Behavioral and synaptic circuit features in a zebrafish model of fragile X syndrome

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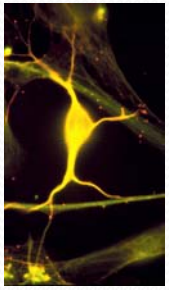
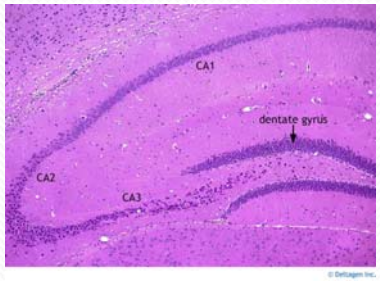
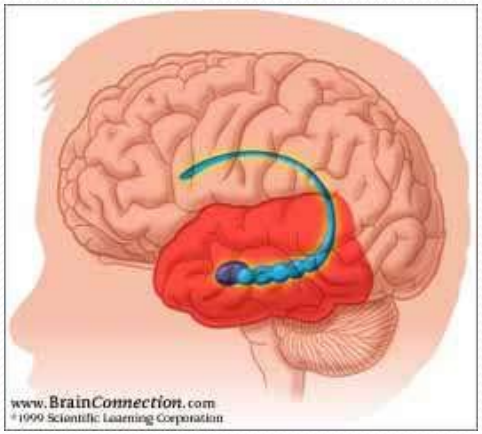
本系現有專任教師32人，具博士學位者31人，師資專長包含現代生命科學之各領域如分子與細胞生物學、神經科學、生命科學及生態與演化等學門。

The nervous system determine

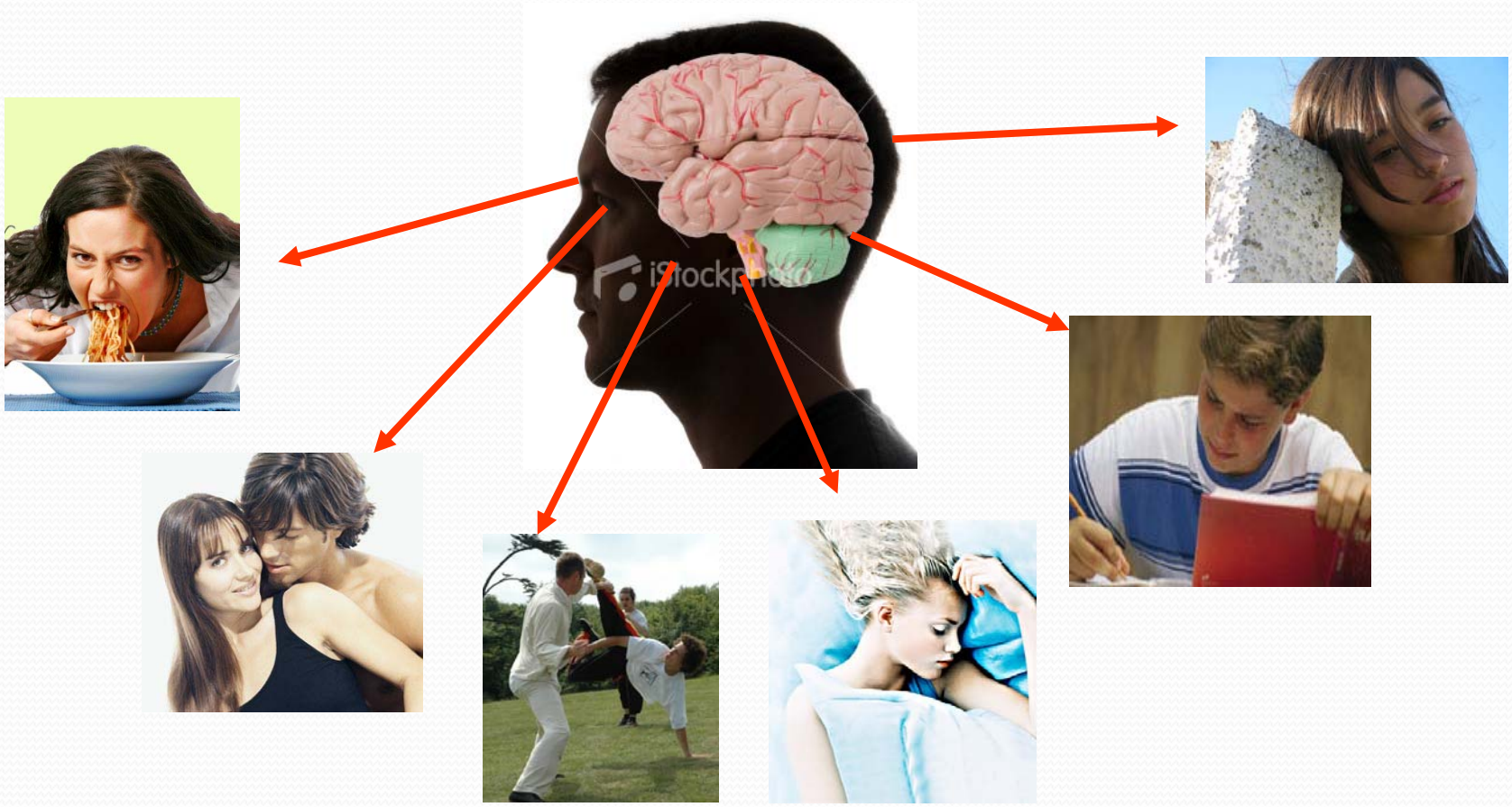
what we perceive, feel, think, say and do.







Perceptual processes, control of movement, sleep and waking, reproductive behaviors, ingestive behaviors, aggressive behaviors learning and memory, language and human pathological conditions.



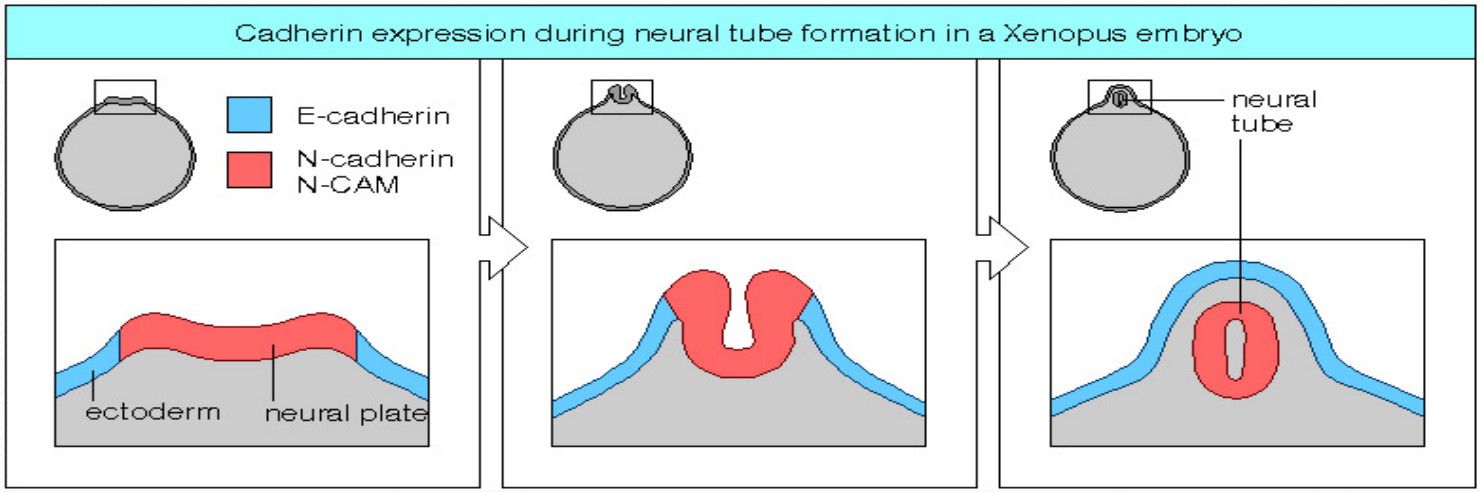
Development of the neural system

神經系統的發展

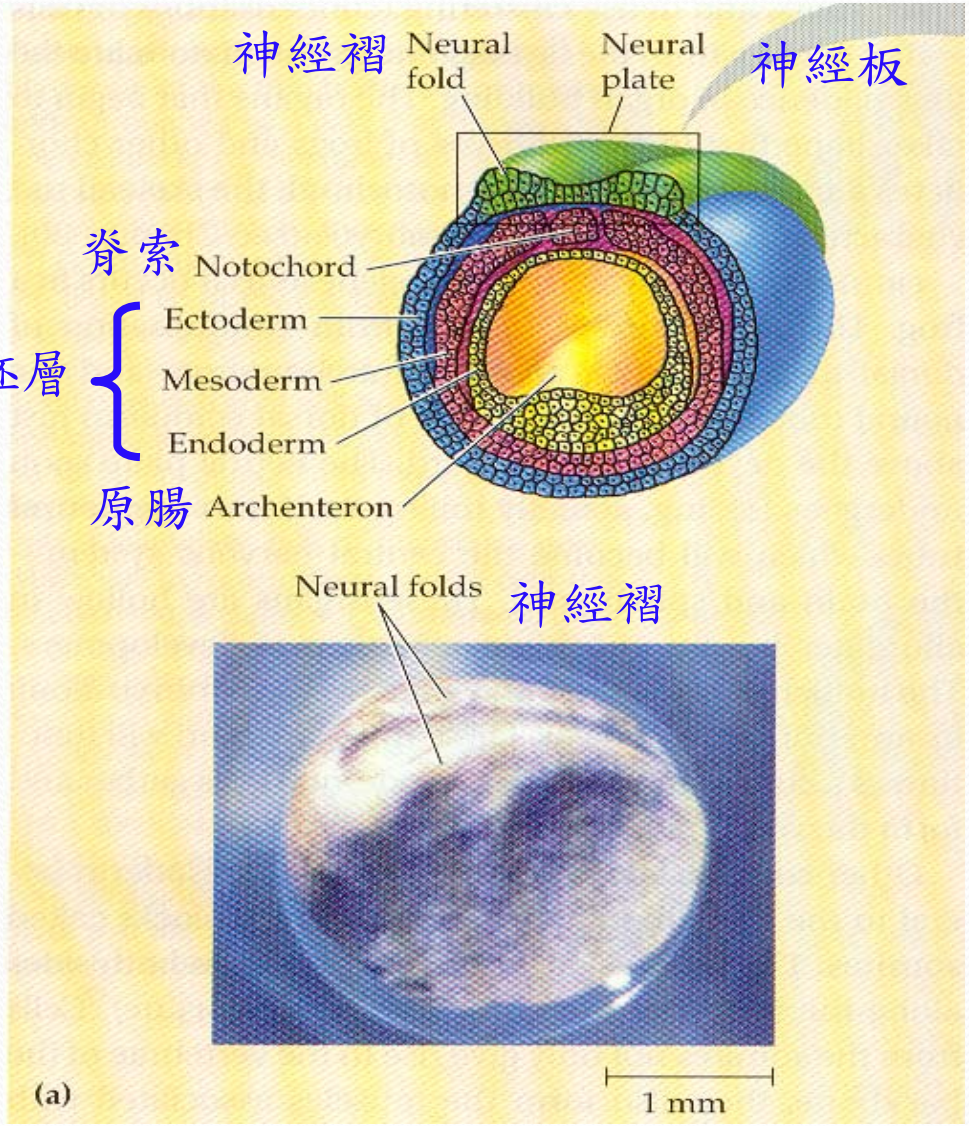
-神經板(neural plate) →神經褶 (neural fold) →神經管(neural tube) 及神經脊(neural crest) 的形成：

神經板(Neural plate)

- 三週大的胚胎(embryo)其外胚層(ectoderm)
  靠近背側的部分細胞會發展成神經板的構造，它有三個特徵
  1. 其外形與其他外胚層的細胞有明顯的差別。
  2. 它們會進一步分化成神經細胞及神經膠細胞(glial cell)。
  3. 失去totipotential的特性。(變成multipotent)

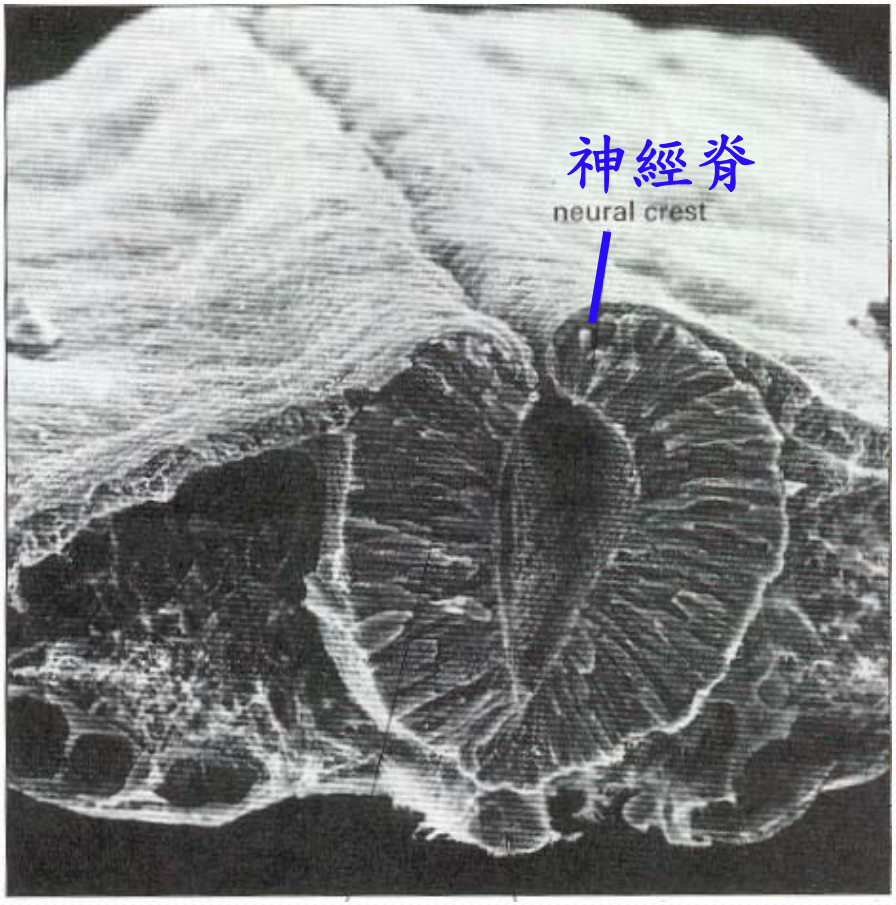


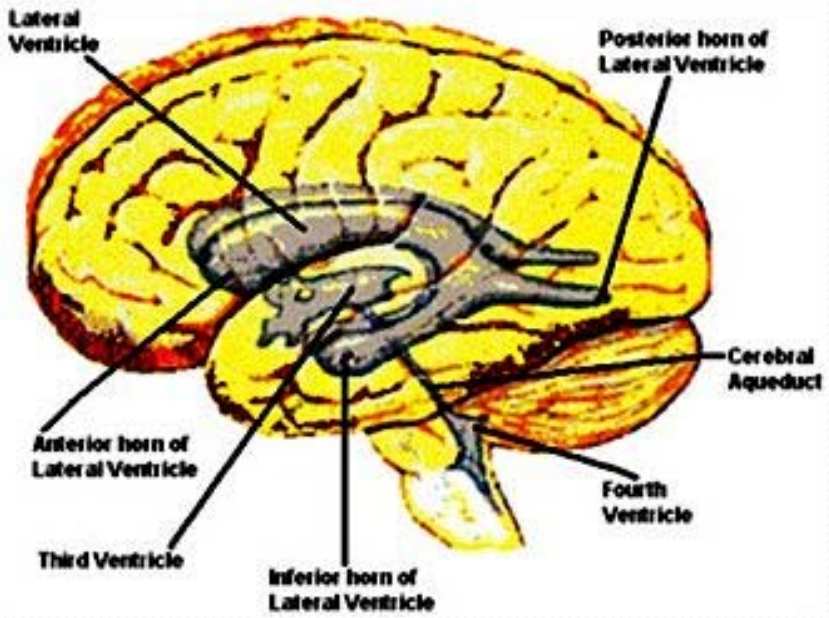
Neural plate 神經板



Neural tube 神經管

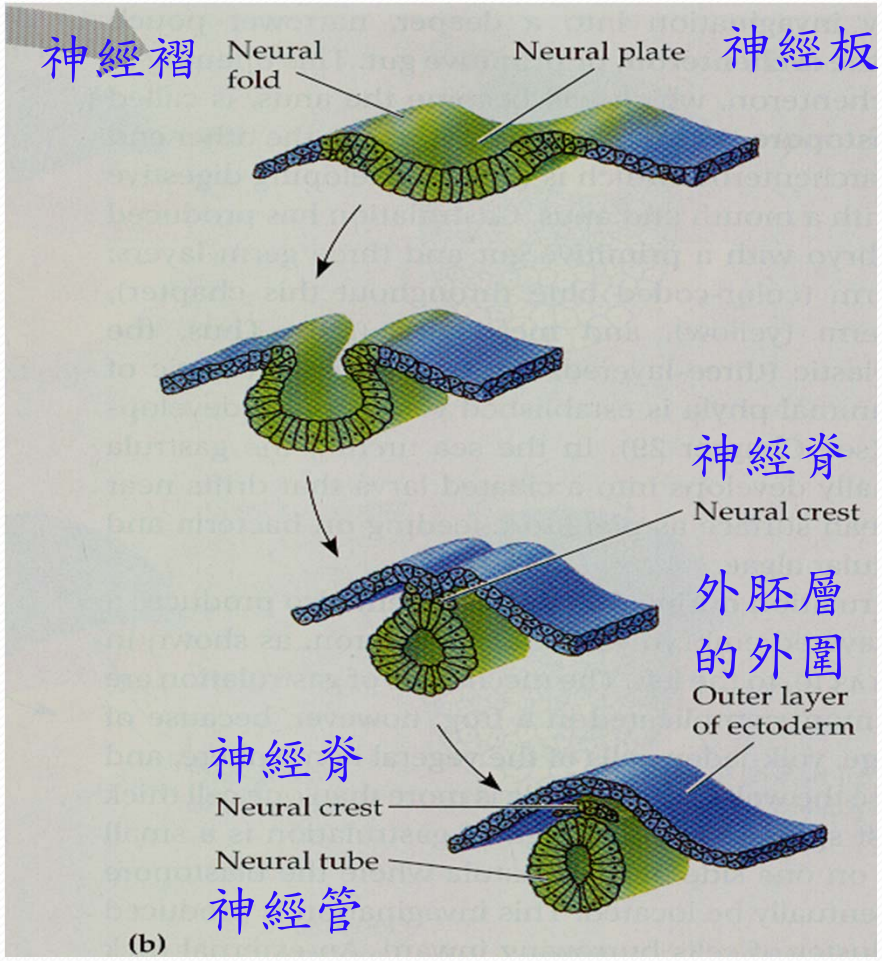
- 神經板會進一步內縮摺合形成神經管(neural tube)的構造（中空的部分形成日後的腦室及中央導水管，其餘的部分形成日後的**CNS**）。



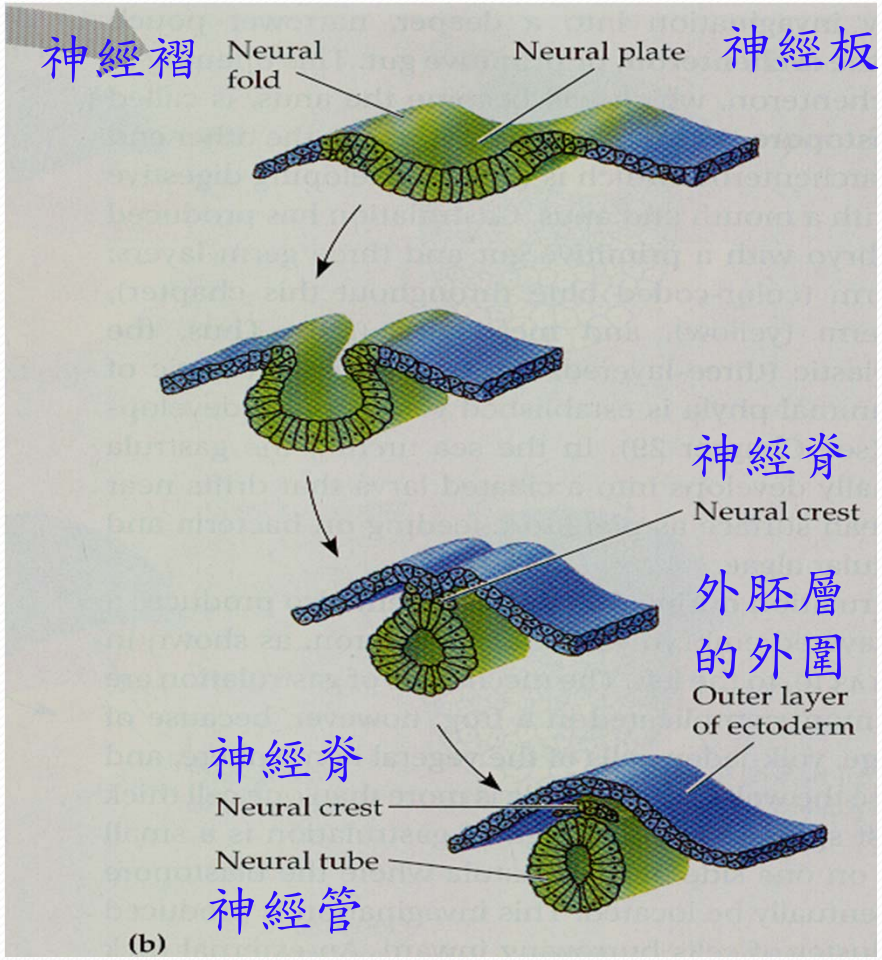


腦室及中央導水管

Neural tube 神經管



Neural tube 神經管

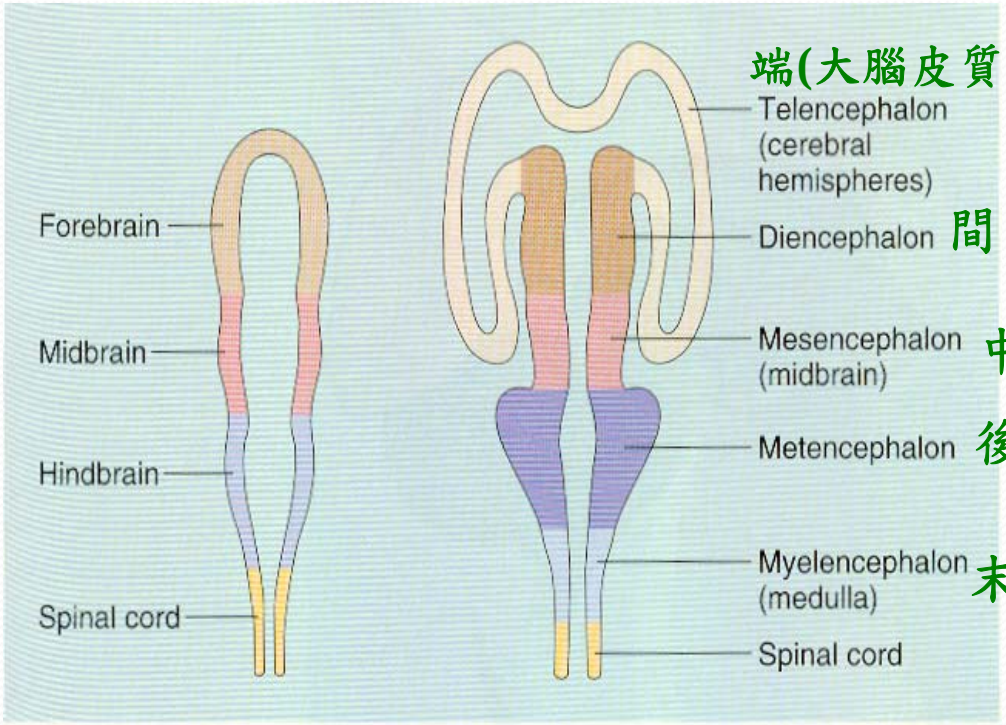


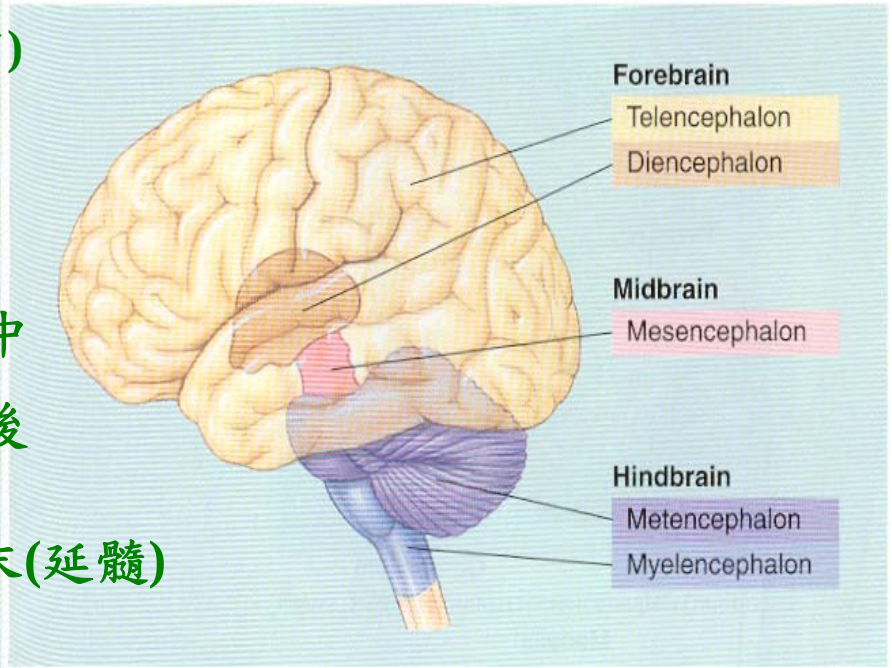
Open neural tube defect

Neural proliferation, migration & aggregation

神經細胞的增生，遷移及聚集

- 當神經管形成後，神經細胞的數量會快速的增加，形成窿起的構造（細胞分裂週期加快，細胞層變厚形成日後的
  前腦(forebrain)(端腦及間腦) 、中腦(midbrain)
  及後腦 (hindbrain)(後腦及末腦)

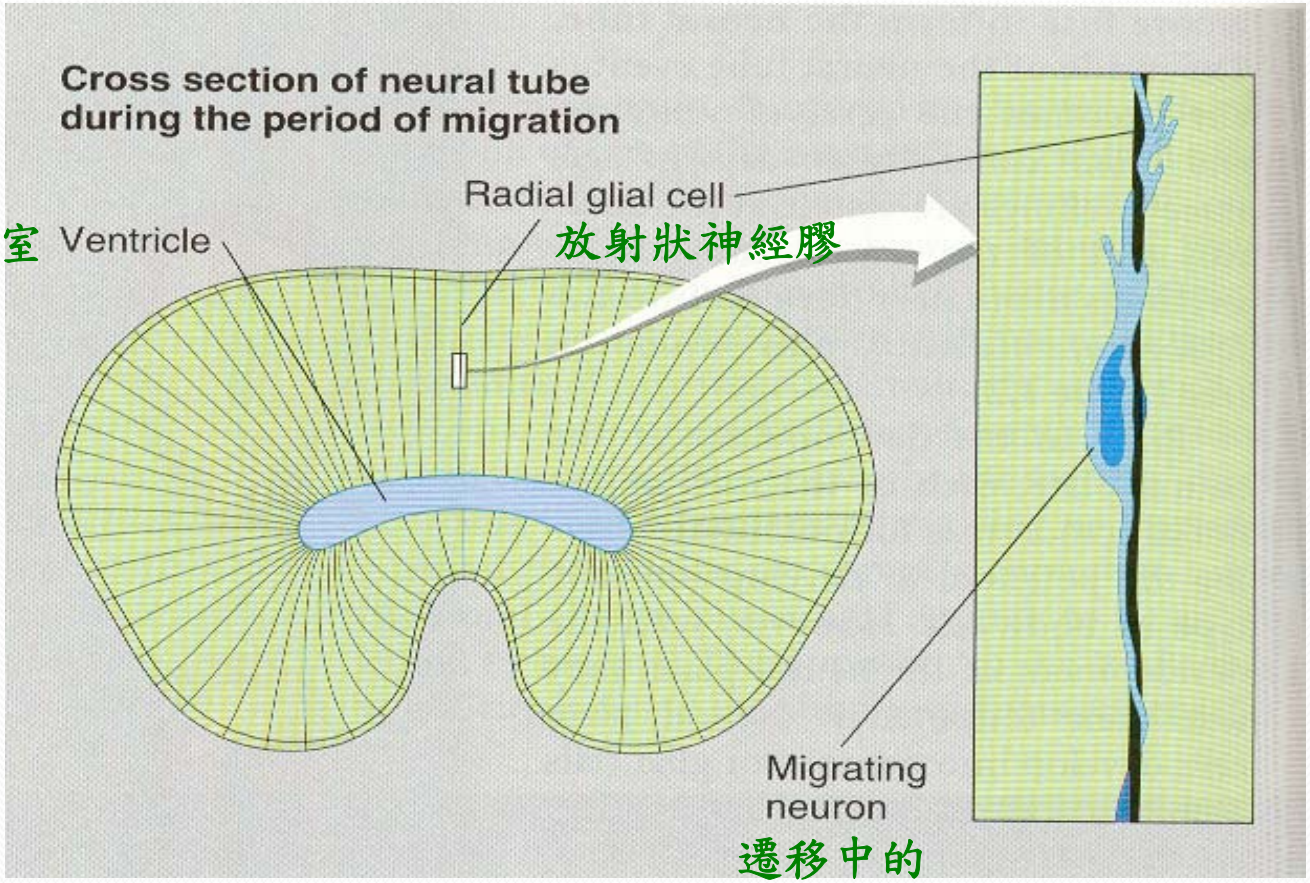




早期　　後期

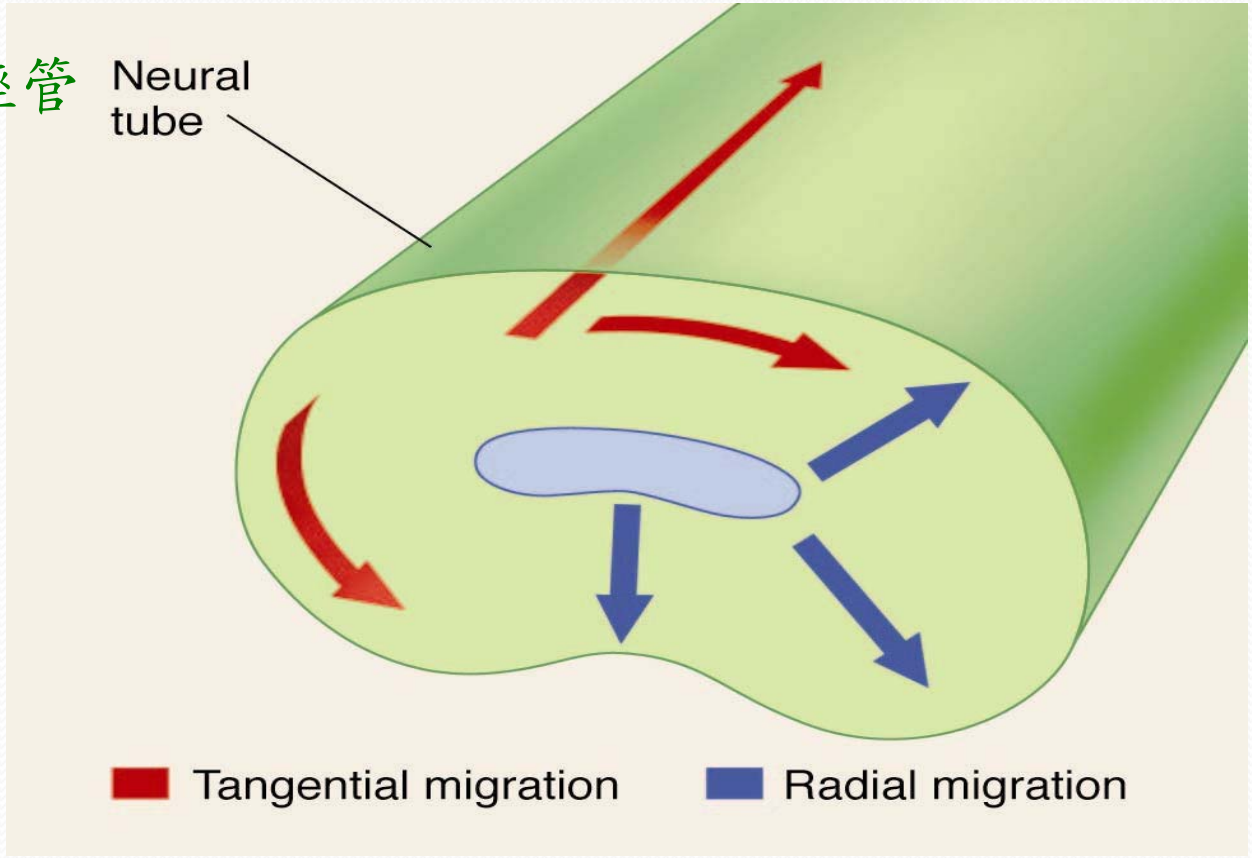
Neural proliferation 神經細胞的增生

- 神經細胞是在神經管旁的腦室帶 **(ventricular zone)** 處分裂增生，當細胞分裂週期完成後，新生的神經細胞會遷移到別的層區 (layer) 中。



Neural migration 神經細胞的遷移 (2)

- Two types of neural tube migration
  - **Radial migration**（放射狀的遷移）– moving out – usually by moving along radial glial cells
  - **Tangential migration**（正切般的遷移）– moving up

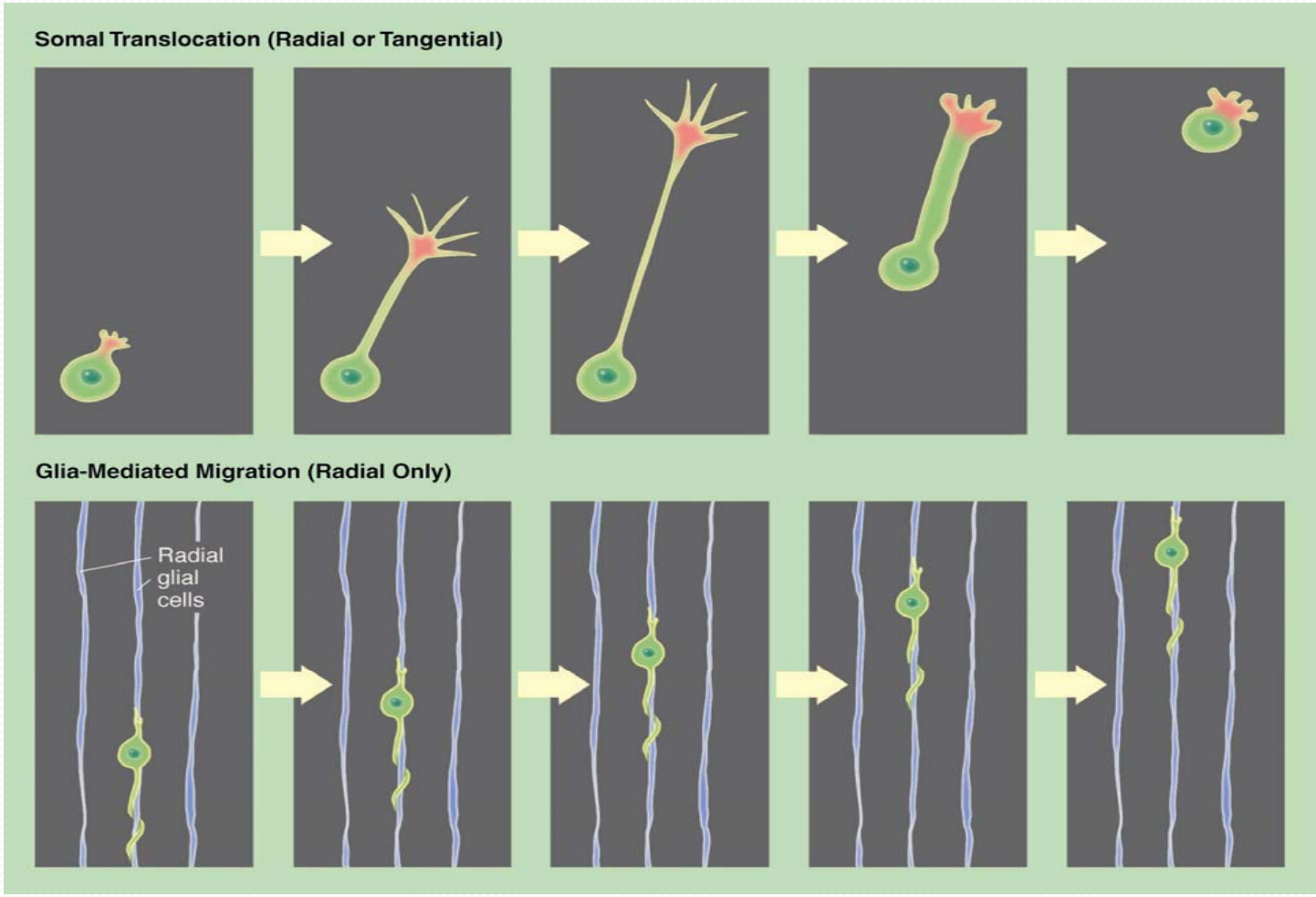


Neural migration 神經細胞的遷移 (3)

Two methods of migration

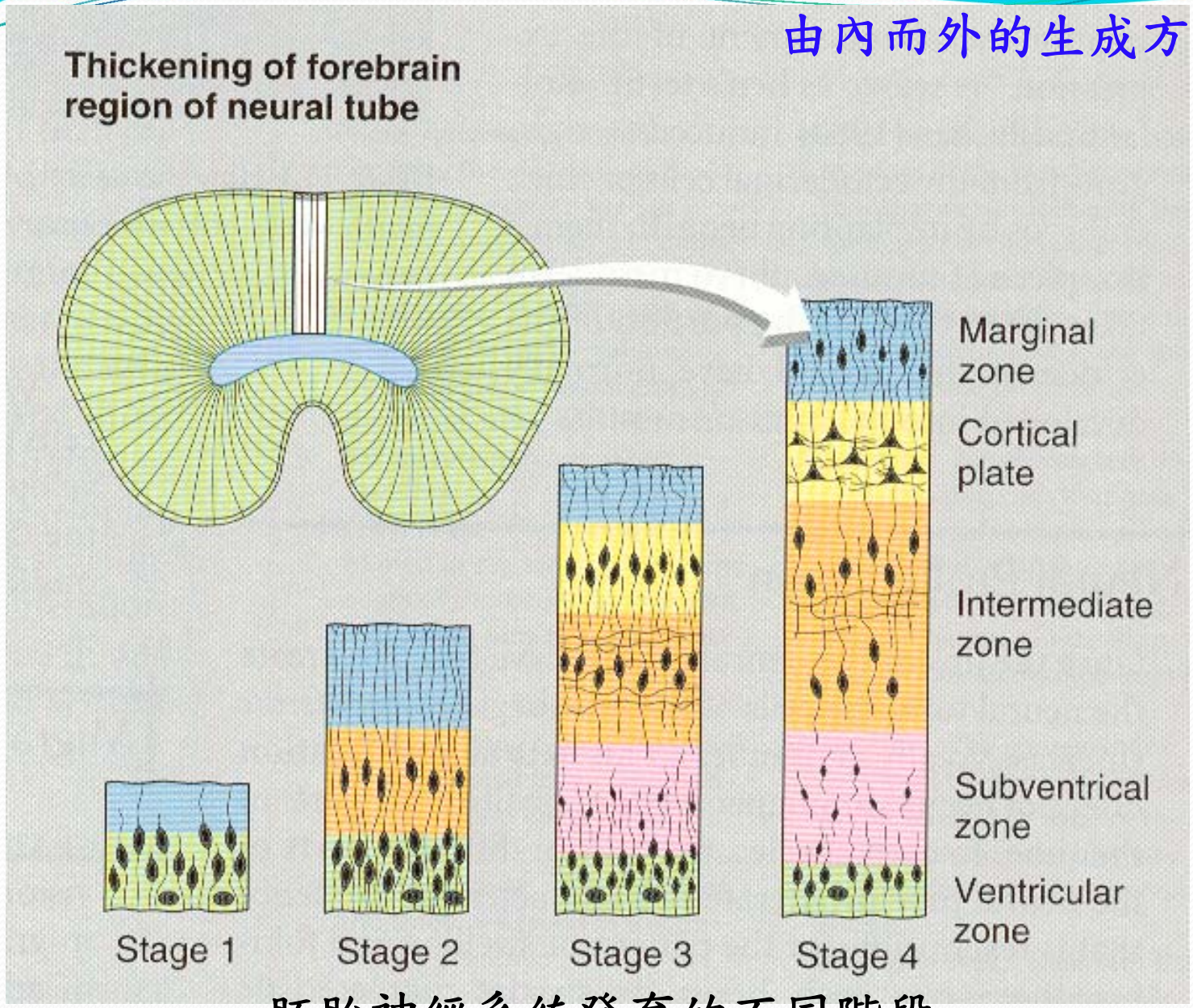
~ **Somal** – an extension develops that leads migration, cell body follows

~ **Glial-mediated migration** – cell moves along a radial glial network



Radical glial cell and inside-out pattern

由內而外的生成方式

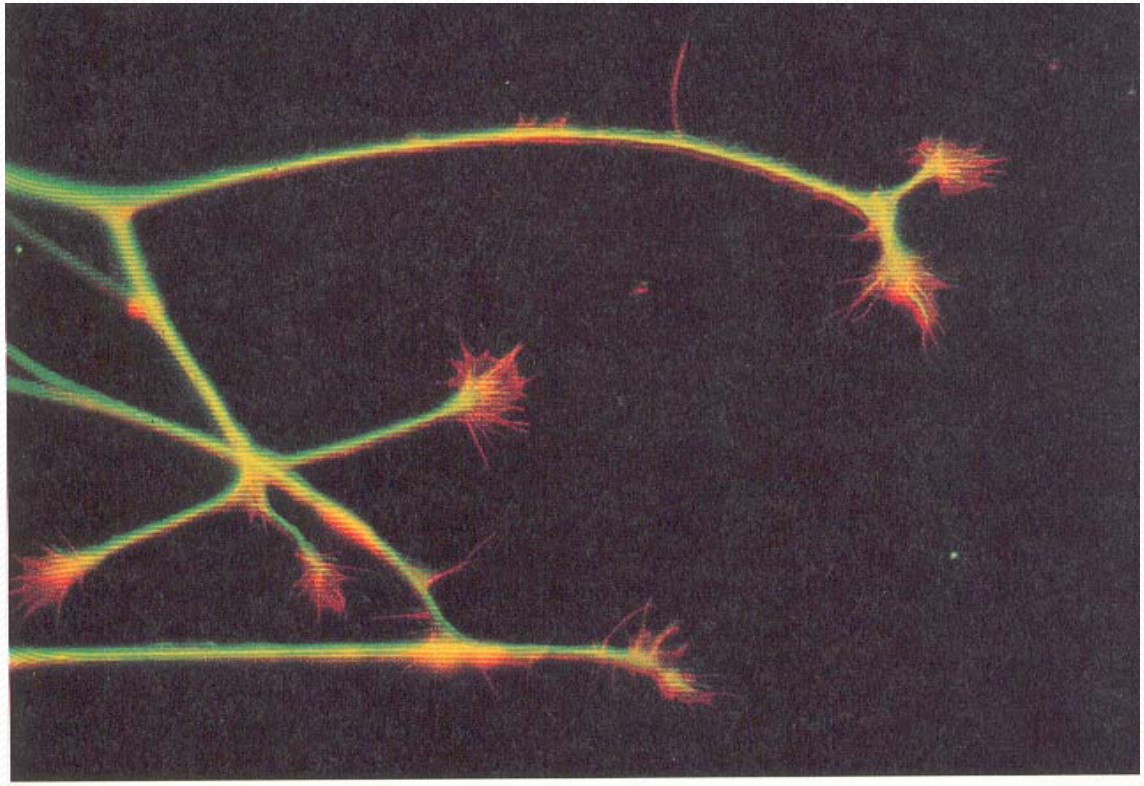


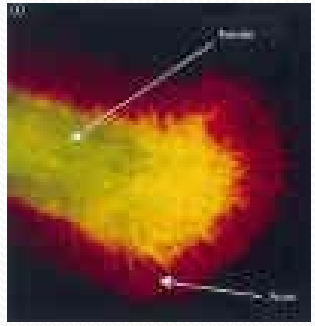
胚胎神經系統發育的不同階段

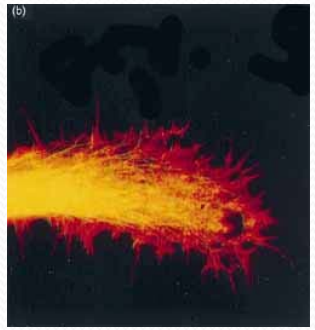
Axon growth & the formation of synapses

軸突的生長及突觸的形成

- 當聚集作用完成後，此時的CNS仍祇是一個粗糙的構造接著一連串細部的調節後（神經細胞伸出軸突並與正確的目標細胞形成突觸），才能形成有功能的神經迴路。

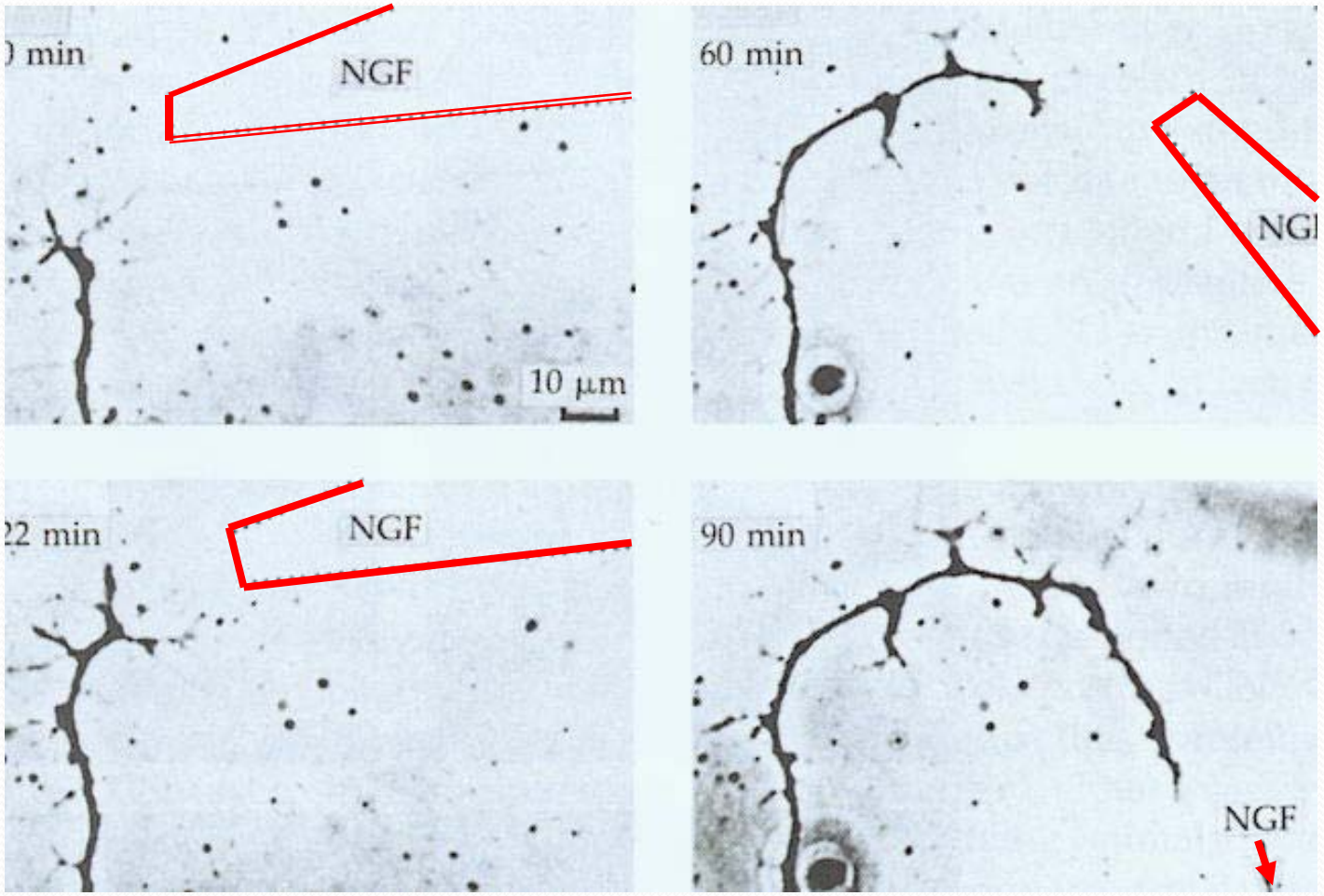






Chemoaffinity hypothesis

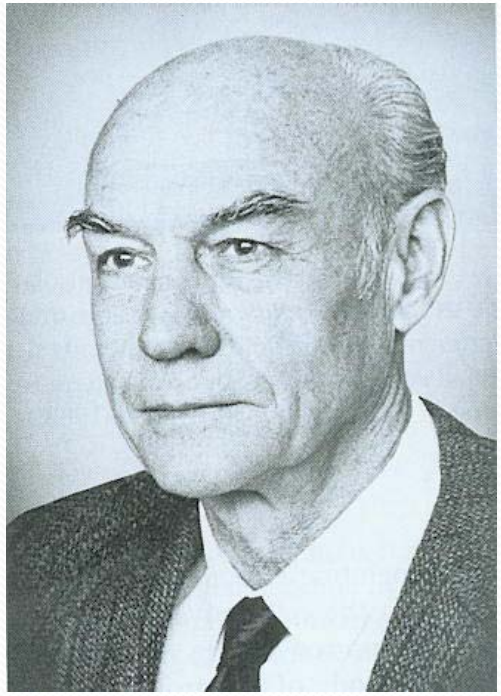
- 主張目標細胞會釋出特定的化學物質，誘導軸突的生長及突觸的形成。



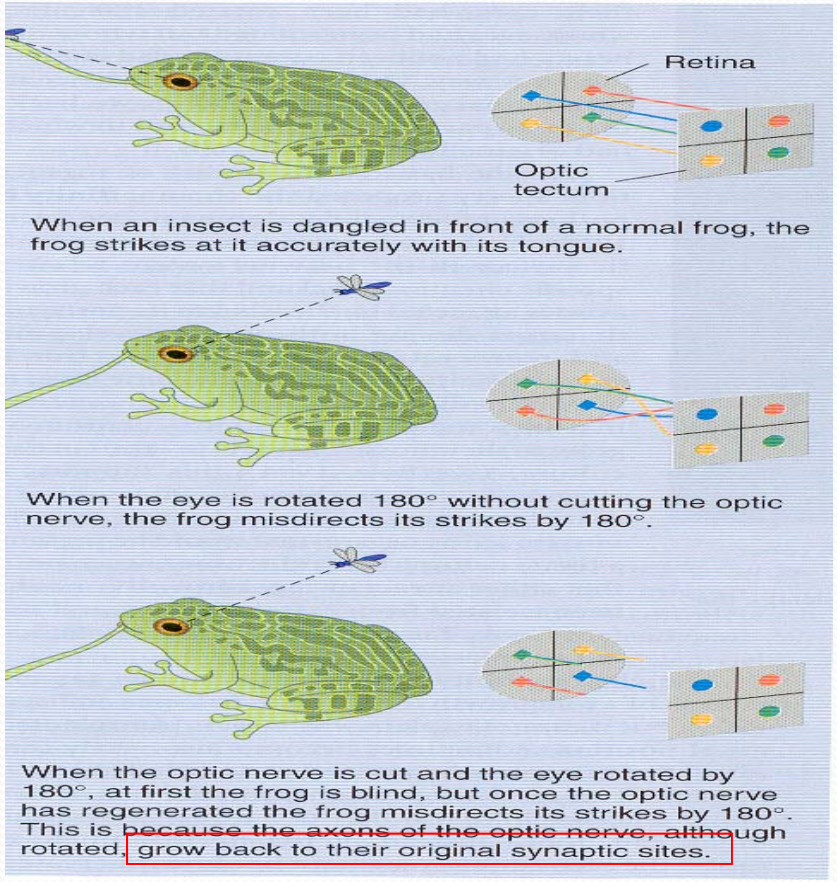
NGF: neural growth factor 神經生長因子

Chemoaffinity hypothesis

- 主要的証據為 **Roger Sperry** 的classic eye-rotation regeneration experiment，把青蛙的視神經切斷，把眼球作固定角度的旋轉，觀察視神經恢復的情形。



Nobel Prize 1981

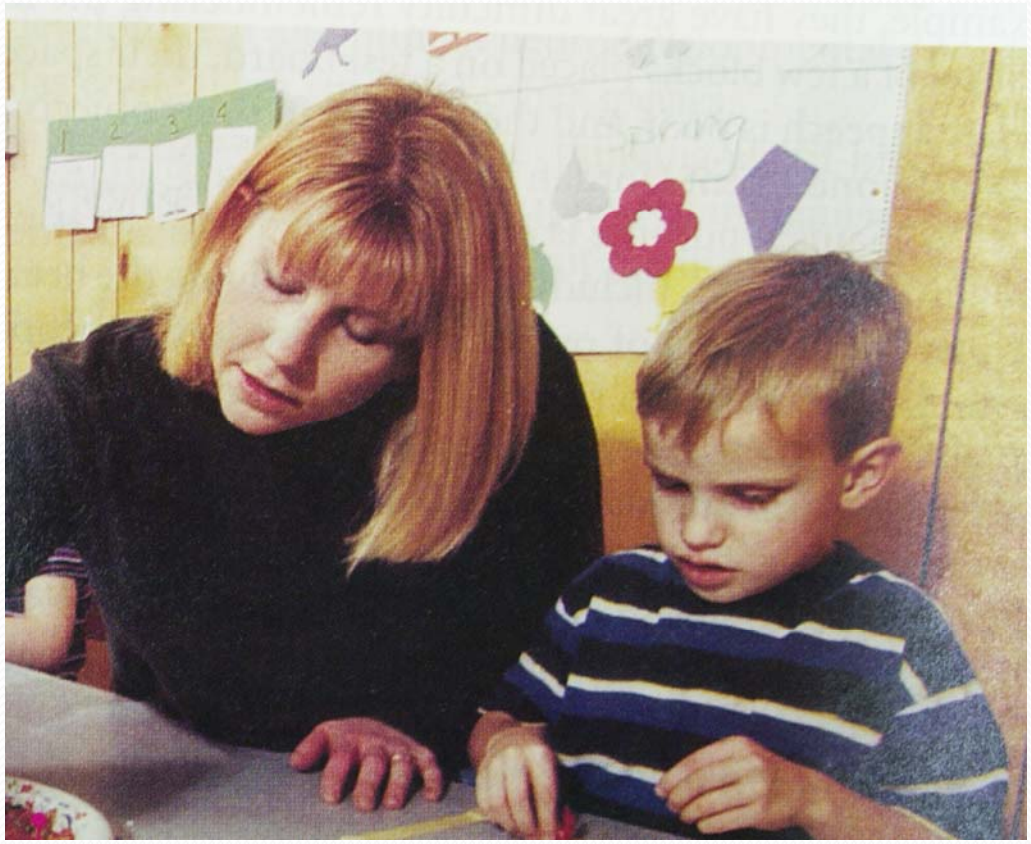


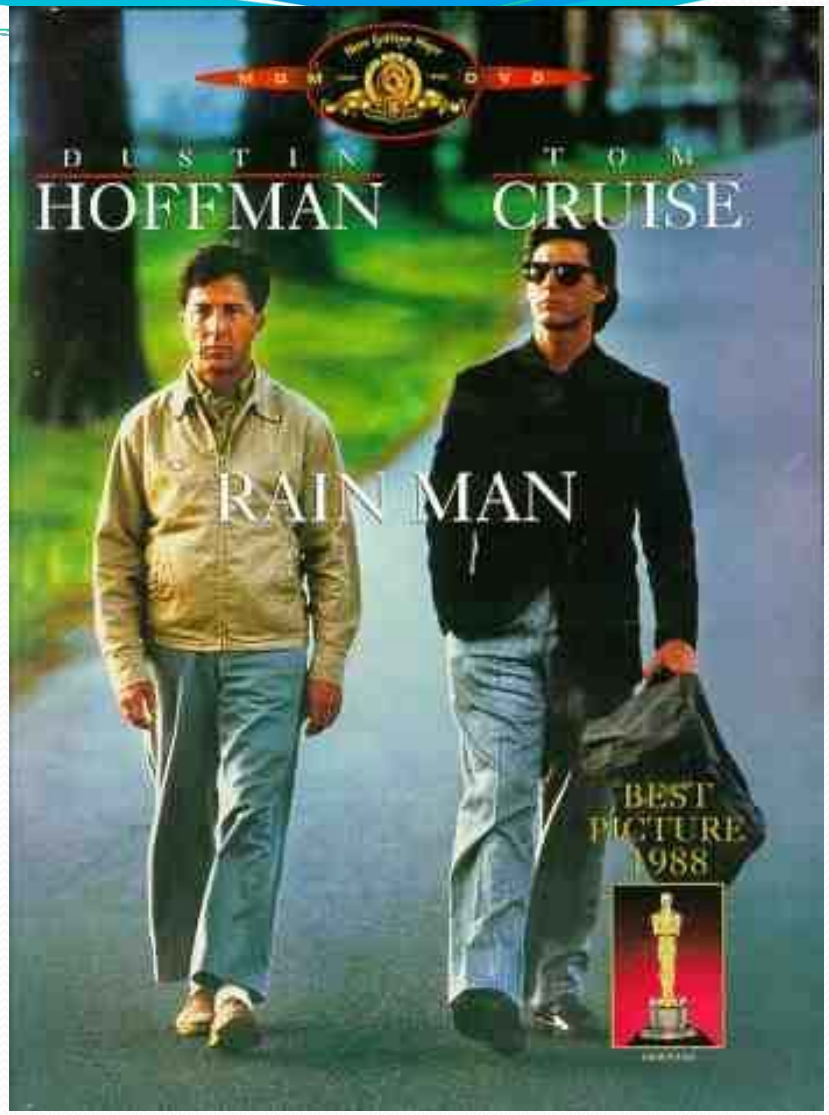
Neurodevelopment

神經系统的發展

- Neural development – an **ongoing process**, the nervous system is **plastic.**
- Complex.
- **Experience** plays a key role.
- Dire consequences when something goes wrong.

Autism 自閉症





Autism

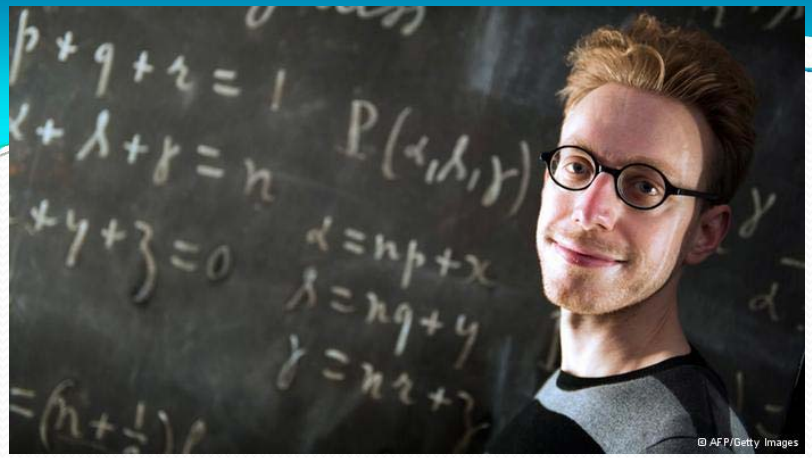
- 4 of every 10,000 individuals – 3 core symptoms:
  - Reduced ability to interpret emotions and intentions
  - Reduced capacity for social interaction
  - Preoccupation with a single subject or activity
- Intensive behavioral therapy may improve function
- Heterogenous – level of brain damage and dysfunction varies

Autism

- Most have some abilities preserved – rote memory, ability to complete jigsaw puzzles, musical ability, artistic ability.
- Savants – intellectually handicapped individuals who display specific cognitive or artistic abilities
- ~1/10 autistic individuals display **savant abilities**
- Perhaps a consequence of compensatory functional improvement in the right hemisphere following damage to the left

Stephen Wiltshire

Ellen Boudreaux



(Daniel Tammett)

Synesthesia

Neural Basis of Autism

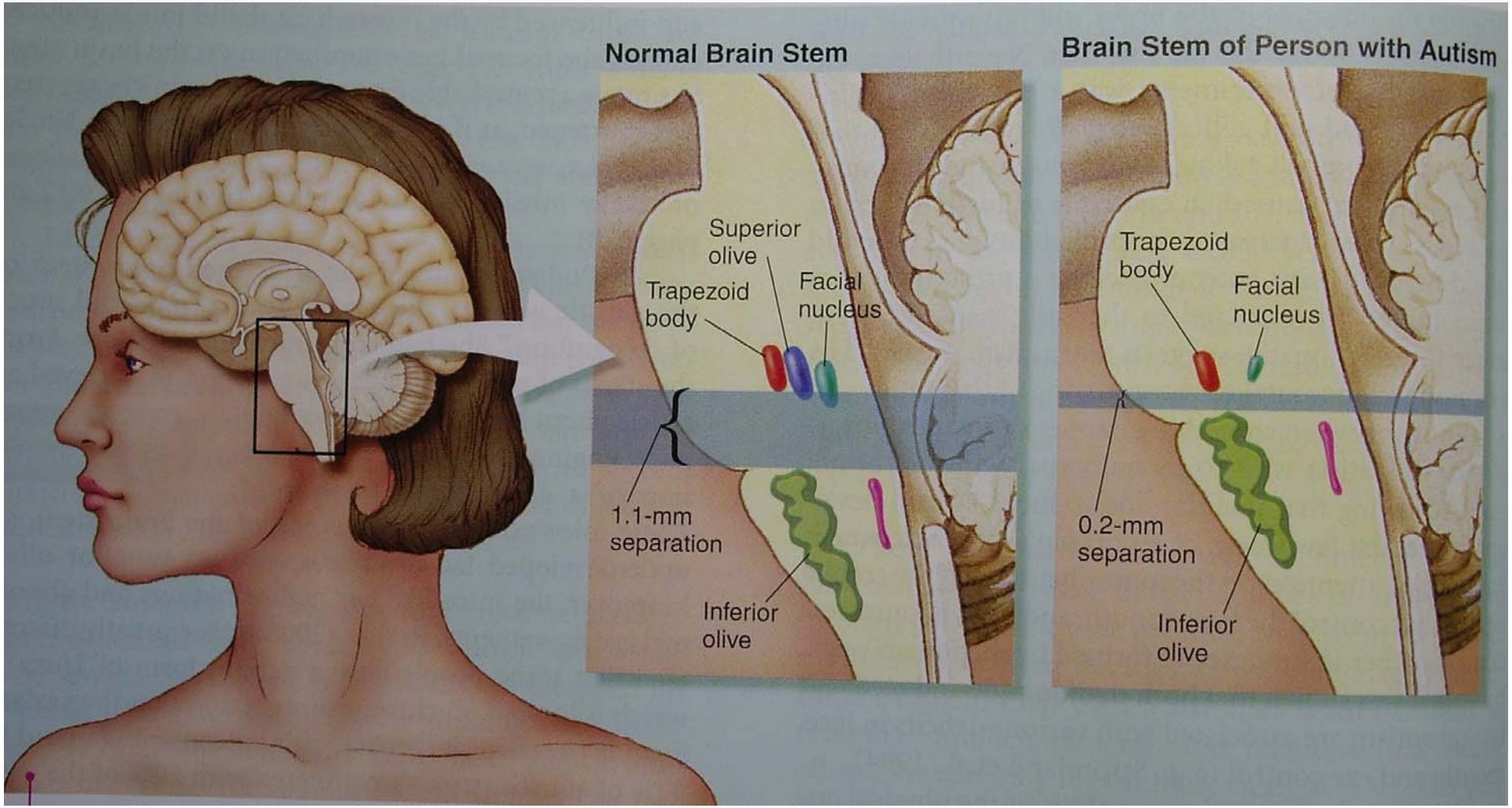
- Genetic basis
  - Siblings of the autistic have a 5% chance of being autistic
  - 60% concordance rate for monozygotic twins
- Several genes interacting with the environment
- Brain damage tends to be widespread, but is most commonly seen in the cerebellum

Neural Basis for Autism

- Thalidomide – given early in pregnancy – increases chance of autism
  - Indicates neurodevelopmental error occurs within 1st few weeks of pregnancy when motor neurons of the cranial nerves are developing
  - Consistent with observed deficits in face, mouth, and eye control
- Anomalies in ear structure indicate damage occurs between 20 and 24 days after conception
- Evidence for a role of a gene on chromosome 7

Adapted from: http://www.vaccinetruth.org/thalidomide.htm

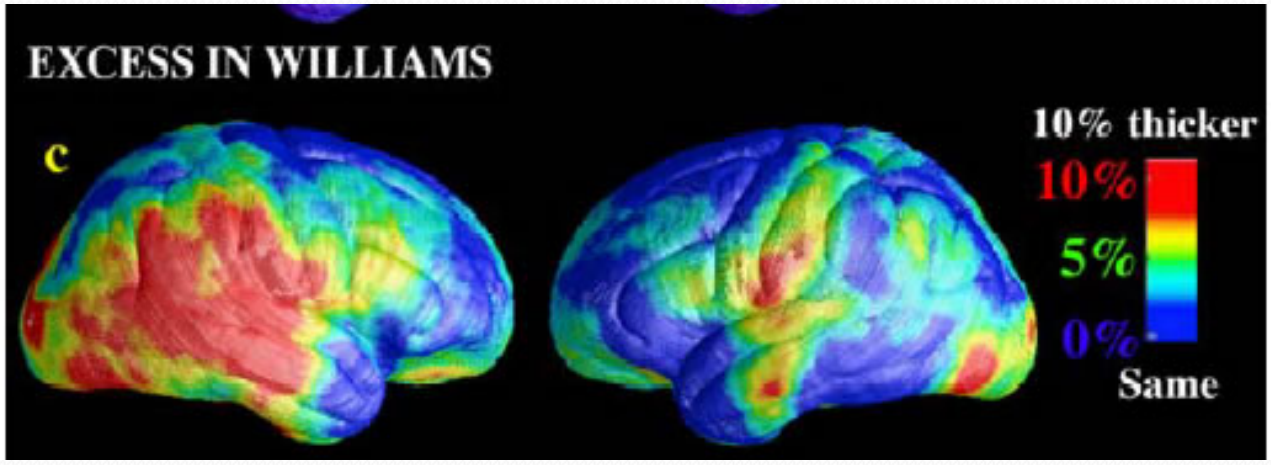
Autism



Adapted from Pinal,

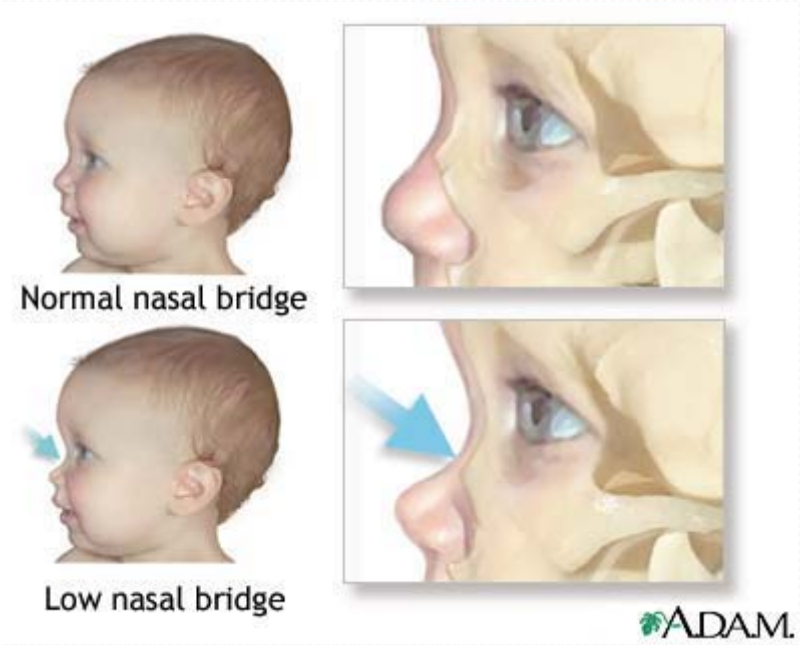
Williams Syndrome

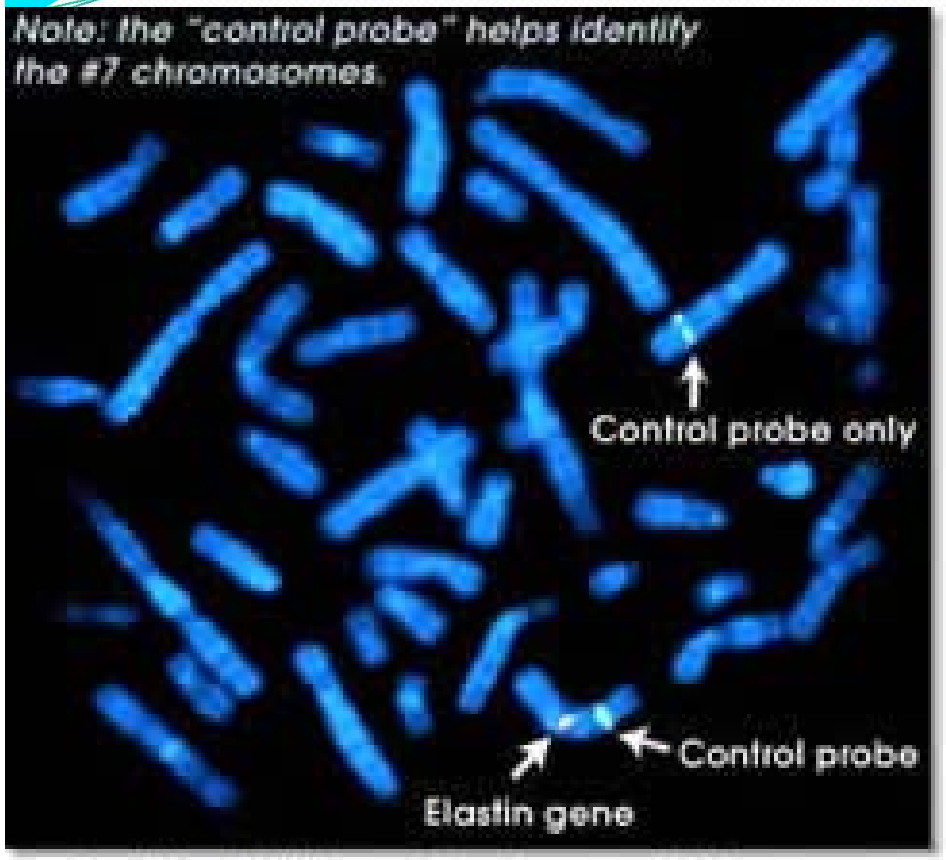
- Variety of abilities – like autistics
- Evidence for a role of chromosome 7 – as in autism
- Underdeveloped occipital and parietal cortex, normal frontal and temporal
- “elfin” appearance – short, small upturned noses, oval ears, broad mouths



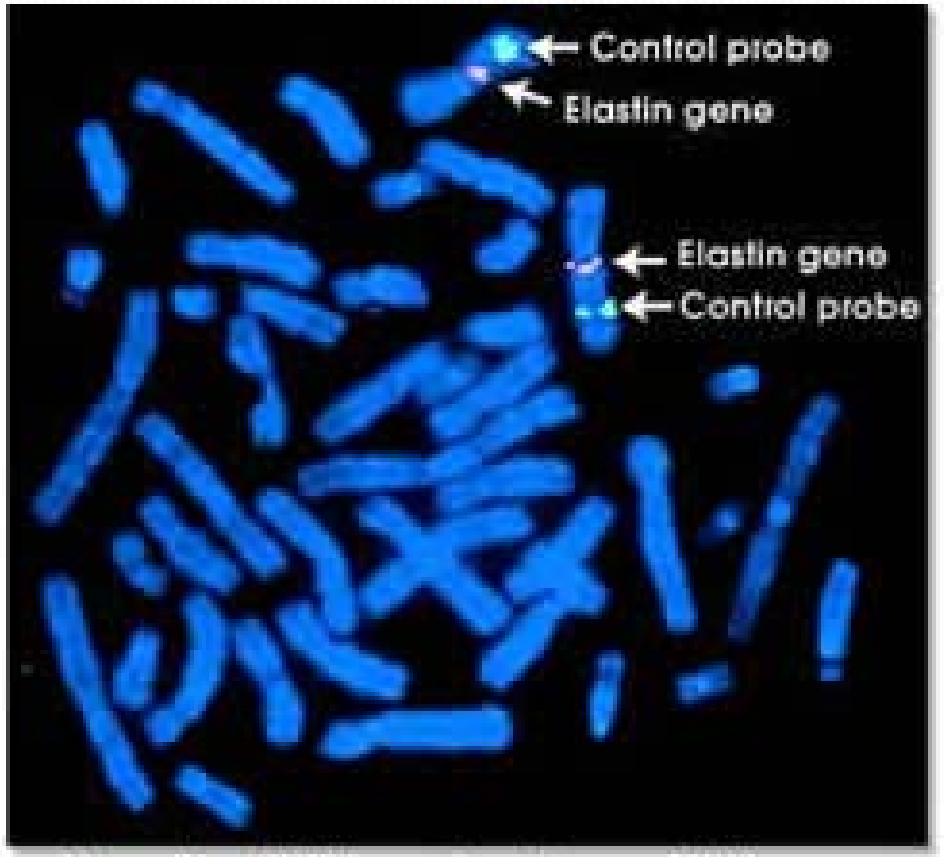
Williams Syndrome

- ~ 1 of every 20,000 births
- Mental retardation and an uneven pattern of abilities and disabilities
- Sociable, empathetic, and talkative – exhibit language skills, music skills and an enhanced ability to recognize faces
- Profound impairments in spatial cognition
- Usually have heart disorders associated with a mutation in a gene on chromosome 7
  - the gene (and others) are absent in 95% of those with Williams

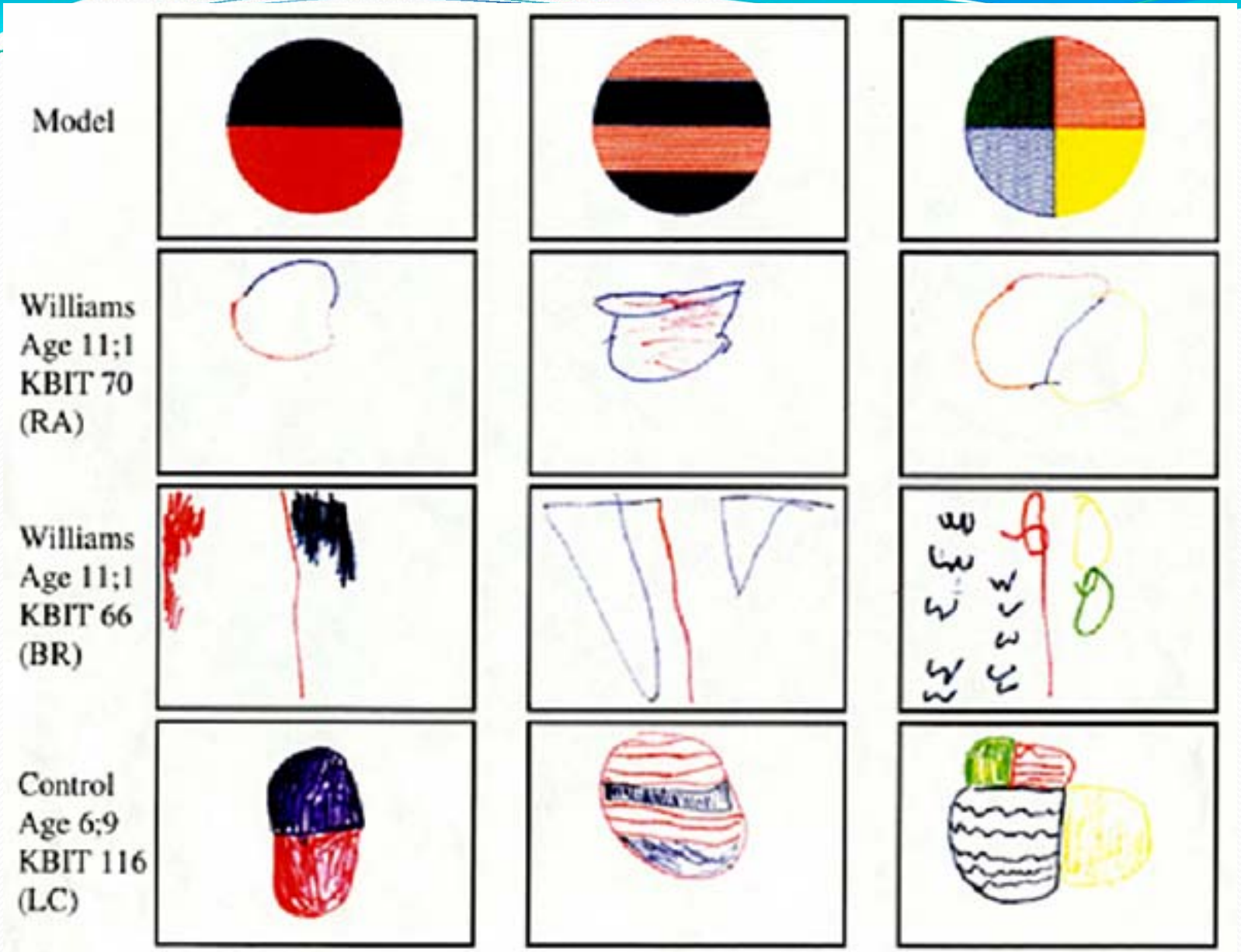




Positive Williams Syndrome FISH assay
(Chromosome 7)
The elastin gene is found on only one chromosome. The other copy carries an elastin gene deletion.



Negative Williams Syndrome FISH assay
(Chromosome 7)
The elastin gene is found on both chromosomes. This individual does not have Williams Syndrome.

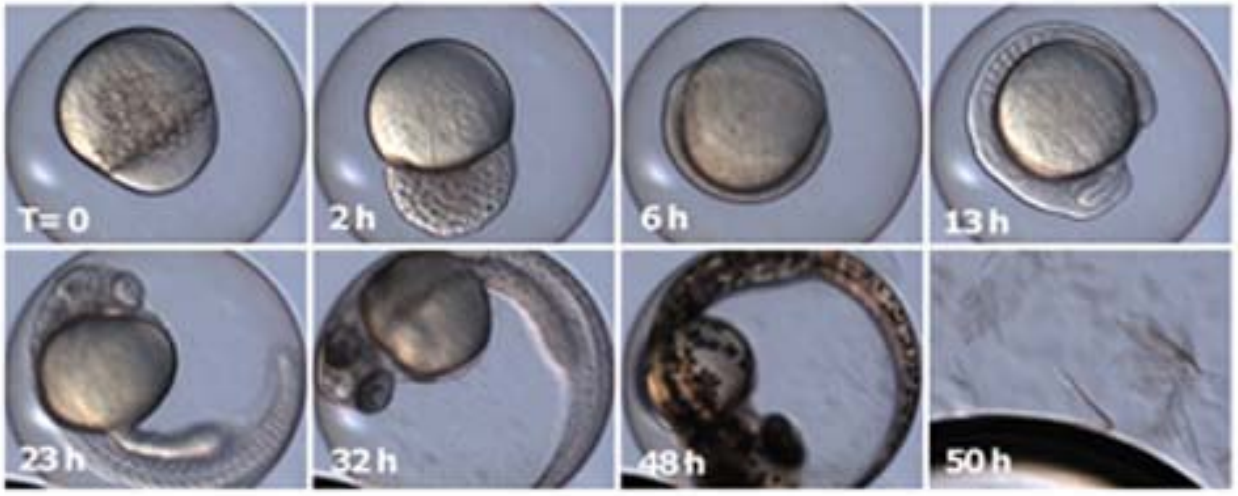

Model
Williams
Age 11;1
KBIT 70
(RA)
Williams
Age 11;1
KBIT 66
(BR)
Control
Age 6;9
KBIT 116
(LC)

斑馬魚(*Danio Rerio*)

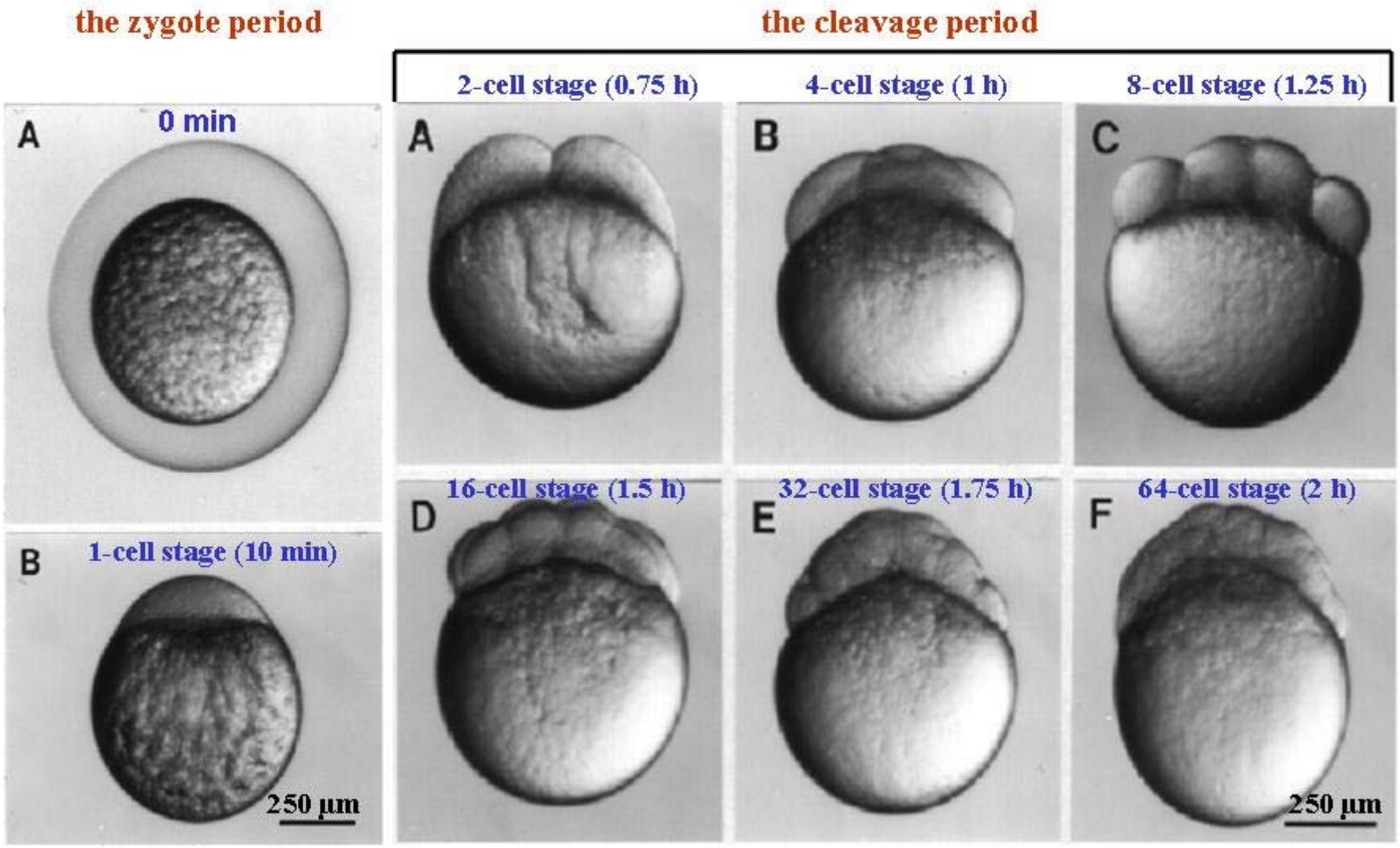
- 小型魚種之脊椎動物
- 產卵數量多
- 卵徑大
- 透明（可觀察胚胎發育）
- 用光即可控制排卵
- 14-hr light and 10-hr dark cycle
- 每天可以排卵（沒有產卵期的限制）
- 轉殖操作簡單
- 成熟期只有2~3個月
- 基因體大小只有哺乳類的20%

為什麼使用斑馬魚？

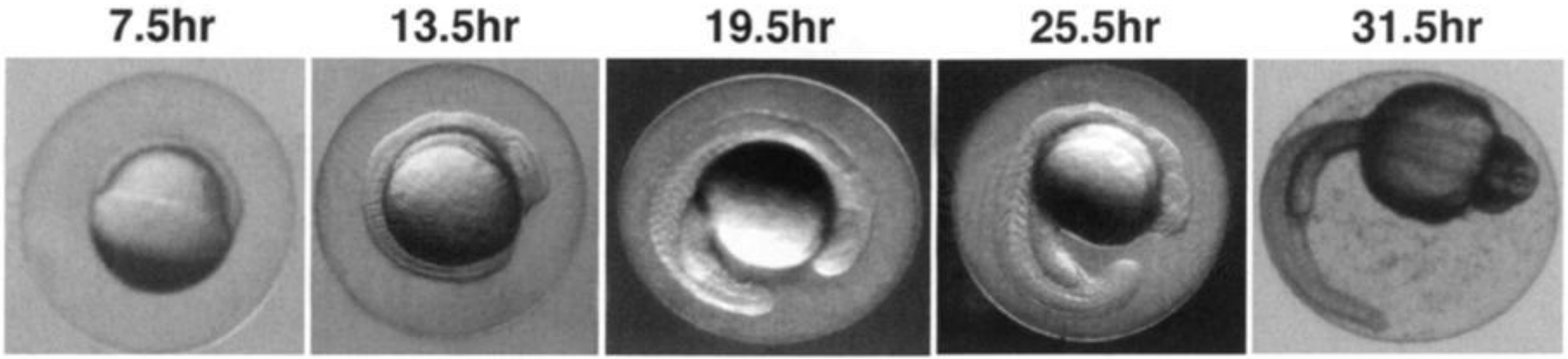
- 基因密碼已完全解讀
- 神經系統構造及不同發生階段之基因調控，已建立完善之資料庫
- 胚胎發育上的機制與哺乳動物相似
- 體外授精體外孵化且胚體完全透明



Stages of embryonic development of the zebrafish



Stages of embryonic development of the zebrafish



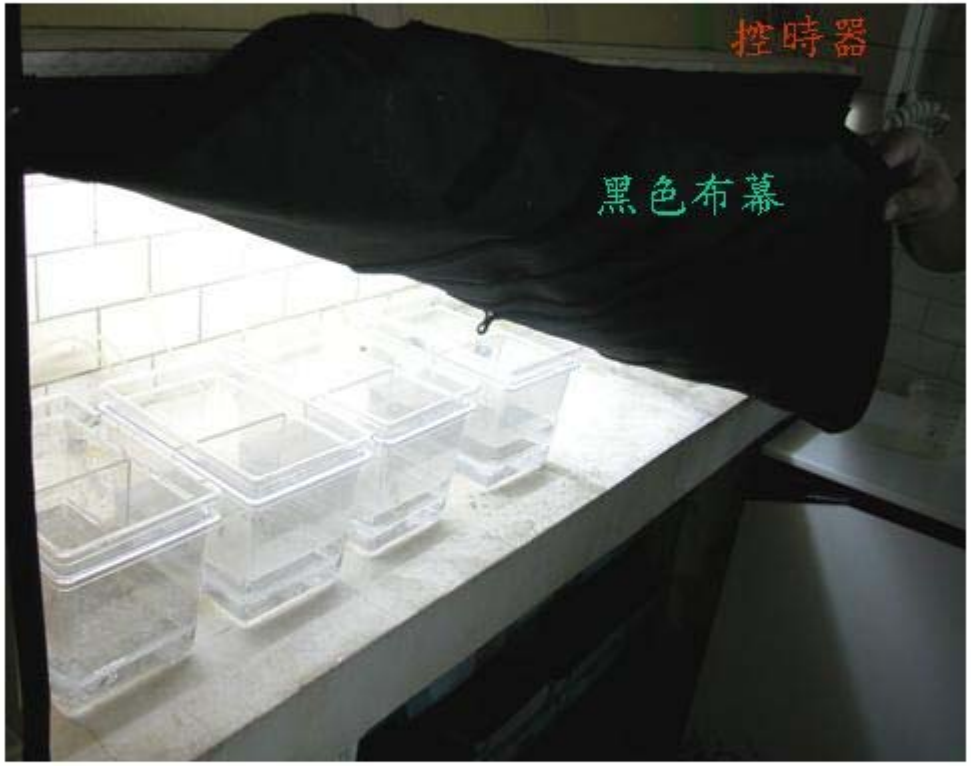
基因轉殖

- 顯微注射技術
- 在斑馬魚的胚胎中注入載體
- 利用一段可產生螢光之序列使特定部位產生螢光以方便觀察

pre-breeding set up (1)

～曾鴻家資料整理

pre-breeding set up (2)

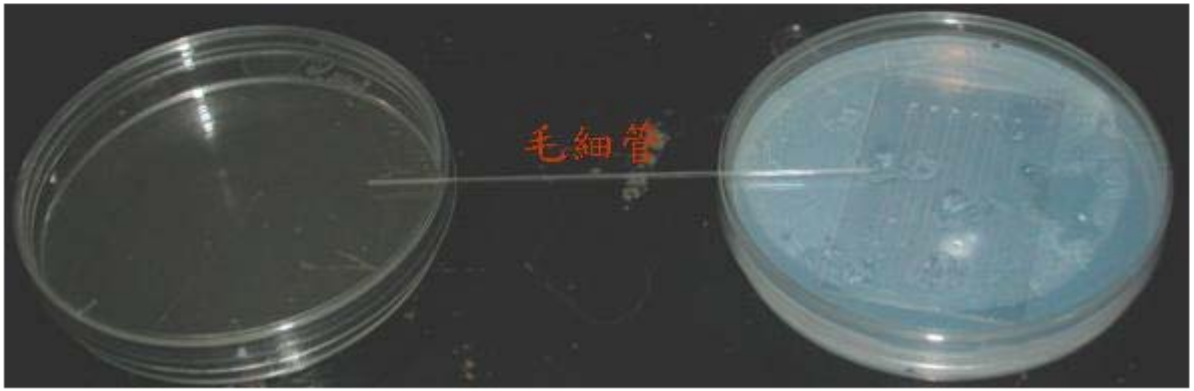


設定14小時照光，10小時暗週期控制排卵

拉針器操作步驟 (1)

2.固定毛細管於拉針器內

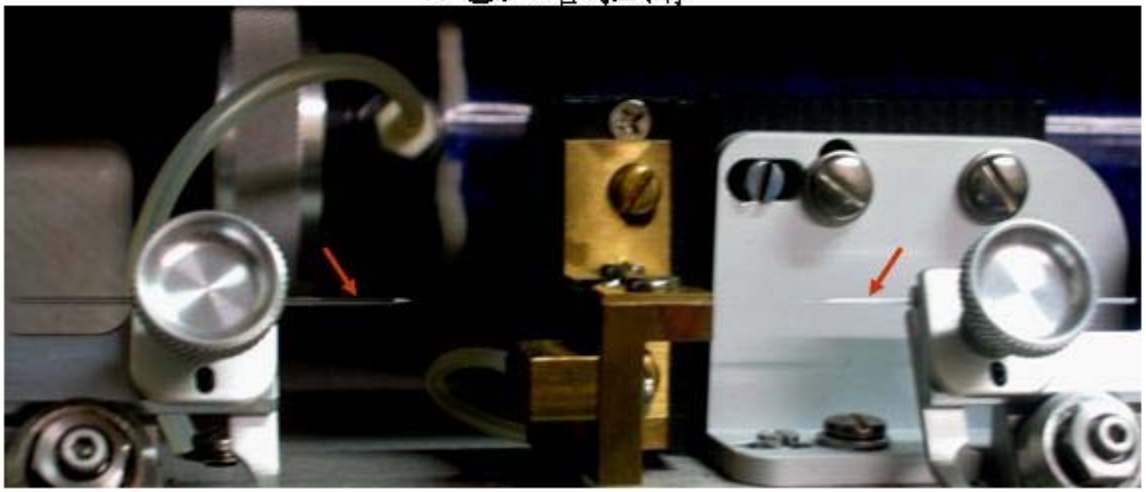
3.蓋上拉針器蓋子後按start



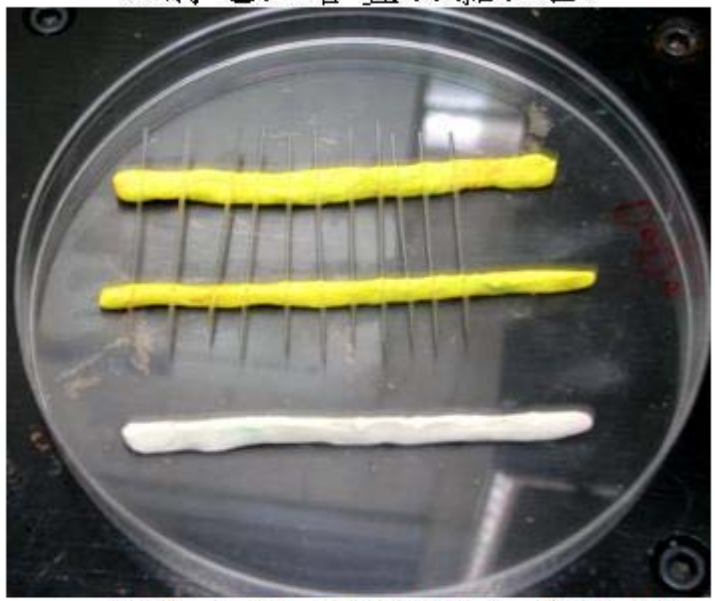
拉針器操作步驟 (2)

3.自毛細管中間加熱，拉開！

4.毛細管拉開

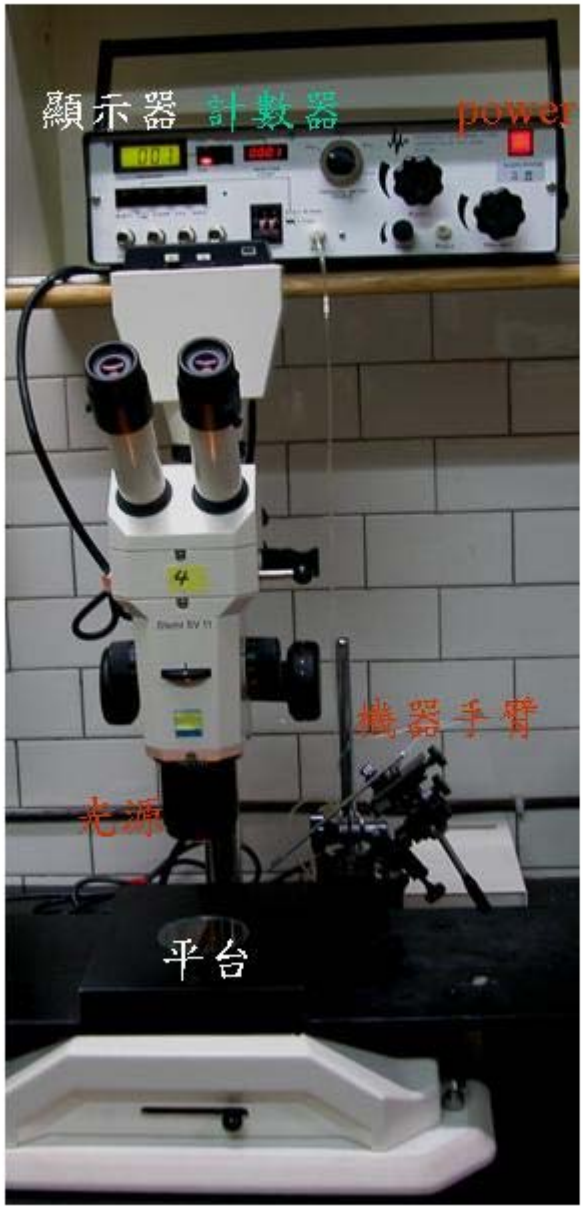


5.將毛細管置於黏土上

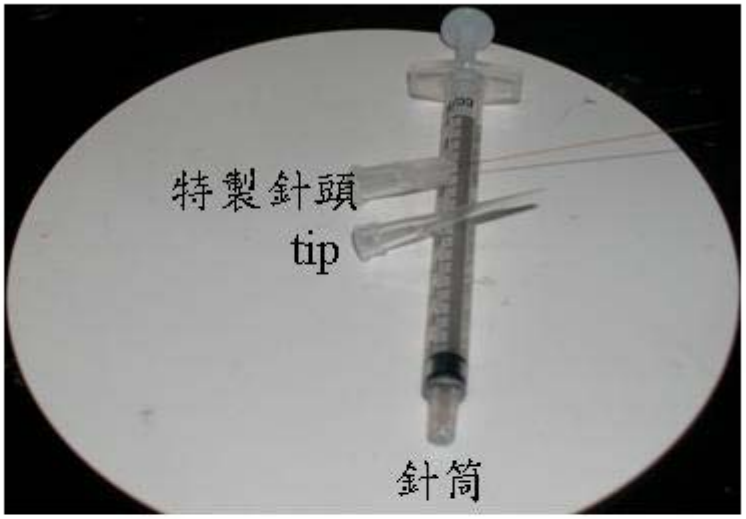


注意！不可碰到毛細管尖端

Microinjection operation (1)



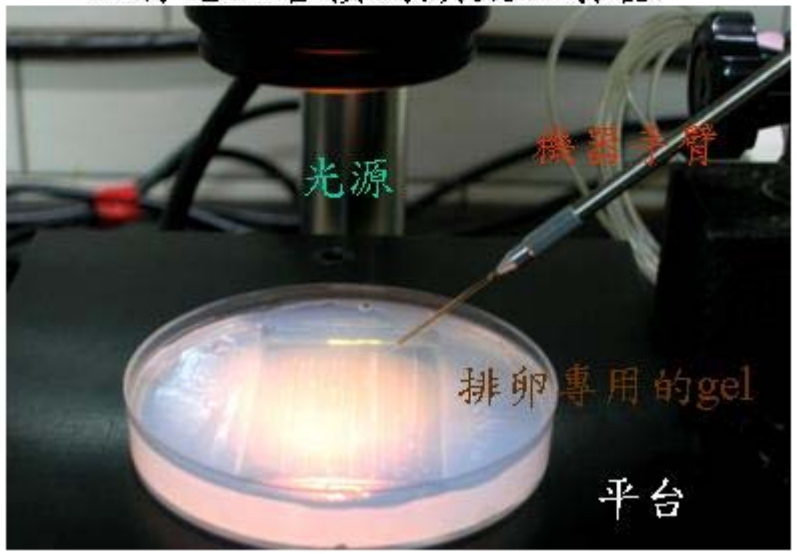
Microinjection operation (2)



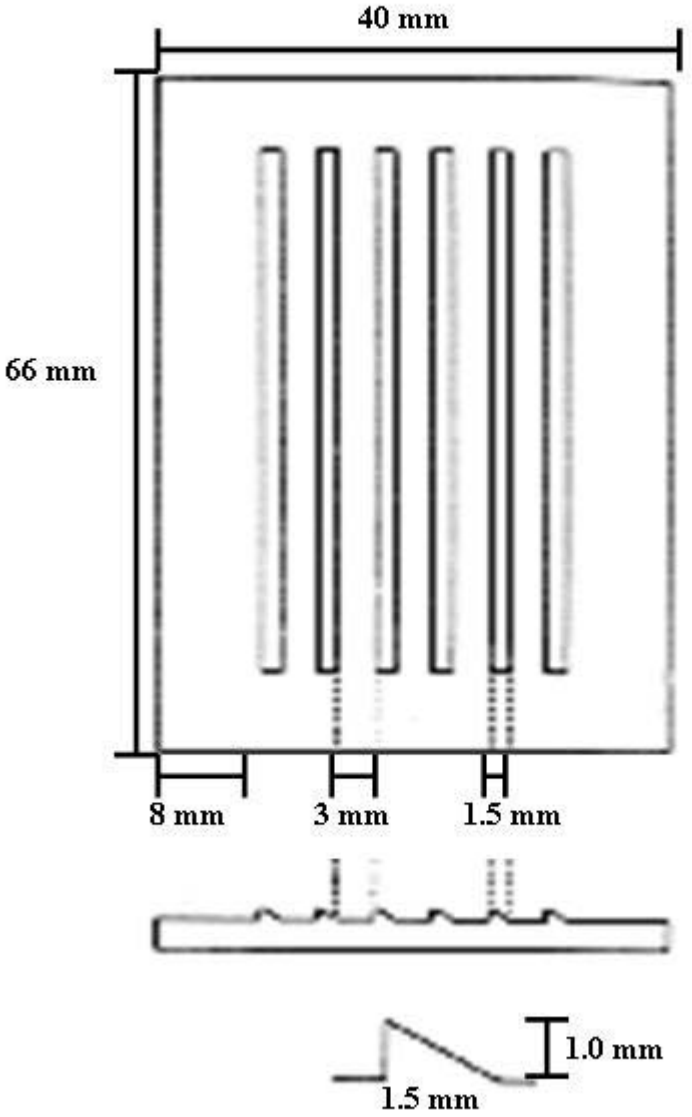
1.將DNA取至特製針頭

2.將特製針頭內的DNA送到毛細管

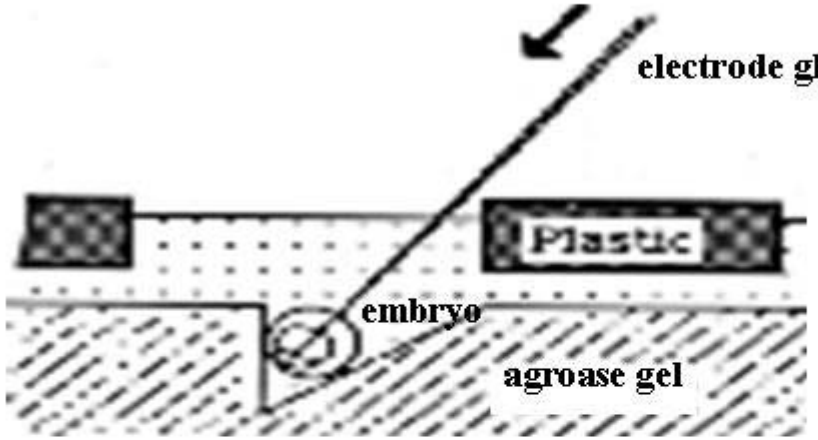
3.將毛細管接到顯微注射器

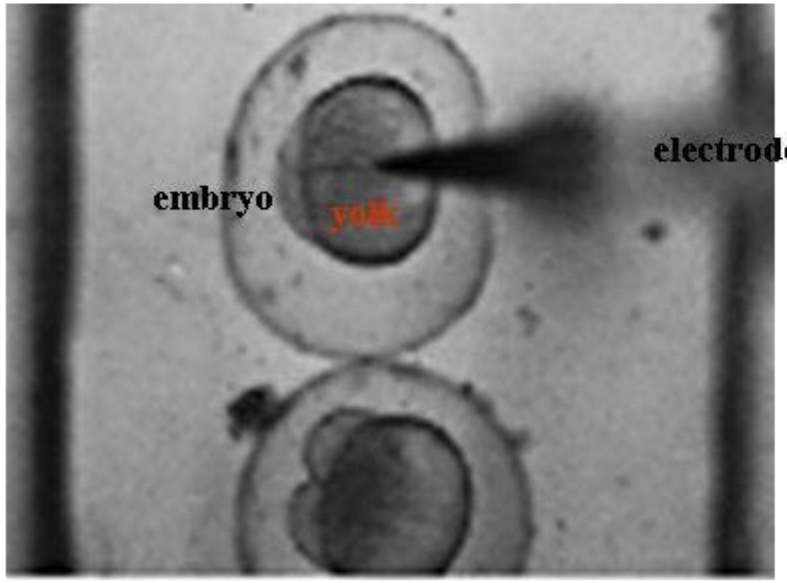


Microinjection



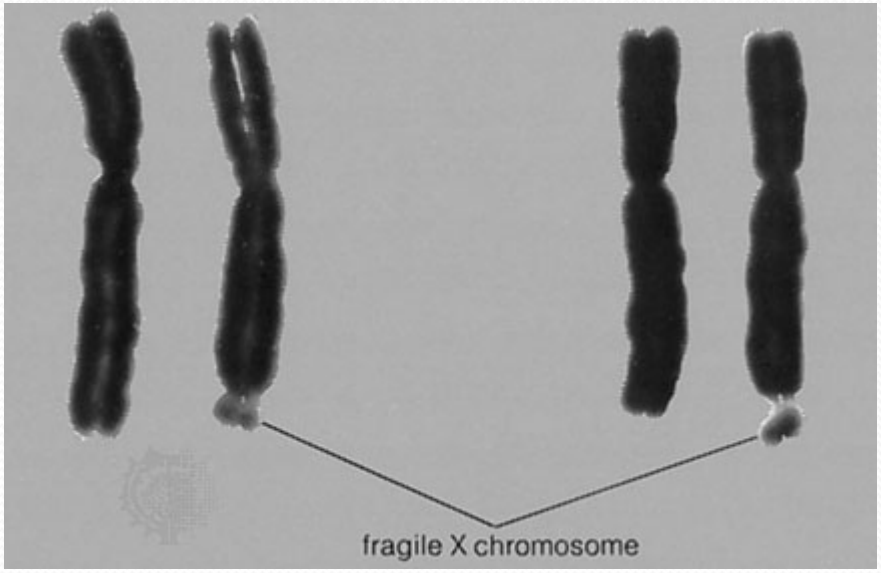
cross-sectional view of the injection set-upas an embryo is injected





Introduction

- **Fragile X syndrome (FXS)** is the most frequent inherited form of human mental retardation, with approximately one in 4,000 males and one in 8,000 females affected (Turner et al., 1996, Garber et al., 2006)



Chromosome karyotyping

BEHAVIOR

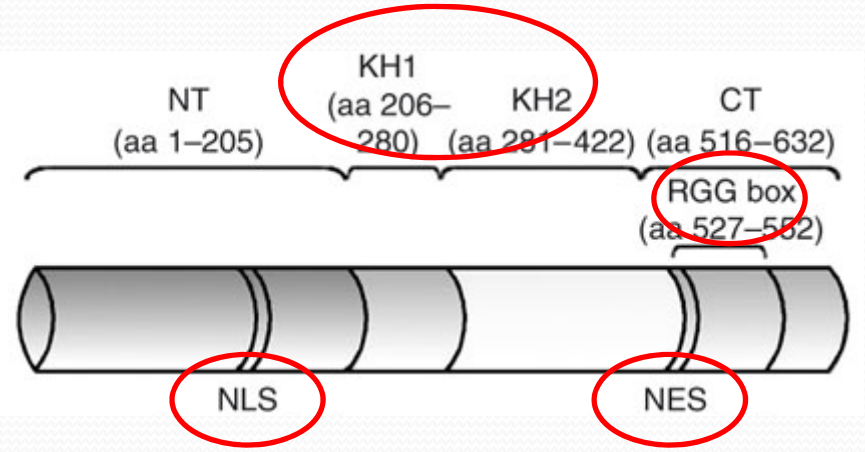
- Learning disabilities
- Attention deficit
- Hyperactivity
- Anxiety disorder
- Aggressiveness

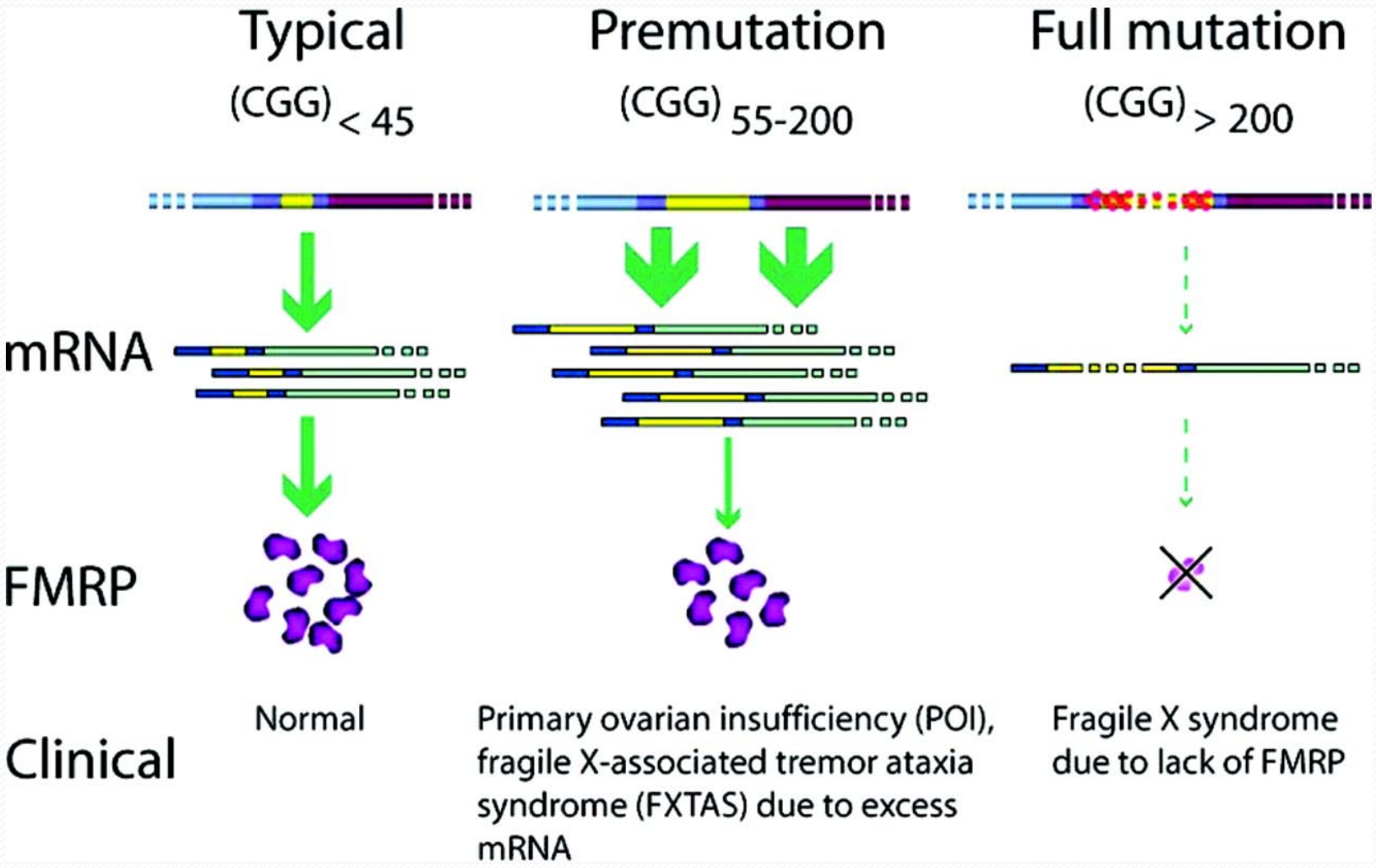
FXS

Fragile X mental retardation protein (FMRP)

- There is an expanded trinucleotide repeat CGG in the ***fmr1*** gene. (Huber et al., 2002)

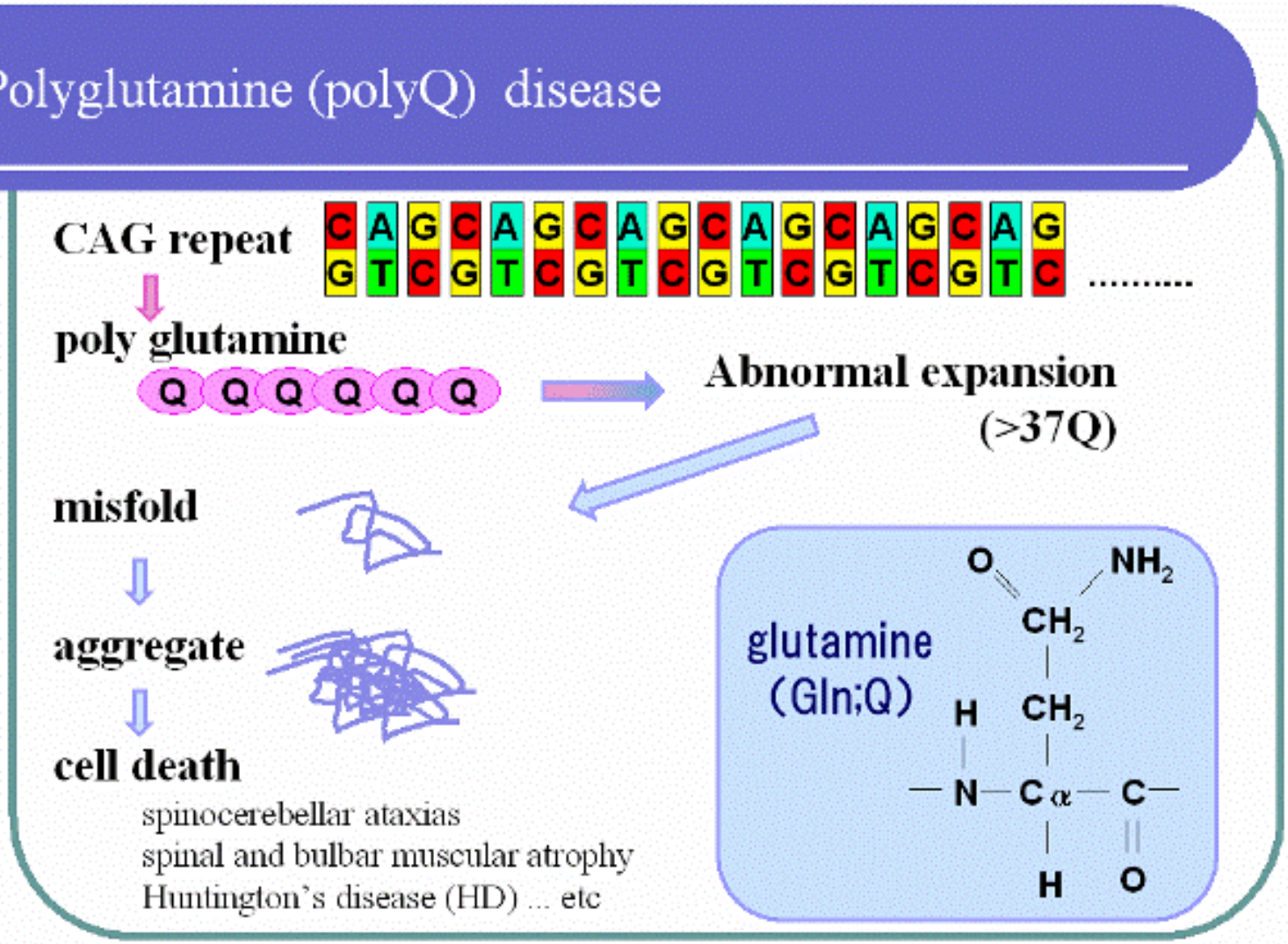
- FMRP is involved in the regulation (repression) of local protein synthesis at the synapse. (Bear et al., 2007)



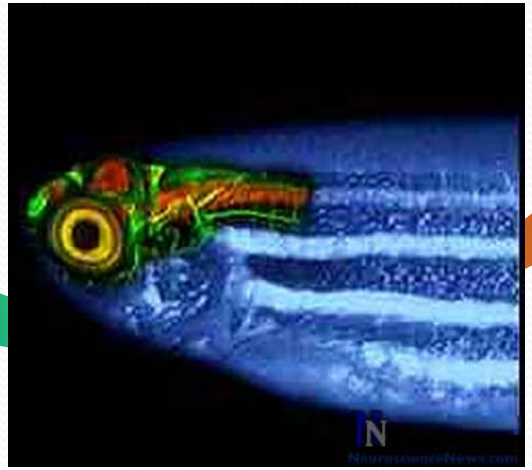


(adapted from Pediatric 123: 378–390, 2009)

Polyglutamine (polyQ) disease



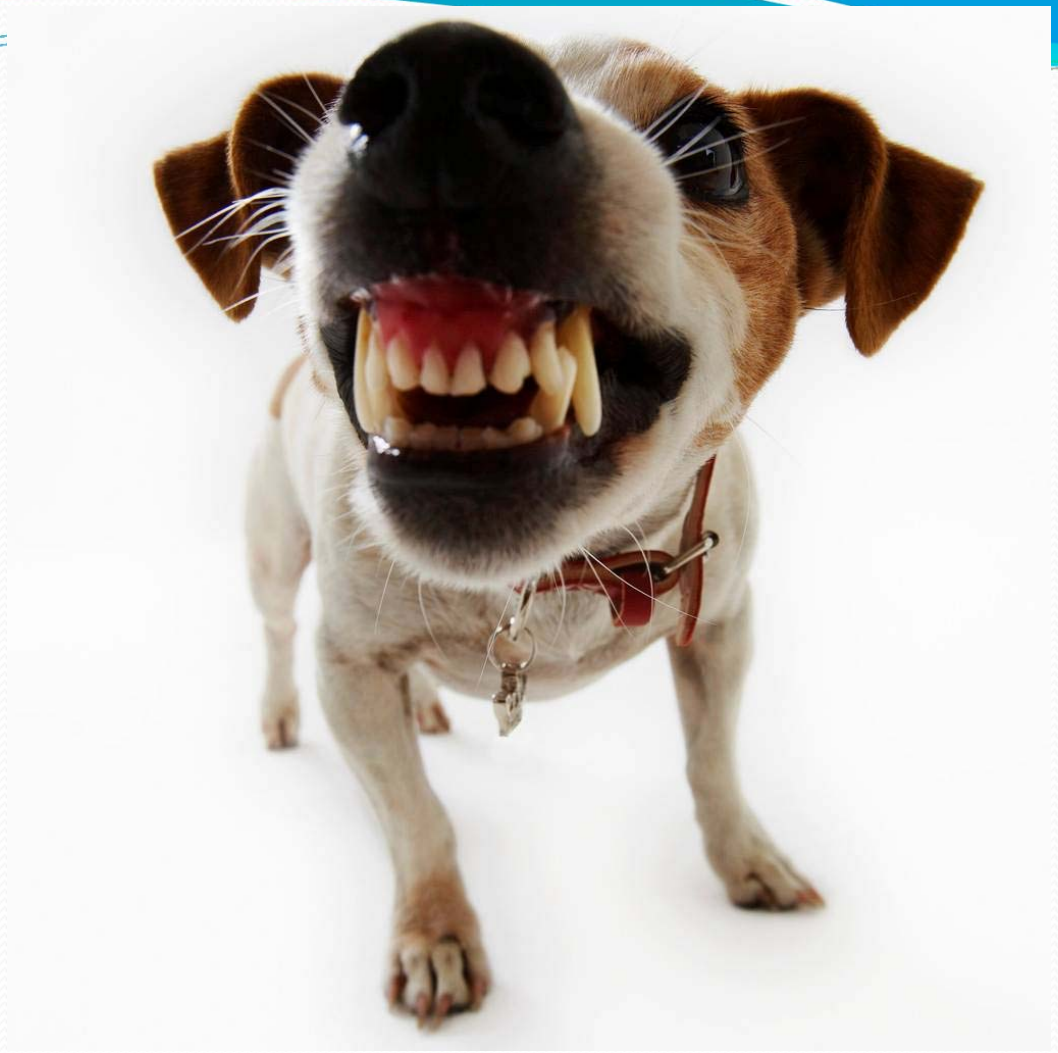
Animal models for studying FXS



The amino acid sequence alignment of FMRP from human, mouse, frog, zebrafish and fruit-fly revealed high conservation at functional domains (shared 72% amino acid identity with human) (van ’t Padje et al., 2005)

Table 1: The Zebrafish Model for Drug Screening

Advantages	• Small embryos are transparent, large number of offspring, short-generation time
	• Inexpensive, easy handling, large-scale screen amenable • A vertebrate, *in vivo* system with combination of forward and reverse genetics • Phenotype-based screening can be performed in wild-type, mutant and promoter-driven reporter transgenic embryos • The screening is robust and high throughput • Relevance to human diseases, high degree of similarity to humans in drug response



They won’t bite

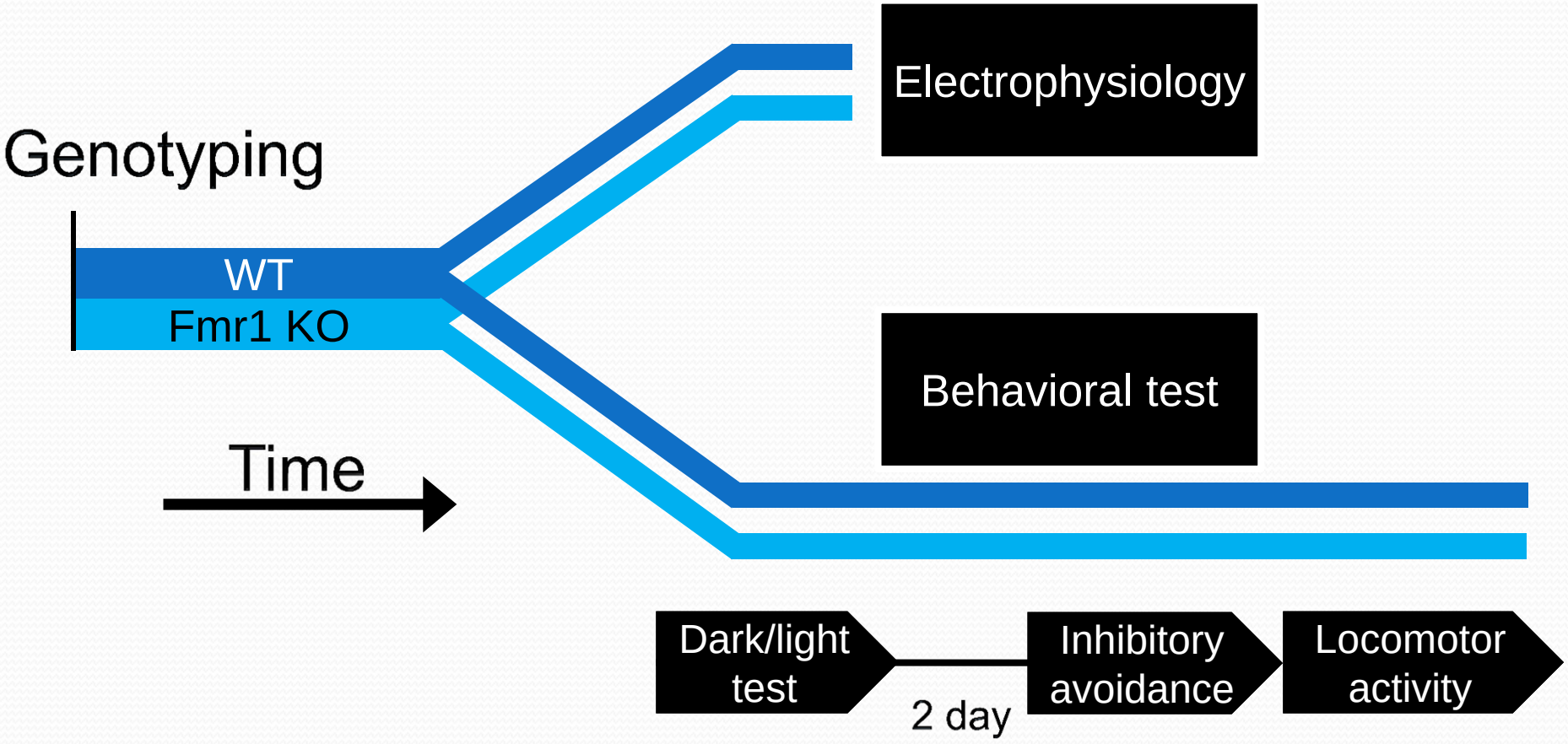
They won't pee on the floor (or your lab coat)

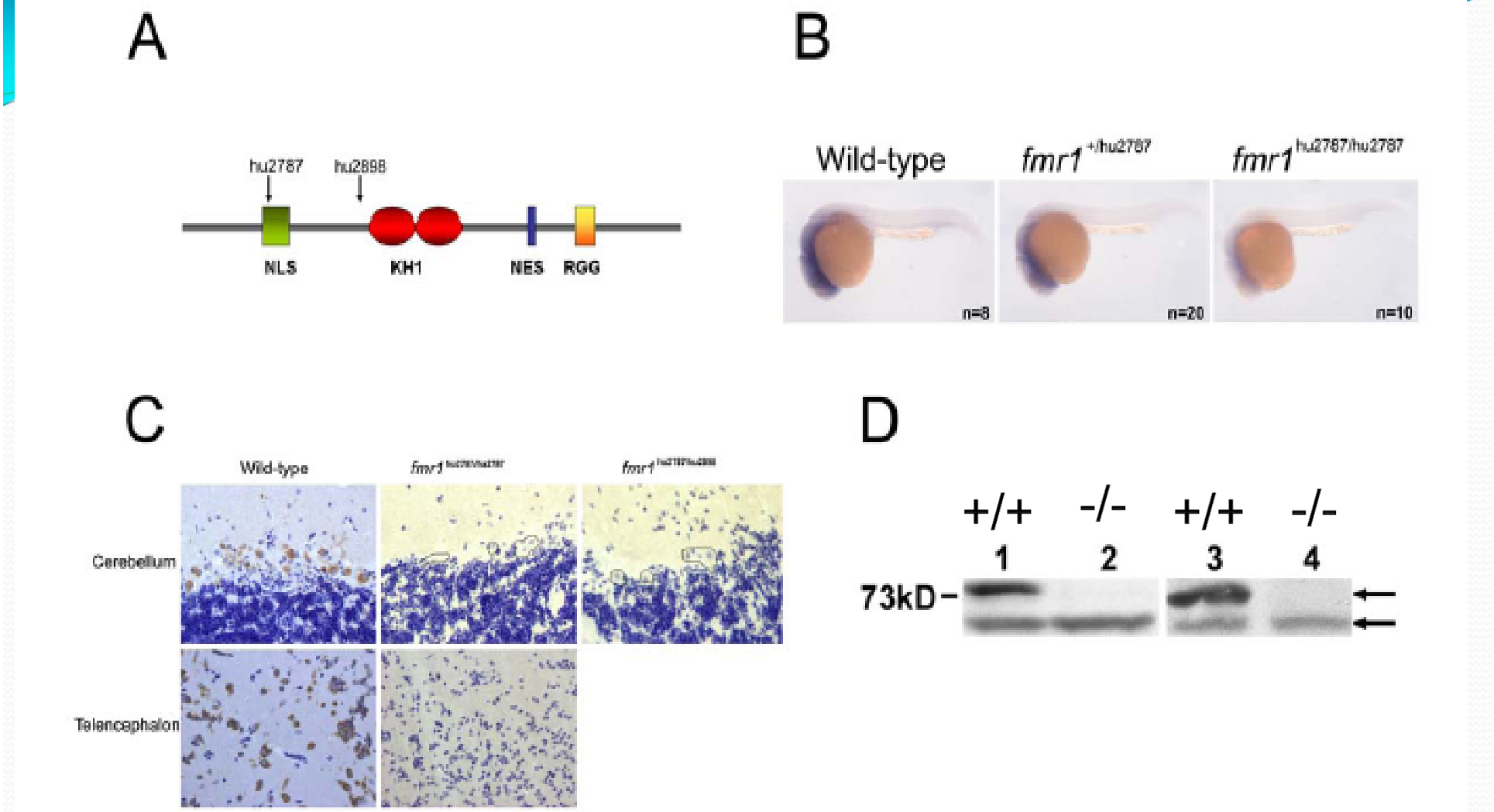
They won’t escape from the cage

Aims

- To study behavioral deficits in Fmr1 KO zebrafish.
- To determine the abnormity on telencephalic synaptic plasticity in Fmr1 KO zebrafish

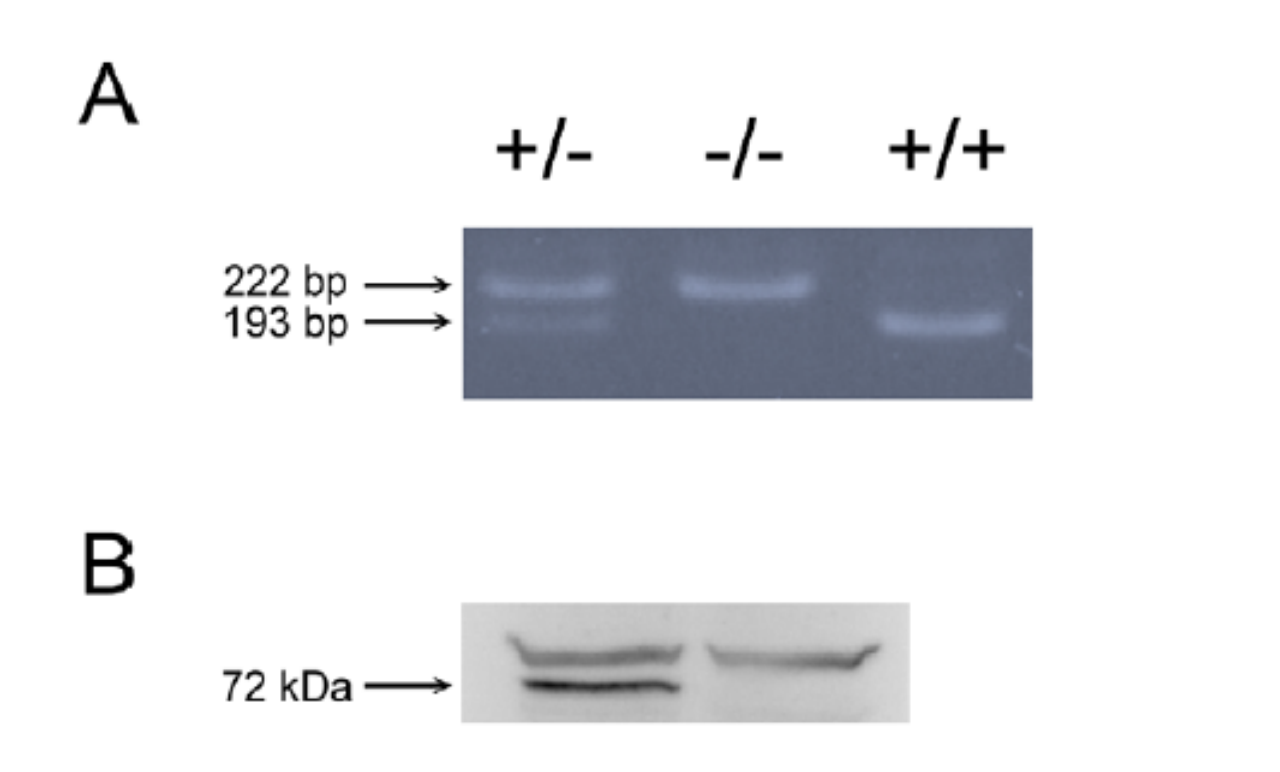
Outline of experimental procedures





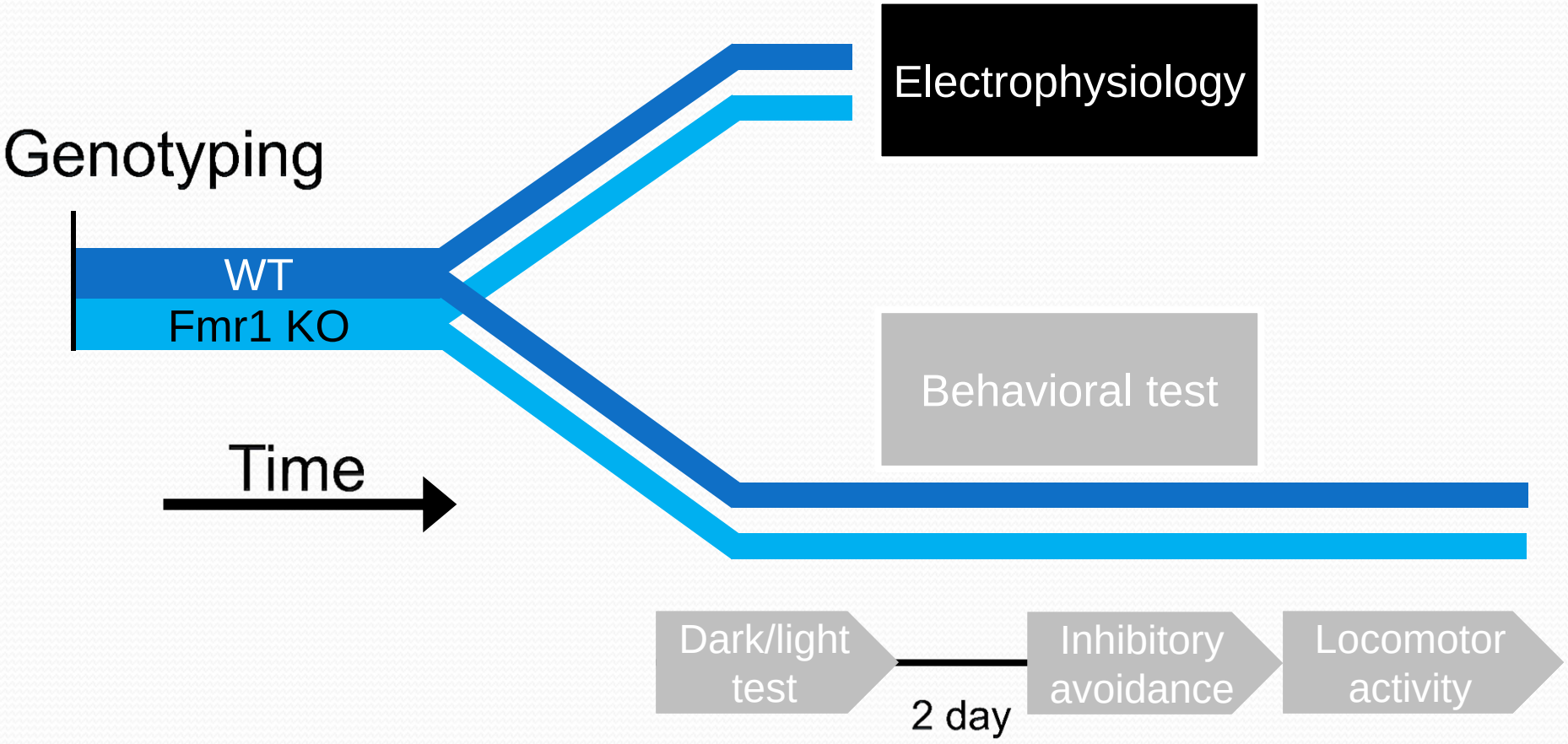
Fmr1 Knockout Zebrafish was generated by Rene´ F. Ketting's lab. (den Broeder et al., 2008)

Genotyping and western blot analysis of Fmr1 knockout zebrafish.

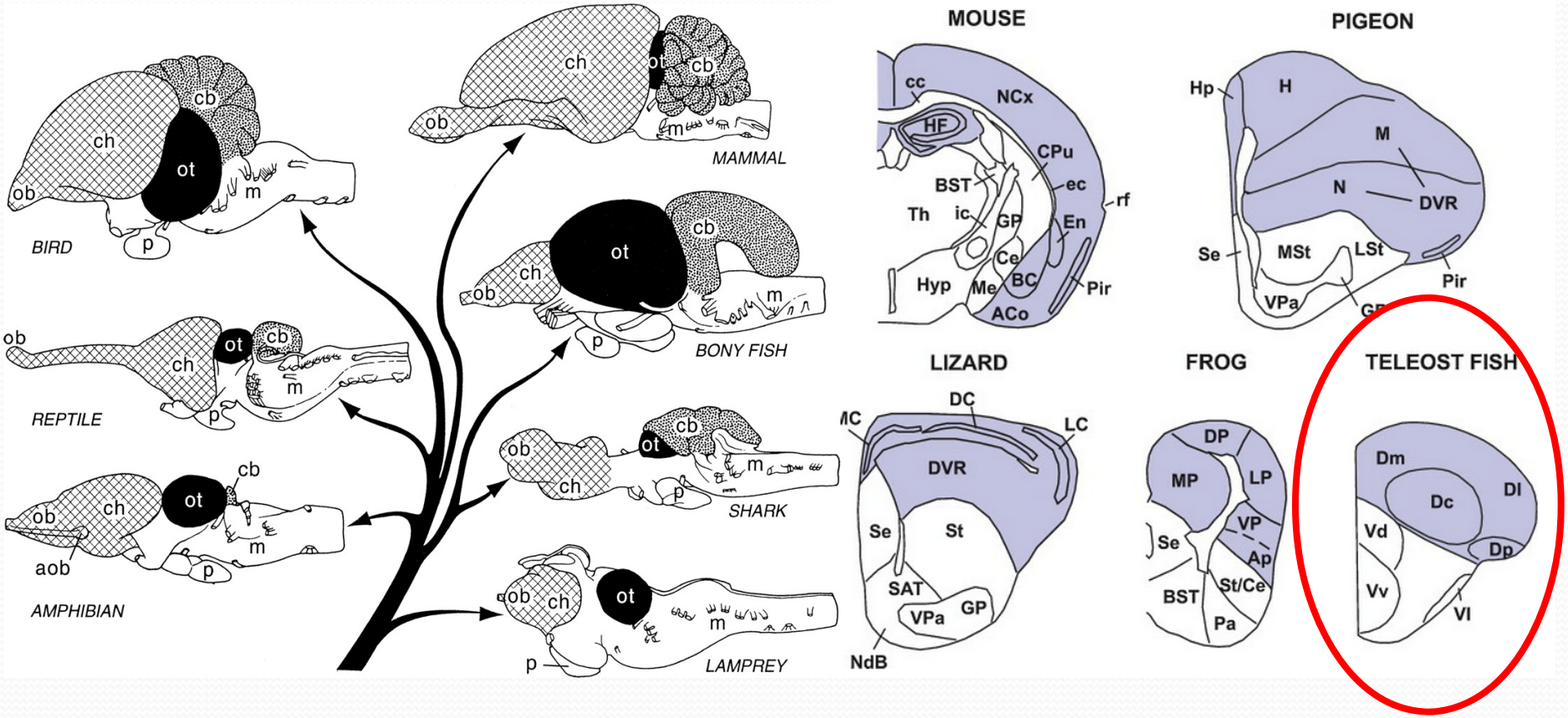


We thank Dr. Rob Willemsen for the kind gift of zebrafish FMR-1 antibody.

Outline of experimental procedures

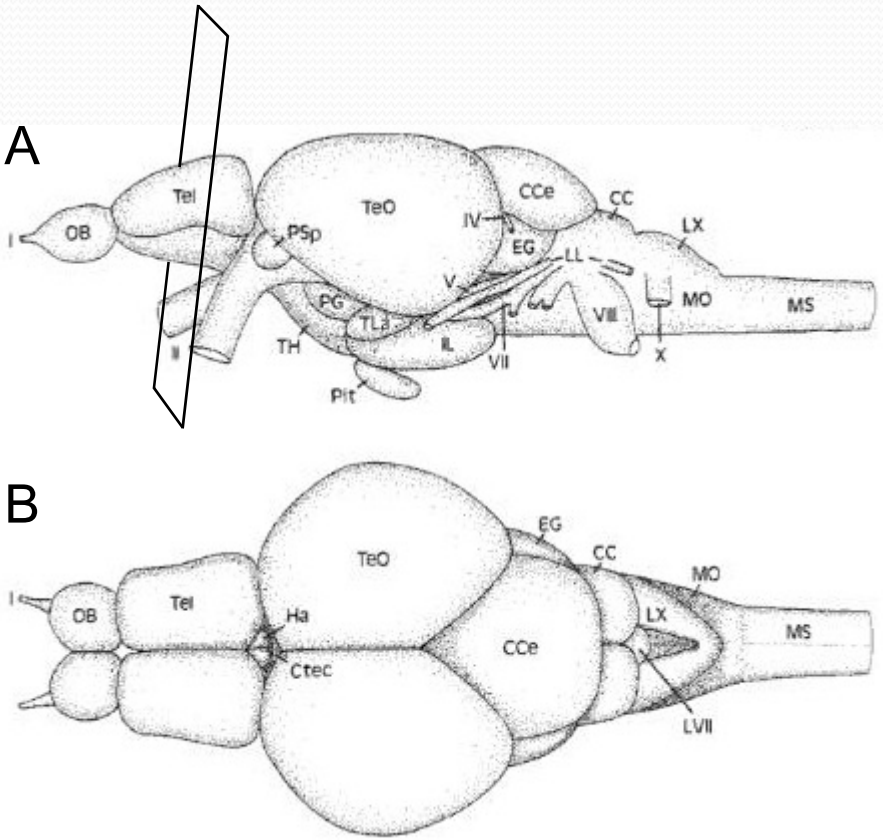


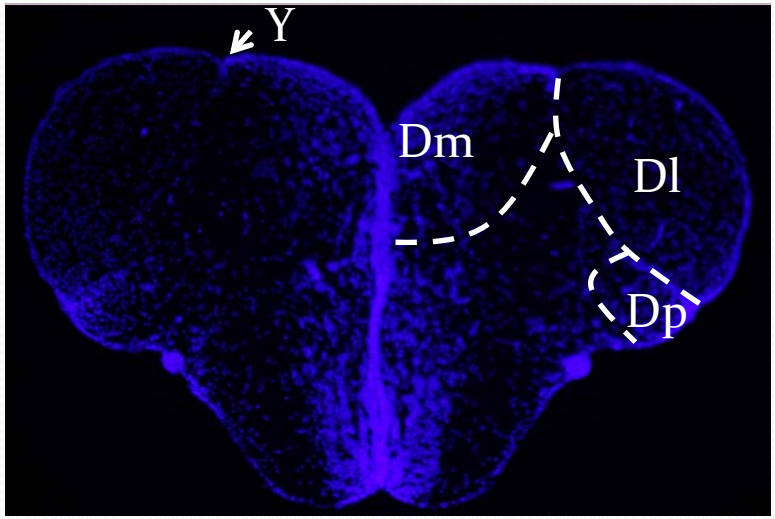
Determine the recording site



(Adapted from Northcutt, 2002; Medina and Abellan, 2009)

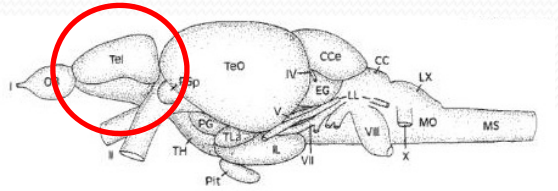
Topography of the telencephalon

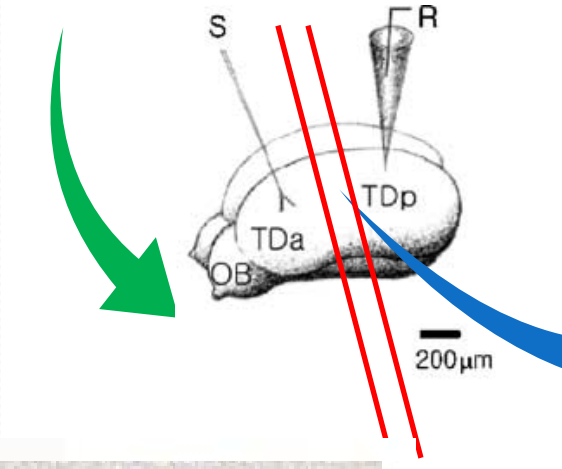


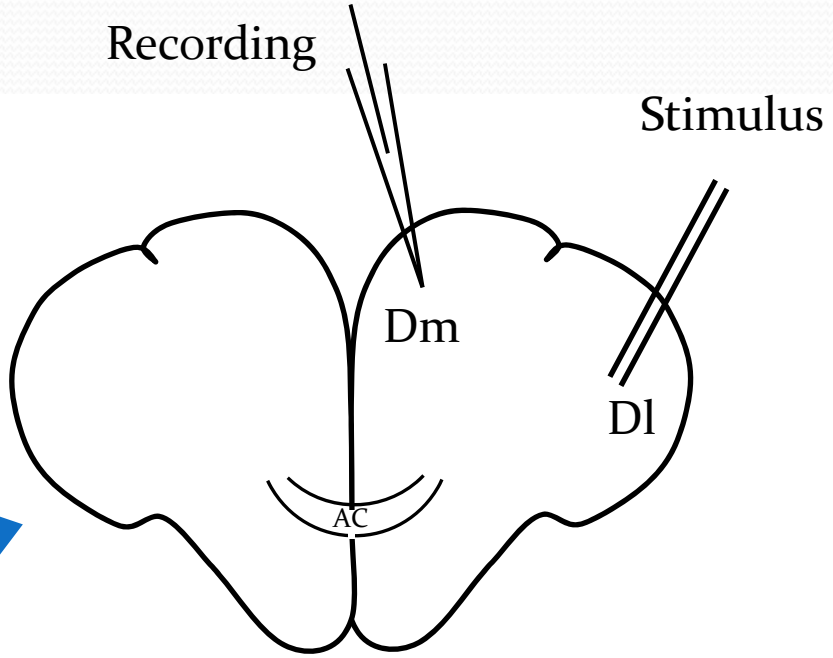


Dm : dorsal medial
Dl : dorsal lateral
Dp : dorsal posterior
Y : sulcus ypsiloniformis

Brain slices preparation





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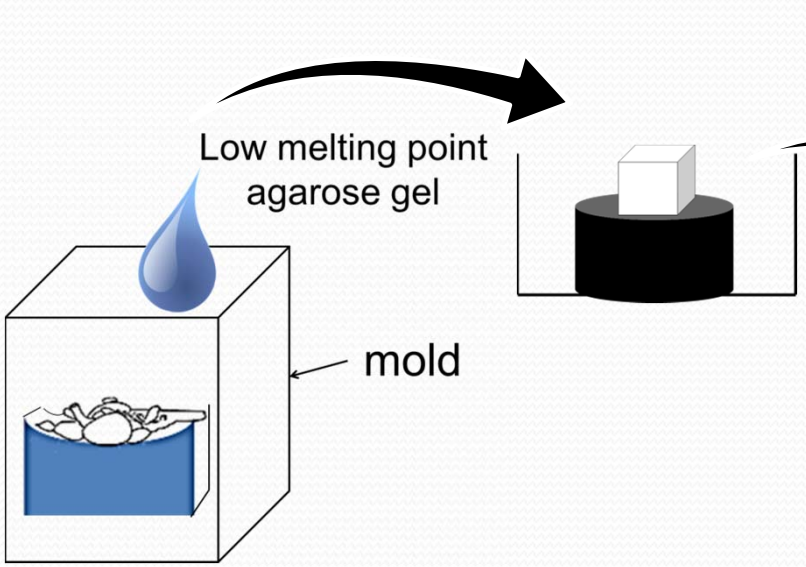
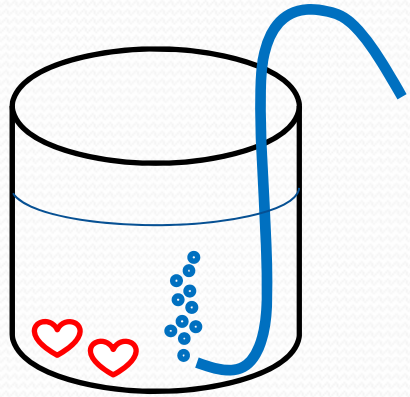
Extracellular recording

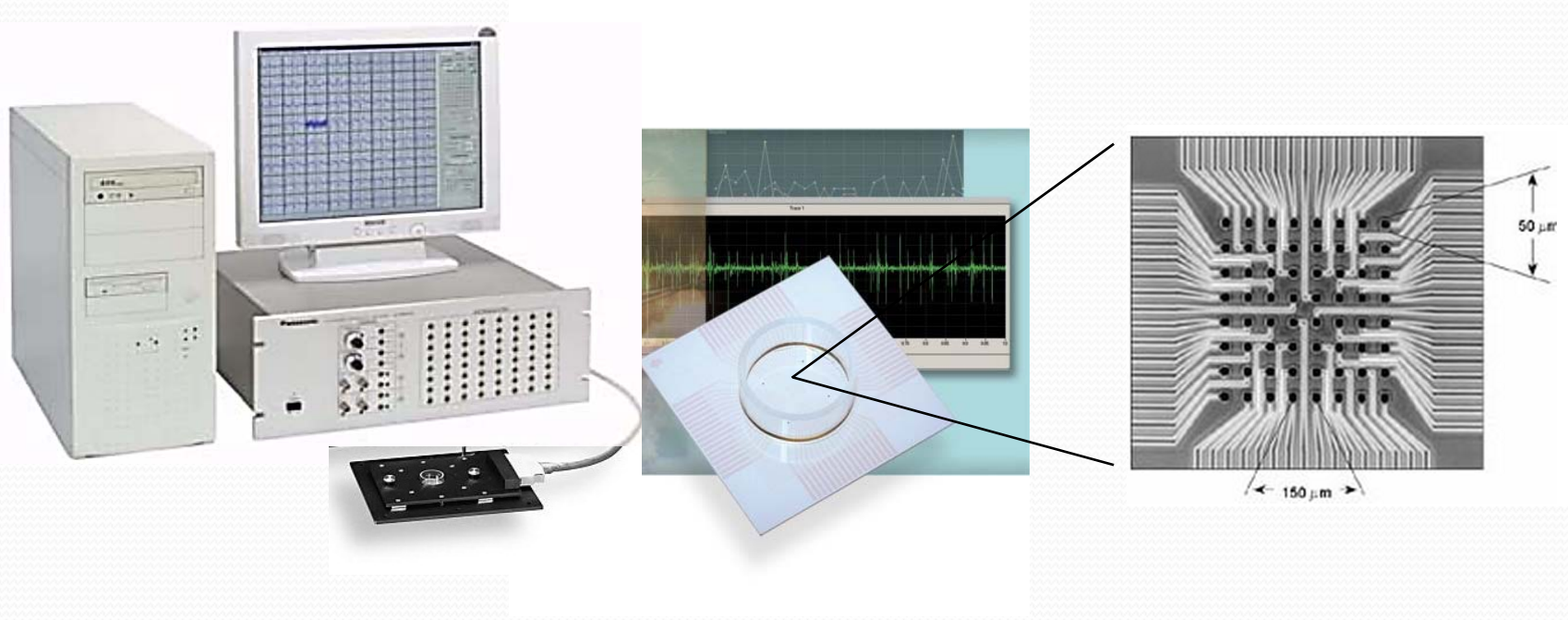
- Animals : zebrafish (3 to 4 months of age)
- Preparation of acute telencephalic slices



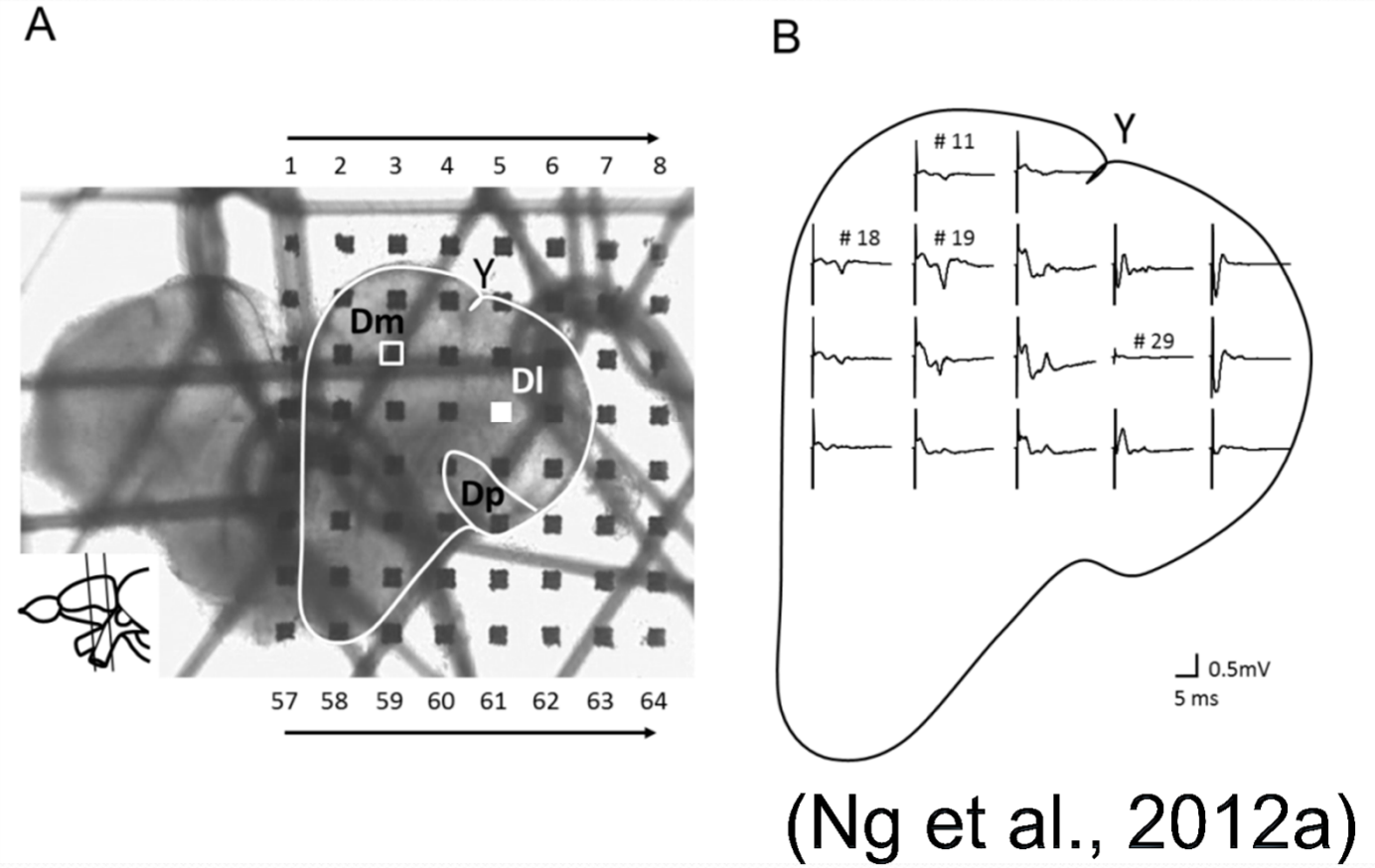


95% O_2/5% CO_2
in RT at least 1 hr

MED64:
multi-electrode recording system

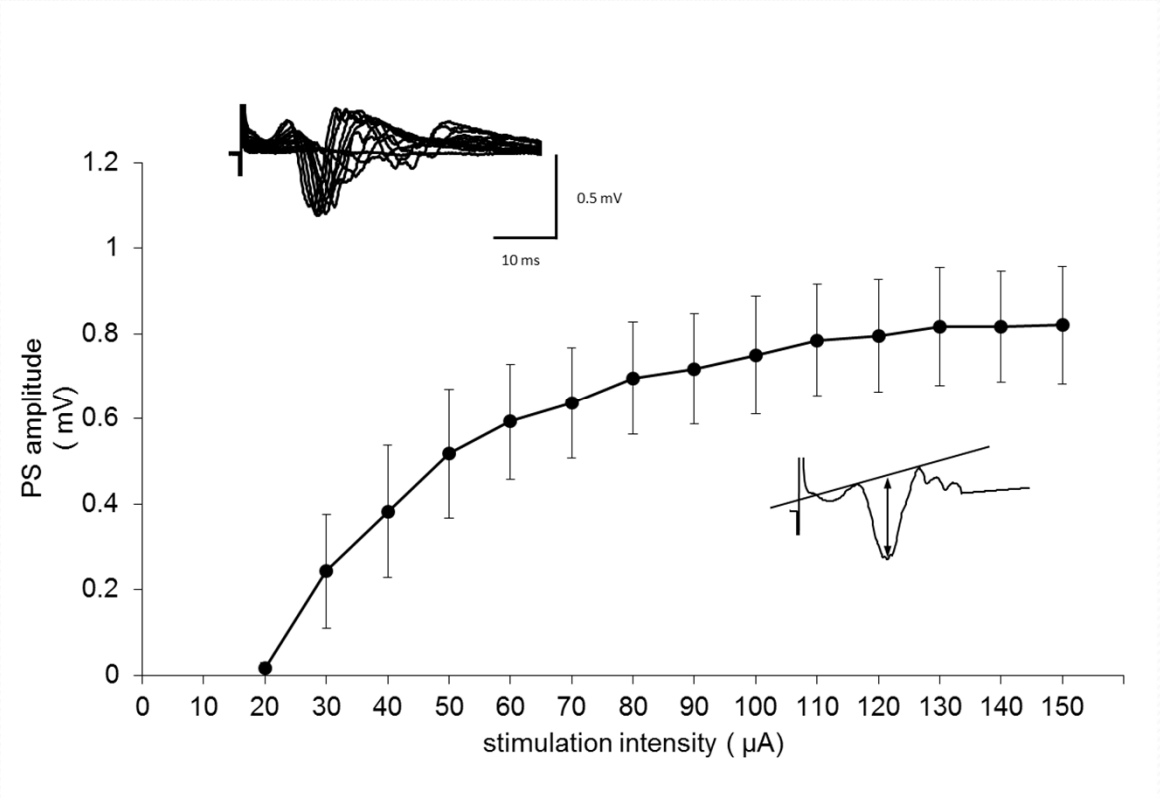


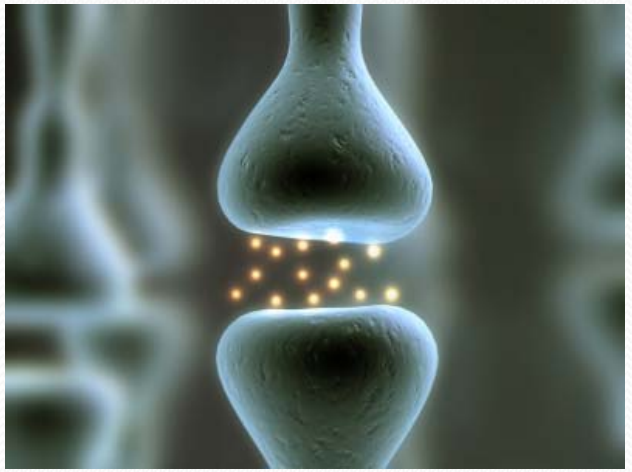
The field potentials evoked in the dorsal pallium by stimulation of the lateral division (Dl) of the pallium.



(Ng et al., 2012a)

The input-output relationship at the Dl-Dm pathway.

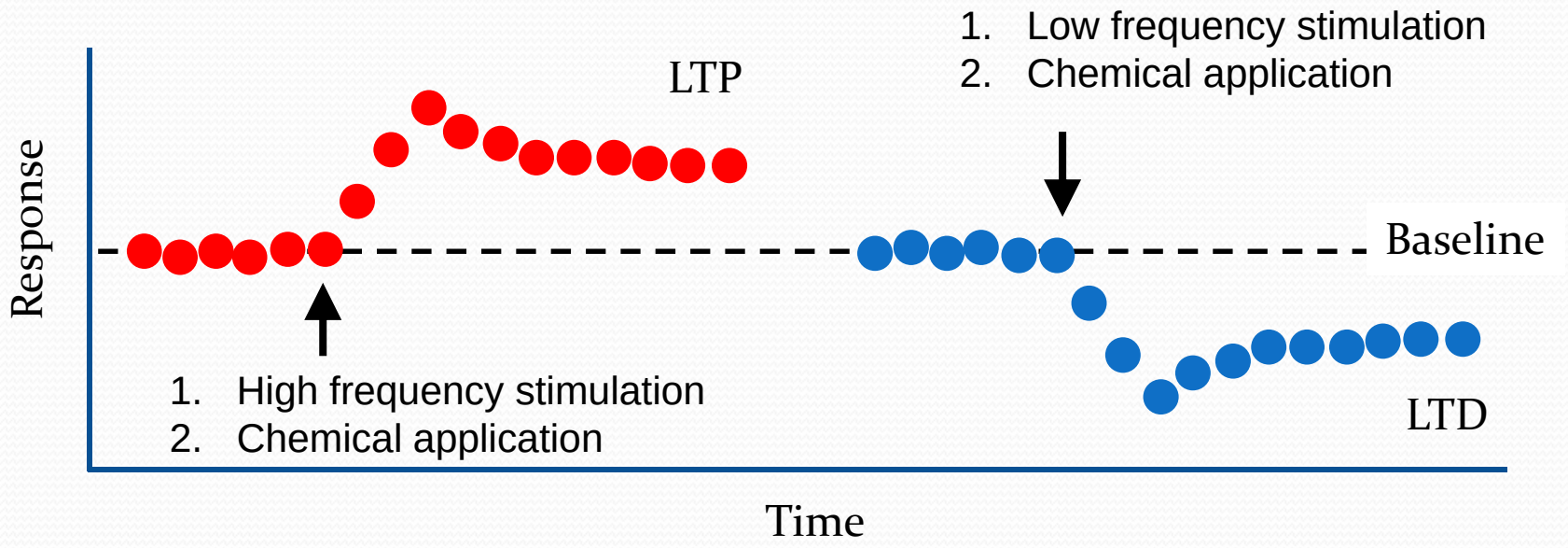




(Ng et al., 2012a)

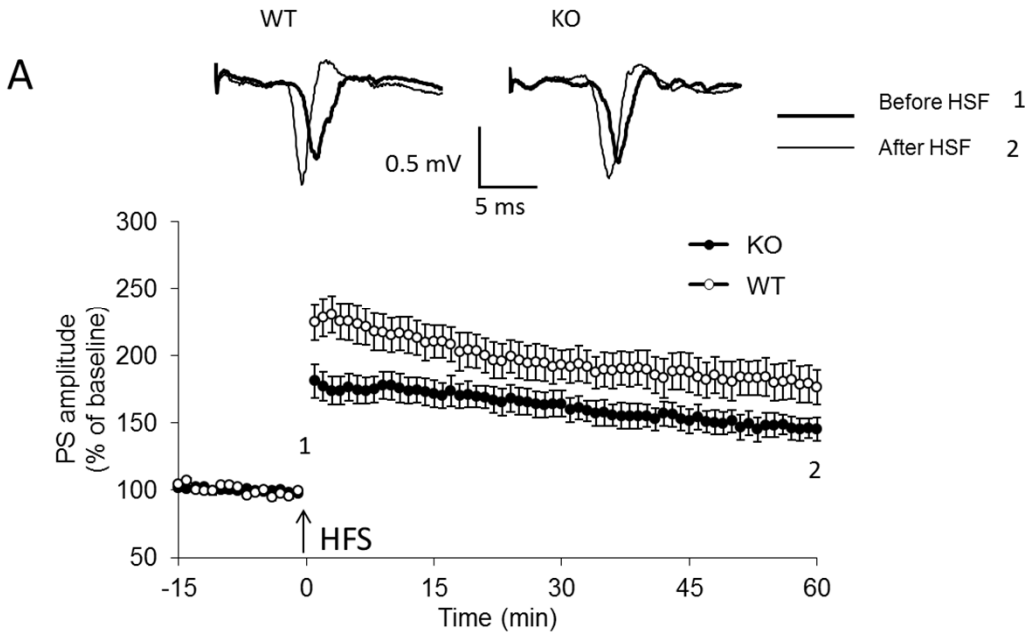
Synaptic plasticity

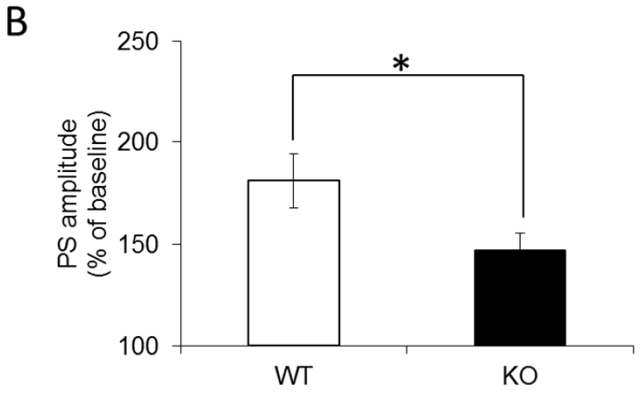
- **Long-term potentiation** (LTP) **and long-term depression** (LTD) of excitatory synaptic transmission, are wide spread phenomena expressed at possibly every excitatory synapse in the mammalian brain.



Results

Reduced long-term potentiation (LTP)

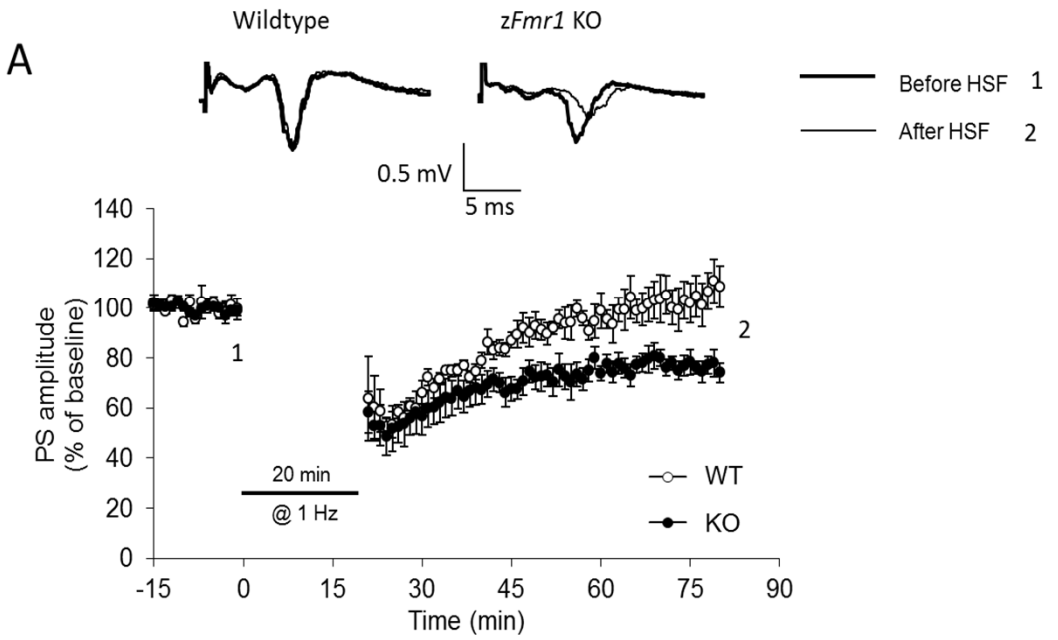


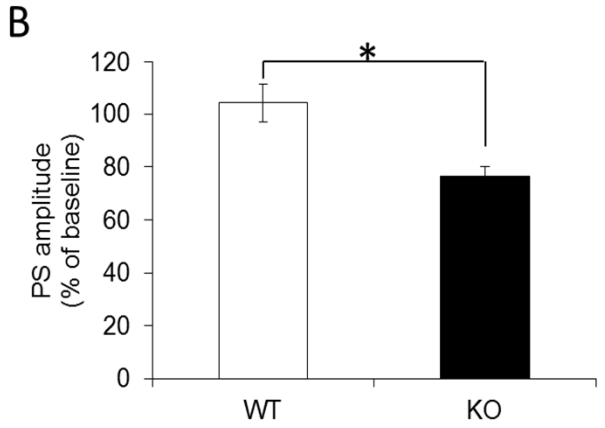


(Ng et al., 2013)

Results

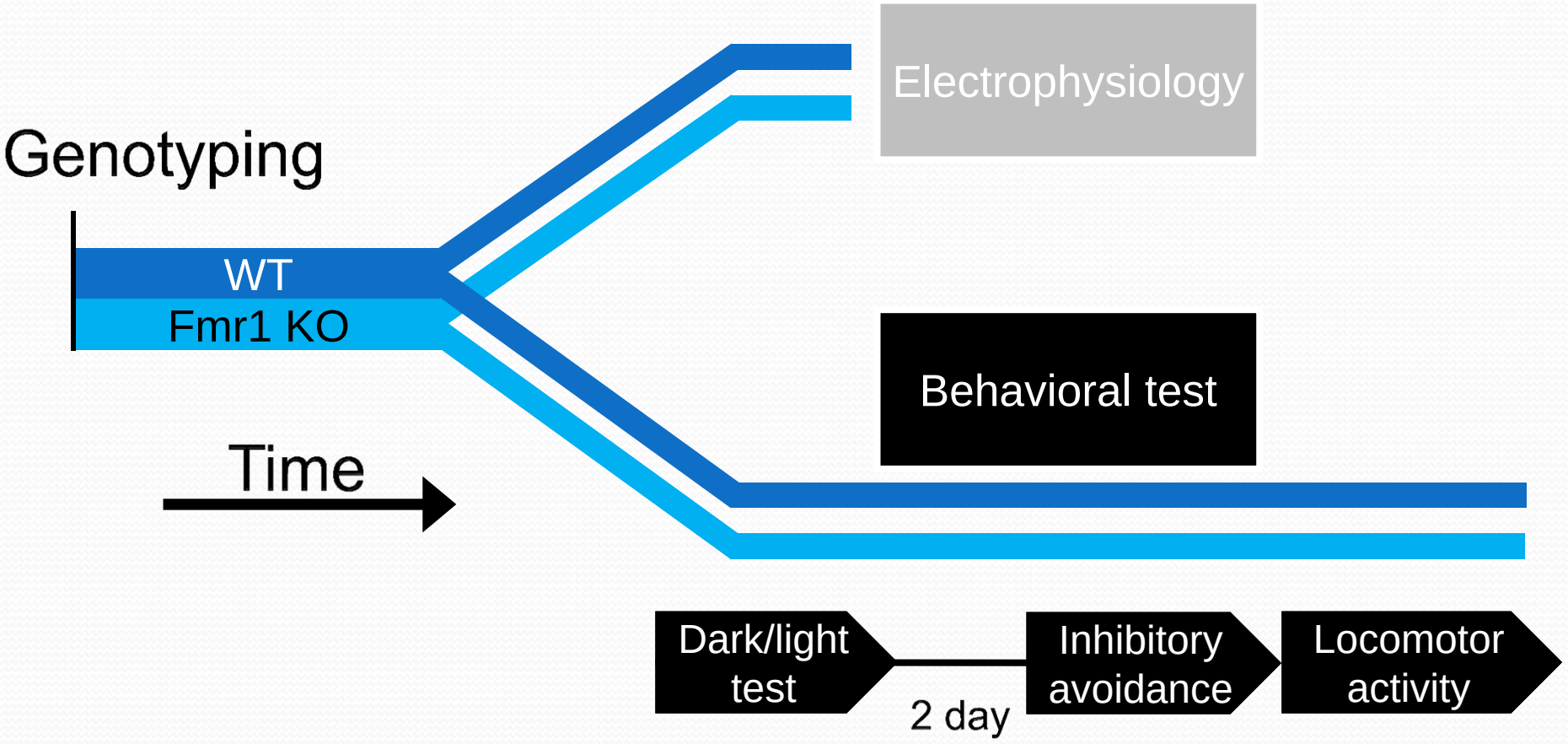
Enhanced long-term depression (LTD)





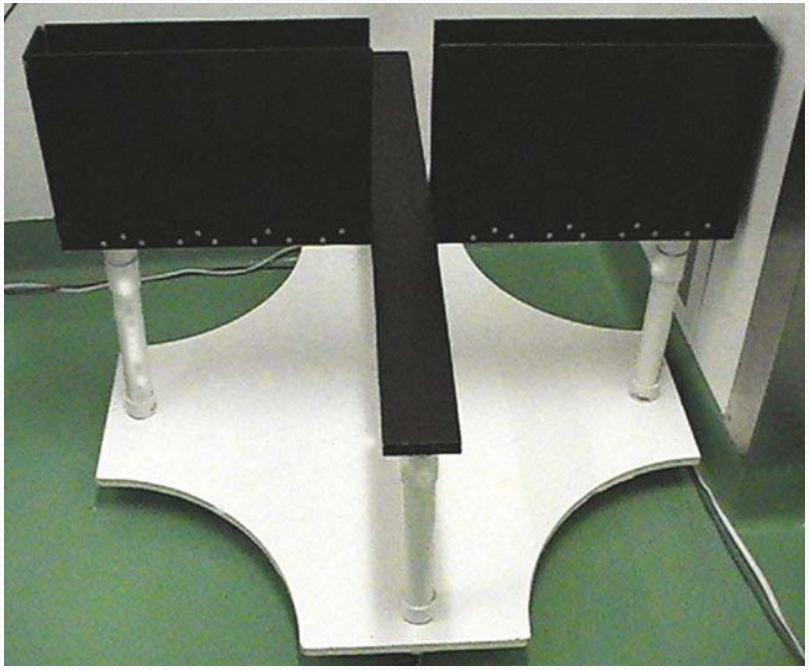
(Ng et al., 2013)

Outline of experimental procedures

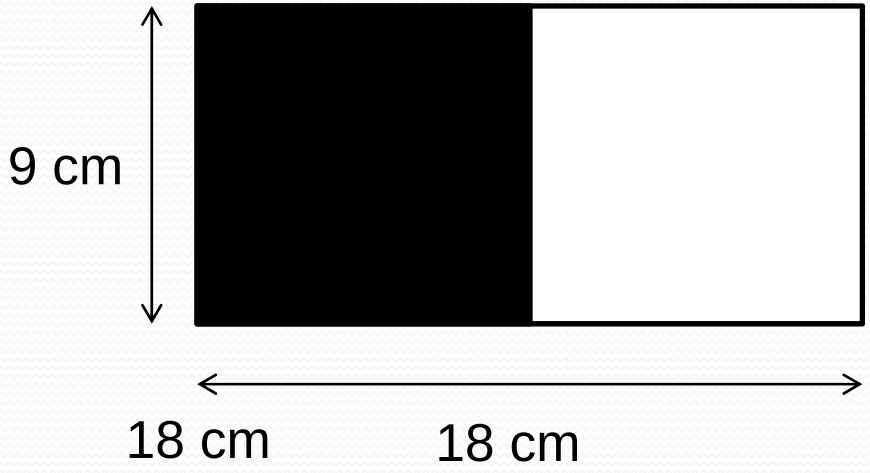


Dark/light

~ Evaluate the anxiolytic-like response in zebrafish

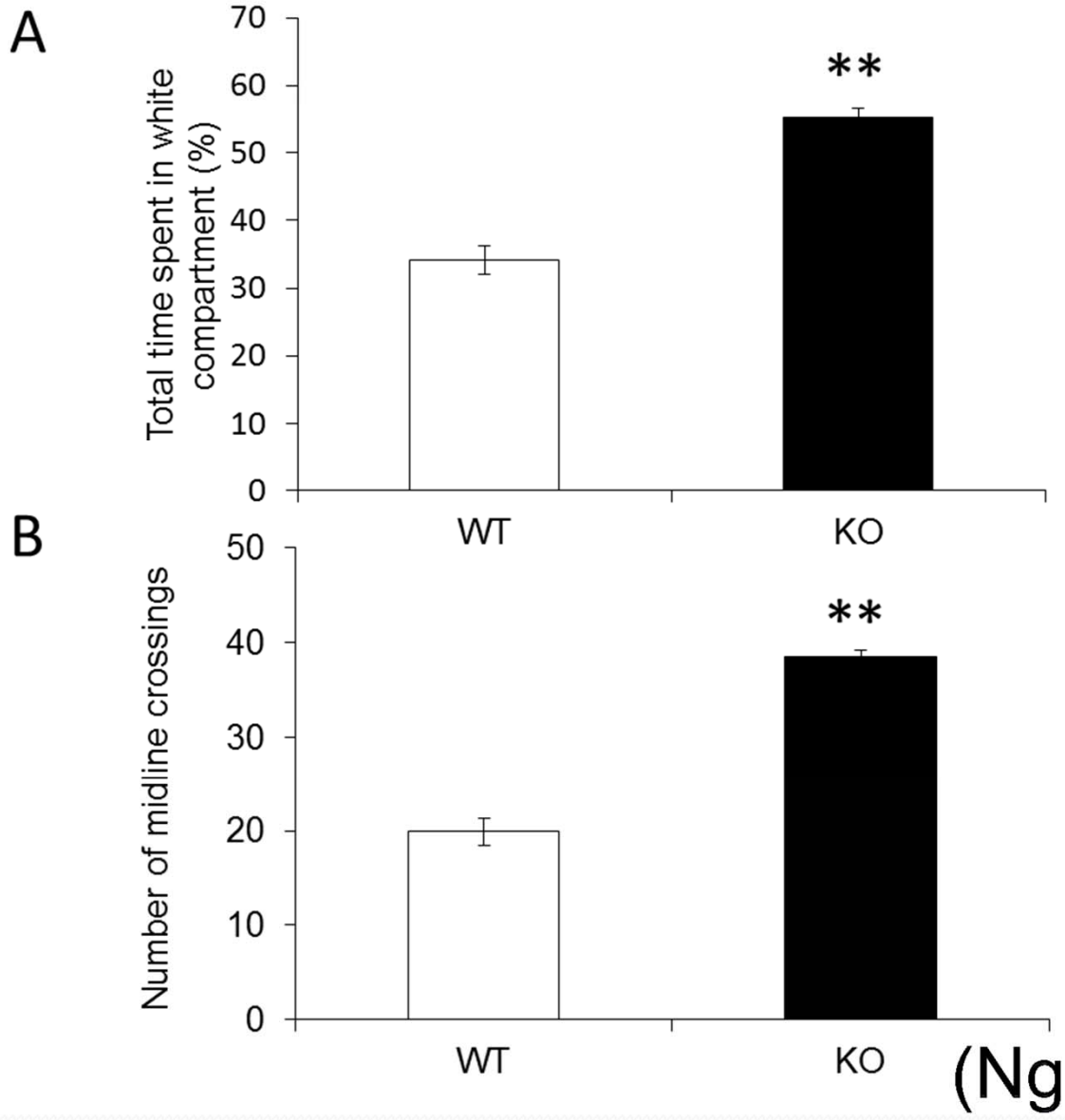


Elevated plus maze
(for rodent)



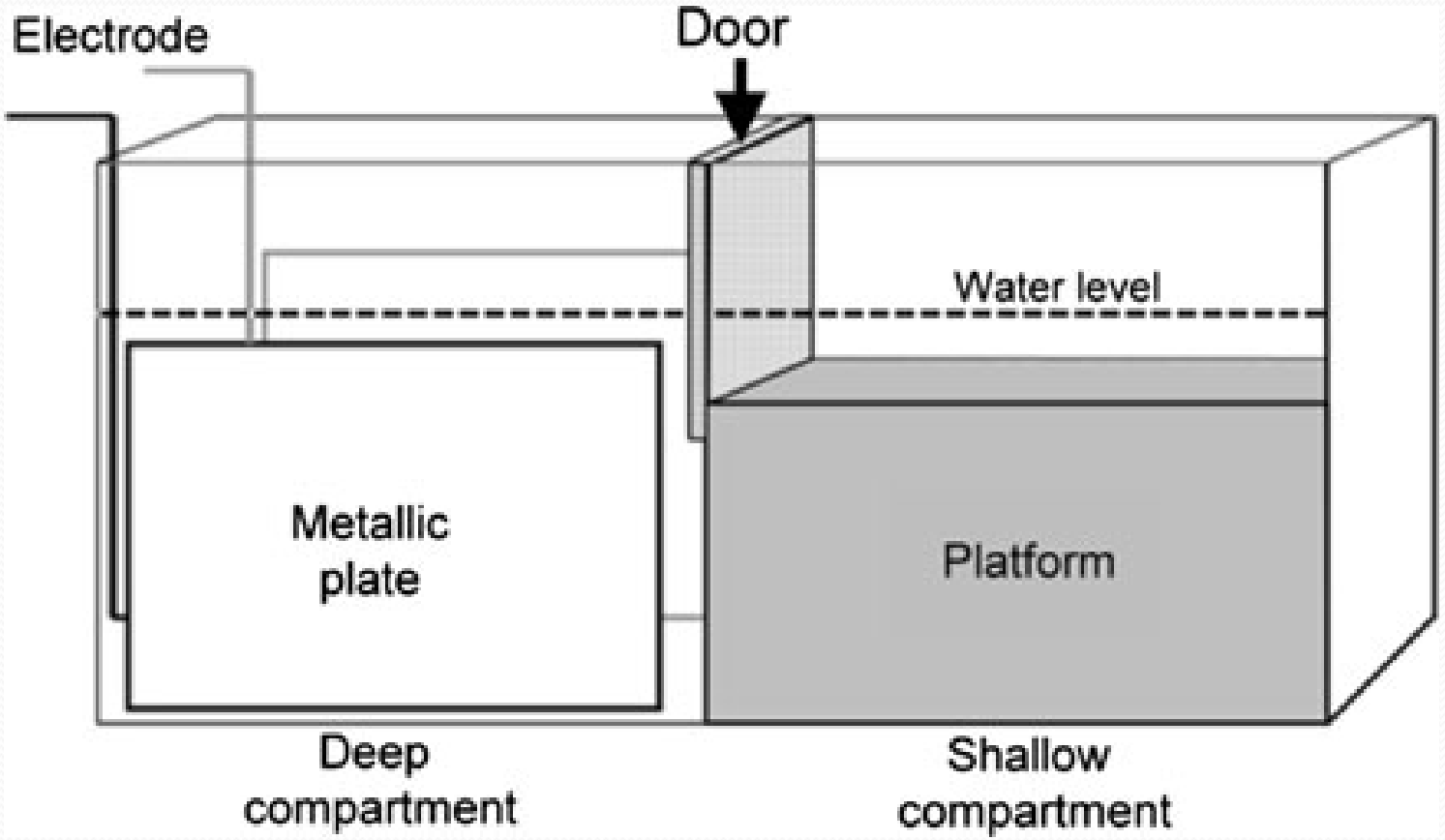
the fish were allowed to swim freely between the two compartments without sliding door for 5 min

Results

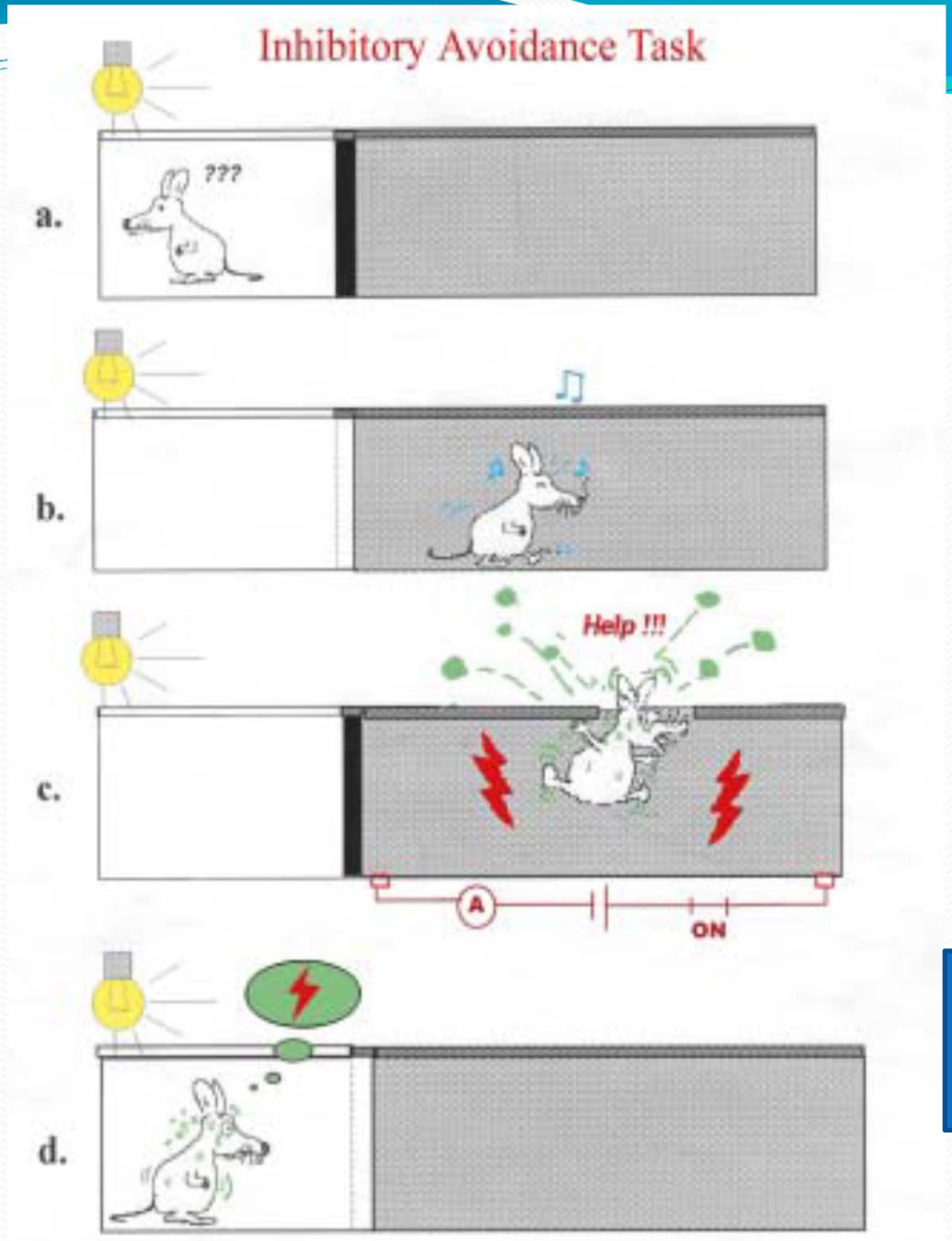


(Ng et al., 2013)

Inhibitory avoidance

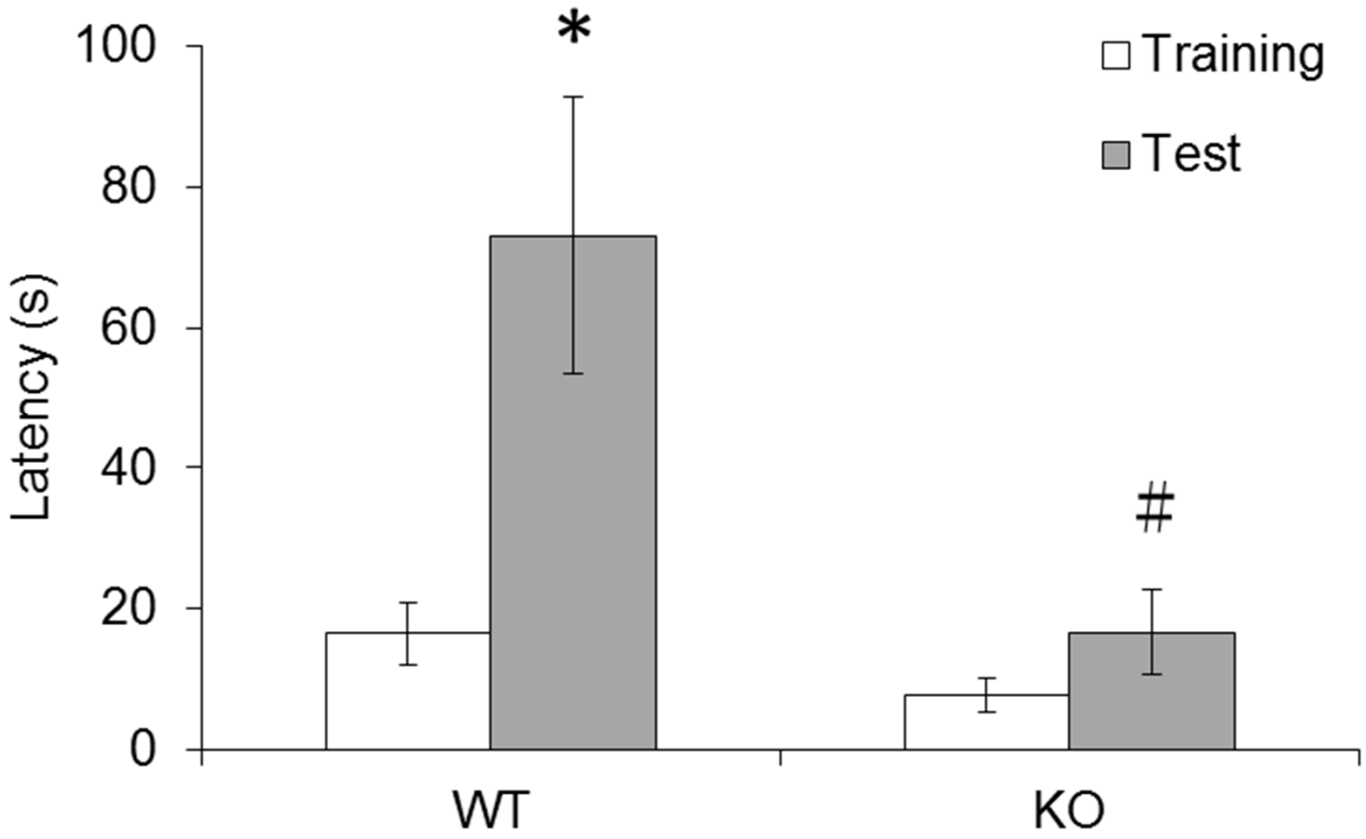


(Ng et al., 2012b)



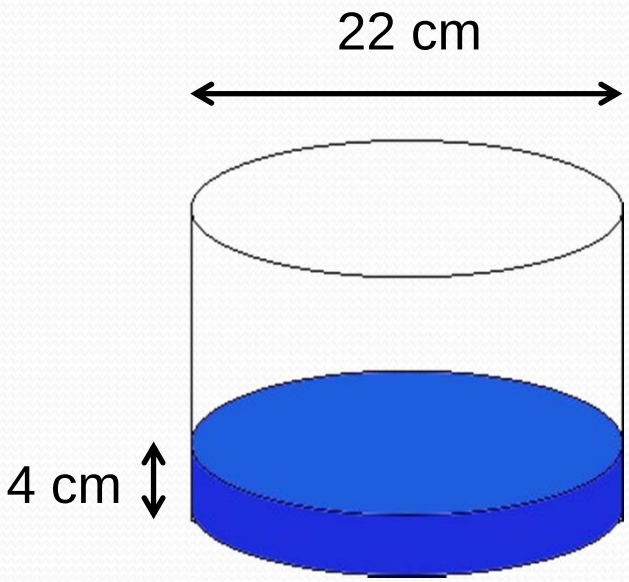
Video

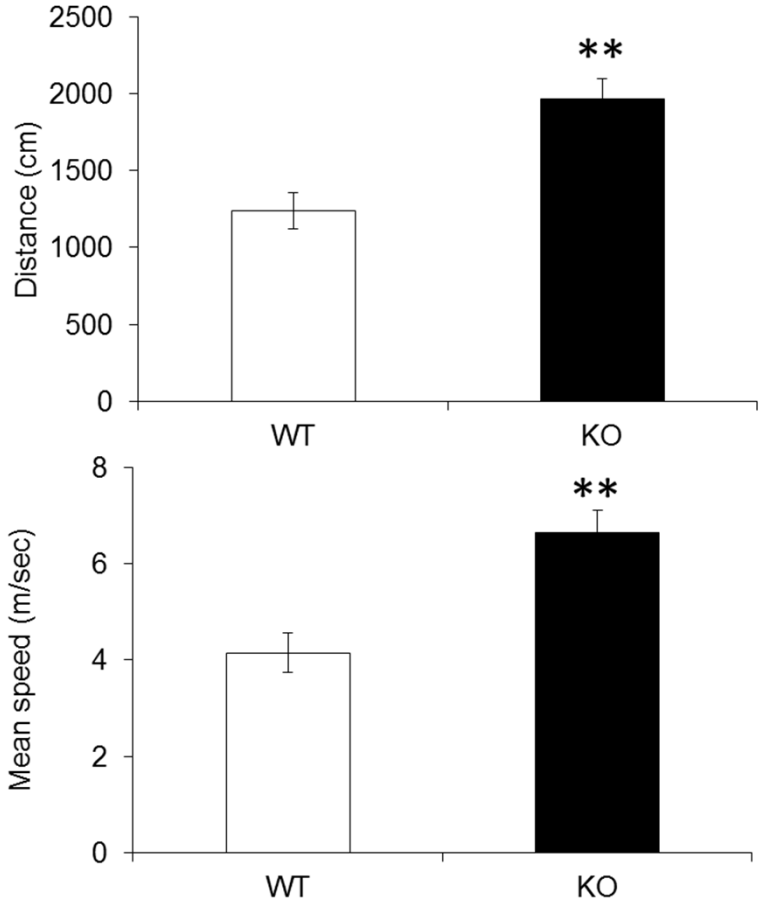
Impaired avoidance learning in Fmr1 KO zebrafishes



(Ng et al., 2013)

Locomotor activities increased





(Ng et al., 2013)

Summary

- Electrophysiological recordings from telencephalic slice preparations of Fmr1 KO fishes showed markedly reduced LTP and enhanced LTD.

- Fmr1 KO fishes exhibit anxiolytic-like behavior, impaired avoidance learning, and hyperactivity.

Acknowledgments

~ Dr. Jen-Leih Wu

~ The Hubrecht laboratory and the zebrafish mutation project from Sanger institute

~ Dr. Rob Willemsen (FMRP antibody)

~ Research grant from National Science Council, Taiwan

Acknowledgments

吳曜如

許竣博

吳民聰

吳世郁

Thanks for your listening