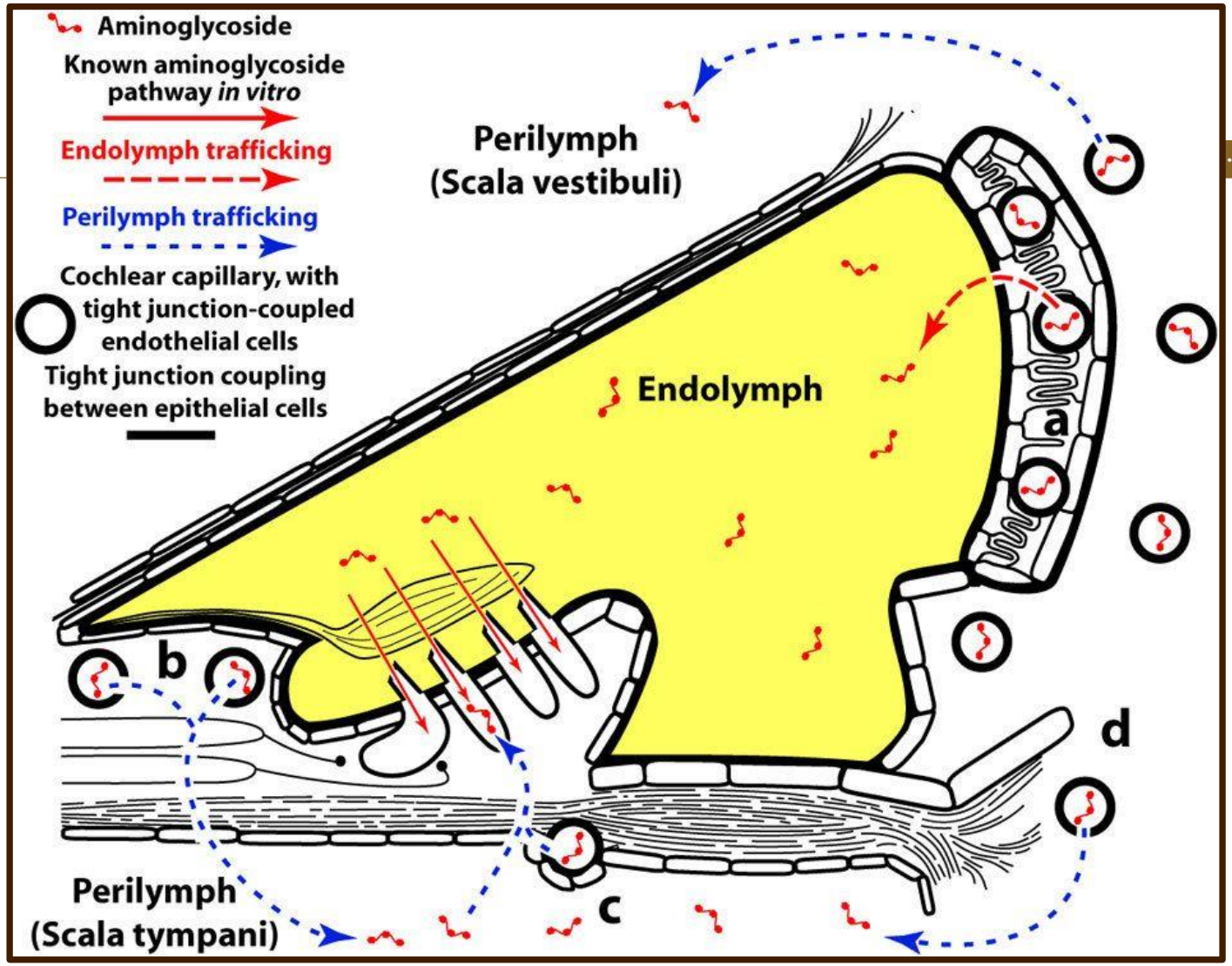


表2：藥物引起的耳毒性機轉

藥物	耳毒性機轉
胺基配糖體類藥	降低螺旋神經節細胞傳導電位由耳蝸到大腦
胺基配糖體類藥 抗腫瘤藥/化學治療藥	減少耳蝸內毛細胞數目
抗腫瘤藥/化學治療藥	改變粒線體功能，直接造成細胞死亡
抗腫瘤藥/化學治療藥 利尿劑 胺基配糖體類藥	降低內耳耳蝸內電位
利尿劑	改變內內耳淋巴液穩定
奎寧 水楊酸類藥	減少耳蝸內血流
水楊酸類藥	活化N-methyl-D-aspartate receptors

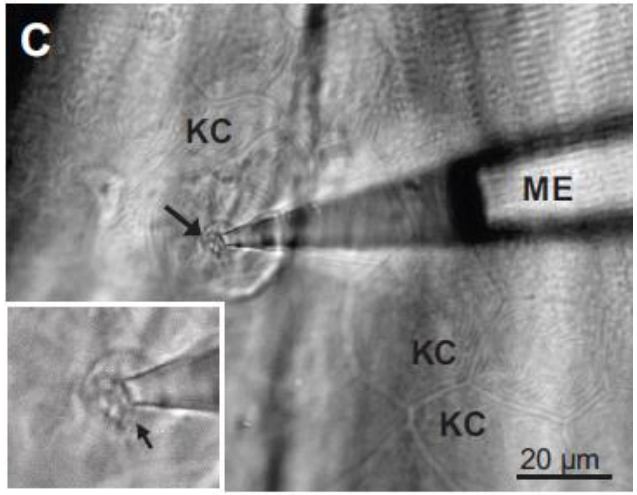
胺基糖酐類抗生素在內耳的循環

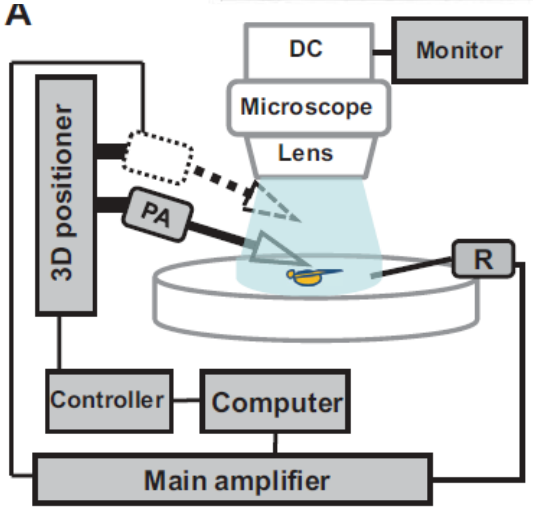


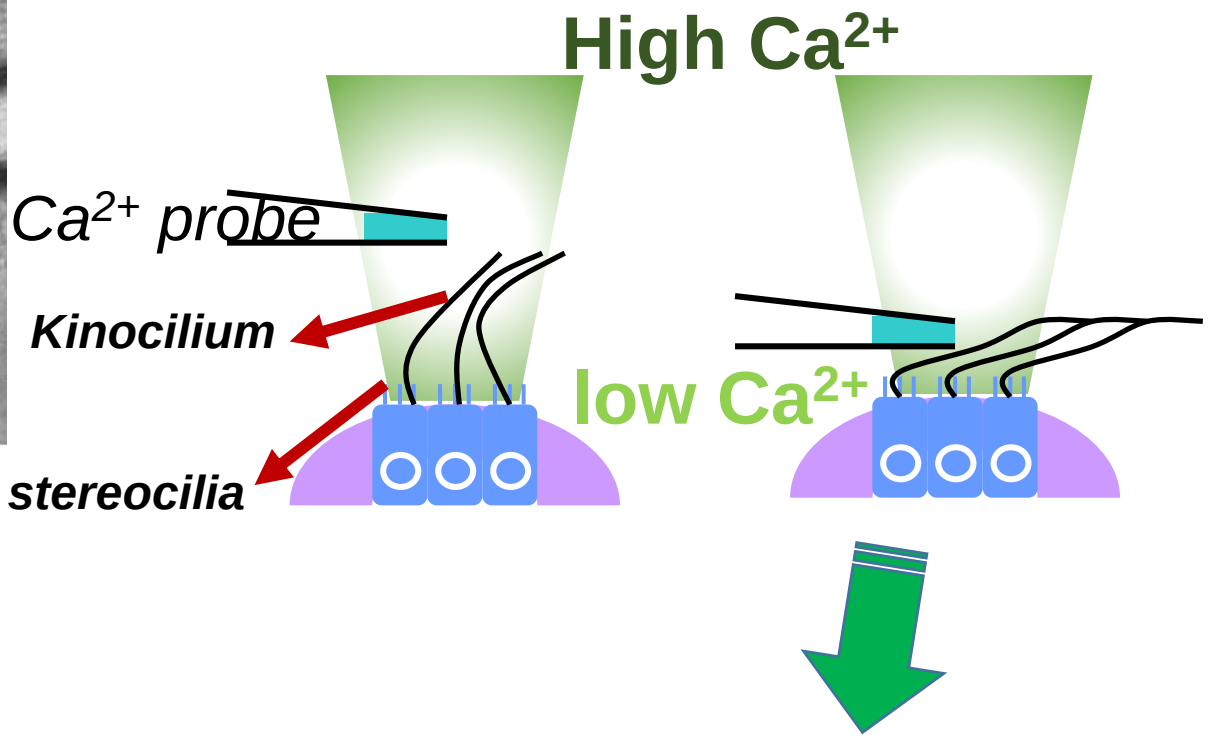
Li H, Steyger PS. Systemic aminoglycosides are trafficked via endolymph into cochlear hair cells. Sci Rep. 2011;1:159

Using NMT to analyze function of zebrafish neuromast hair cell

- **Non-invasive Micro-test Technology**
- **SIET** (noninvasive electrophysiological scanning ion-electrode technique)





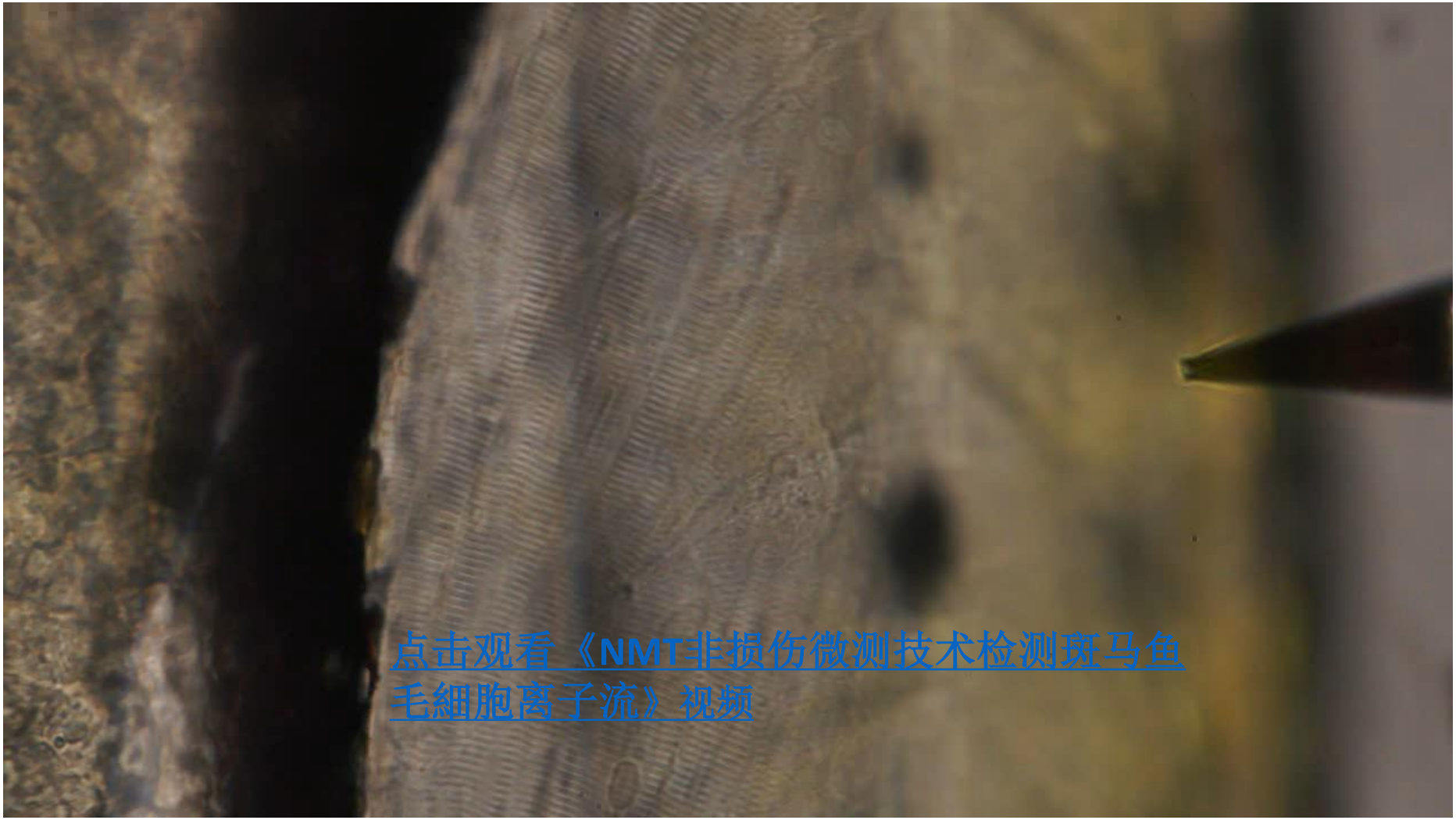


Ca^{2+} influx detected

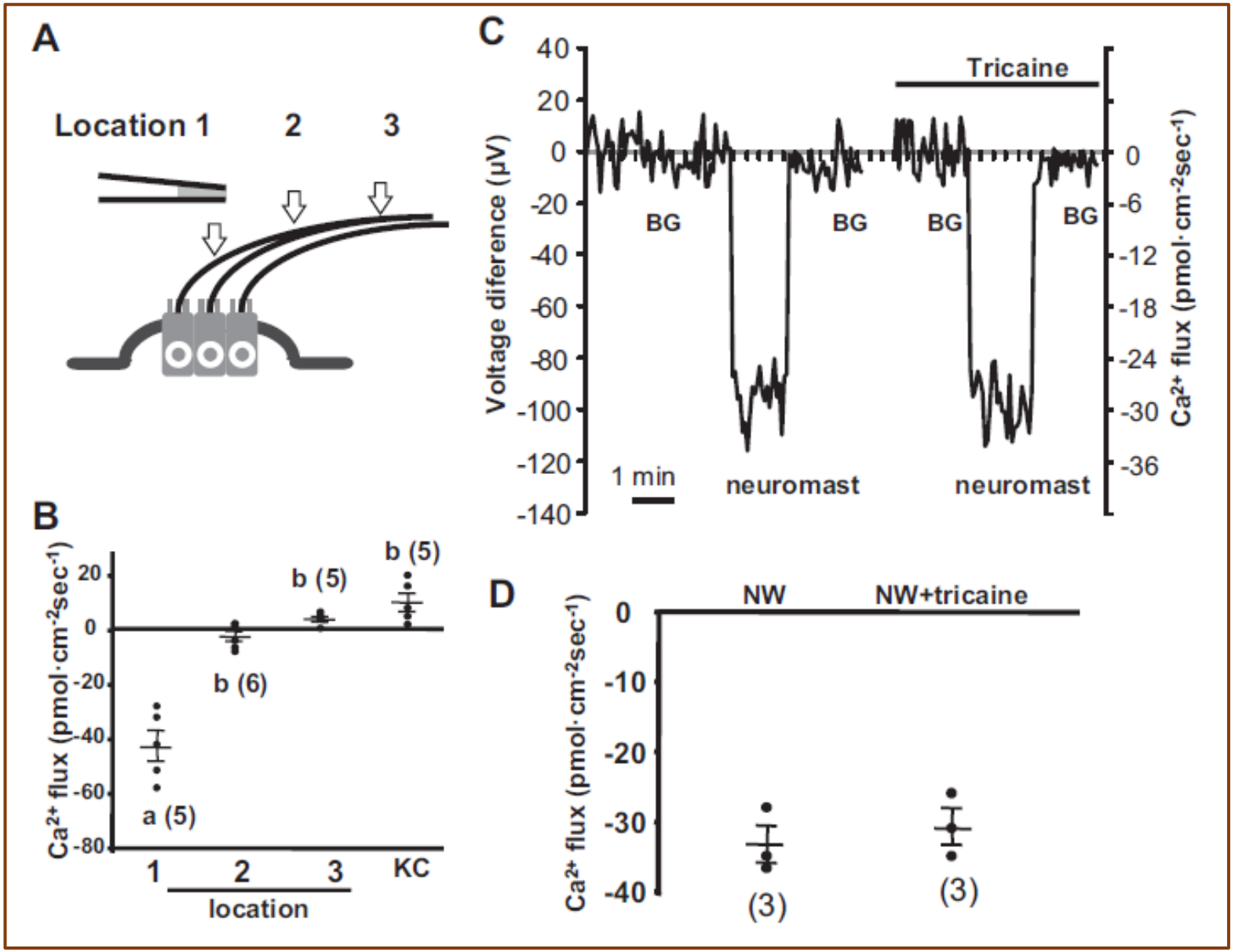
(Lin LY, et al. AJP 2013)

Using NMT to detect Ca^{2+} influx of zebrafish neuromast hair cell

https://www.youtube.com/watch?v=j5gw8mJraxI

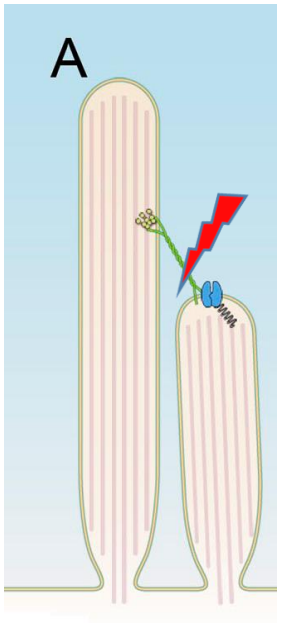


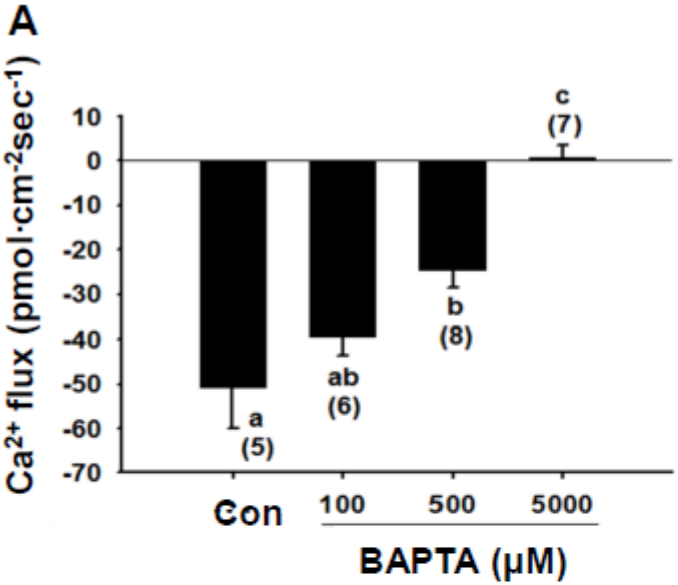
Detection of Ca^{2+} flux in neuromasts of zebrafish embryos

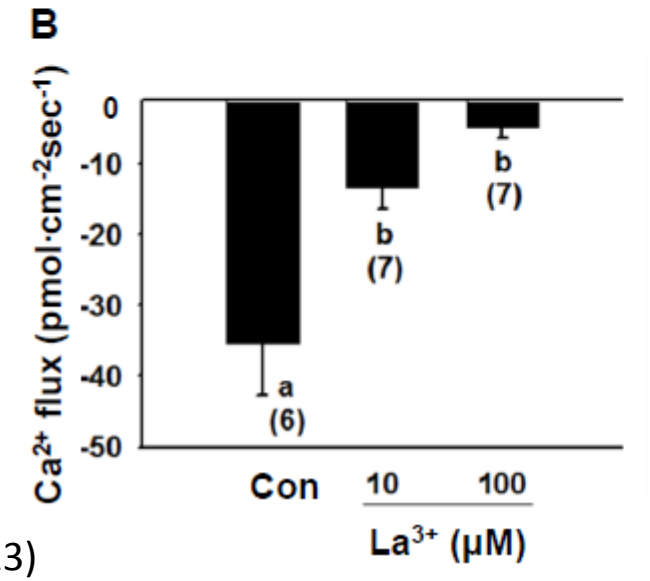


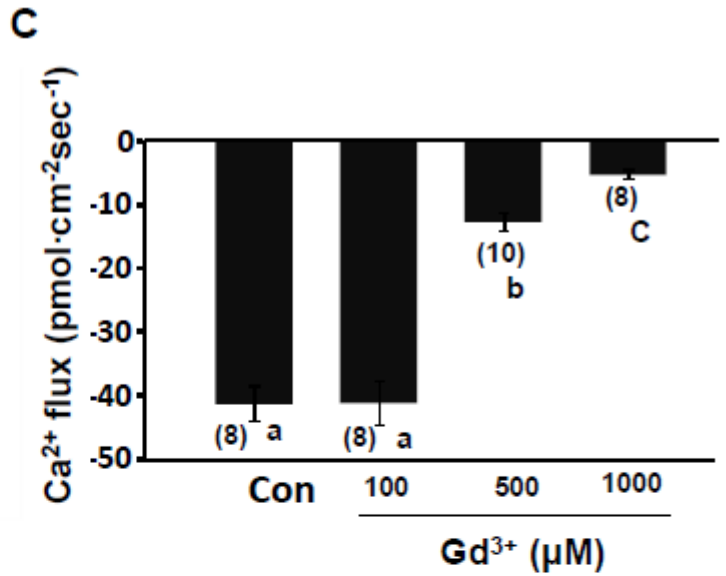
(Lin LY, et al. AJP 2013)

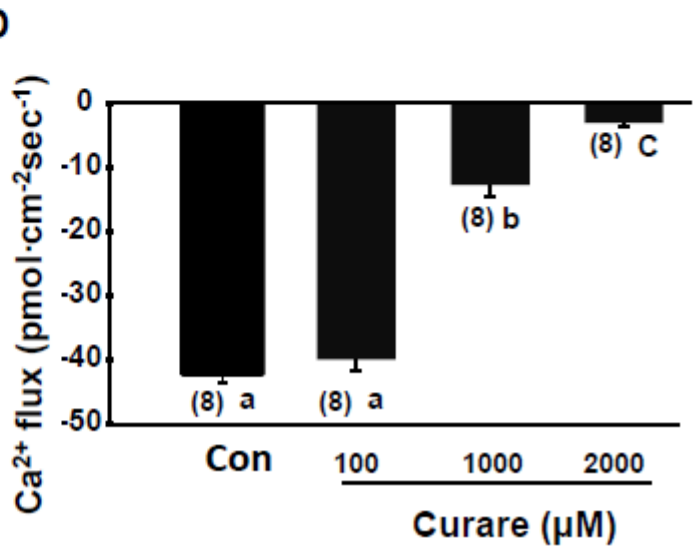
MET channel blockers inhibit MET channel mediated Ca^{2+} influx





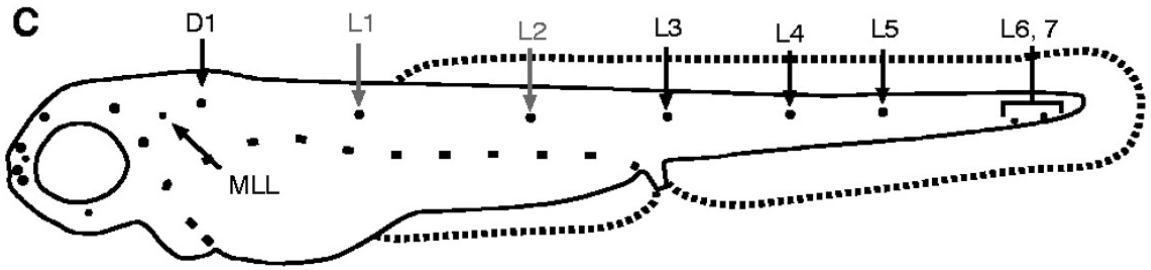


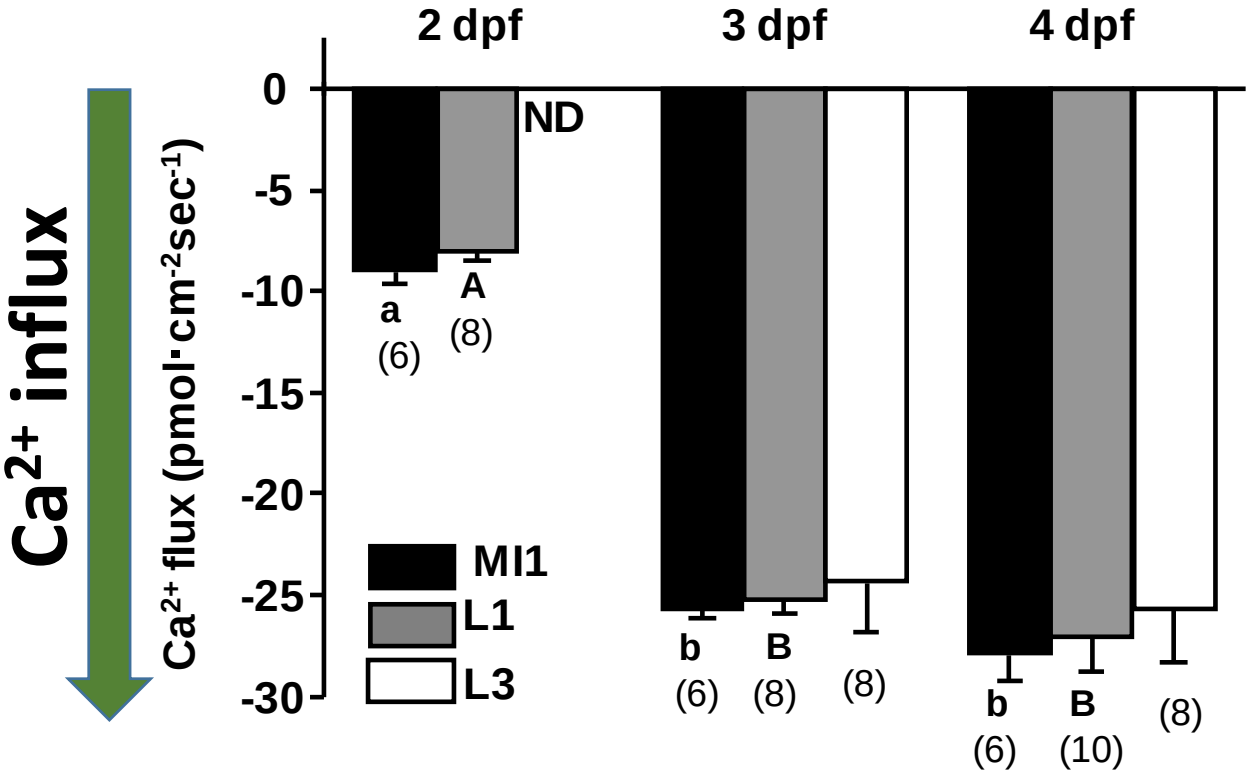


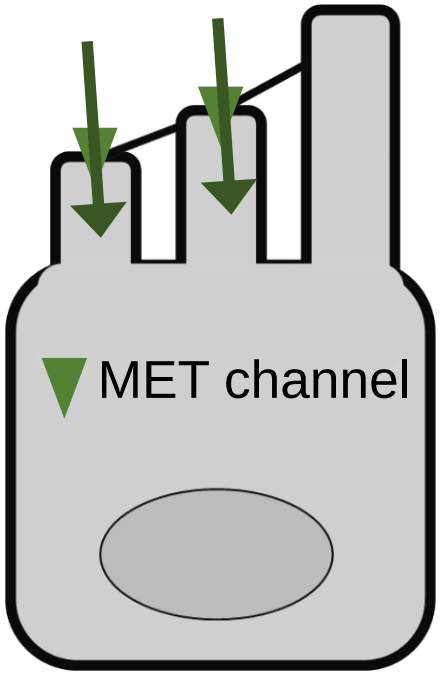


(Lin LY, et al. AJP 2013)

The MET channel mediates Ca^{2+} influx during embryonic development



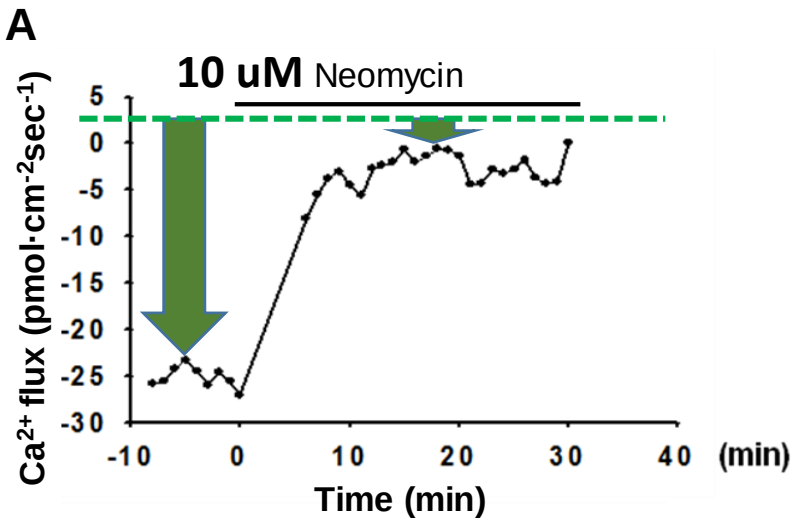


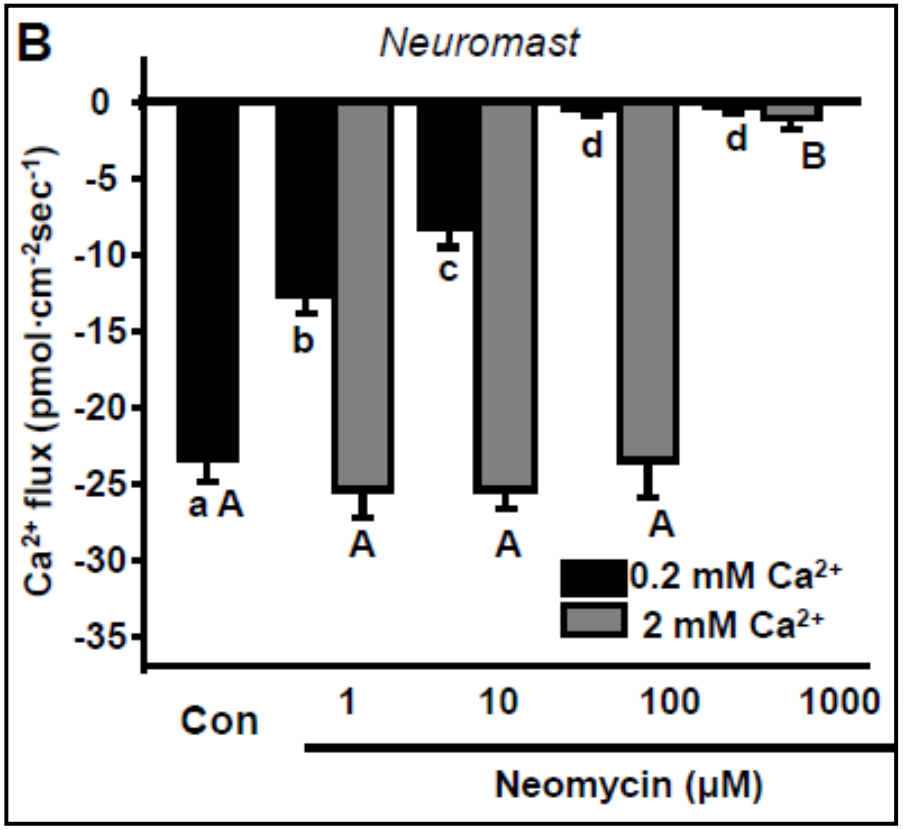


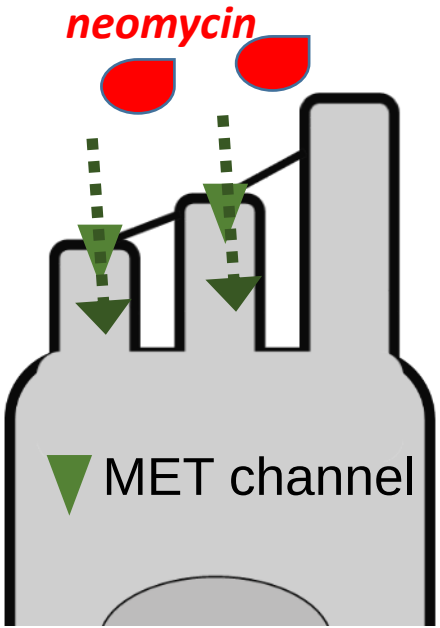
✓ **Functions of MI1, L1 and L3 were begin at 2 dpf**

(Lin LY, et al. AJP 2013)

Neomycin(新黴素) suppress MET channel mediated Ca^{2+} influx





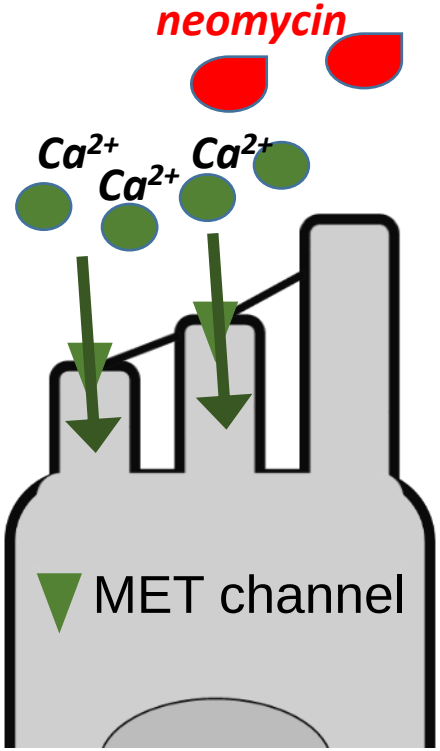


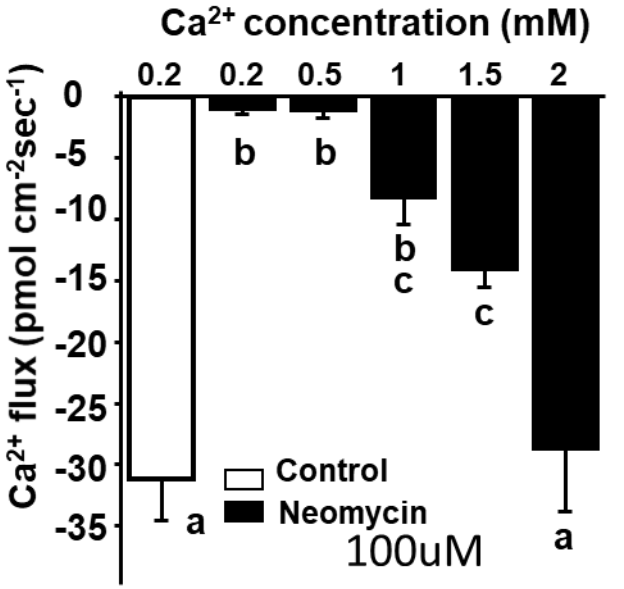
Neomycin	10 μM	24 h
	50, 100, 150, 200, 400 μM	1 h
	10, 50, 100, 125, 250, 300, 500 μM	1 h
	10 μM	1, 5 h
	100 μM	1 h
	25, 50, 75, 100, 200, 400 μM	30 min
	25, 50, 100, 200, 400 μM	1 h

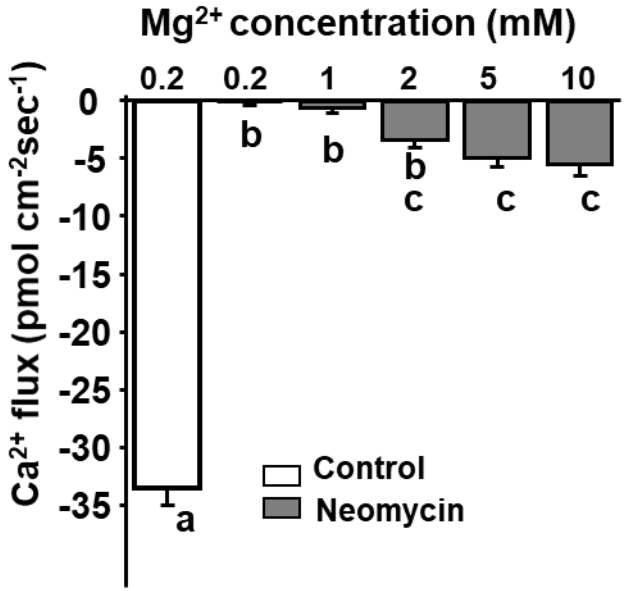
(Lin LY, et al. AJP 2013)

Addition of Ca^{2+} neutralized the inhibition of neomycin

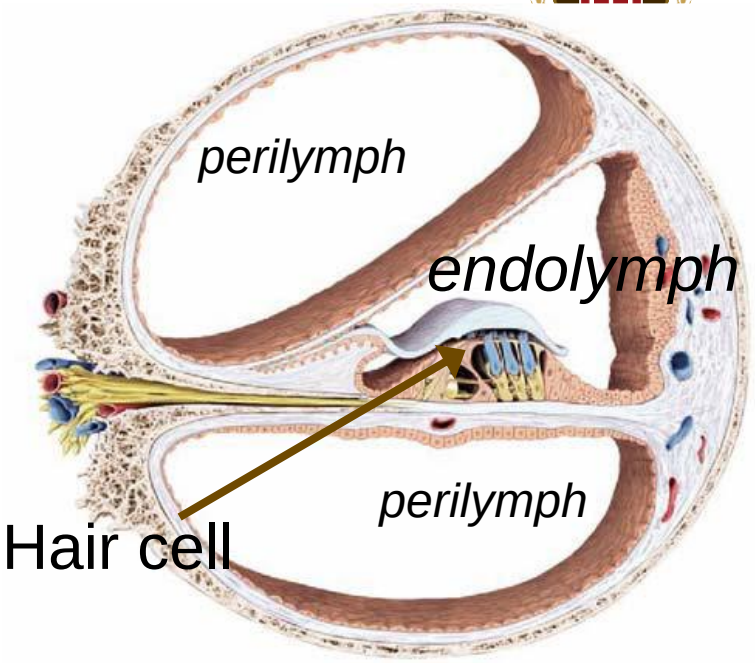
高鈣中和了新黴素對毛細胞機械性通道的抑制







(Lin LY, et al. AJP 2013)



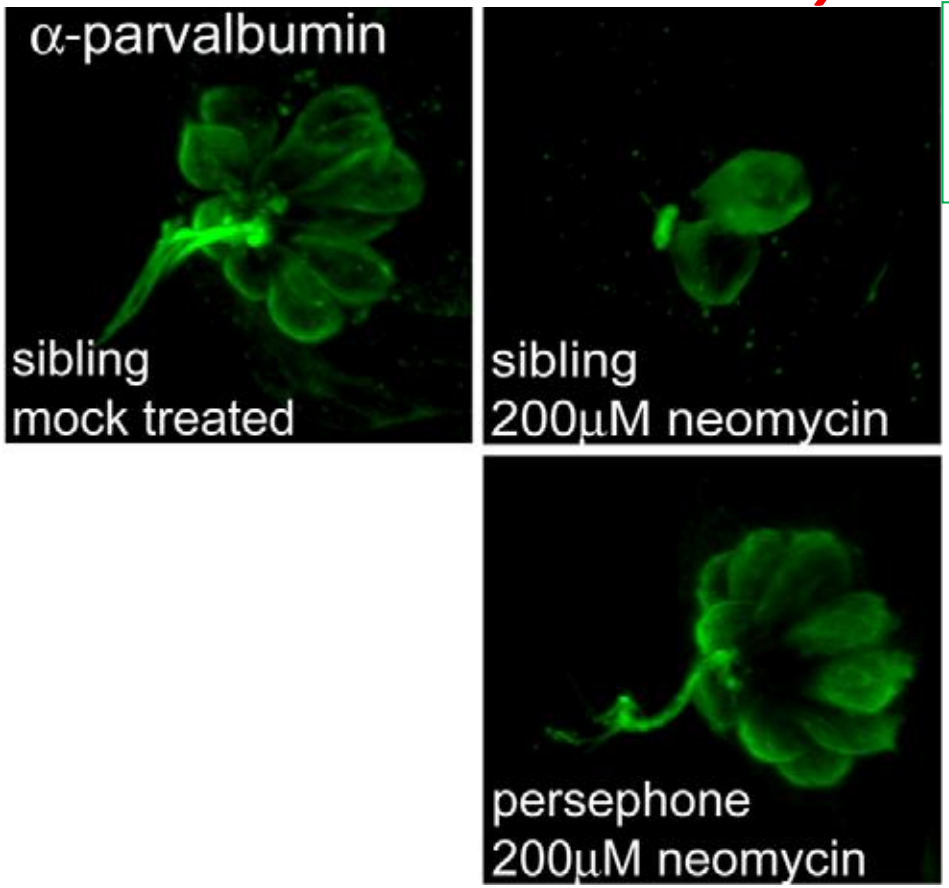
	FW	E3 embryo medium	SW	endolymph	plasma
Na+ (mM)	0.5	5	500	1.3	145
K+ (mM)	0.2	0.13	10	157	5
Ca2+ (mM)	0.2	0.5	10	0.02	2.6
Cl- (mM)	0.5	5	500	132	106
HCO3- (mM)	0.2	0	1.6	31	18
pH	7	7	8	7.4	7.3

PLoS Genet. 2012;8(10):e1002971. doi: 10.1371/journal.pgen.1002971. Epub 2012 Oct 11.

Loss of Slc4a1b chloride/bicarbonate exchanger function protects mechanosensory hair cells from aminoglycoside damage in the zebrafish mutant persephone.

Hailey DW[1], Roberts B, Owens KN, Stewart AK, Linbo T, Pujol R, Alper SL, Rubel EW, Raible DW.

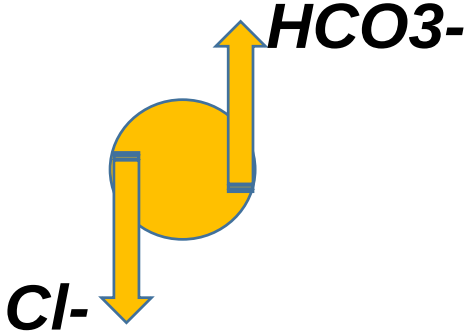
WT **WT + neomycin**



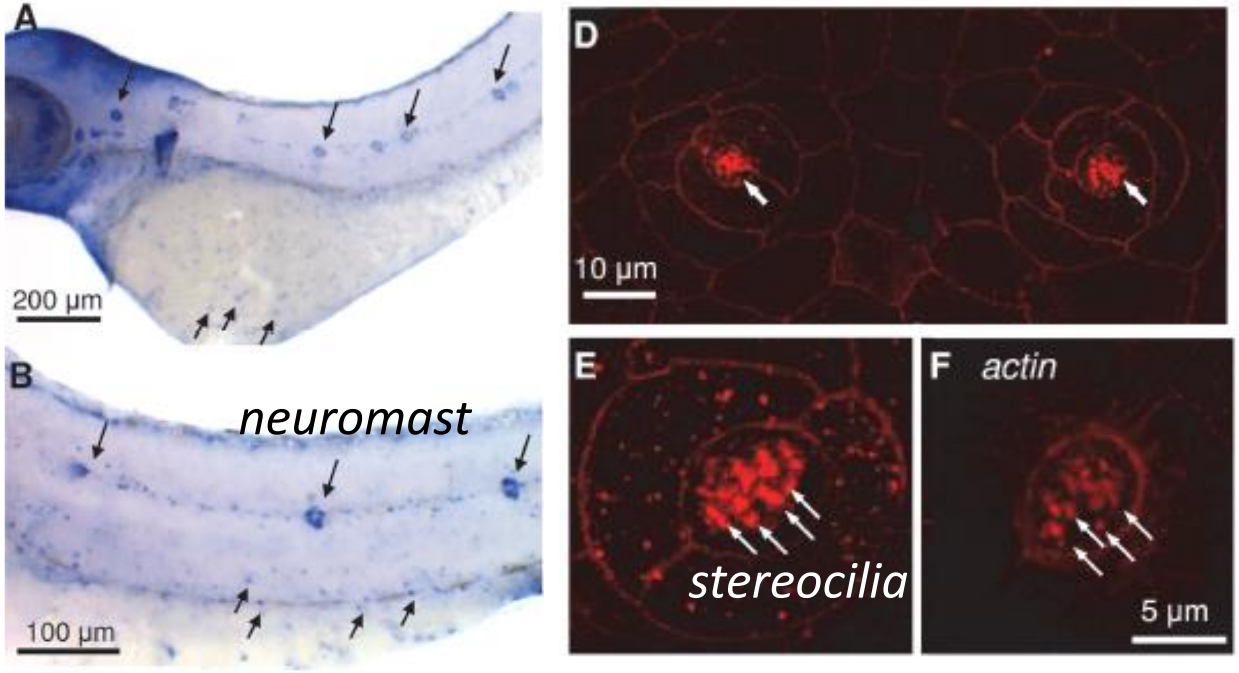
AE1b mutant + neomycin

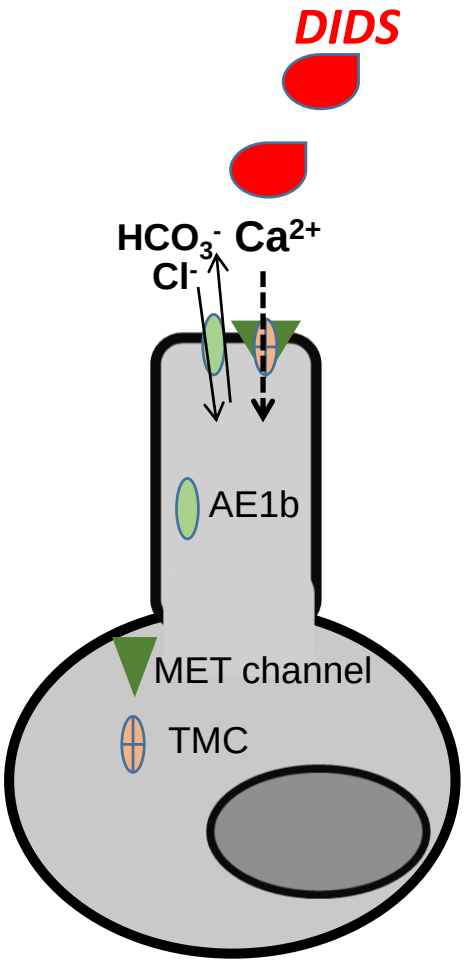
Anion exchanger (AE1b)

陰離子交換蛋白

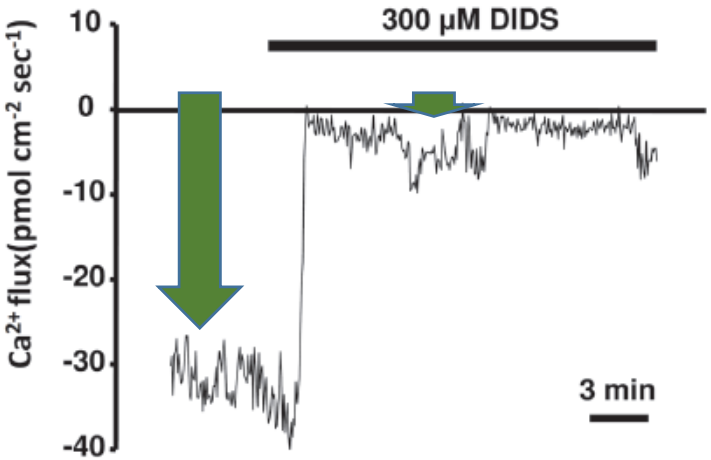


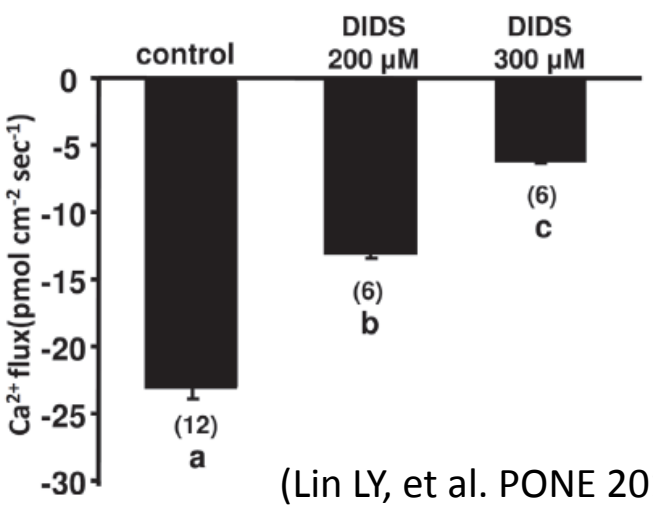
Expression of AE1b in stereocilia of hair cell





AE1b inhibitor, DIDS, supresses hair cell Ca^{2+} influx

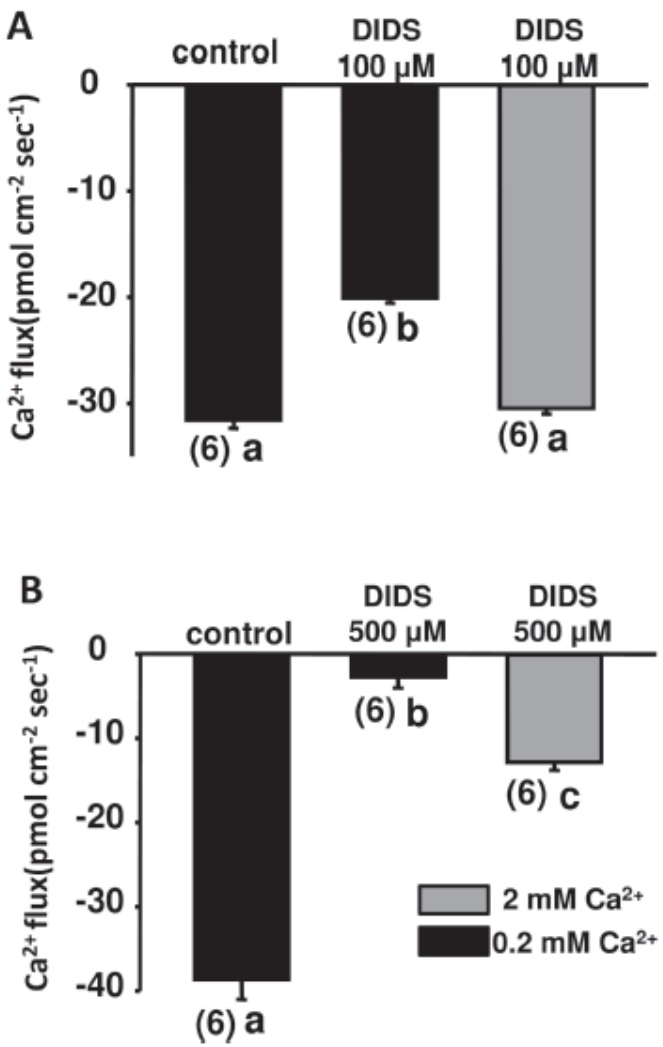




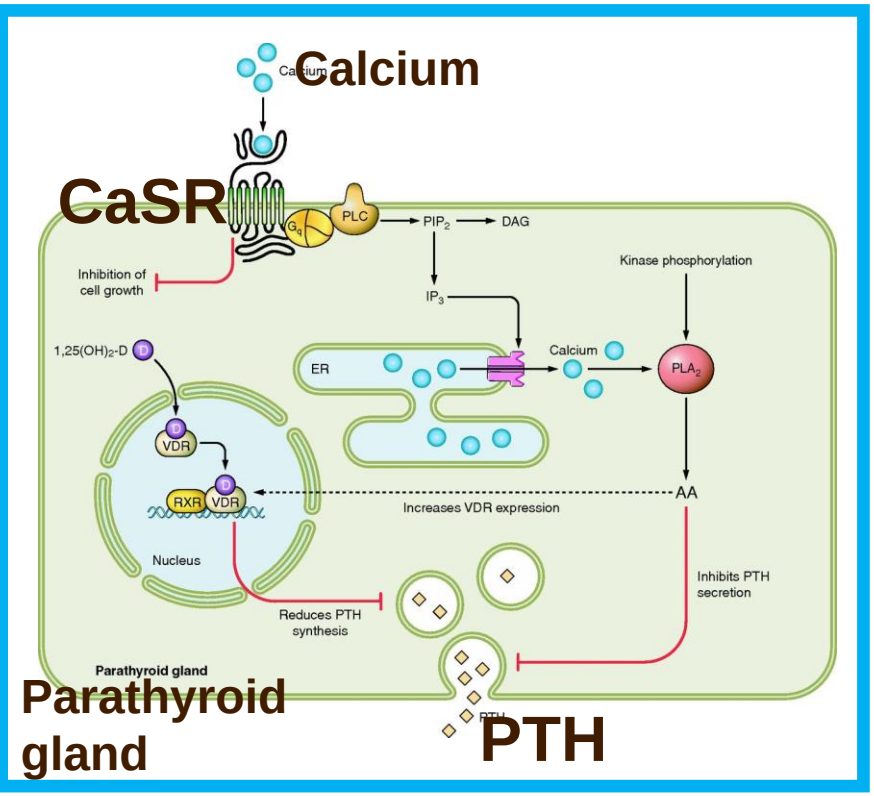
(Lin LY, et al. PONE 2015)

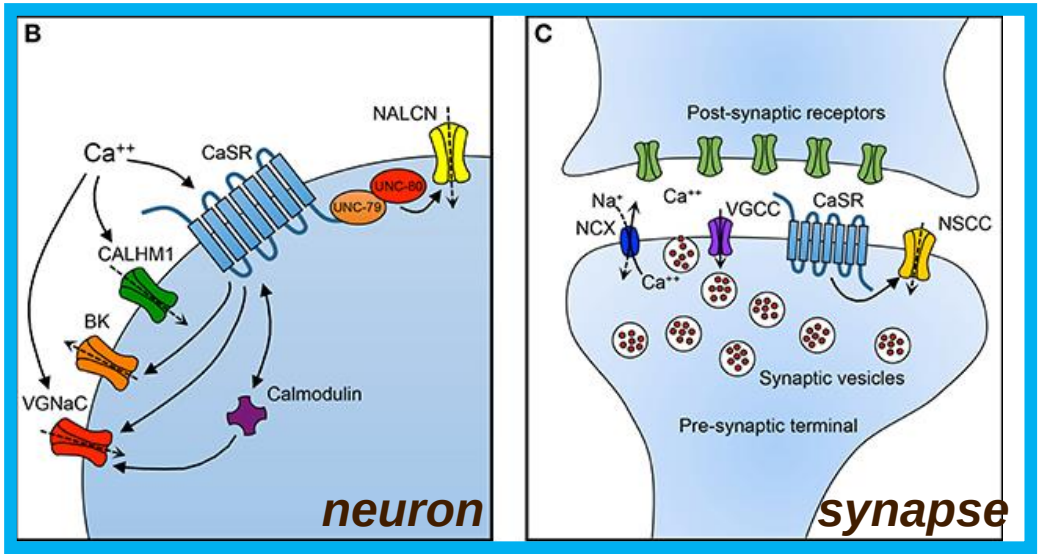
Addition of Ca^{2+} neutralized the inhibition of DIDS

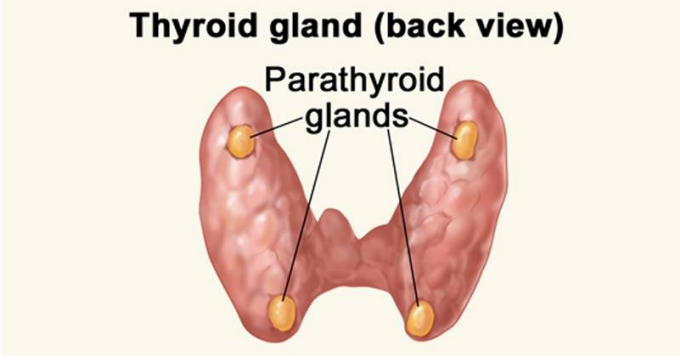
- Extracellular Ca^{2+} is important for function of MET channel
- How are hair cells sense environmental Ca^{2+}?



Ca^{2+} sensing receptor (CaSR)鈣離子感測器
-highly expressed in parathyroid gland and kidney

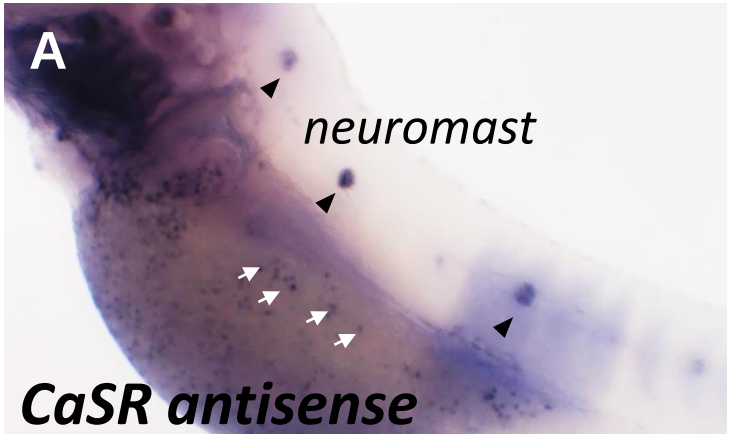


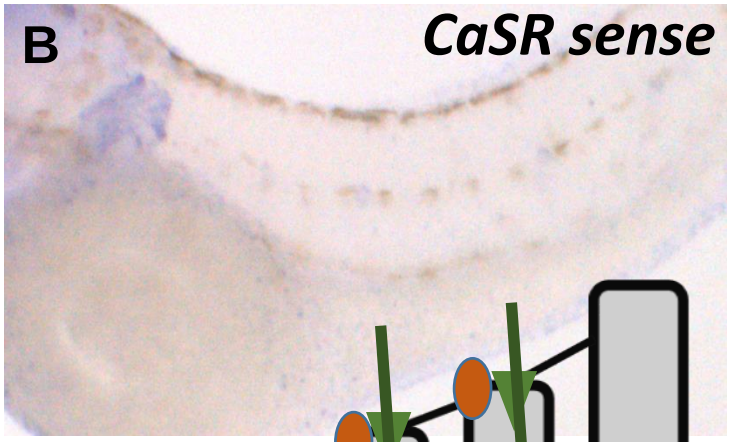


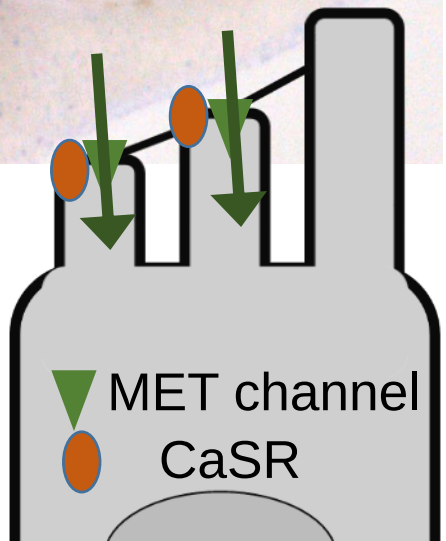


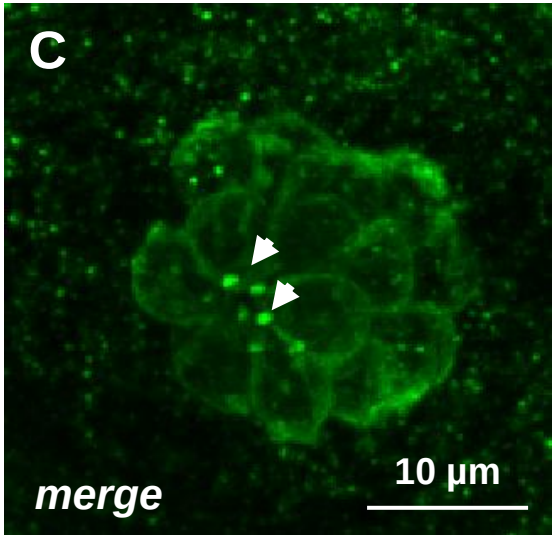
Goltzman et al. Nature Re. 2015

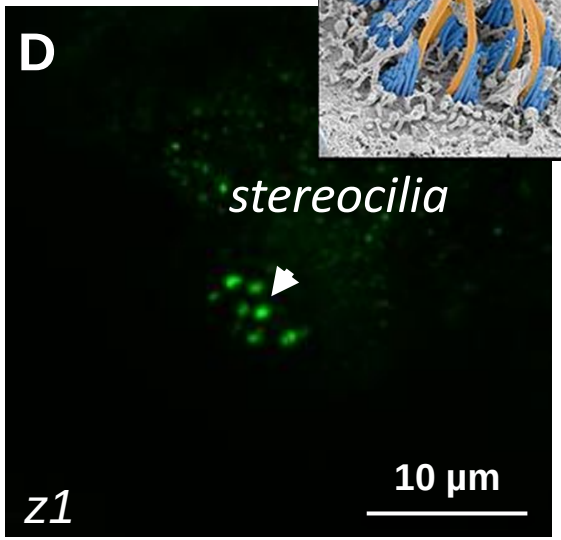
Expression of Ca^{2+} sensing receptor in zebrafish hair cell

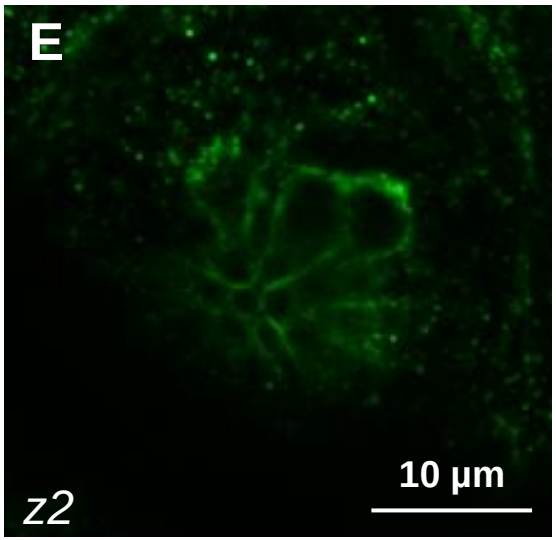


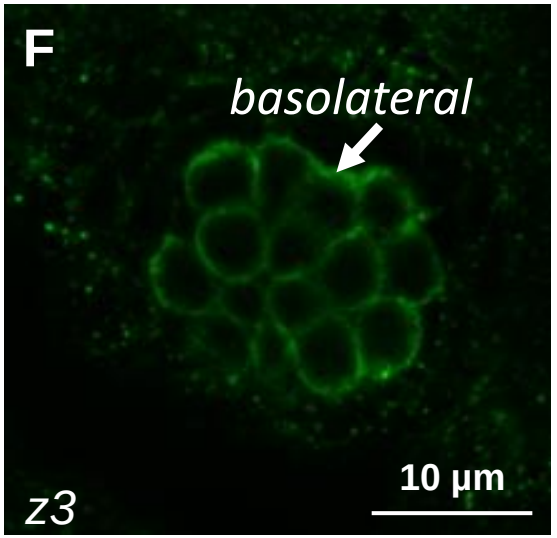


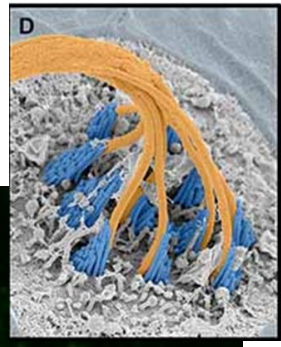






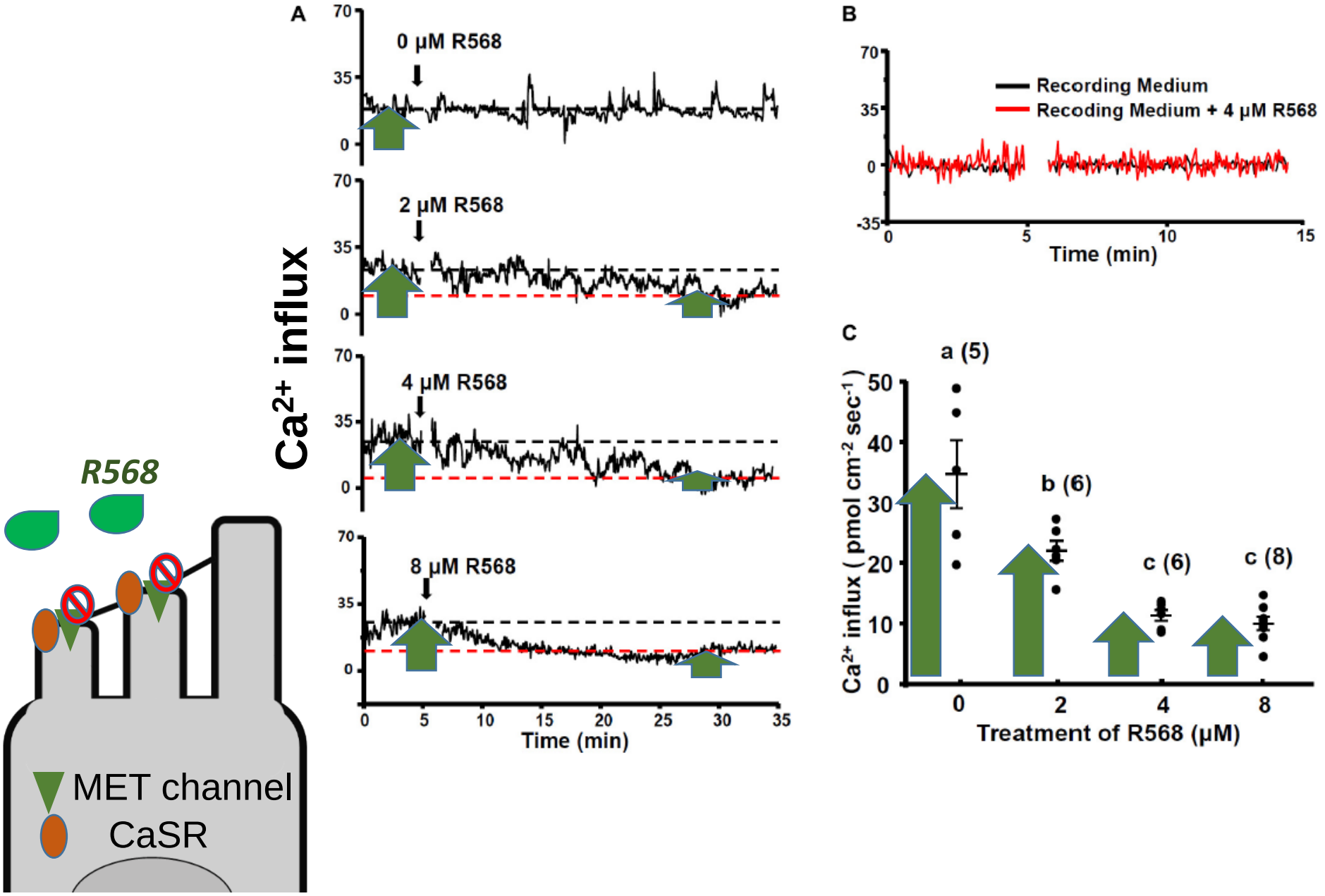




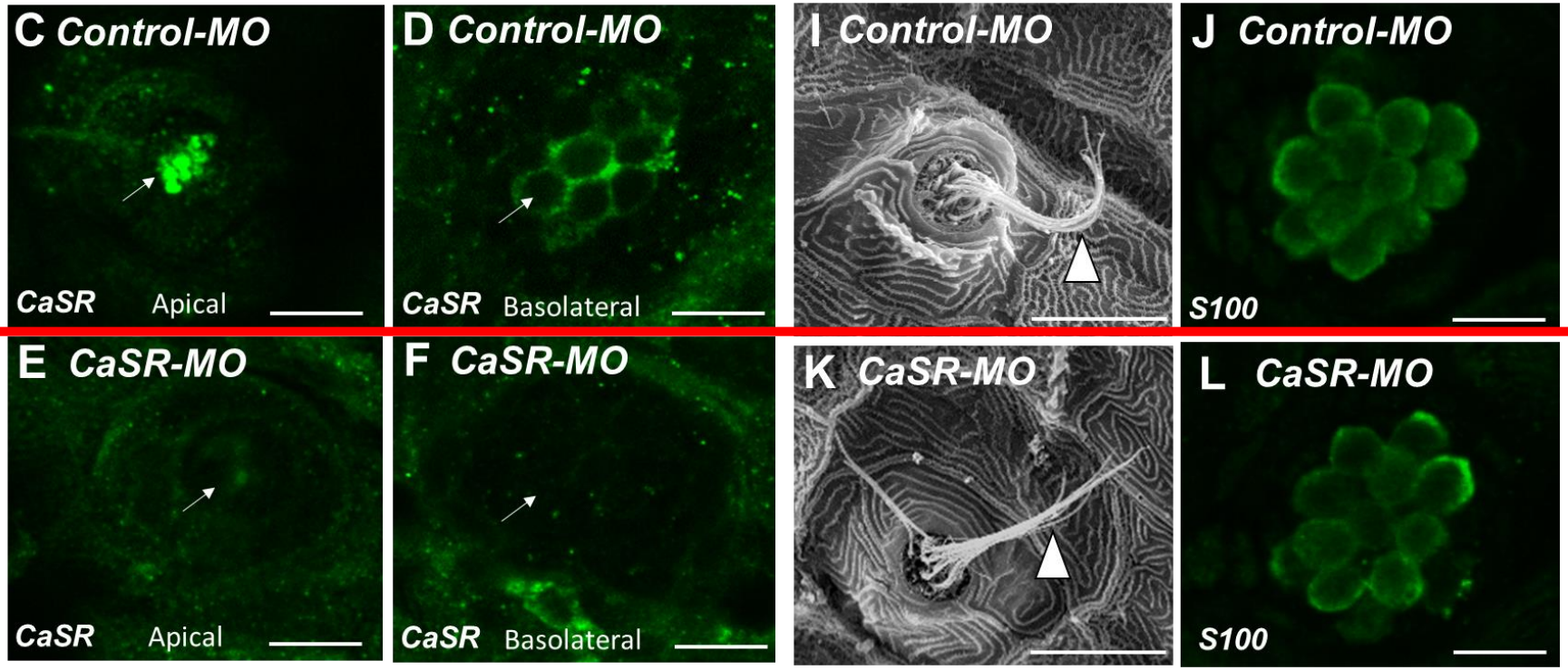


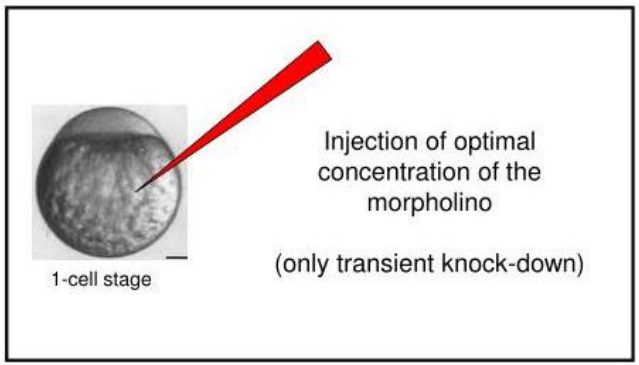
Lin LY, et al. Frontier Physiol 2018

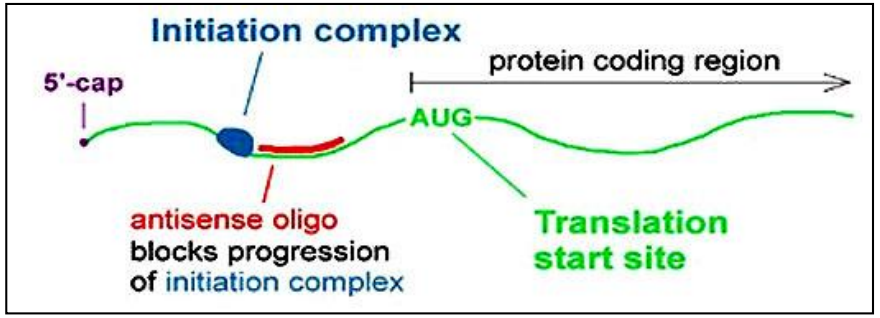
R-568 (CaSR agonist) suppress Ca^{2+} influx of neuromasts.



CaSR protein expression in neuromast hair cell is reduced in CaSR morphant







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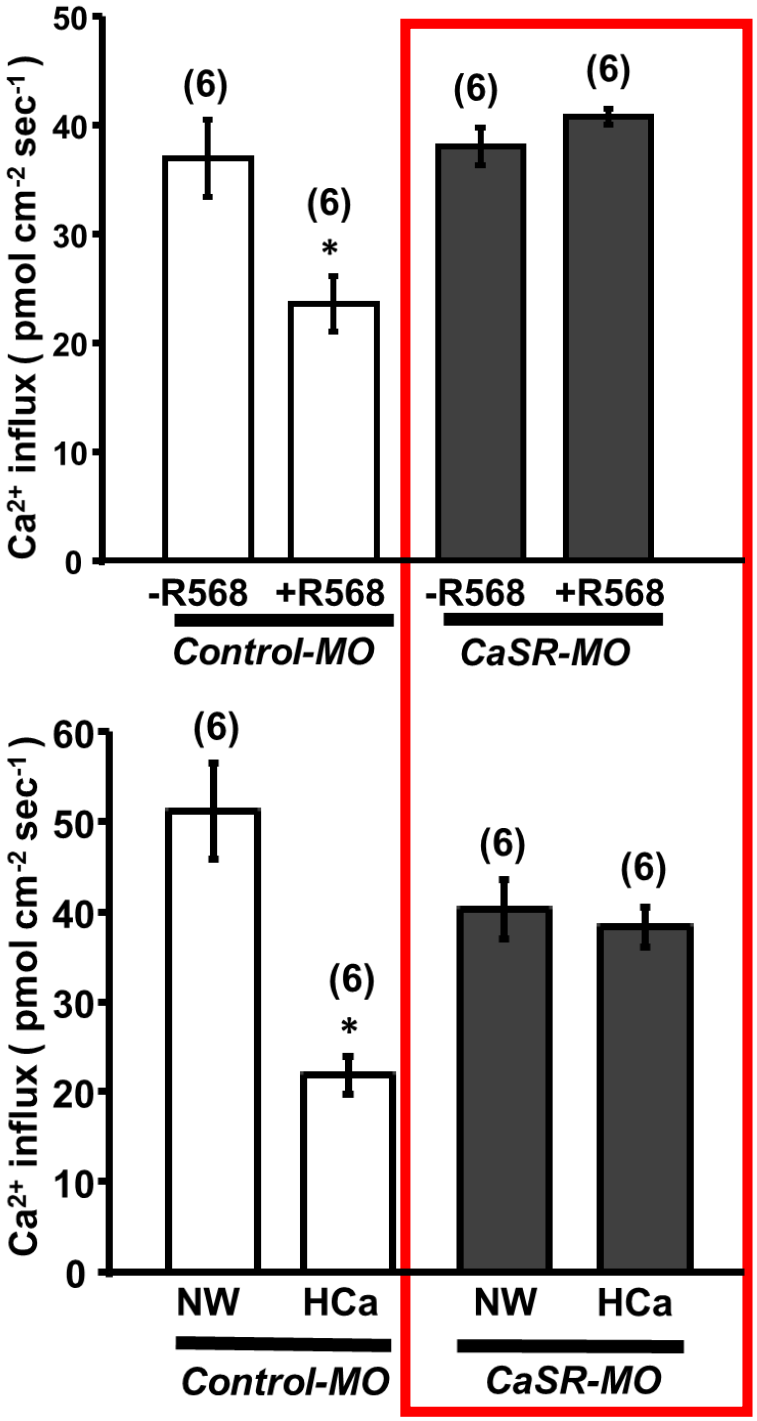
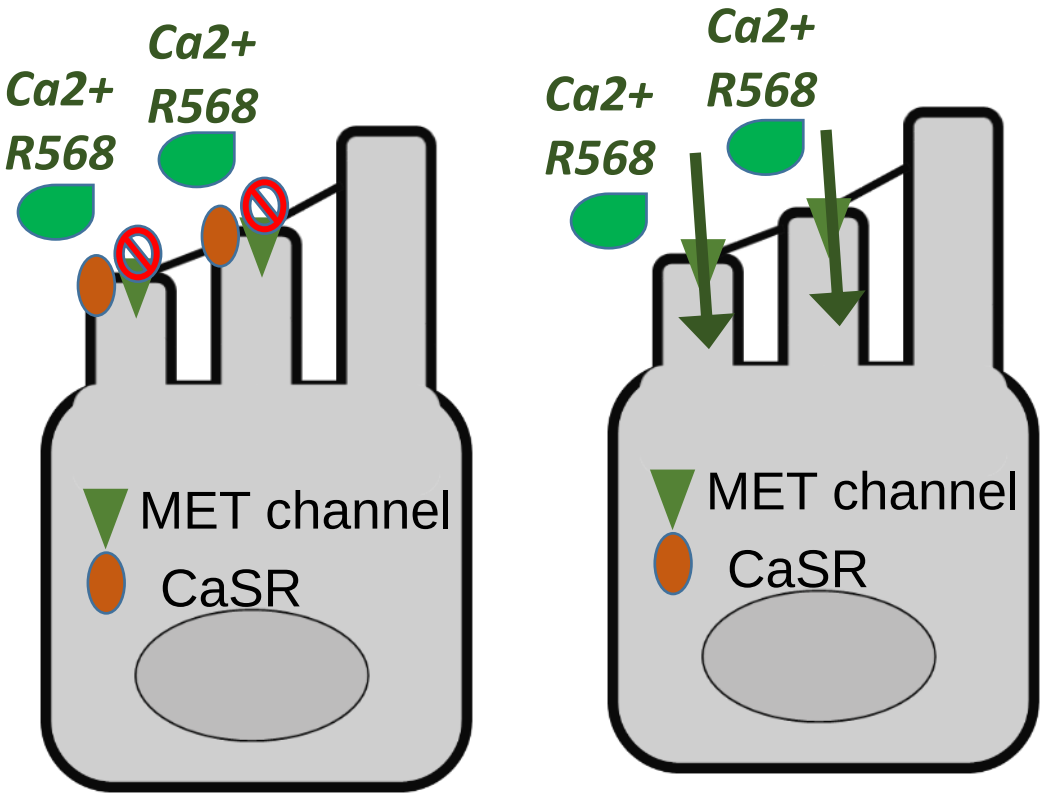


Effects of R-568 and HCa (0.2→2 mM) on MET channel mediated Ca^{2+} influx are neutralized in CaSR MO larvae.



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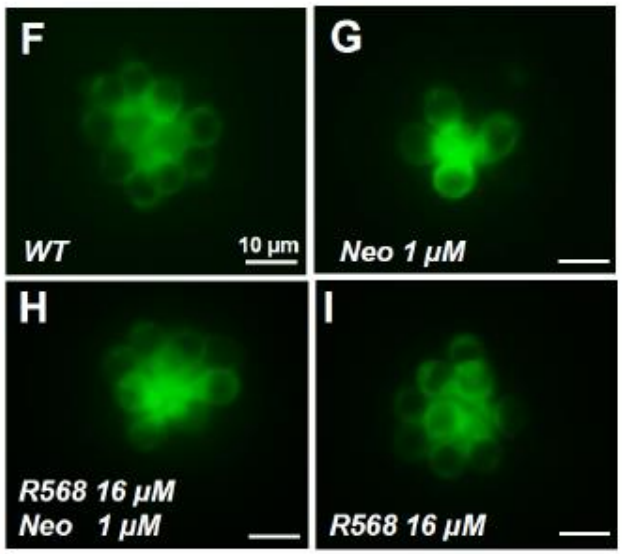
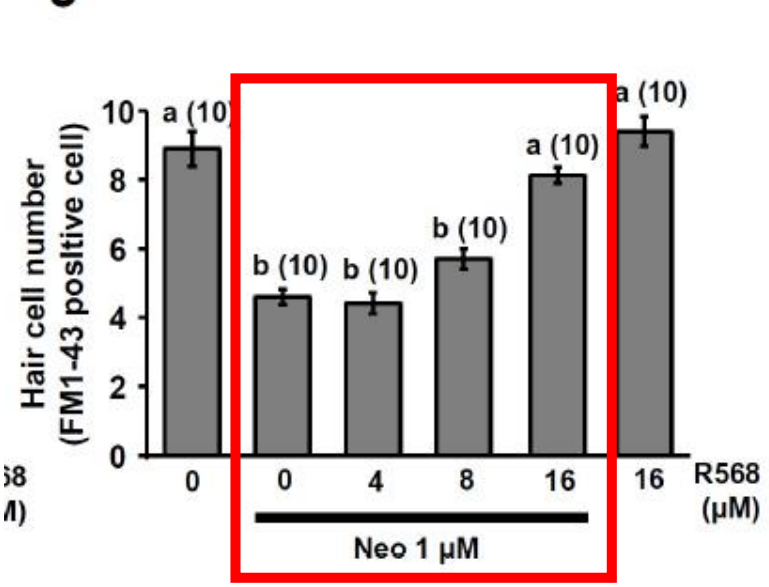
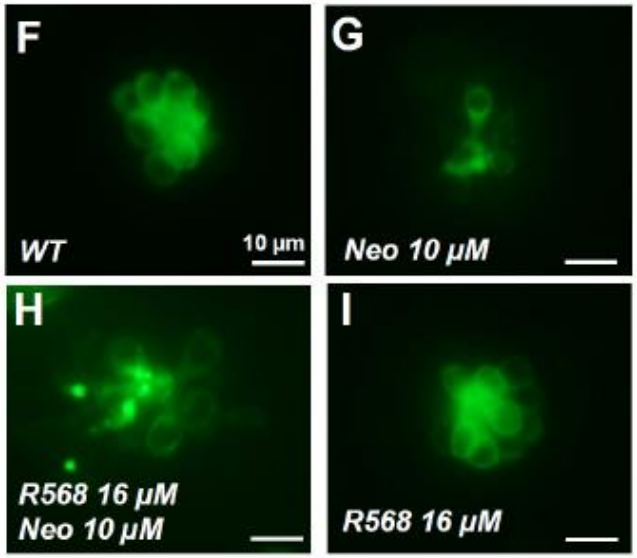
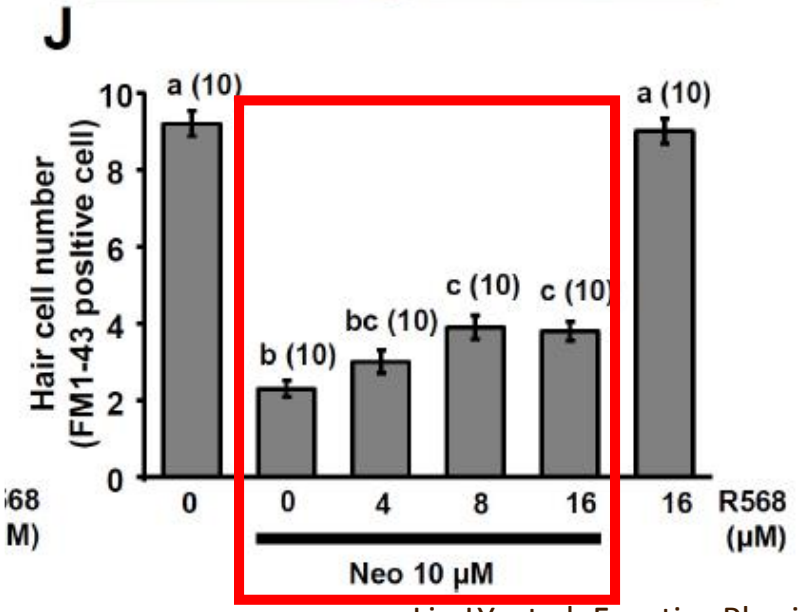
The addition of R-568 partial neutralize the neuromast hair cell death induced by neomycin





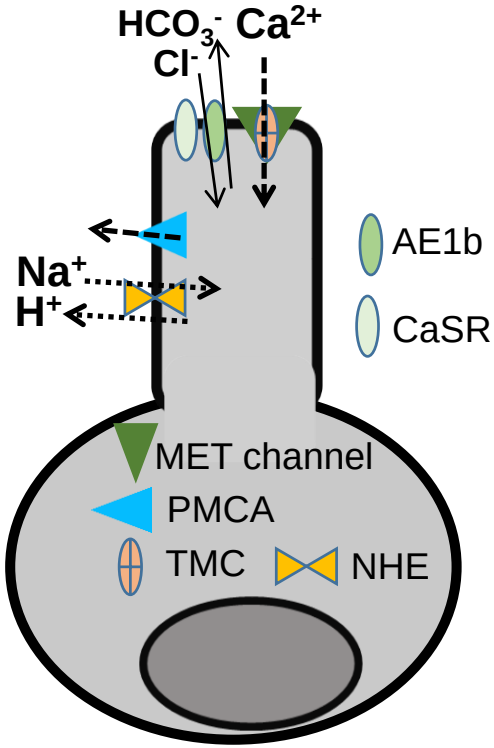


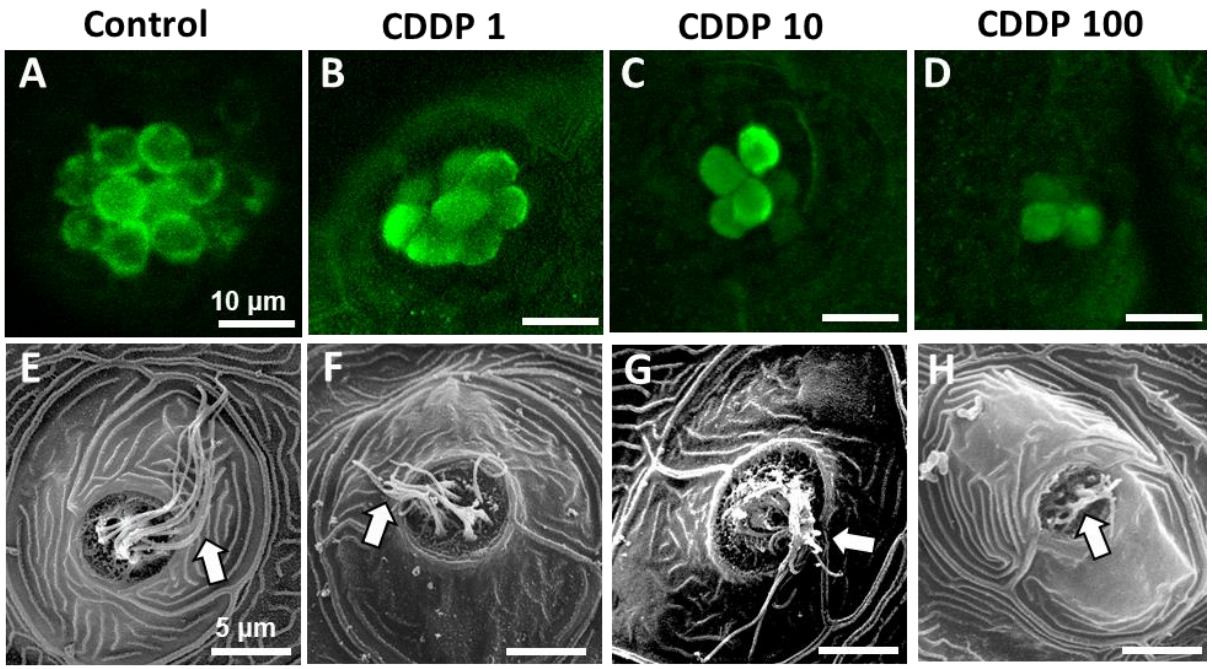


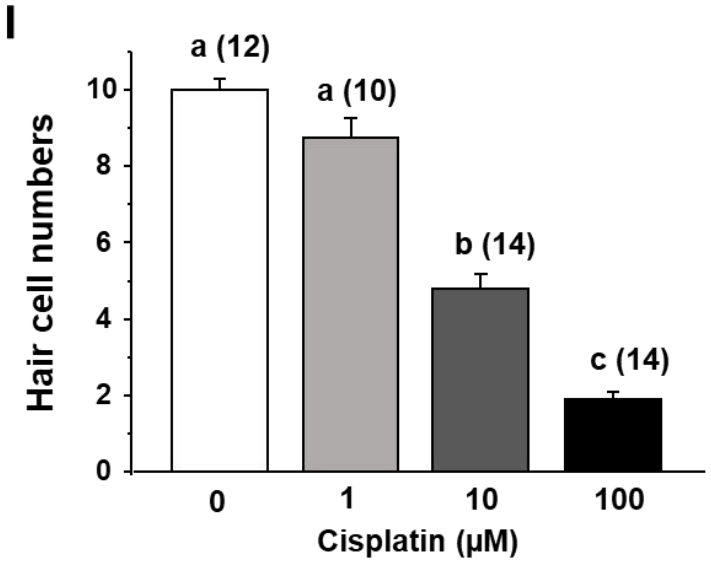
Lin LY, et al. Frontier Physiol 2018

Summary

- 建立以NMT偵測毛細胞MET channel的功能
- 胞外鈣離子可以保護MET channel功能
- CaSR偵測環境鈣濃度並調節MET channel的功能

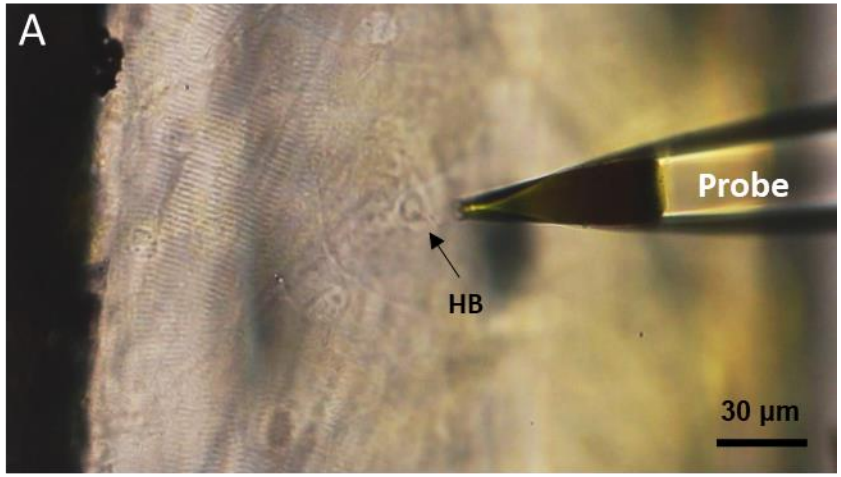


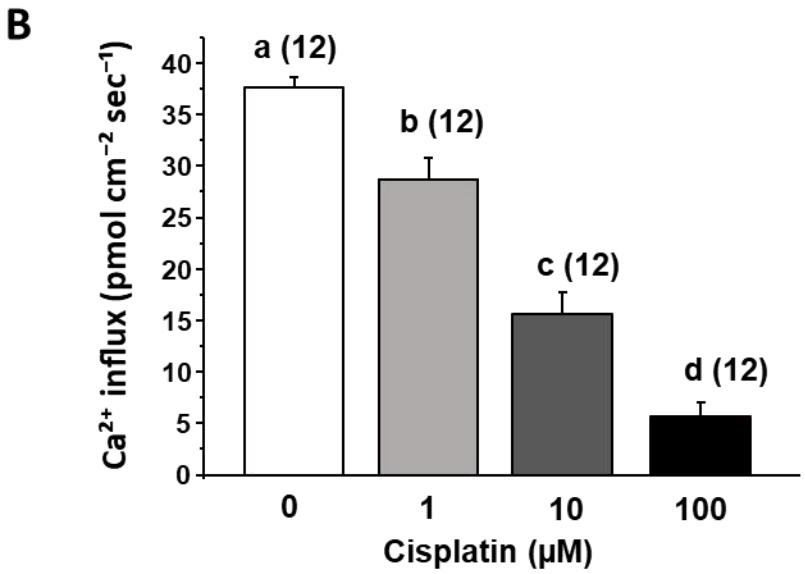


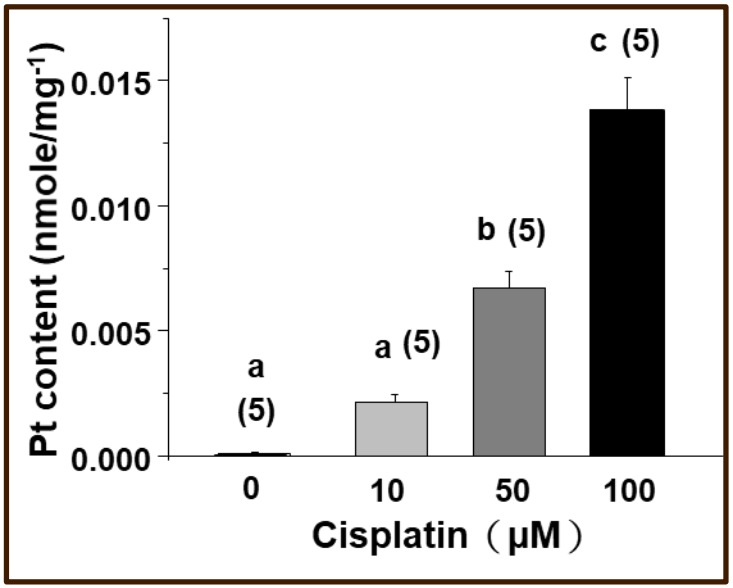


Effects of cisplatin on the hair cell number and function in zebrafish embryos

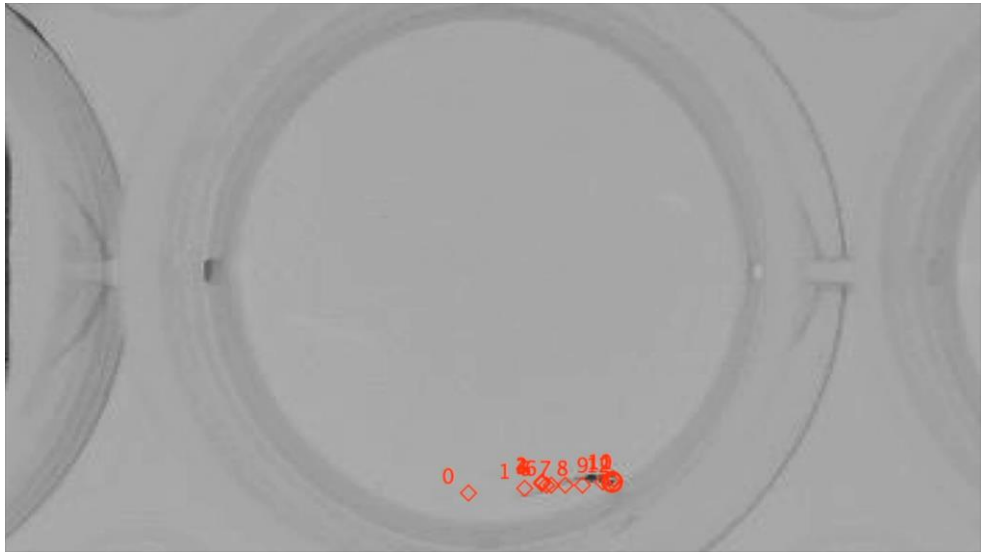
Effects of cisplatin on the hair cell number and function in zebrafish embryos

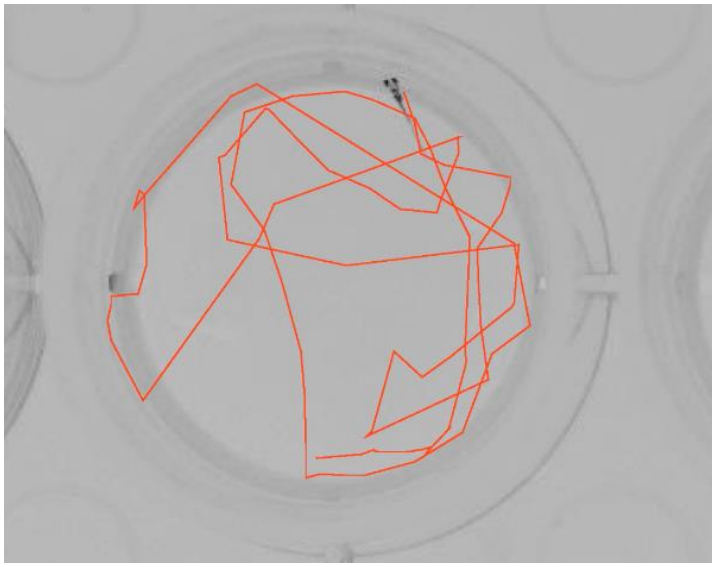




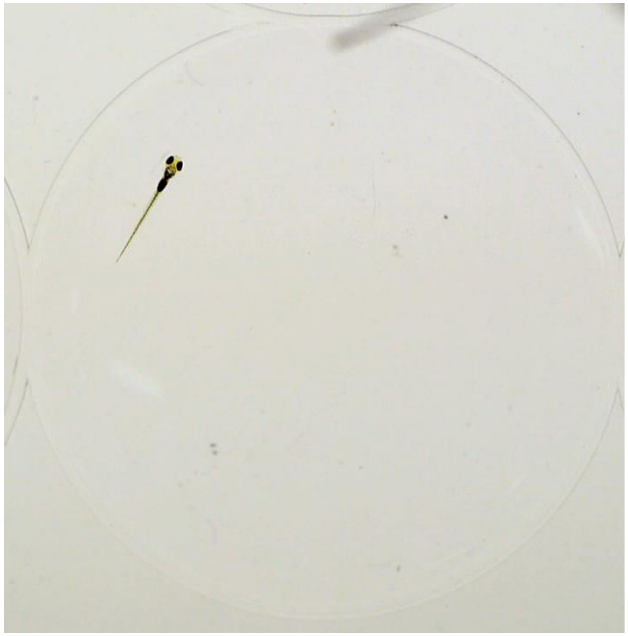


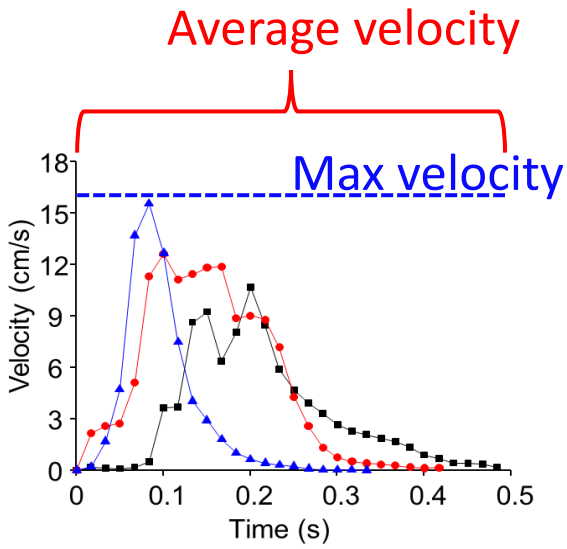
活動力分析(運動軌跡)



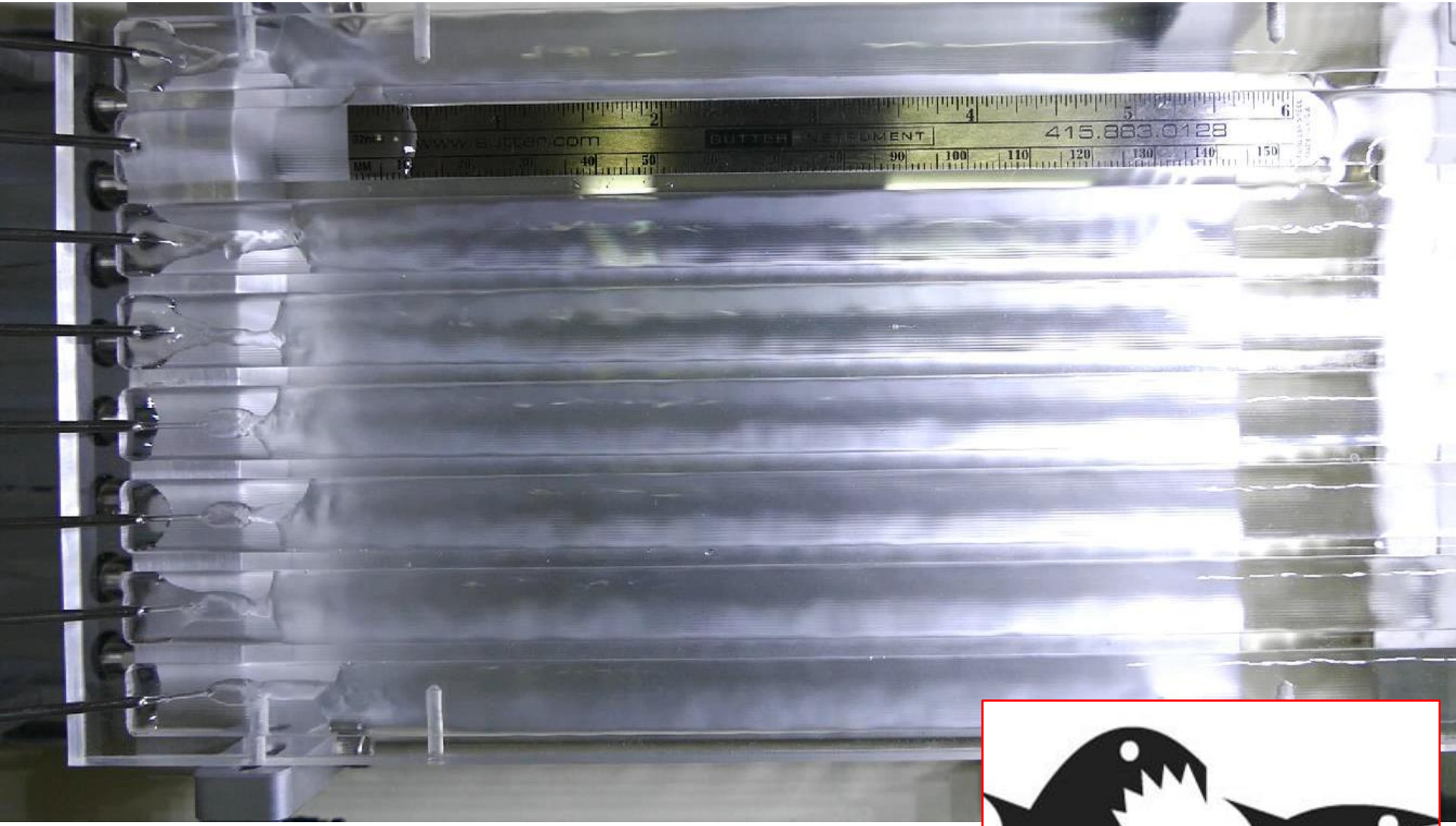


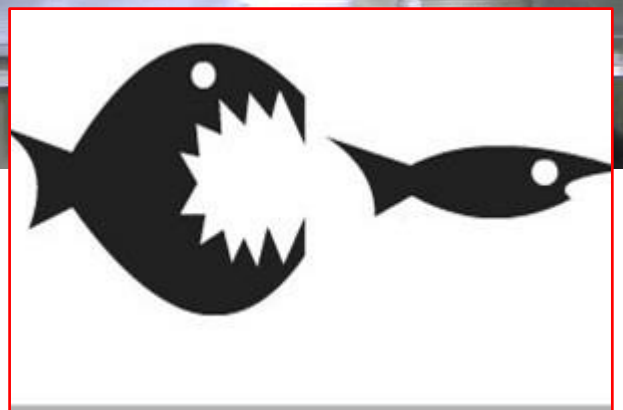
逃避運動速度分析



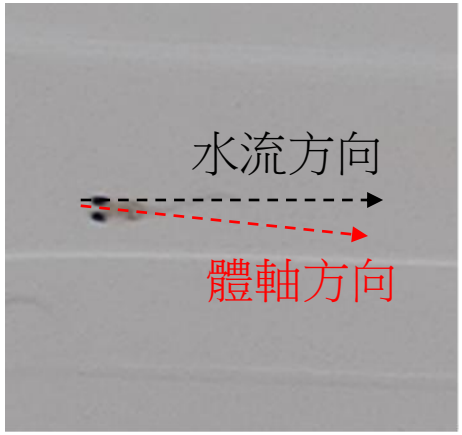


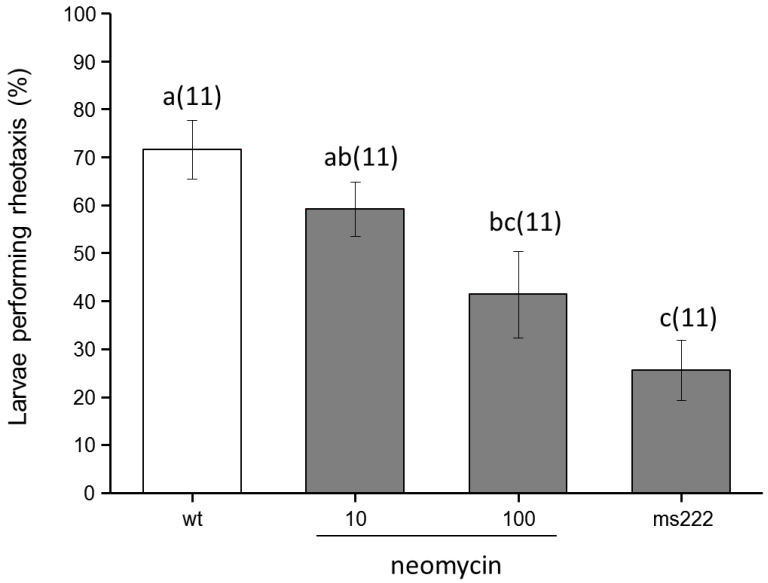
逆流行為分析

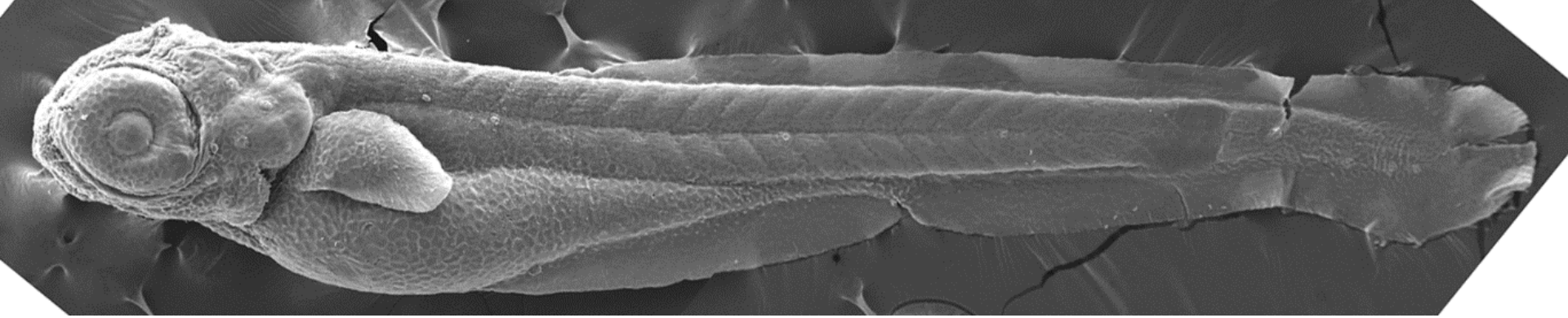




逆流行為分析


水流方向
體軸方向


Larvae performing rheotaxis (%)
100
90
80
70
60
50
40
30
20
10
0
a(11)
ab(11)
bc(11)
c(11)
wt
10
100
ms222
neomycin



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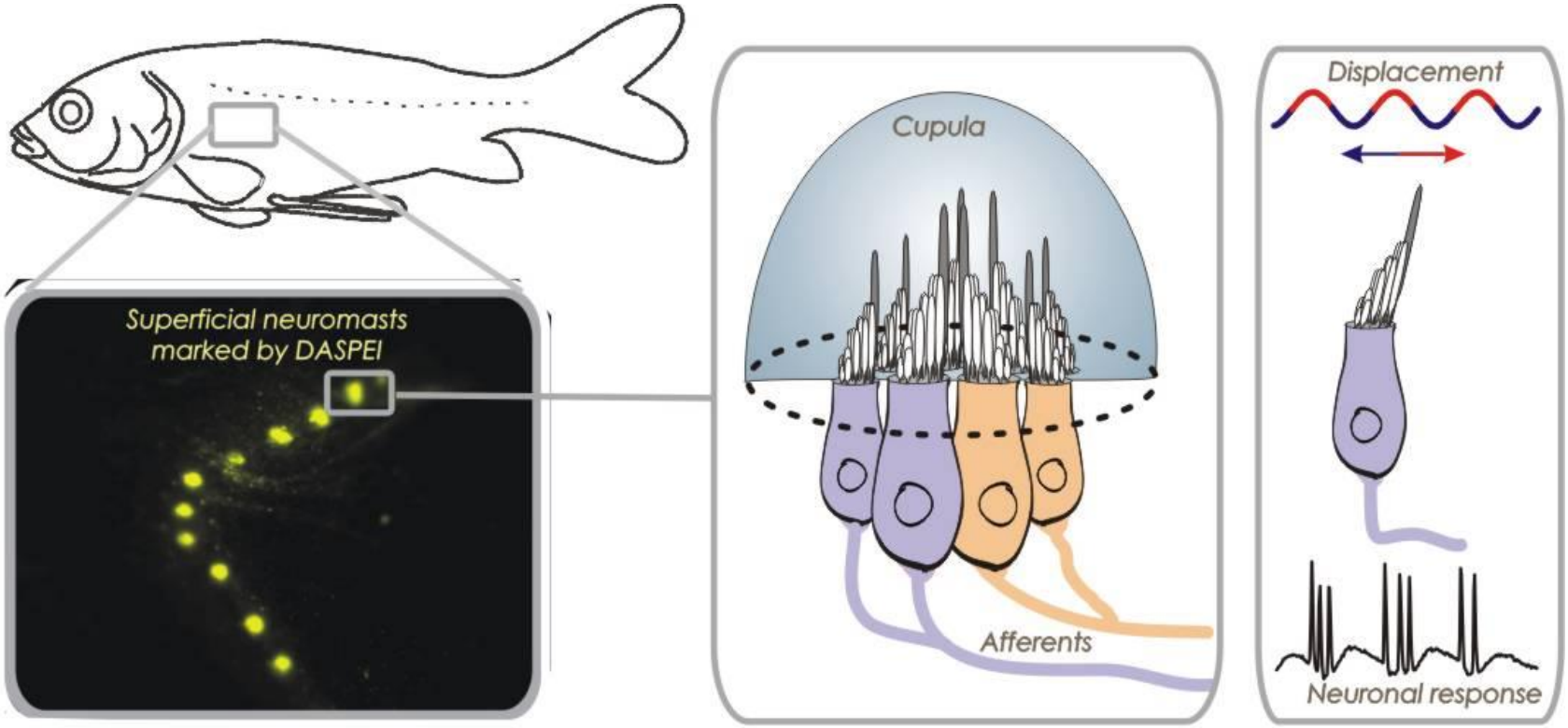

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