斑馬魚在生物醫學研究之應用

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神經科學的發展

- 史前時代:

  1.腦對生命是必須的

  2.顱骨穿孔術(Trepanation): 顱骨上有癒合的痕跡

- 古埃及時代

  1.埃伯斯紙草卷:專門記述人類心臟運動的內容

  2.心臟掌管靈魂與記憶(不是腦)

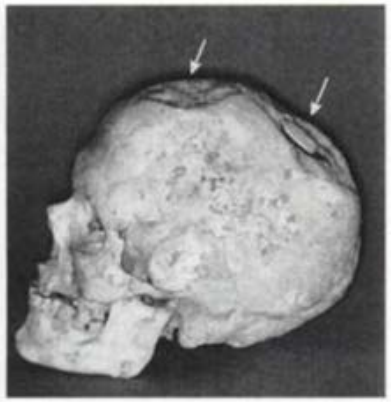
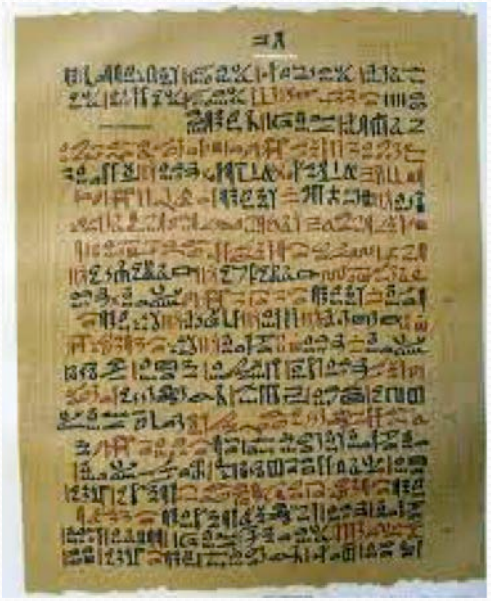


FIGURE 1.1
Evidence of prehistoric brain surgery.
This skull of a man over 7000 years old was surgically opened while he was still alive. The arrows indicate two sites of trepanation.
(Source: Alt et al., 1997, Fig. 1a.)



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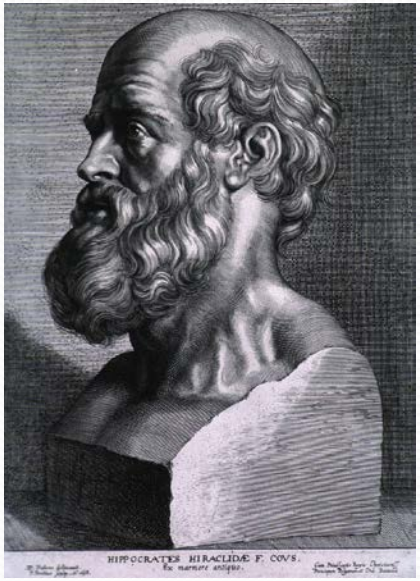
神經科學的發展



- 古希臘時代

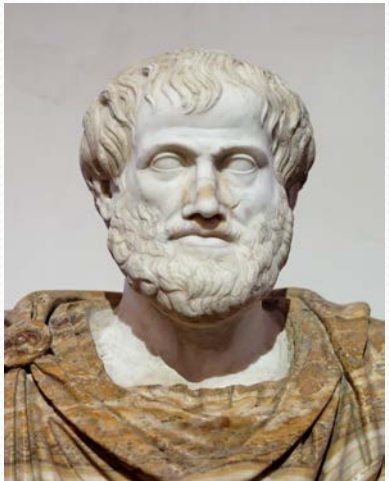
1.構造與功能間的關聯

2.希波克拉提斯(460-379 B.C., 西方醫學之父)

(1)醫師誓詞

(2)大腦是所有感覺及智慧的中樞

Men ought to know that from nothing else but the brain come joys, delights, laughter and sport, and sorrows, griefs, despondency, and lamentations.



3.亞里斯多德 (384-322 B.C.):

(1) 心臟是智慧的中樞

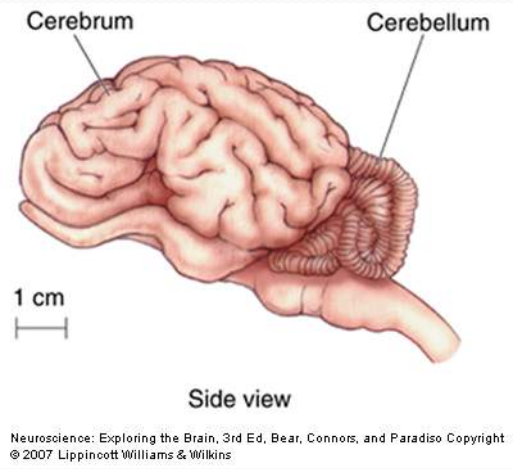
(2) 大腦: 冷卻器

神經科學的發展

- 羅馬帝國

  神鬼戰士(Gladiator) 醫師: Galen (129 AD-216 AD)

  - 大腦(Cerebrum)
  - 小腦(Cerebellum)
  - 腦室(Ventricles)



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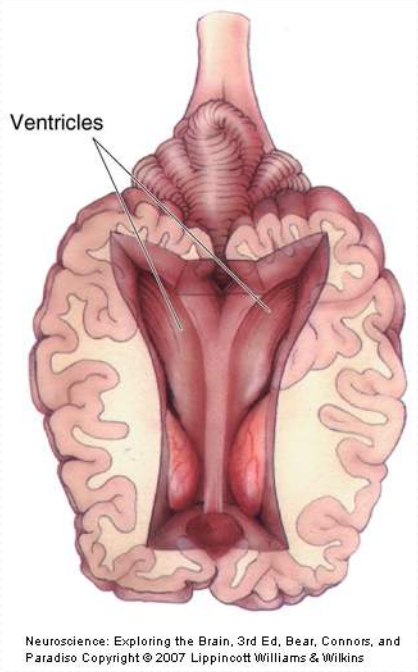

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神經科學的發展

- 文藝復興時期：**René Descartes** (1596-1650)
  - Fluid-mechanical theory of brain function
  - Philosophical mind-brain problem

    (1) people possess intellect and a God-given soul

    (2) the mind is a spiritual that receives sensations and commands movements by communicating with the machinery of the brain via the pineal gland

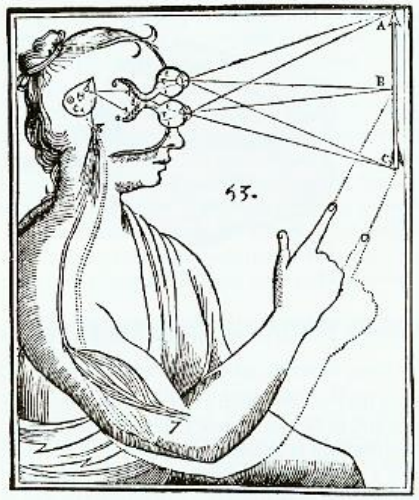

Figure 1.5
The brain according to Descartes. This drawing appeared in a 1662 publication by Descartes. Hollow nerves from the eyes project to the brain ventricles. The mind influences the motor response by controlling the pineal gland (H), which works like a valve to control the movement of animal spirits through the nerves that inflate the muscles. (Source: Finger, 1994, Fig. 2.16.)

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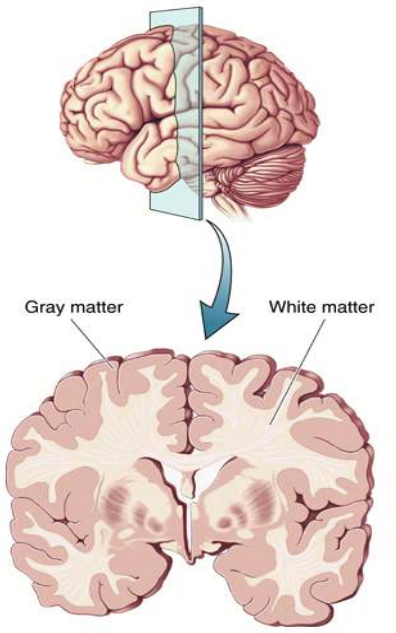
神經科學的發展

- 17-18世紀
  - 灰質 v.s. 白質
  - 週邊神經系統v.s.中樞神經系統



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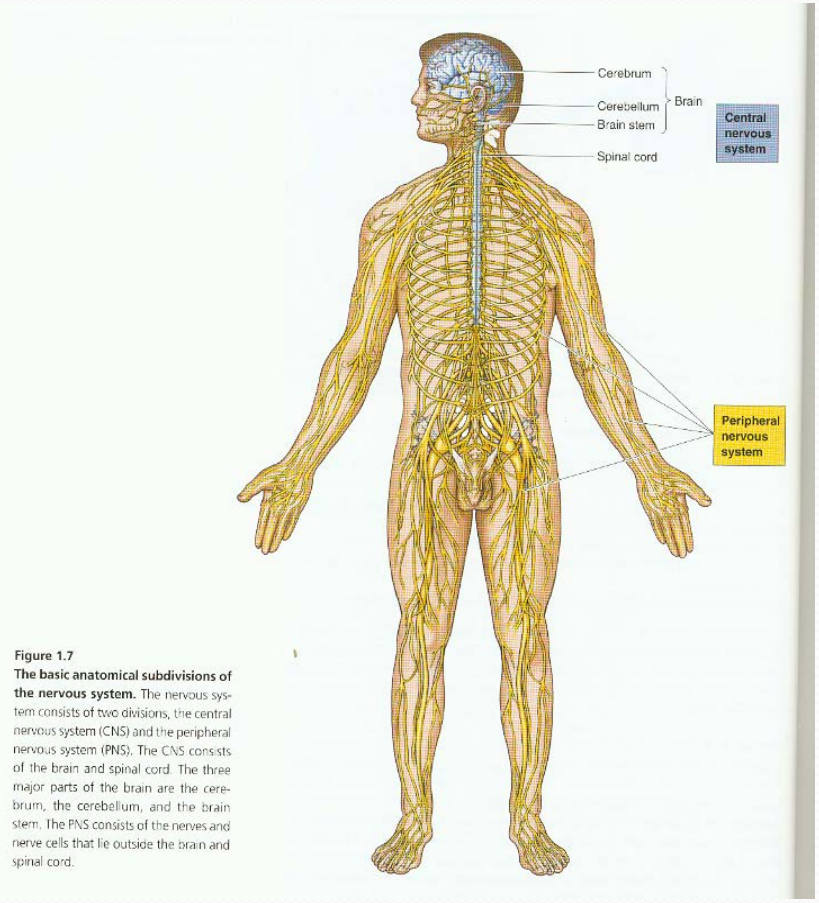

Figure 1.7
The basic anatomical subdivisions of the nervous system. The nervous system consists of two divisions, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS consists of the brain and spinal cord. The three major parts of the brain are the cerebrum, the cerebellum, and the brain stem. The PNS consists of the nerves and nerve cells that lie outside the brain and spinal cord.

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- *Nineteenth-Century views of the Brain*

  At the end of 18th century:

  (1) Injury to the brain can disrupt sensations, movement, and thought and can cause death.

  (2) The brain communicates with the body via the nerves.

  (3) The brain has different identifiable parts, which probably perform different functions.

  (4) The brain operates like a machine and follow the law of nature

神經科學的發展

- 19世紀

  神經像電線，會發電並傳遞下去

- 大腦各部位與脊髓之功能

  Charles Bell (1810); Francois Maagendie

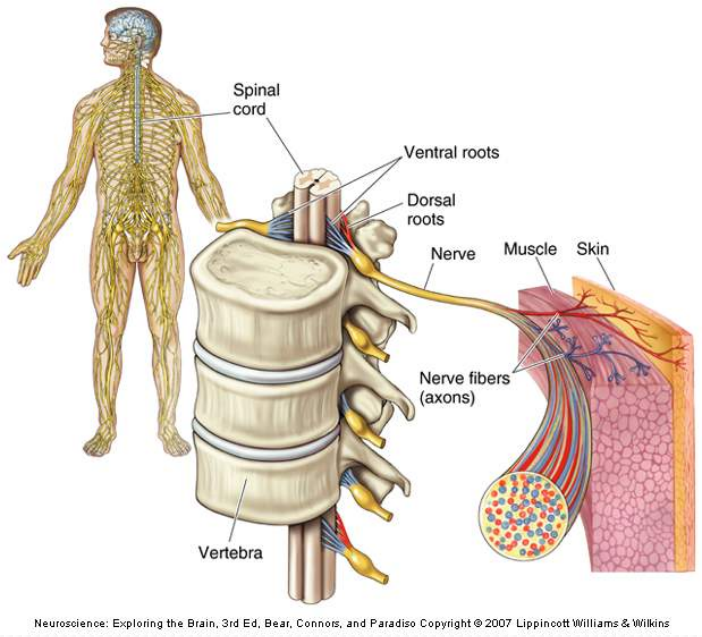
  1. 脊髓背根與腹根有不同功能

     背根

     腹根

  2.小腦: 動作神經元起點

  3. 大腦：感覺神經纖維之終點



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神經科學的發展

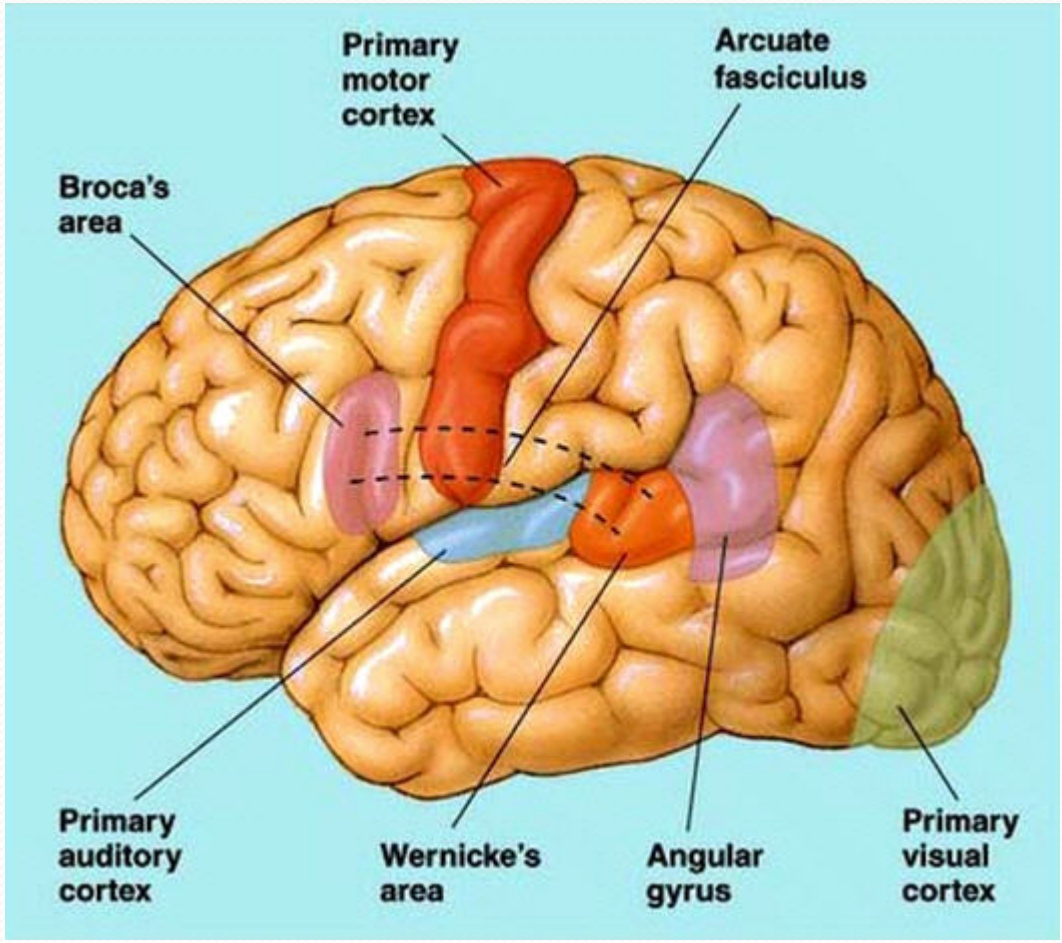
- Paul Broca

  Discrete regions of the human cerebrum for speech (Broca's area)

- Gustav Fritsch and Eduard Hitzig
  - exposed surface of the brain of a dog could elicit discrete movements

- Hermann Munk: occipital lobe vision

Broca's area v.s. Wernicke's area



常見之神經系統疾病

- Epilepsy
- Parkinson's disease
- Alzheimer's disease
- Depression
- Schizophrenia
- Stroke
- Alcohol and drug addiction
- Traumatic brain injury

Epilepsy 癲癇

- 因腦中部分神經細胞異常放電，引發部分或全身性運動功能異常，部分伴隨暫時性的昏睡狀態。

P/S 成人或小孩會因中毒或發燒而產生暫時的癲癇，這種急性症狀與我們目前所討論的因長期腦部病變，所產生的會重覆發作的癲癇不同。

長期腦部病變可分為下列兩大類：

1. Partial seizures 部分發作
2. Generalized seizures 全身性發作

Partial seizures 部分發作

- 異常放電的部位祇限於腦中一小部分，不會擴散至整個大腦，又可細分為兩種：

a. Simple partial seizure簡單型部分發作:
又名Jacksonian Seizures
  - 常發於腦中感覺或運動區，臨床症狀端視發病的區域，而伴有短暫的知覺或運動障礙，但十分輕微甚至患者本身亦不自覺。

b. Complex partial seizure 複雜型部分發作：
又名temporal lobe seizure
  - 常發temporal lobe, 會呈現aura 先兆，然後產生**psychomotor attack** 心理運動異常，常見的是**automatisms**自動化行為，患者會在不自知的情況下做出一種或一連串的動作。

Generalized seizures 全身性發作

- 異常放電的部位擴散至整個大腦，常伴隨運動功能及意識的喪失，有下列兩個主要階段：

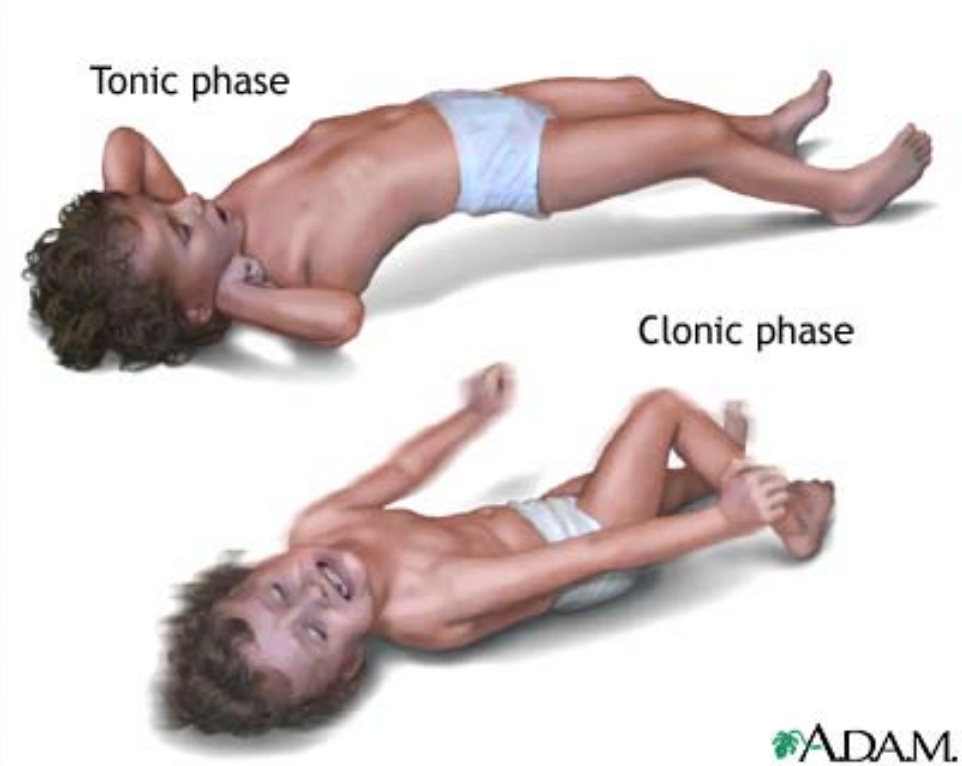
a. Grand mal 大發作

臨床症狀包括，失去意識、平衡、小便失禁、強烈肌肉痙攣。

b. Petit Mal 小發作

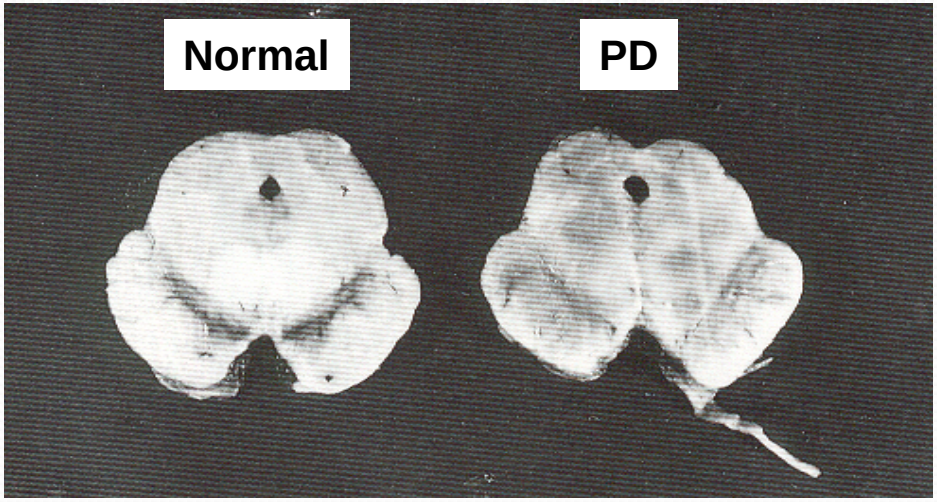
臨床症狀包括petit mal absence 失神，
EEG中出現3-per-second spike-and-wave 圖形。

Generalized seizures 全身性發作

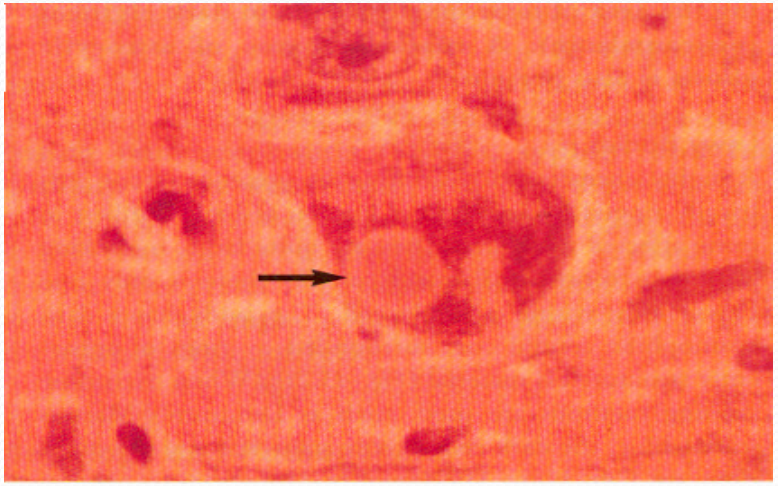


Parkinson's disease 柏金森氏症

- 因腦中黑質紋狀體路徑中多巴胺神經原大量壞死，所引發的運動功能異常，如靜止時震抖，起始運動不能，肌肉僵直等，可用L-DOPA or Deprenyl (MAO inhibitor) 作治療。
  (L-DOPA is the precursor of catecholamines)



Nigrostriatal pathway
(destain of the Substantia Nigra)

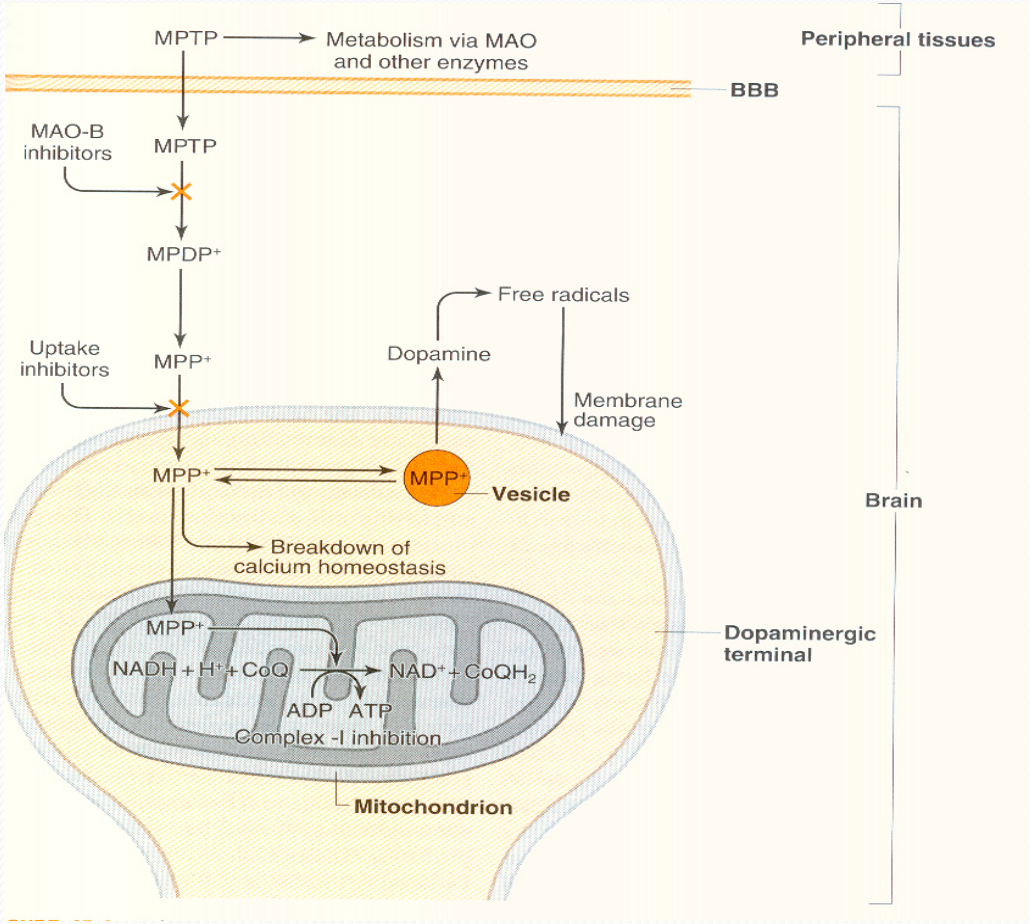


Loweys bodies

MPTP Model of Parkinson's disease

利用MPTP引發類柏金森氏症動物模式

- **MPTP** 是一種具選擇性的神經毒物，可專一性地破壞黑質紋狀體路徑中的多巴胺神經原。

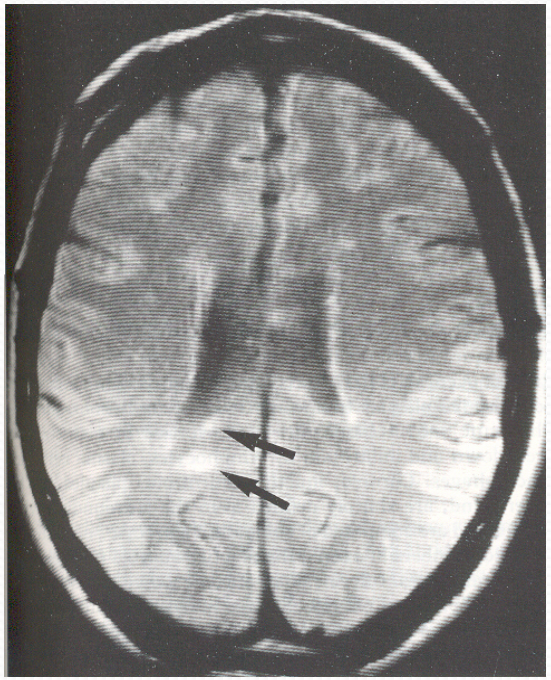


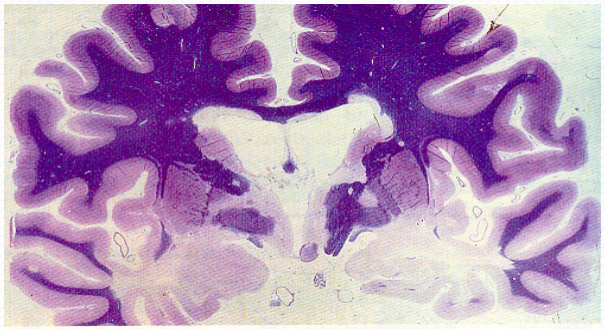
Huntington's disease 亨丁頓舞蹈症

- 為一種遺傳性疾病，患者的子女有一半的機會得病，約在四十歲時開始發病，約十至十五年後死亡，目前沒有治療方法，病人會呈現不自主如跳舞的運動，

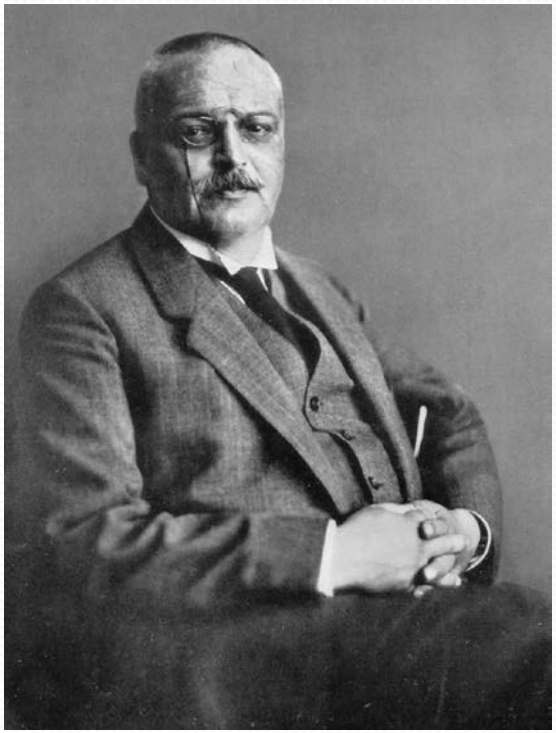
Multiple Sclerosis 多發性神經硬化

- 為一種自體免疫性疾病，因血腦屏障**BBB**功能異常，使體內產生會攻擊myelin 的抗体，引發神經傷害。





Alzheimer's disease



Alois Alzheimer (Jun. 14, 1864 – Dec. 19, 1915)

Psychiatrist and neuropathologist who first defined Alzheimer's Disease on Nov. 3, 1906.

Mrs. Auguste Deter (51 years old)

loss of short-term memory (1901)

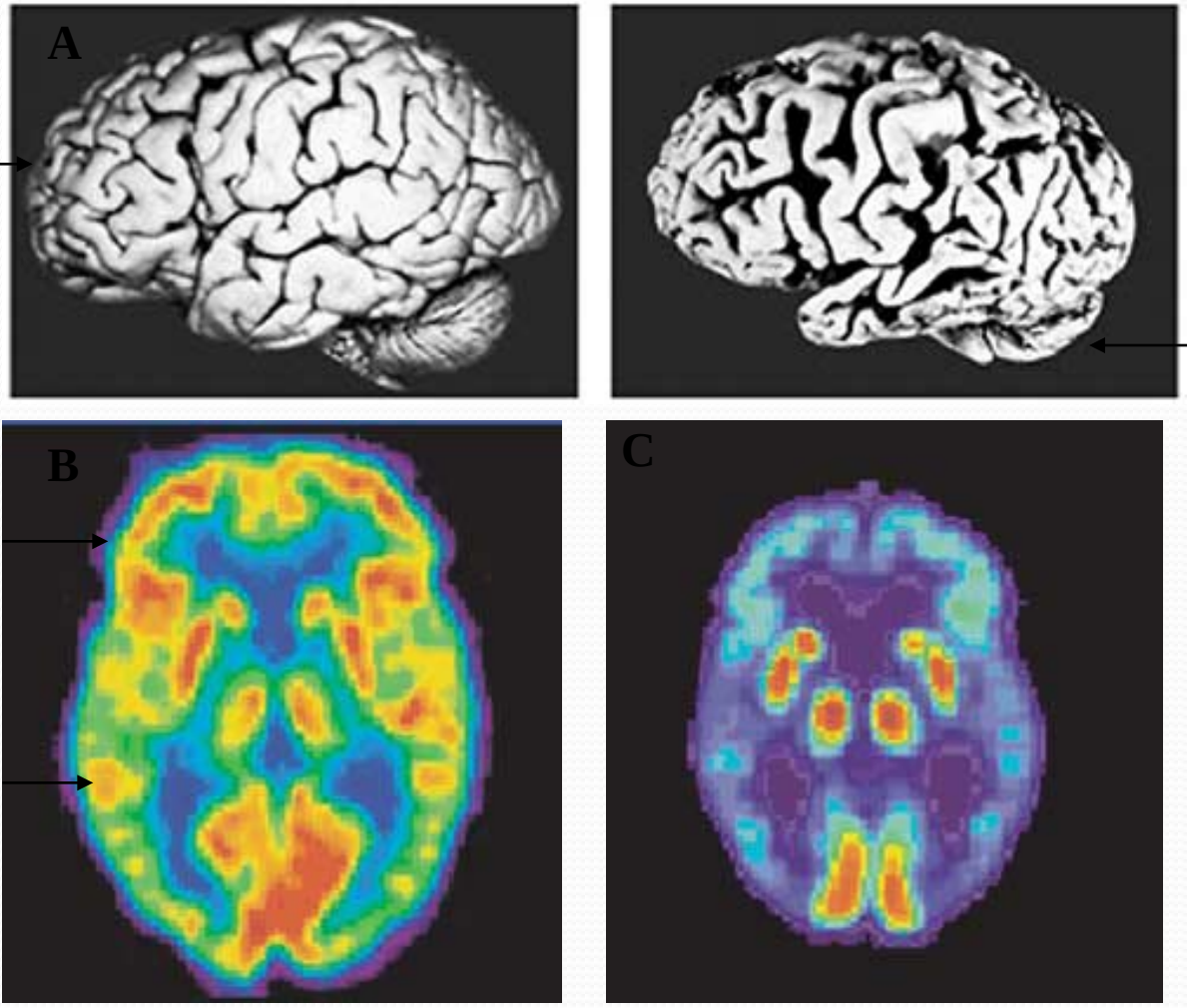
Amyloid plaques and neurofibrillary tangles were identified.

(Alzheimer's disease International)

Alzheimer’s disease

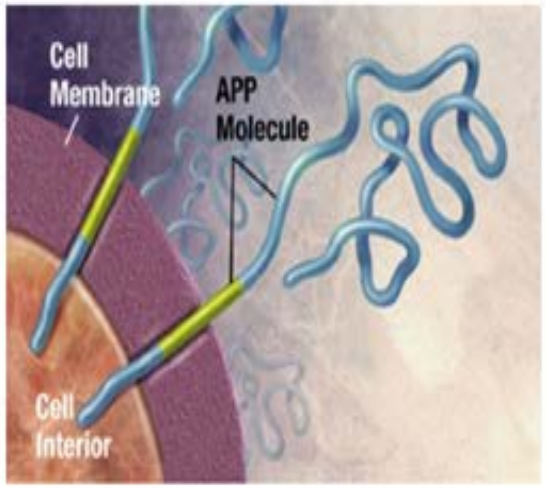
1. A neurodegenerative disorder: characterized by progressive memory loss, deterioration of cognitive functions and loss of synapses and neurons in the cerebral cortex and hippocampus (van Marum, 2008).

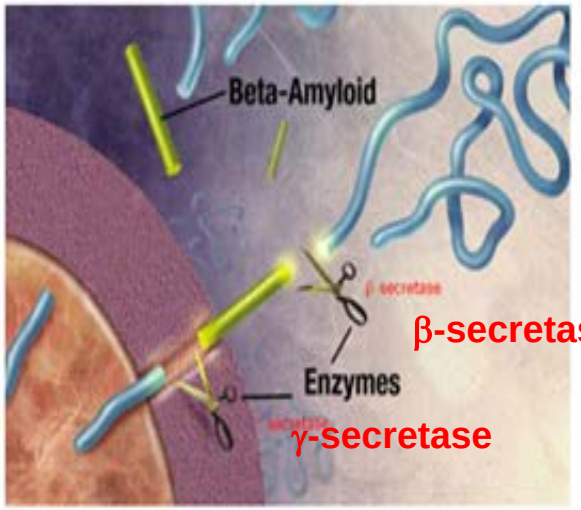
2. A serious health problem : In 2000, 25 million people in the world were diagnosed to suffer from AD, and this number is expected to increase to 114 million by 2050 (Wimo et al., 2003).

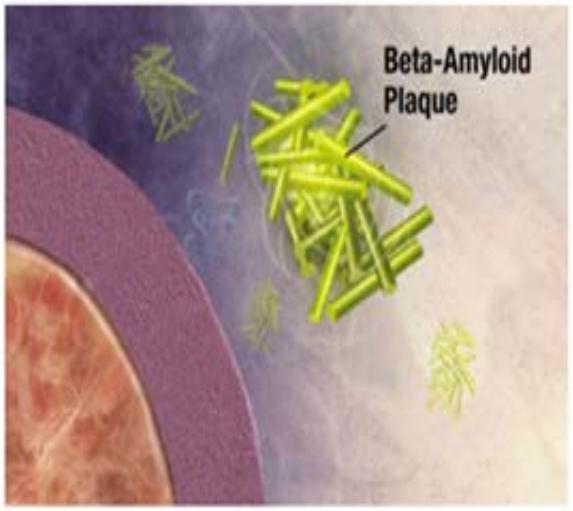


(Mattson, 2004)

Alzheimer's disease results in shrinkage of brain regions which is correlated with major reductions in cellular energy metabolism in living patients. a, Alzheimer's disease patient exhibits marked shrinkage of gyri in the temporal lobe and frontal lobes. b, PET images showing large decreases in energy metabolism in the frontal cortex and temporal lobes.







http://www.web-books.com/eLibrary/Medicine/Neurological/Alzheimer_Amyloid.htm

What is AD

3. AD pathogenesis: triggered by the accumulation of the amyloid-β peptide (Aβ), which is due to overproduction of Aβ and/or the failure of clearance mechanisms (Roberson and Mucke 2006).

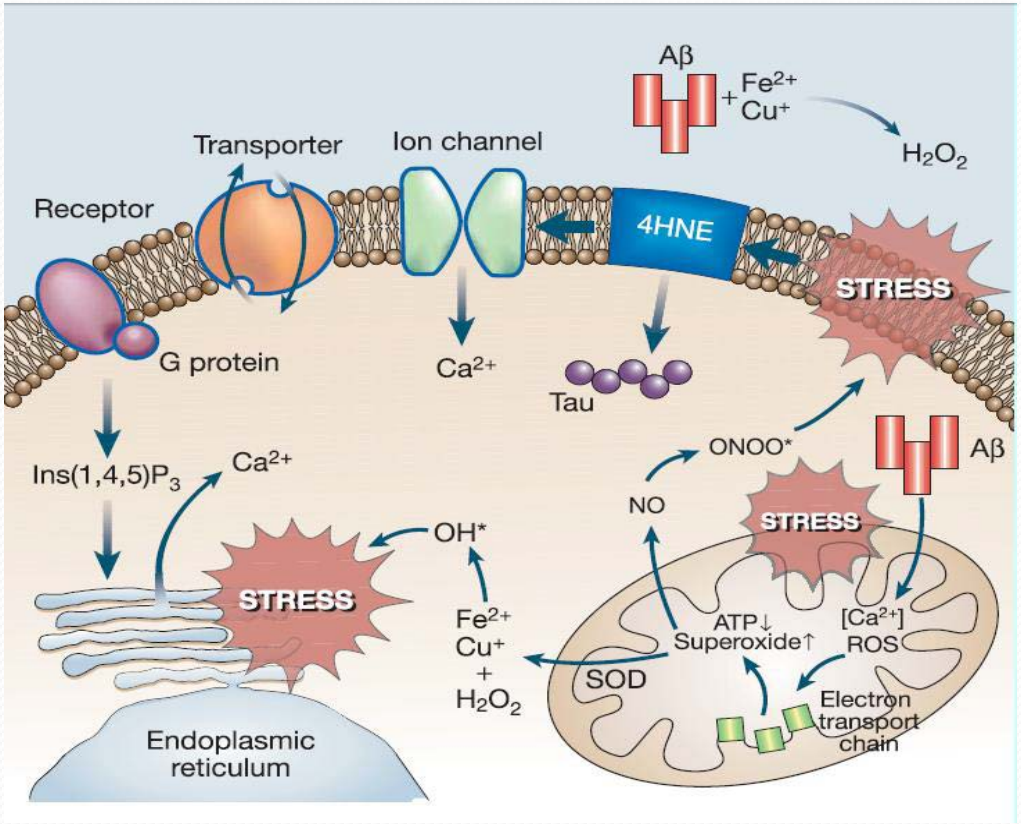
4. Amyloid precursor protein (APP):

   widely expressed throughout the body

   integral membrane protein

   α–, β–, γ– secretase

Neurotoxic action of Aβ



1. Lipid peroxidation, generation of 4-HNE (4-hydroxynoneal)
2. Activated some receptor, ion channel
3. Cause mitochondrial oxidative stress and alter Ca^{2+} homeostasis, increase superoxide production, interact with NO to produce peroxinitrite (Mattson, 2004)

Traumatic brain injury (TBI)

1. A major cause of morbidity and mortality all over the world (Goldstein, 2000).
2. TBI produces long lasting disabilities in 25% of cases and with a high socioeconomic impact: 1.7 million new cases of TBI occur in the United States every year with an estimated annual cost of $77 billion

Traumatic brain injury (TBI)

1. Primary injury:

a. Cerebral contusion

b. Traumatic intracranial hemorrhage

c. Skull fracture

2. Secondary injury:

a. Stroke

b. Meningitis

c. Brain edema

TRAUMATIC BRAIN INJURIES

TRAUMATIC BRAIN INJURIES IN THE UNITED STATES OF AMERICA

1,700,000 TBIs PER YEAR

PLUS ALL THE TBIS THAT GO UNTREATED!

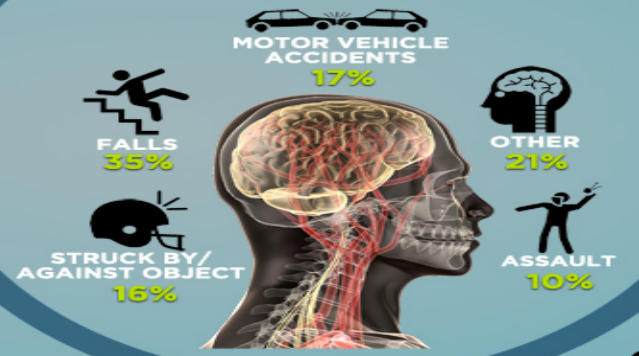
SYMPTOMS OF TRAUMATIC BRAIN INJURY (TBI)

- FATIGUE
- SEIZURES
- HEADACHES
- MEMORY LOSS
- VISUAL DISTURBANCES
- POOR ATTENTION/CONCENTRATION
- SLEEP DISTURBANCES
- DIZZINESS/LOSS OF BALANCE
- IRRITABILITY-EMOTIONAL DISTURBANCES
- FEELINGS OF DEPRESSION

OTHER SYMPTOMS ASSOCIATED WITH MILD TBI

- NAUSEA
- LOSS OF SMELL
- MOOD CHANGES
- SENSITIVITY TO LIGHT AND SOUNDS
- SLOWNESS IN THINKING
- GETTING LOST OR CONFUSED

CAUSES OF TRAUMATIC BRAIN INJURY (TBI)



LOST PRODUCTIVITY COST

$76.5 Billion

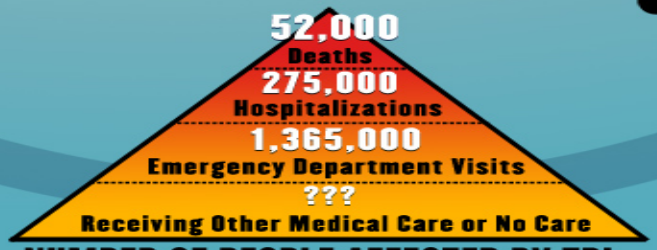
DUE TO TRAUMATIC BRAIN INJURIES IN THE UNITED STATES, YEAR 2000.

473,947 ANNUAL ER VISITS

FOR TBI MADE ANNUALLY *BY* CHILDREN AGED 0 TO 14 YEARS

ADULTS AGED 75 YEARS AND OLDER

HIGHEST RATES OF TBI-RELATED HOSPITALIZATION AND DEATH

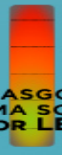


NUMBER OF PEOPLE AFFECTED BY TBIs.

GLASGOW COMA SCORE 9 – 12

MODERATE TBI

A loss of consciousness that lasts for more than 30 minutes but less than 24 hours. Memory loss after the traumatic event, called post-traumatic amnesia or PTA, lasting for 24 hours to 7 days



GLASGOW COMA SCORE 8 OR LESS

SEVERE TBI

A loss of consciousness that lasts for more than 24 hours PTA lasting for 7 days or longer. A Glasgow Coma Score of 8 or less, which indicates that the patient is in a coma.

Shulman DuBois LLC
1553 SE Tolman St. Portland, OR 97202
Phone 503-222-4411 • Fax 503-224-6424

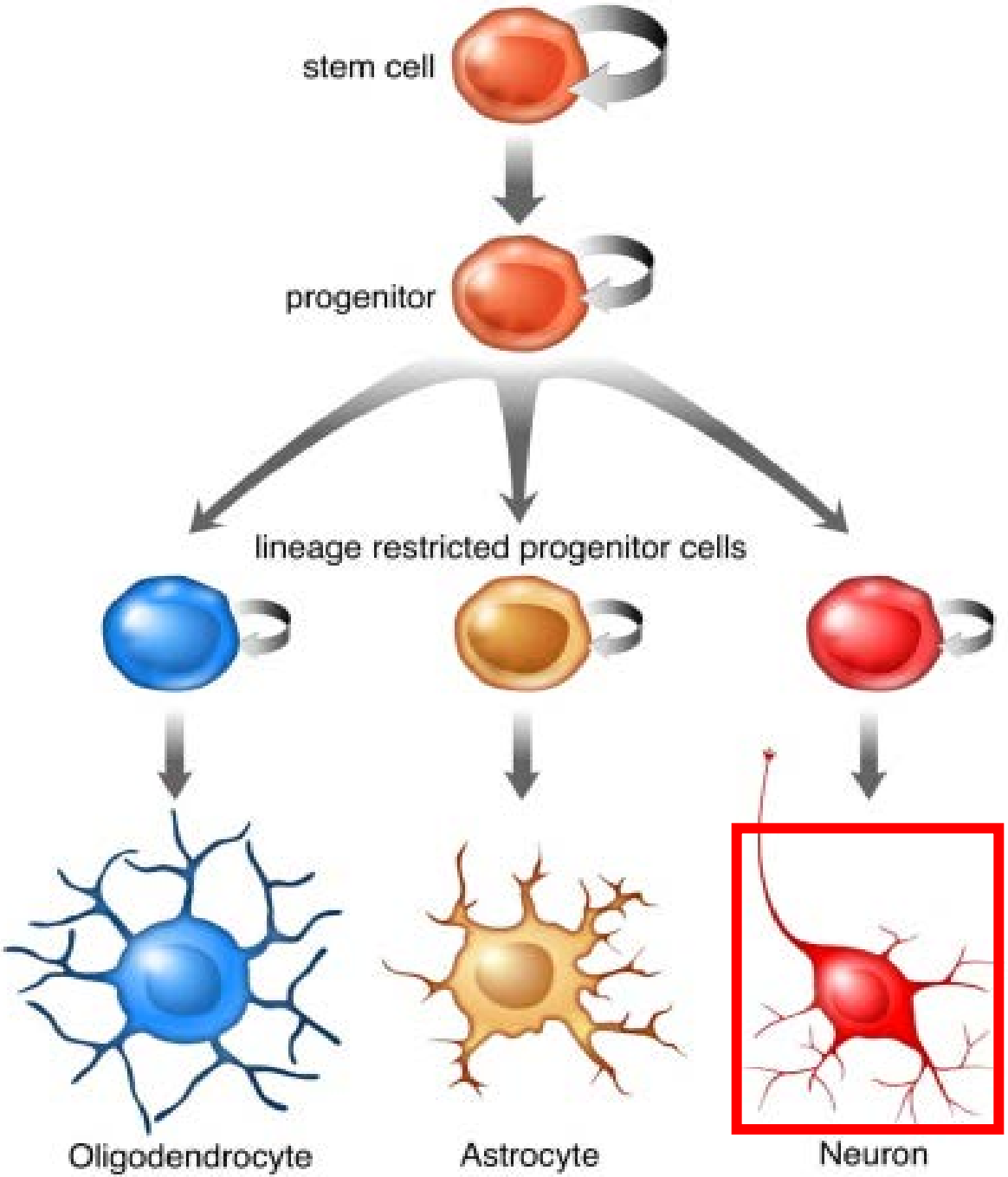
Sources: http://www.cdc.gov/traumaticbraininjury/
http://www.biausa.org/
http://www.mayoclinic.com/health/traumatic-brain-injury/DS00552

www.pdxinjurylaw.com

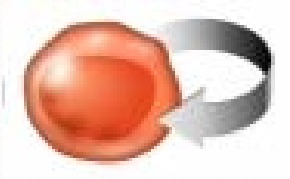
Neurogenesis

1. Characteristic: endogenous progenitors proliferate in response to various stimulus (Nakatomi et al., 2002).

2. Location: subgranular zone and rostral subventricular zone (Jin et al., 2001).

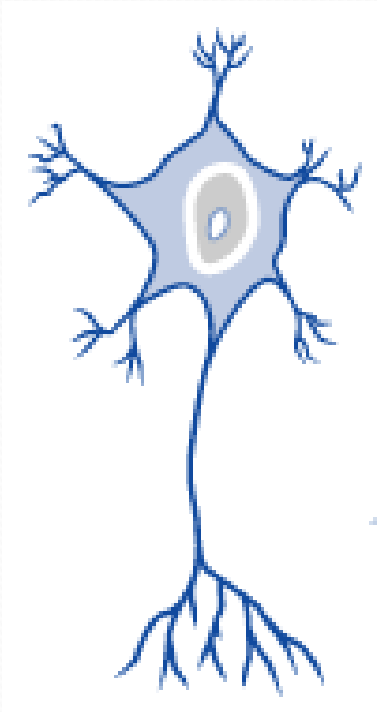

stem cell
progenitor
lineage restricted progenitor cells
Oligodendrocyte
Astrocyte
Neuron

Neurogenesis

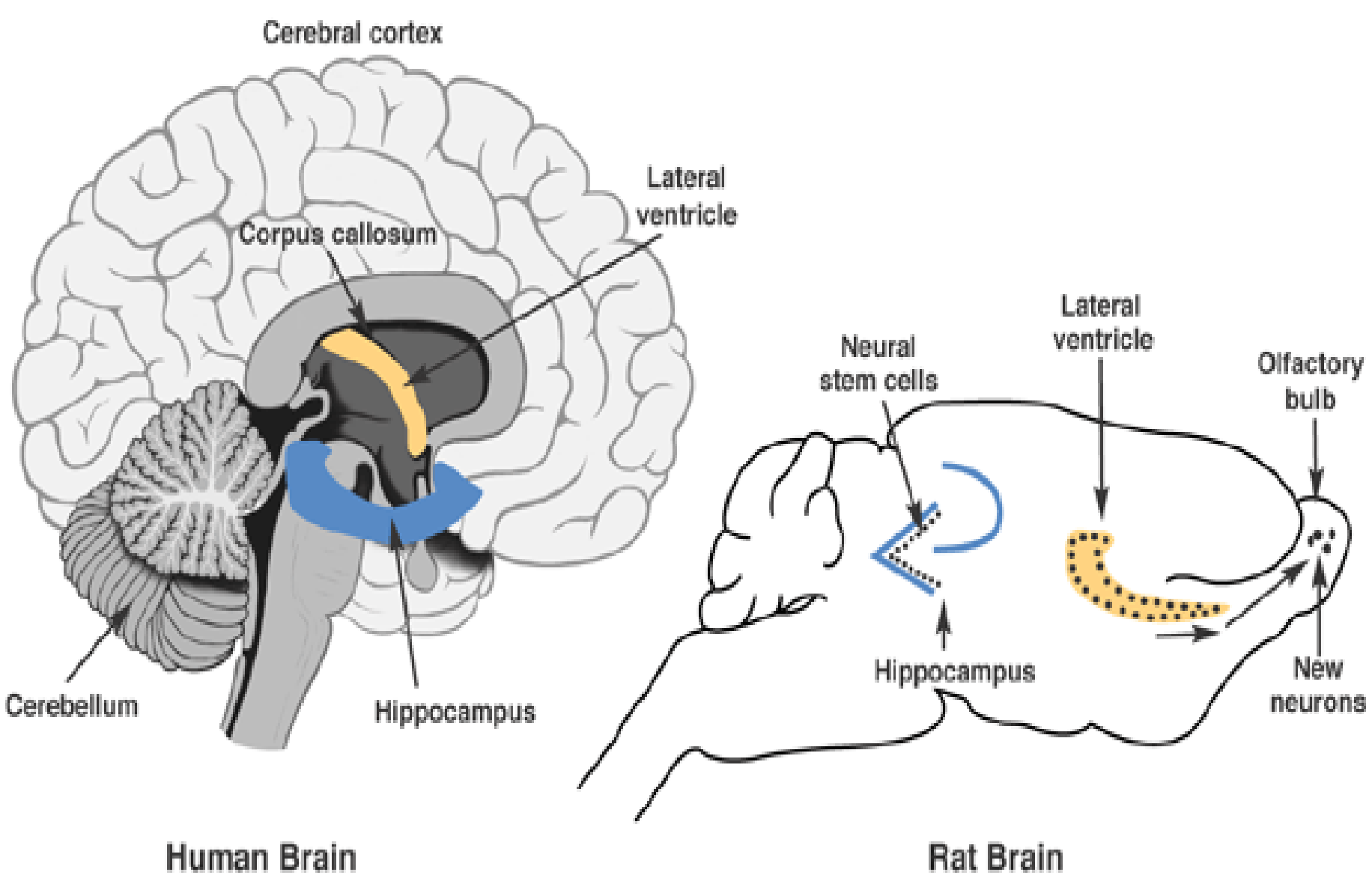


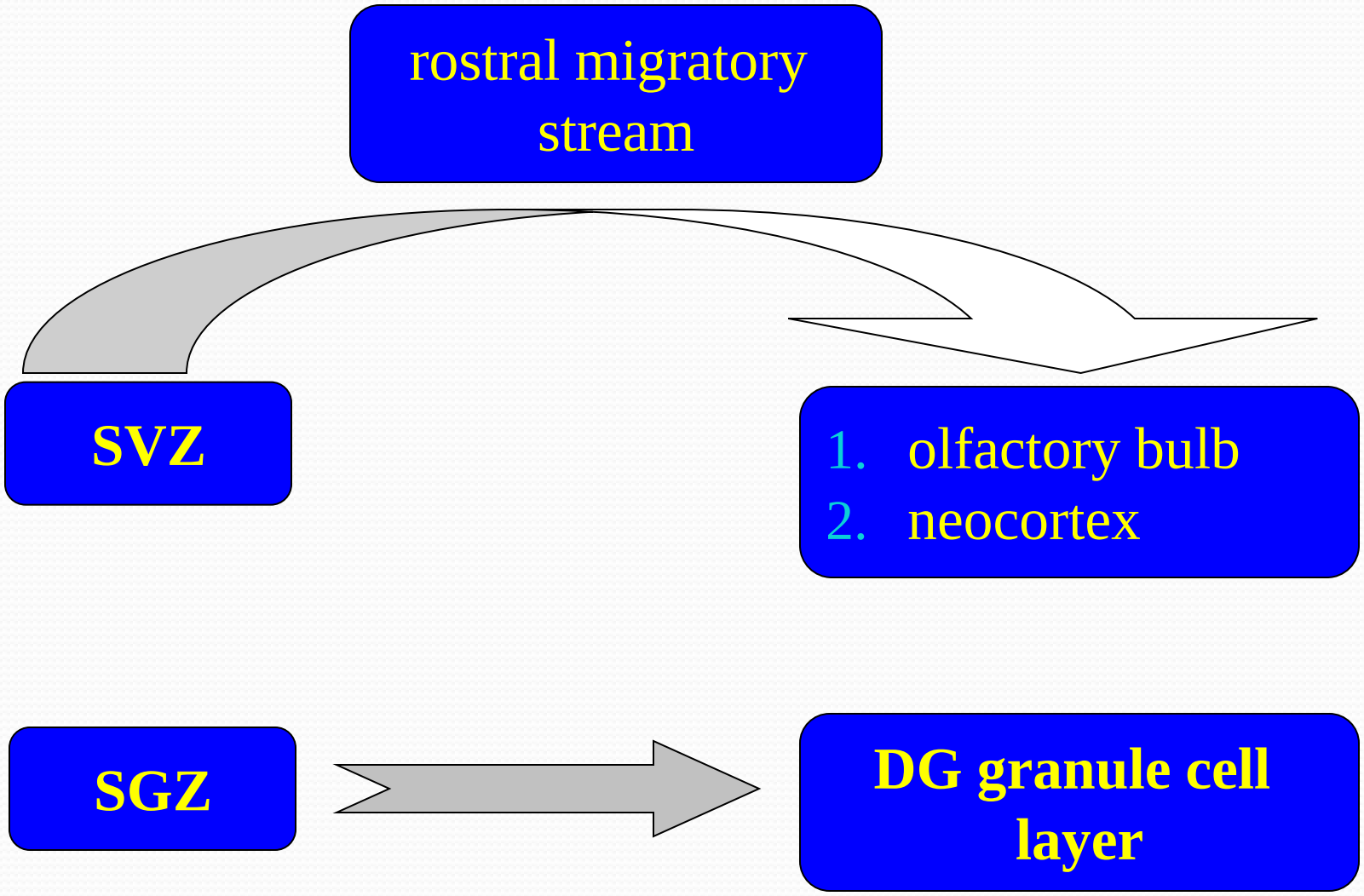
Self-renewing neural progenitor

- asymmetric or symmetric division
- fate choice decision
- cell-cell contacts
- survival
- neuronal membrane
- dendrite extension
- axon extension
- synaptogenesis
- neuronal transmitter
- network integration
- functional maturation



neuron


Cerebral cortex
Lateral ventricle
Corpus callosum
Cerebellum
Hippocampus
Human Brain
Neural stem cells
Lateral ventricle
Olfactory bulb
Hippocampus
New neurons
Rat Brain


rostral migratory stream
SVZ
1. olfactory bulb
2. neocortex
SGZ
DG granule cell layer

Factors of neurogenesis

1. **Enrichment environment; exercise (**Ehninger and Kempermann, 2003; Van Praag et al., 2002).
2. **Diseases :**

   ischemia (Jin et al., 2001)

   subarachnoid hemorrhage (Mino et al., 2003)

   epilepsy (Parent et al., 2002).
3. **Stem cell transplant** (Munoz et al., 2005)
4. **Growth factor effect: VEGF** (Jin et al., 2000)
5. **Drugs or neurotransmitter: glucocorticoid; glutamate (**Cameron et al., 1998; Gould et al., 1999; Gould and Tanapat, 1999; O'Kusky et al., 2000)

動物實驗之意義與原則

(1) 無知的代價

(2)我們為什麼要做動物實驗:

(3)動物實驗的原則: 3R

Replacement: Methods that avoid or replace the use of animals

Reduction: Methods that minimise the number of animals used per experiment or study

Refinement: Methods that minimise the pain, suffering, distress or lasting harm that may be experienced by the animals.


Laboratory Animal Center of NHRI
國家衛生研究院
National Health Research Institutes
實驗動物中心
Laboratory Animal Center of NHRI

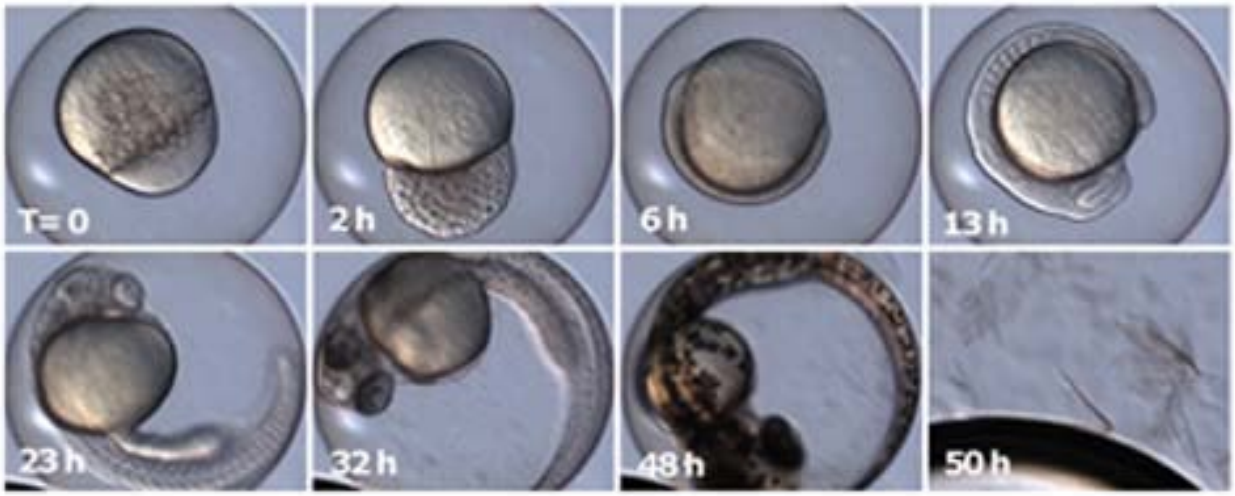
- The Use of Animals in Neuroscience Research
  - Animals: Renewable natural resources
  - The more basic the process under investigation, the more distant the evolutionary relationship with humans
    - Examples (from simple to more complex) - nematodes, insects, snails, squid, rodents, monkeys, etc.

斑馬魚(*Danio Rerio*)

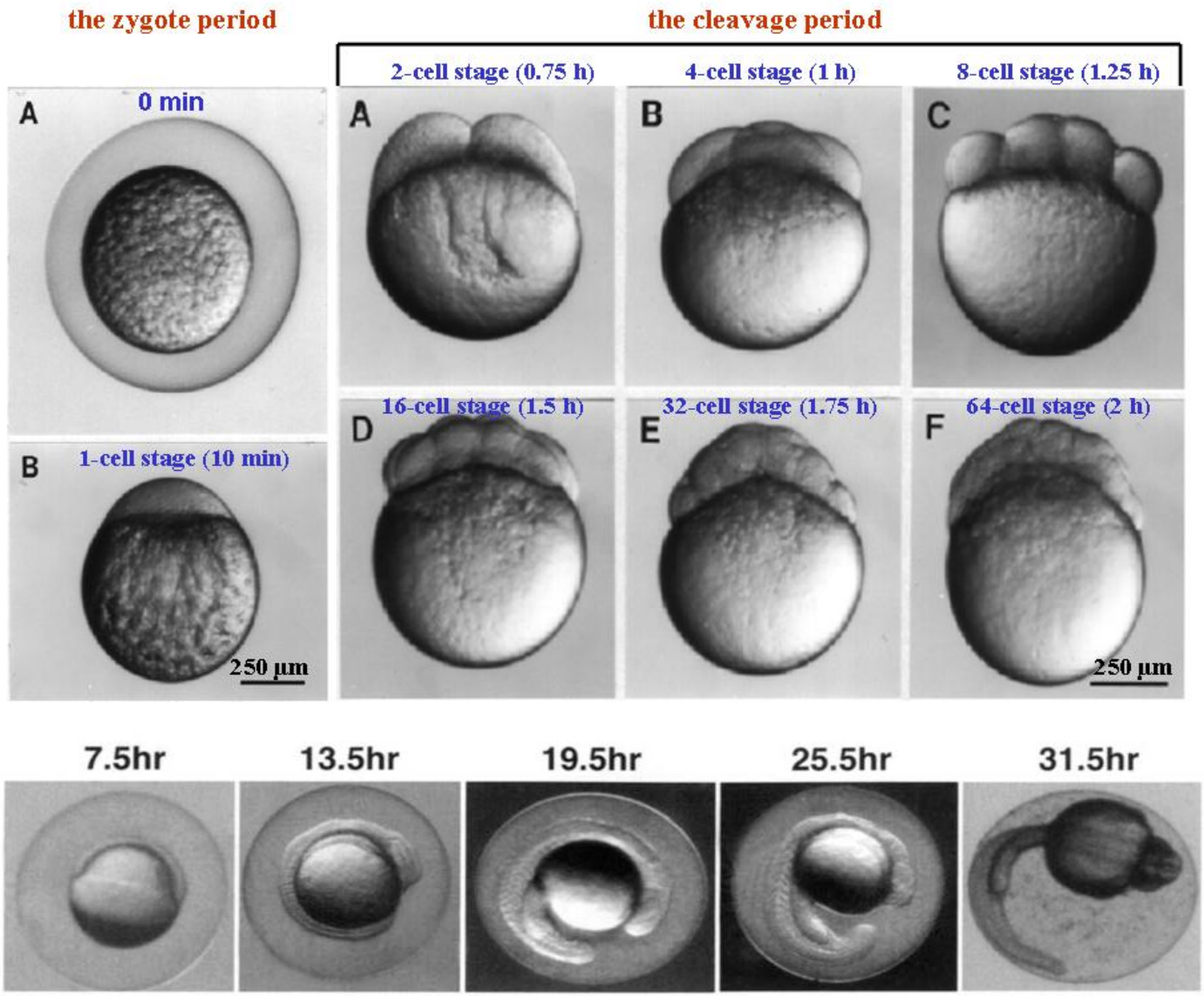
- 小型魚種之脊椎動物
- 產卵數量多
- 卵徑大
- 透明（可觀察胚胎發育）
- 用光即可控制排卵
- 14-hr light and 10-hr dark cycle
- 每天可以排卵（沒有產卵期的限制）
- 轉殖操作簡單
- 成熟期只有2~3個月
- 基因體大小只有哺乳類的20%

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



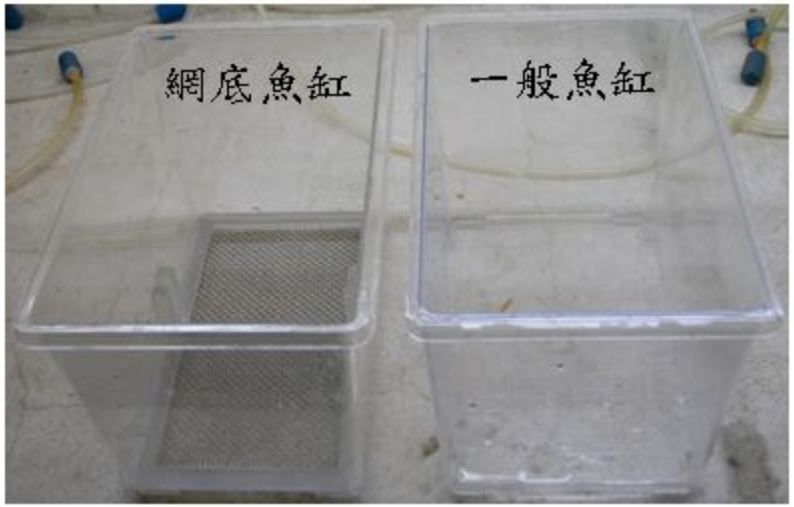
Stages of embryonic development of the zebrafish

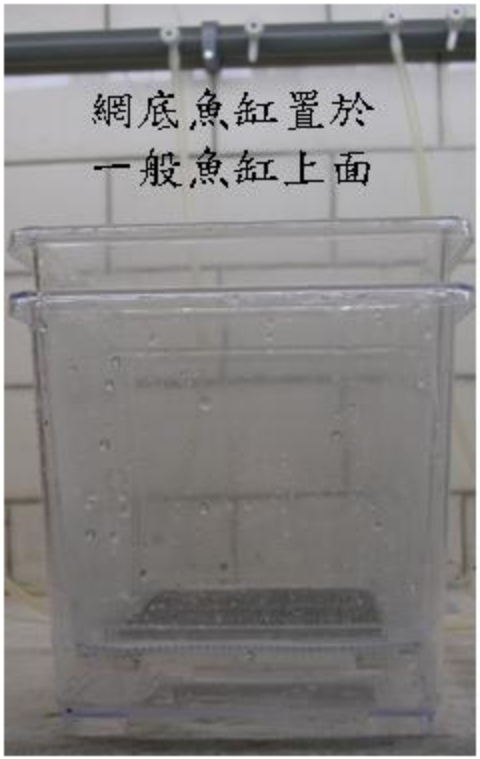


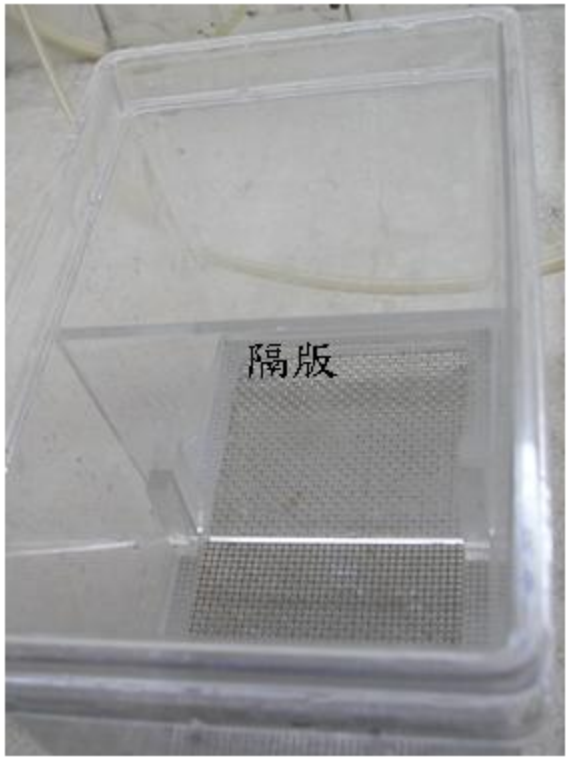
基因轉殖

- 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)

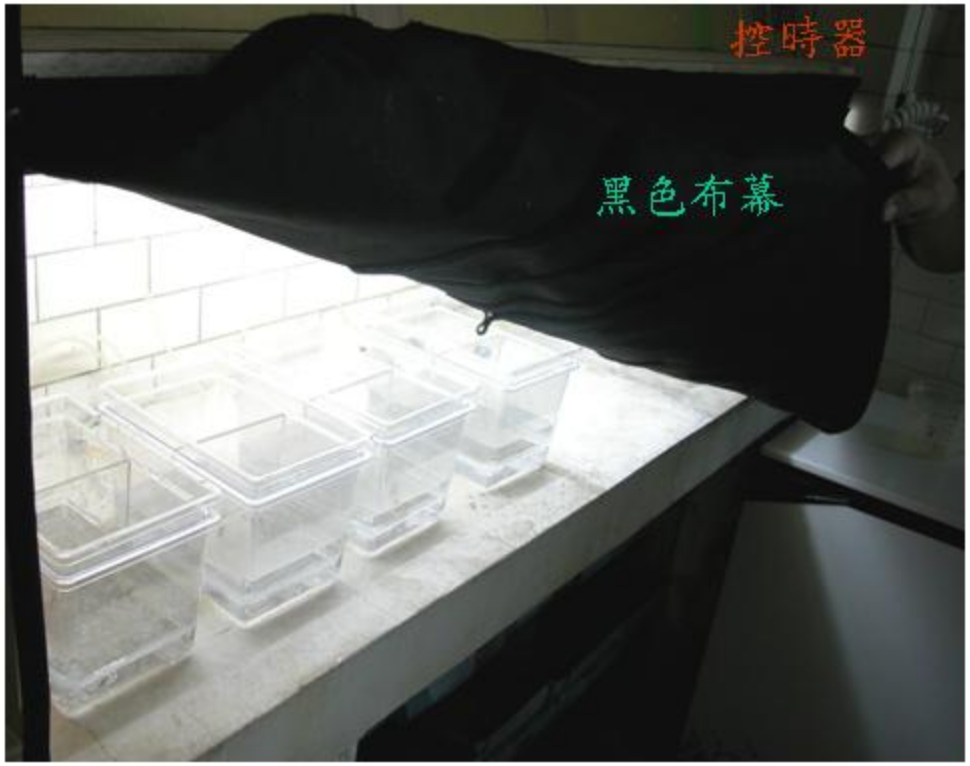






～曾鴻家資料整理

pre-breeding set up (2)



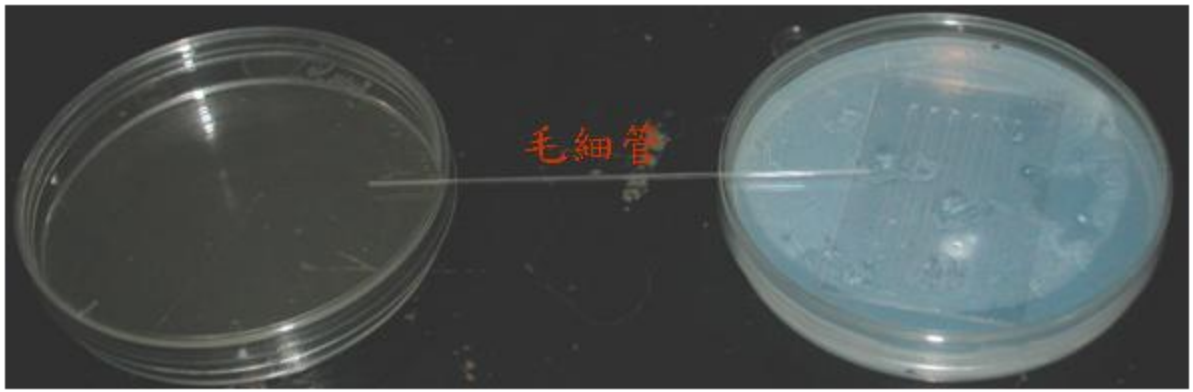
設定14小時照光，10小時暗週期控制排卵

拉針器操作步驟 (1)

1.打開拉針器蓋子

2.固定毛細管於拉針器內

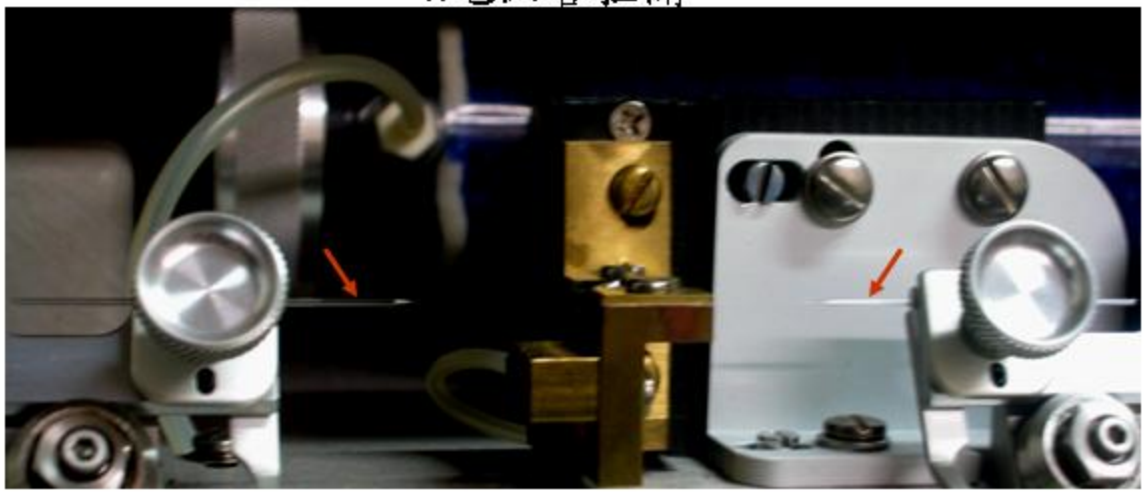
3.蓋上拉針器蓋子後按start



拉針器操作步驟 (2)

3.自毛細管中間加熱，拉開！

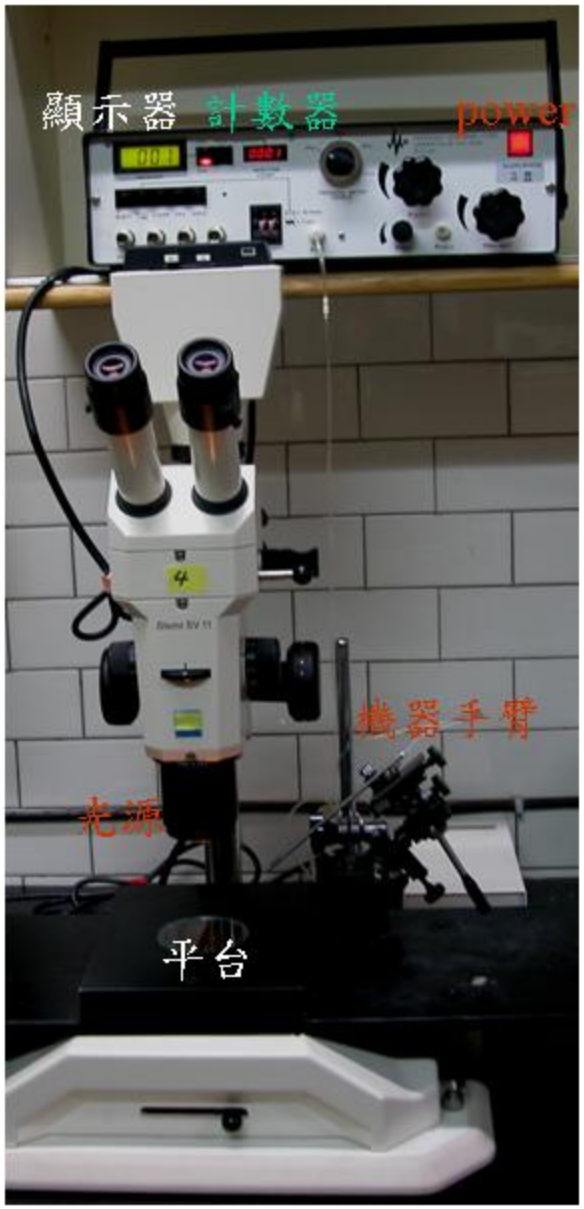
4.毛細管拉開

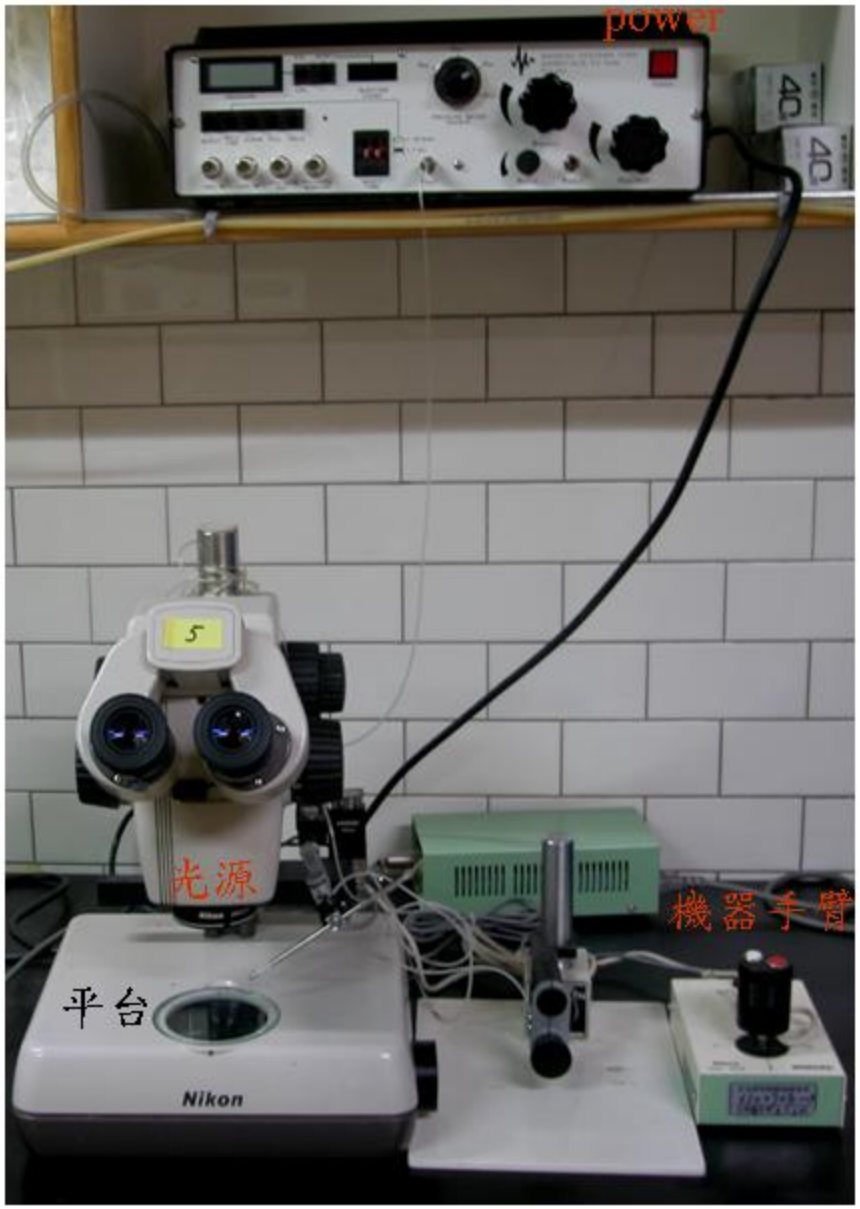


5.將毛細管置於黏土上

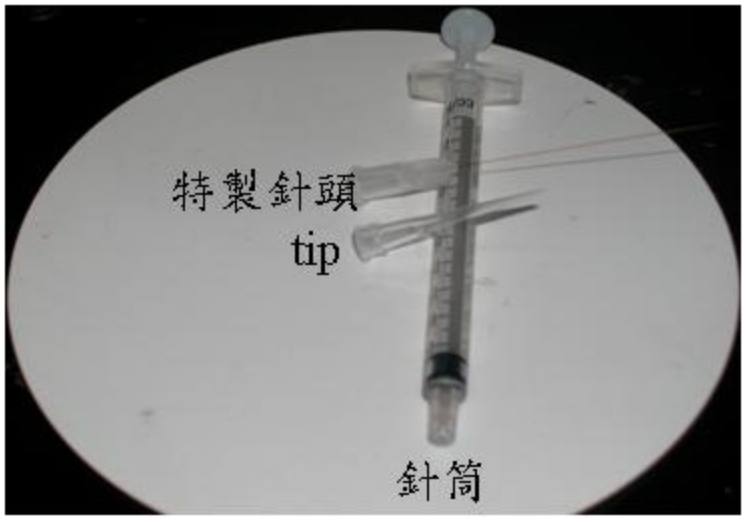
注意！不可碰到毛細管尖端

Microinjection operation (1)

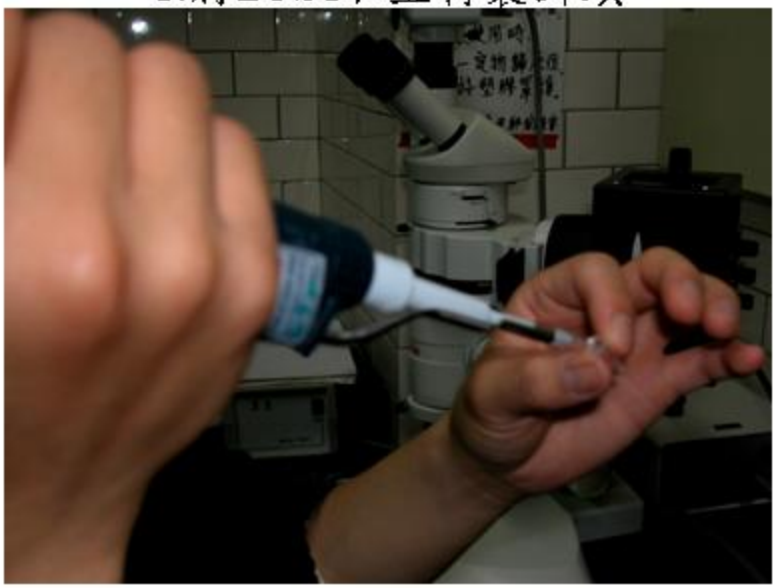




Microinjection operation (2)

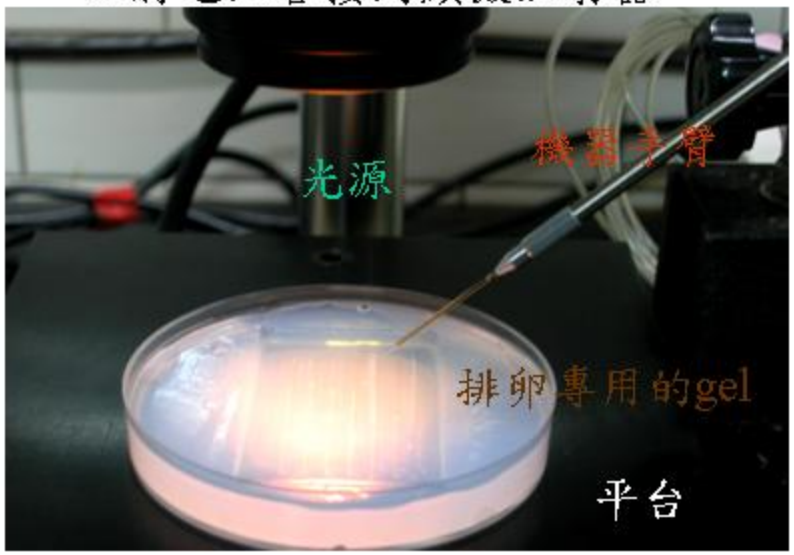


1.將DNA取至特製針頭

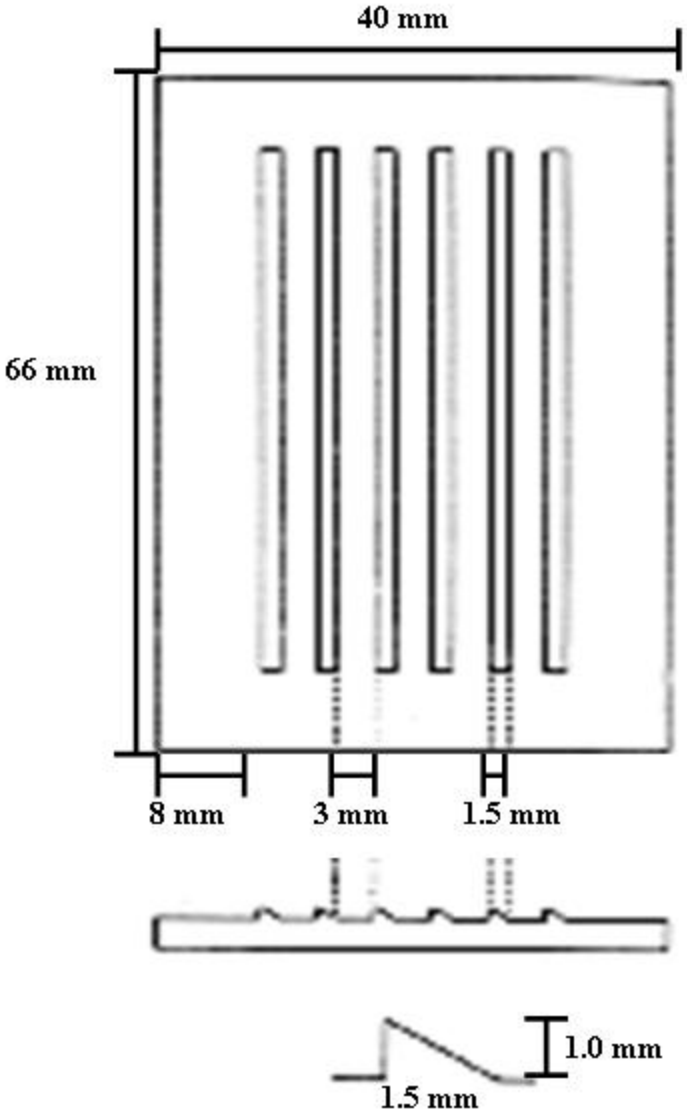


2.將特製針頭內的DNA送到毛細管

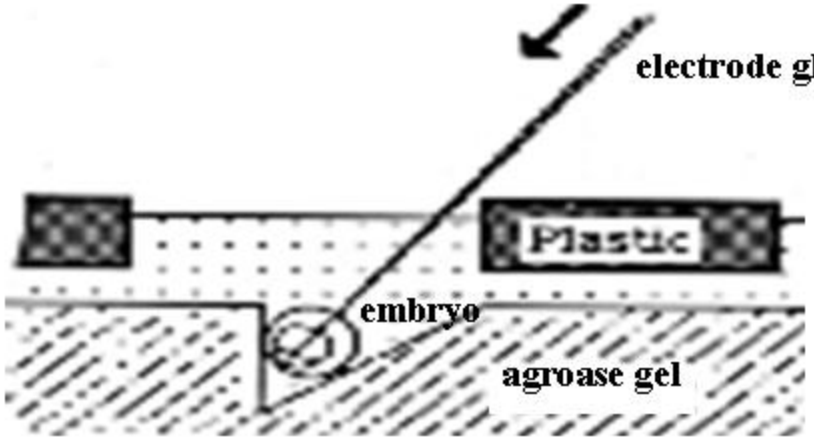
3.將毛細管接到顯微注射器

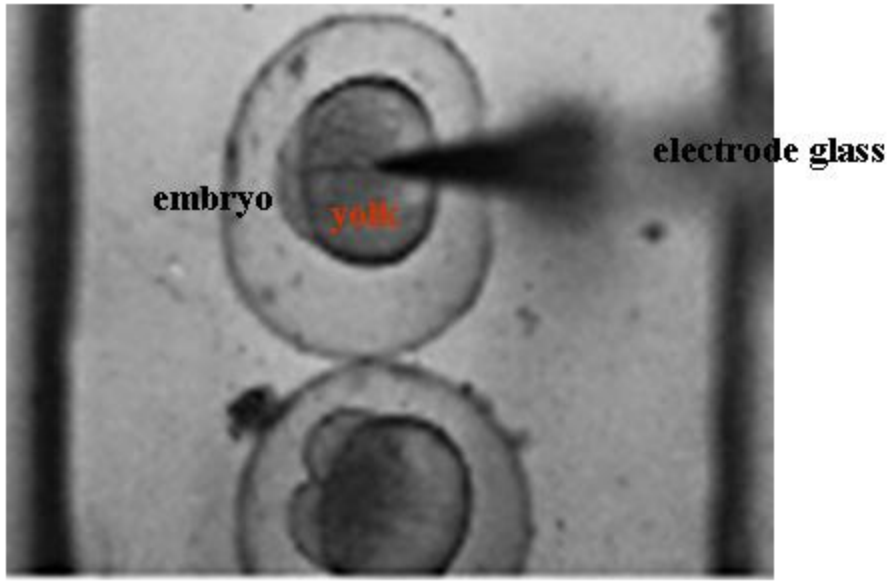


Microinjection



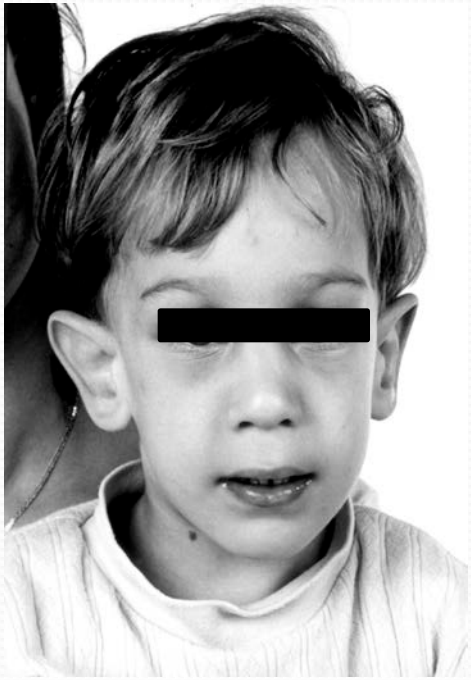
cross-sectional view of the injection set-upas an embryo is injected

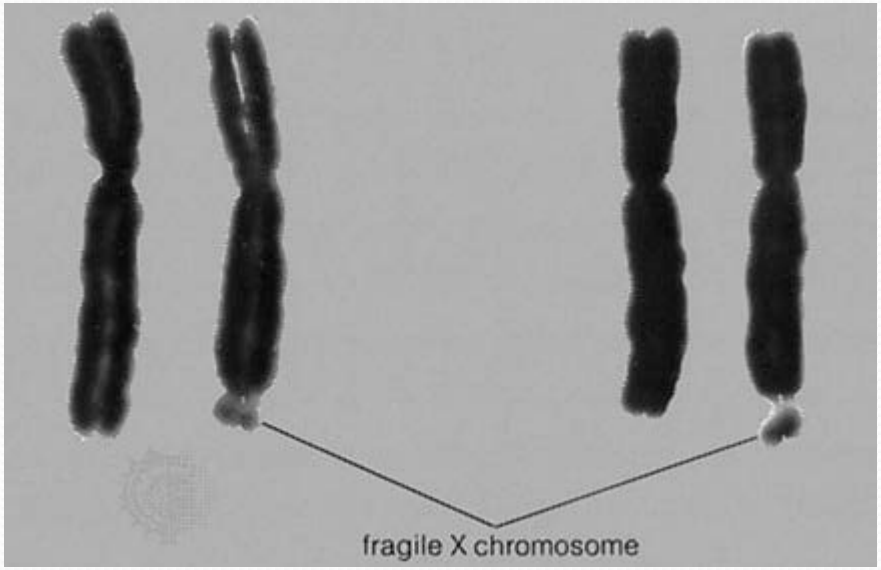




Introduction

- **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

FXS

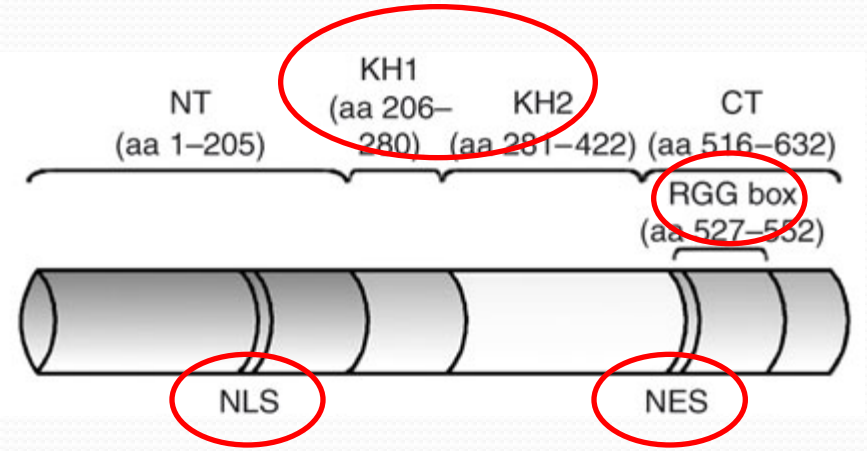
BEHAVIOR

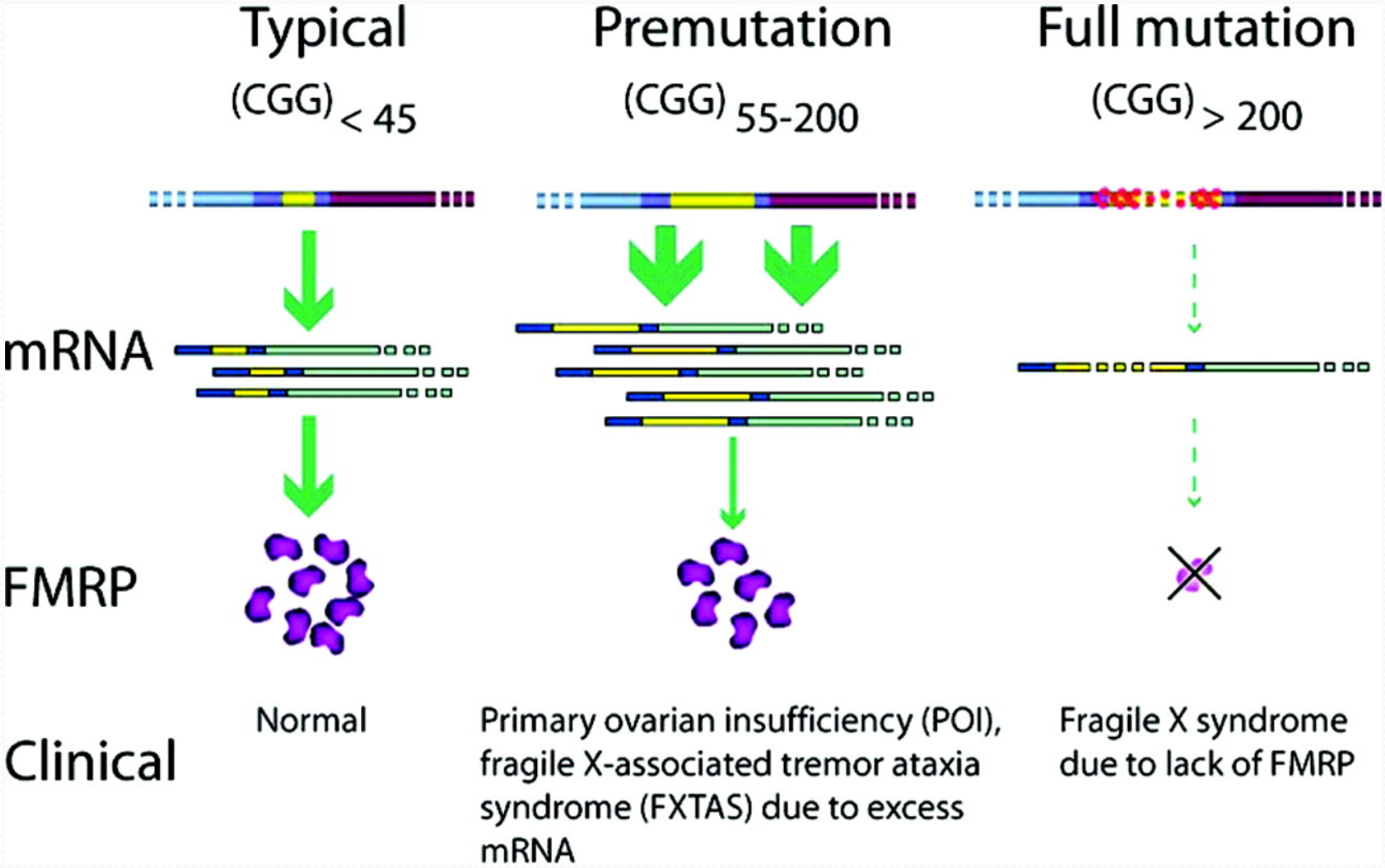
- Learning disabilities
- Attention deficit
- Hyperactivity
- Anxiety disorder
- Aggressiveness

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

CAG repeat

.....

poly glutamine

Q Q Q Q Q Q

Abnormal expansion (>37Q)

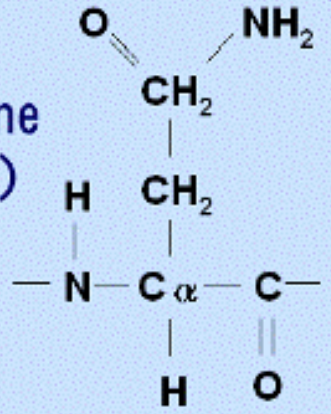
misfold

aggregate

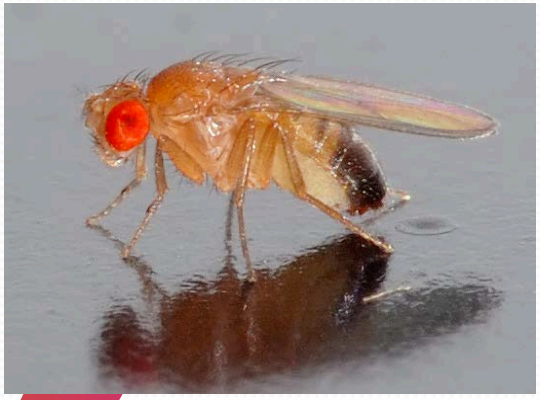
cell death

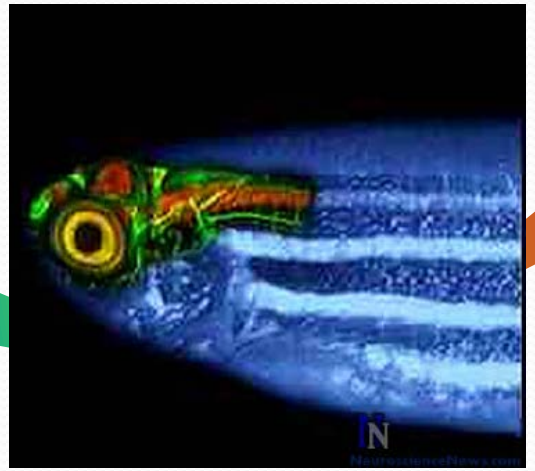
spinocerebellar ataxias
spinal and bulbar muscular atrophy
Huntington's disease (HD) ... etc

glutamine (Gln;Q)



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)

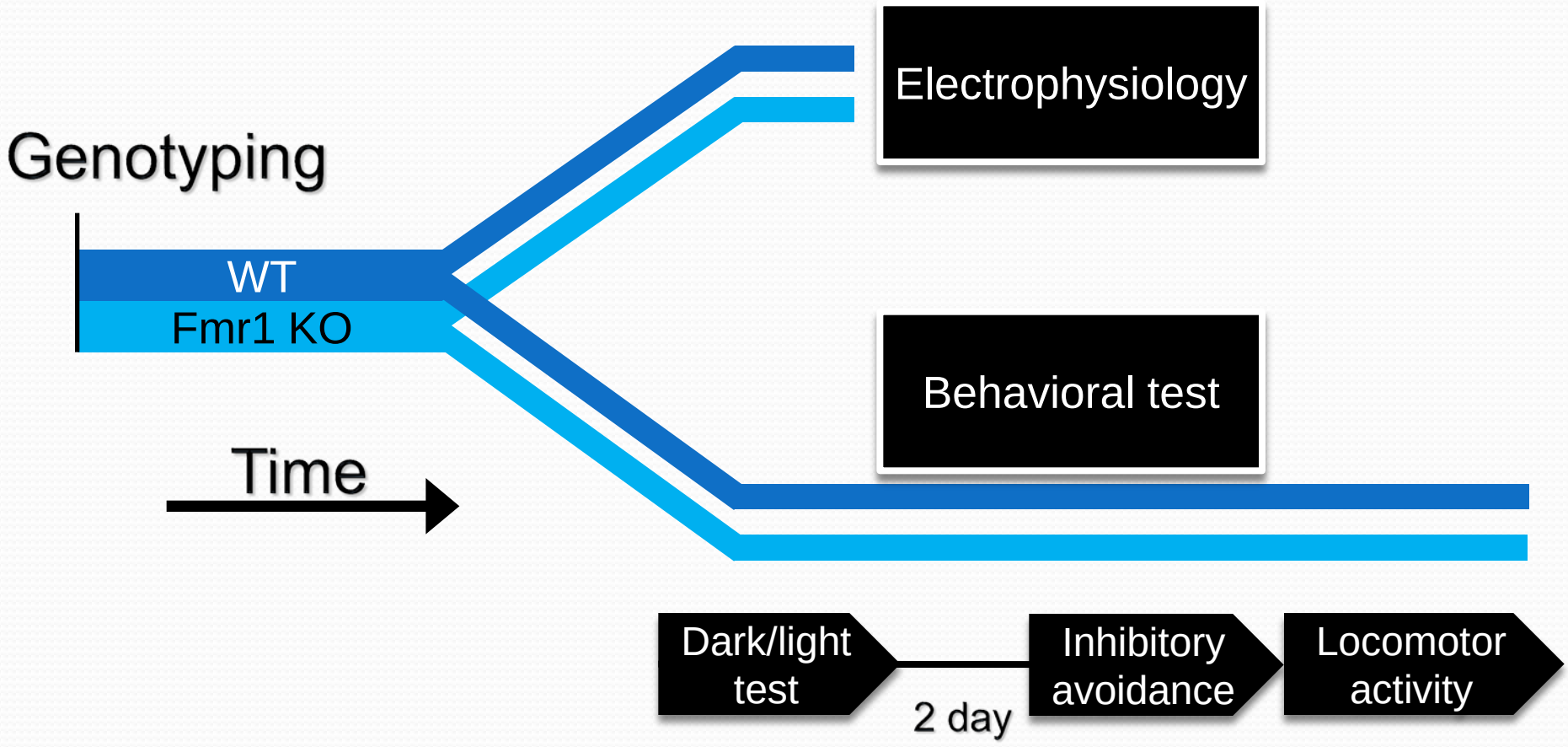
They won’t escape from the cage

Aims

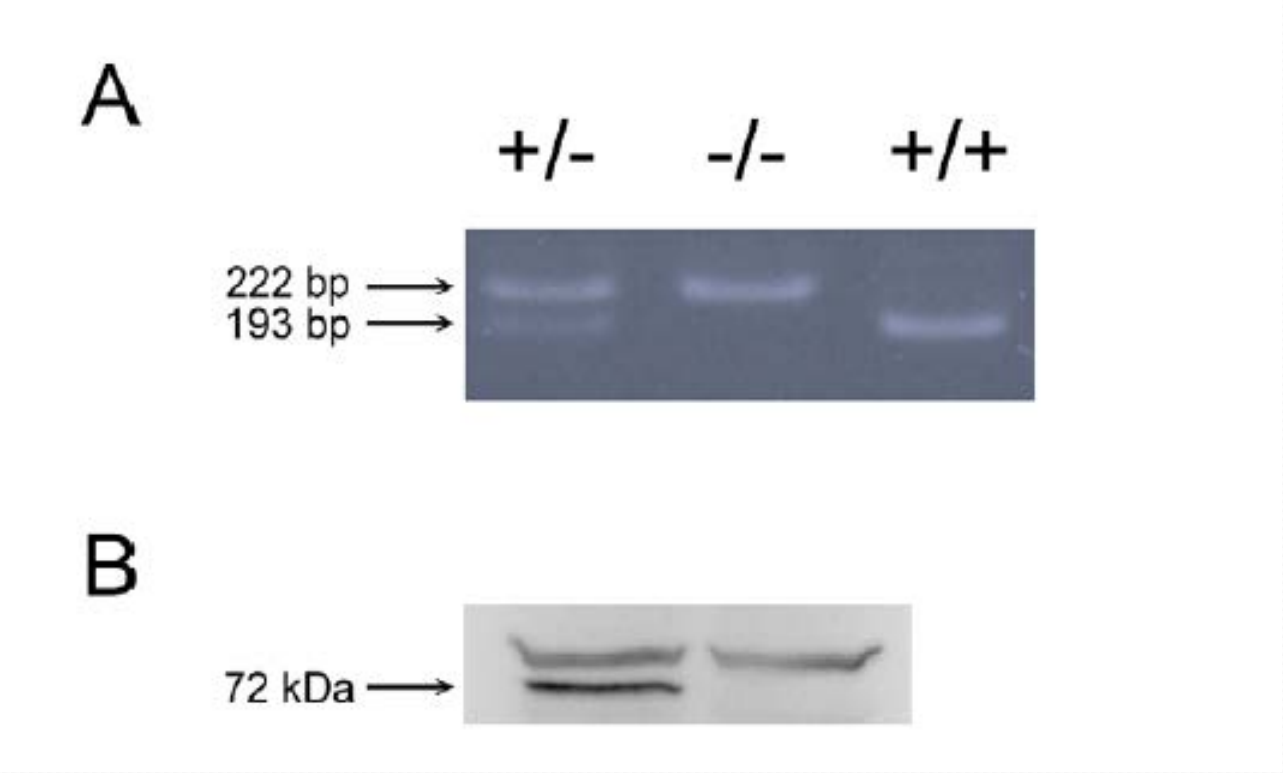
- To study behavioral deficits in Fmr1 KO zebrafish.
- To determine the abnormity on telencephalic synaptic plasticity in Fmr1 KO zebrafish

- Scientific Process
  - Observation
  - Replication
  - Interpretation
  - Verification

Outline of experimental procedures

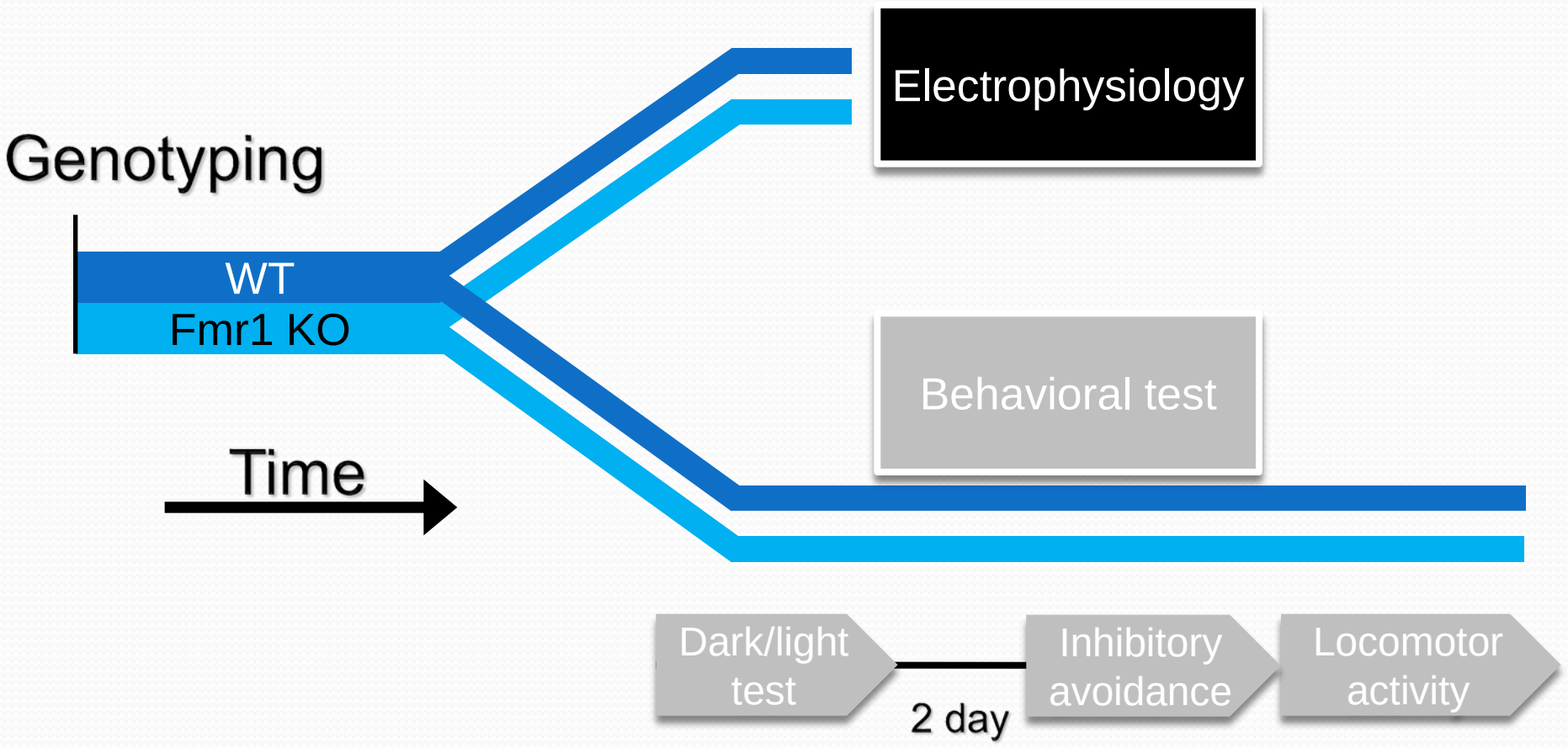


Genotyping and western blot analysis of Fmr1 knockout zebrafish.

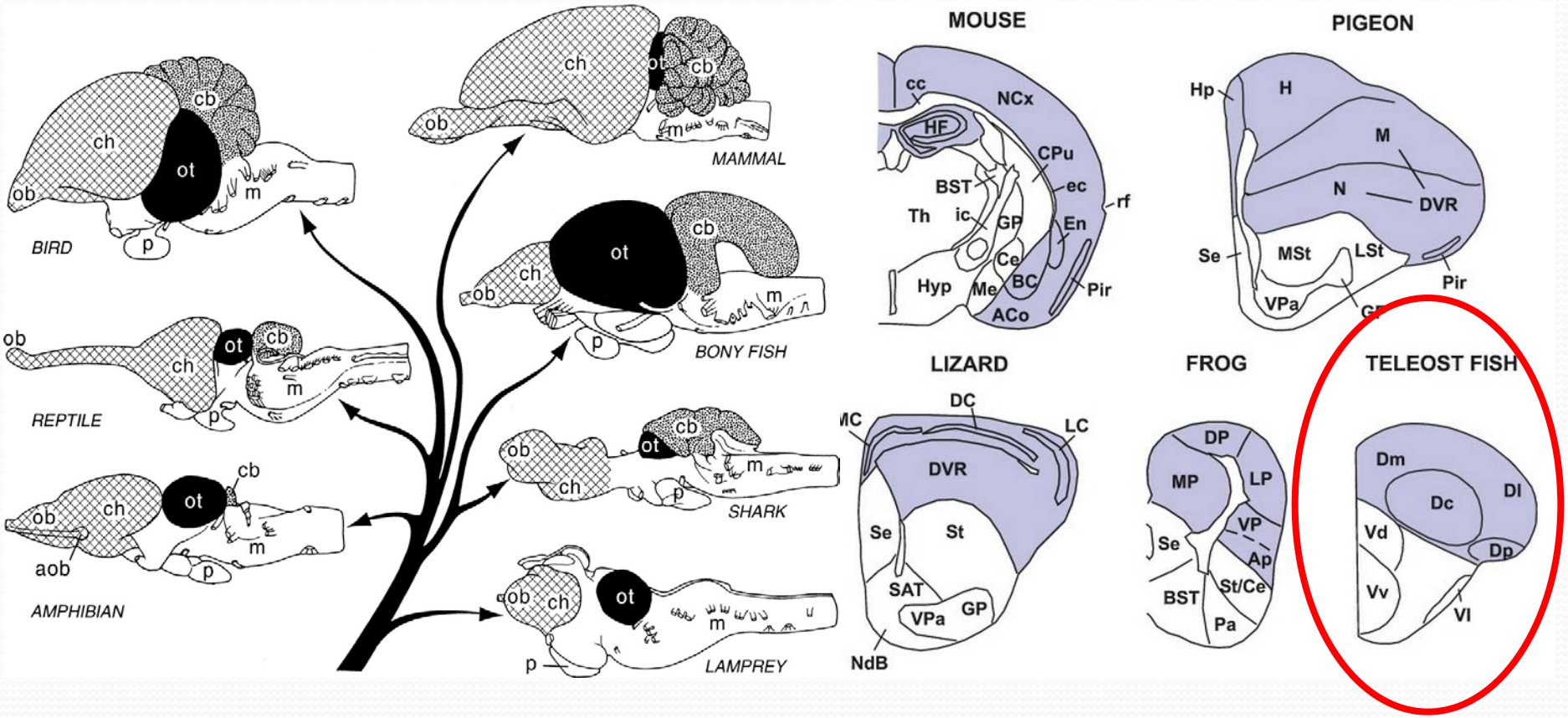


We thank Dr. Rob Willemsen for the kind gift of zebrafish FMR-1 antibody.

Outline of experimental procedures

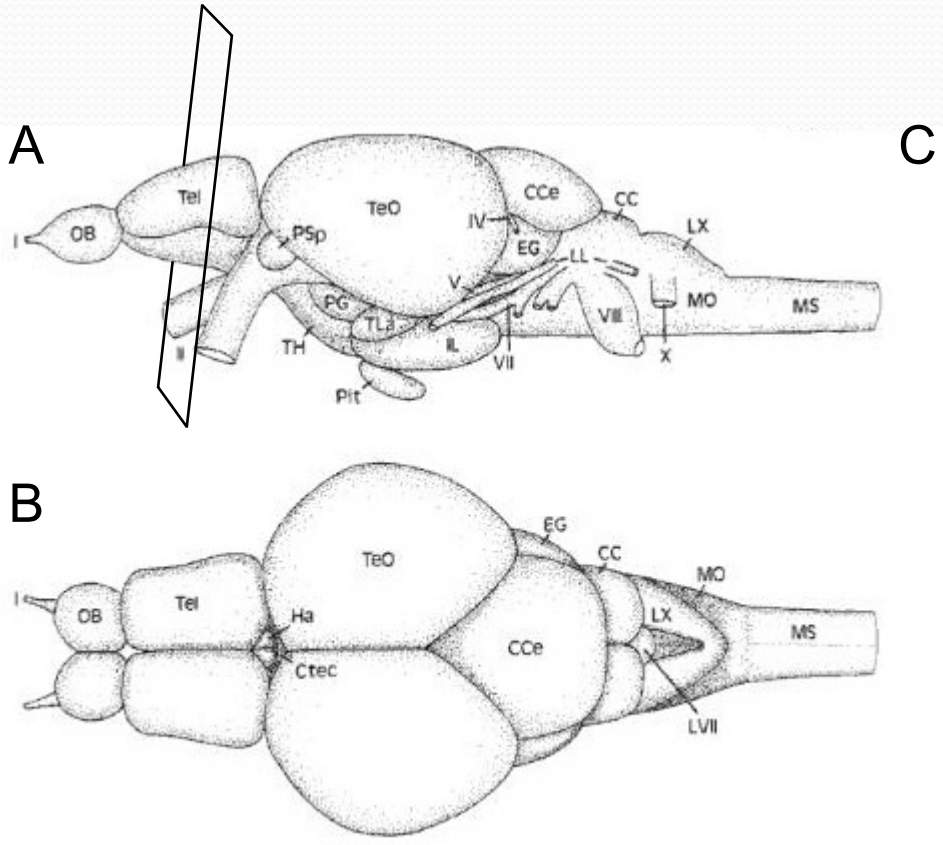


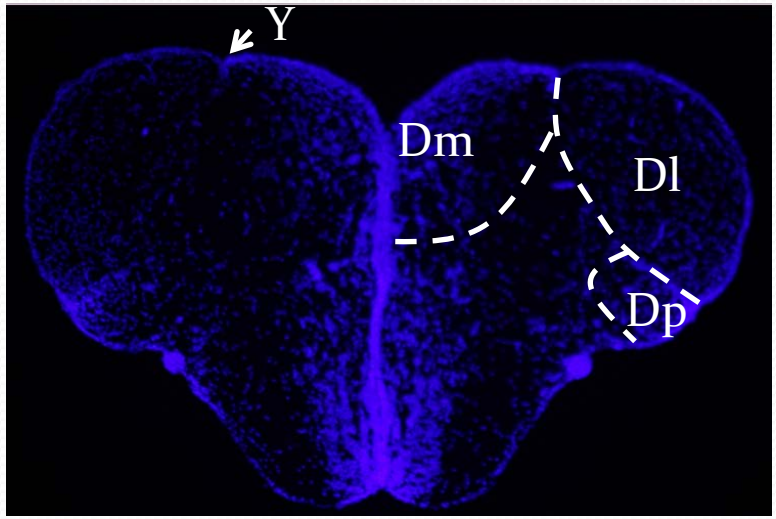
Determine the recording site



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

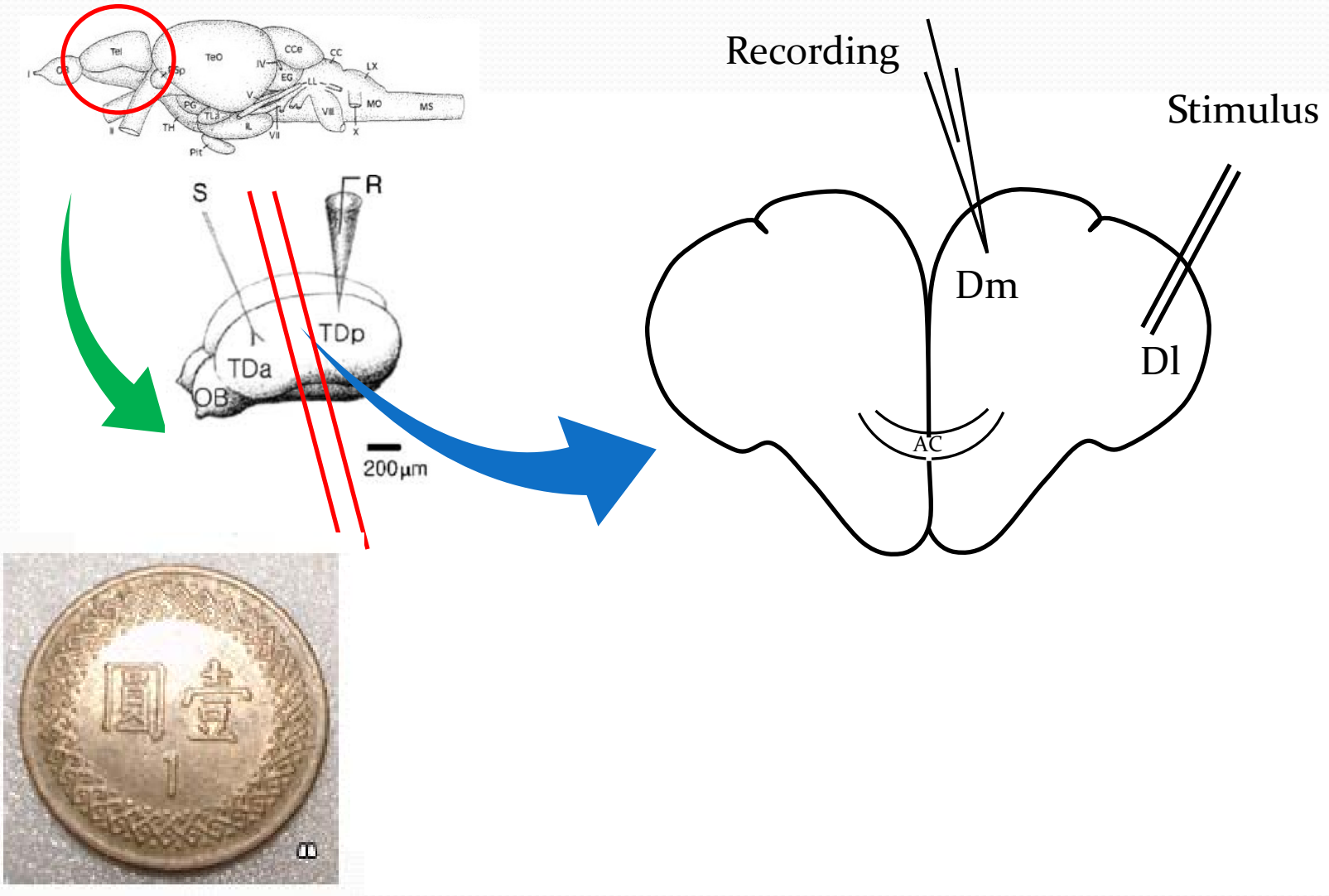
Topography of the telencephalon



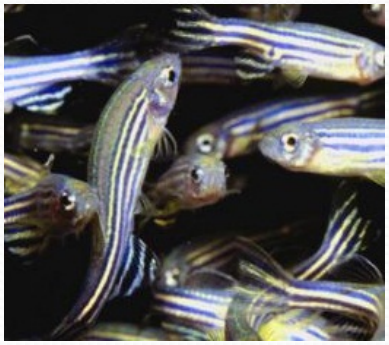


Dm : dorsal medial
Dl : dorsal lateral
Dp : dorsal posterior
Y : sulcus ypsiloniformis

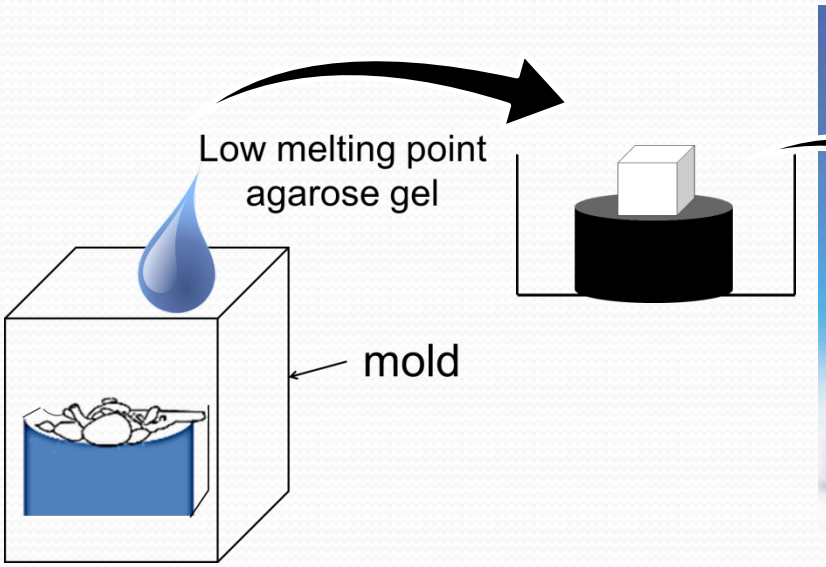
Brain slices preparation

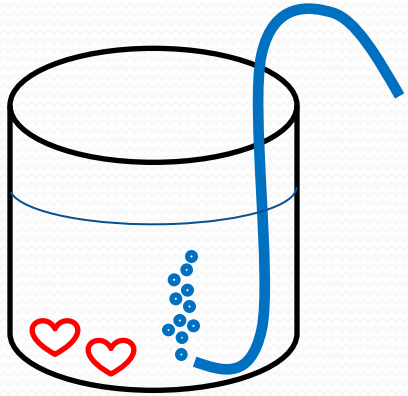


Extracellular recording



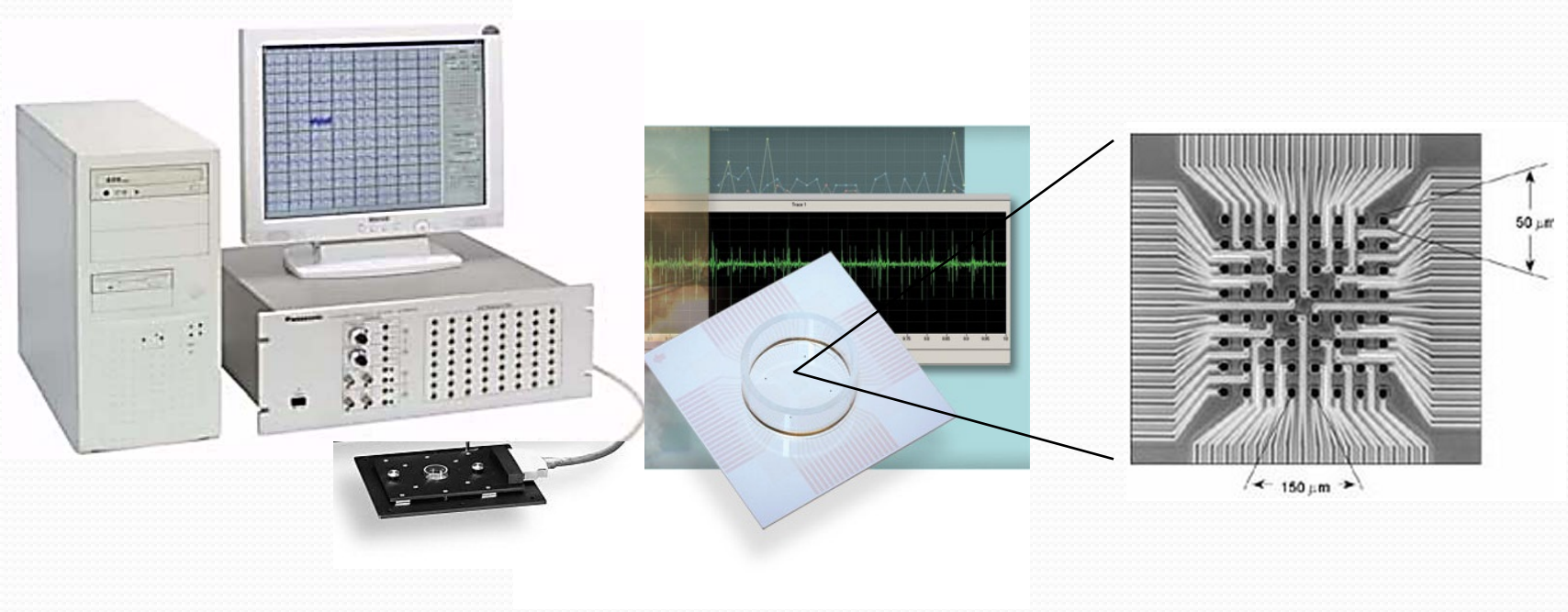
- Animals : zebrafish (3 to 4 months of age)
- Preparation of acute telencephalic slices





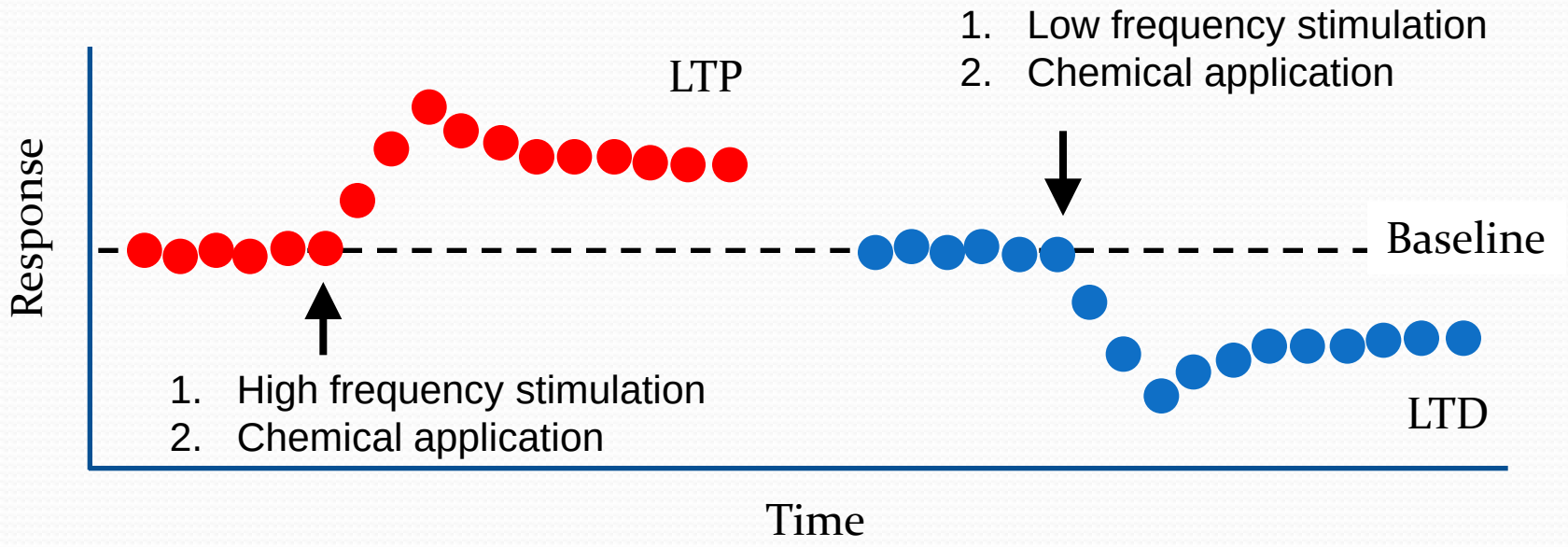
95% O_2/5% CO_2
in RT at least 1 hr

MED64:
multi-electrode recording system



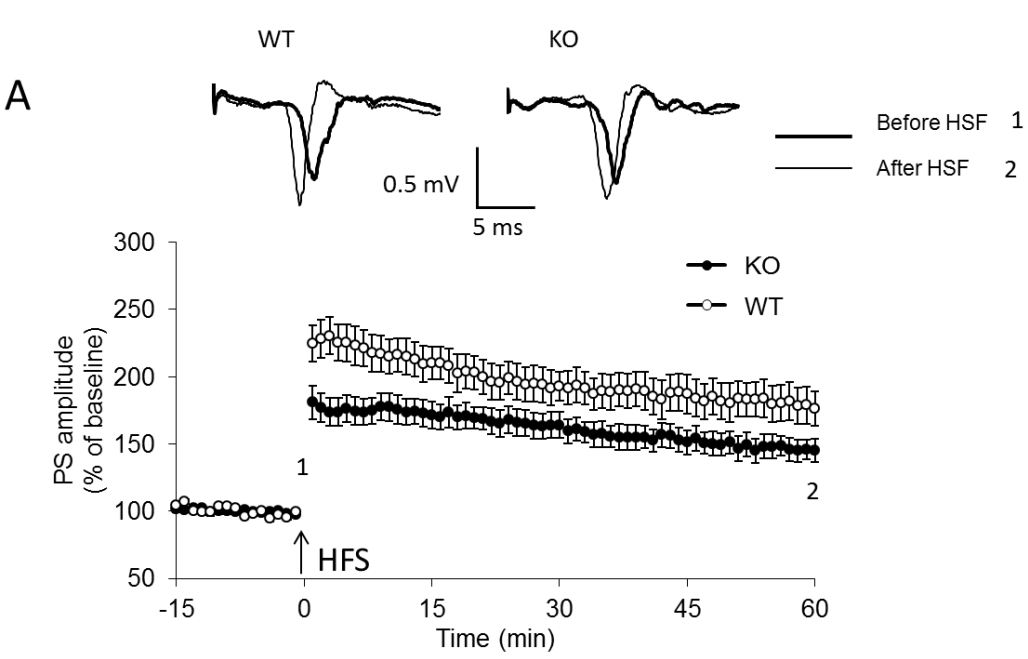
Synaptic plasticity

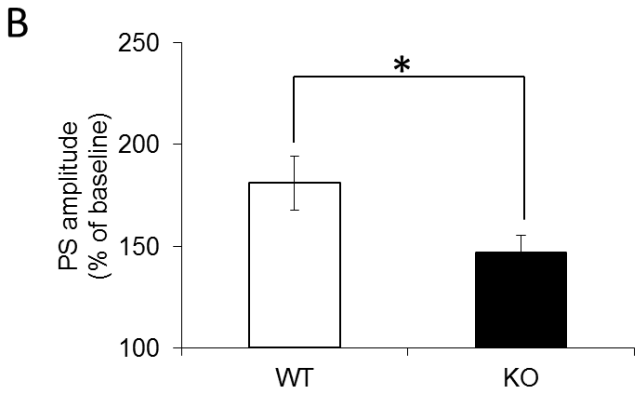
- **Long-term potentiation** (LTP) **and long-term depression** (LTD) of excitatory synaptic transmission, are wide spread phenomena expressed at possibly every excitatory synapse in the mammalian brain.



Results

Reduced long-term potentiation (LTP)

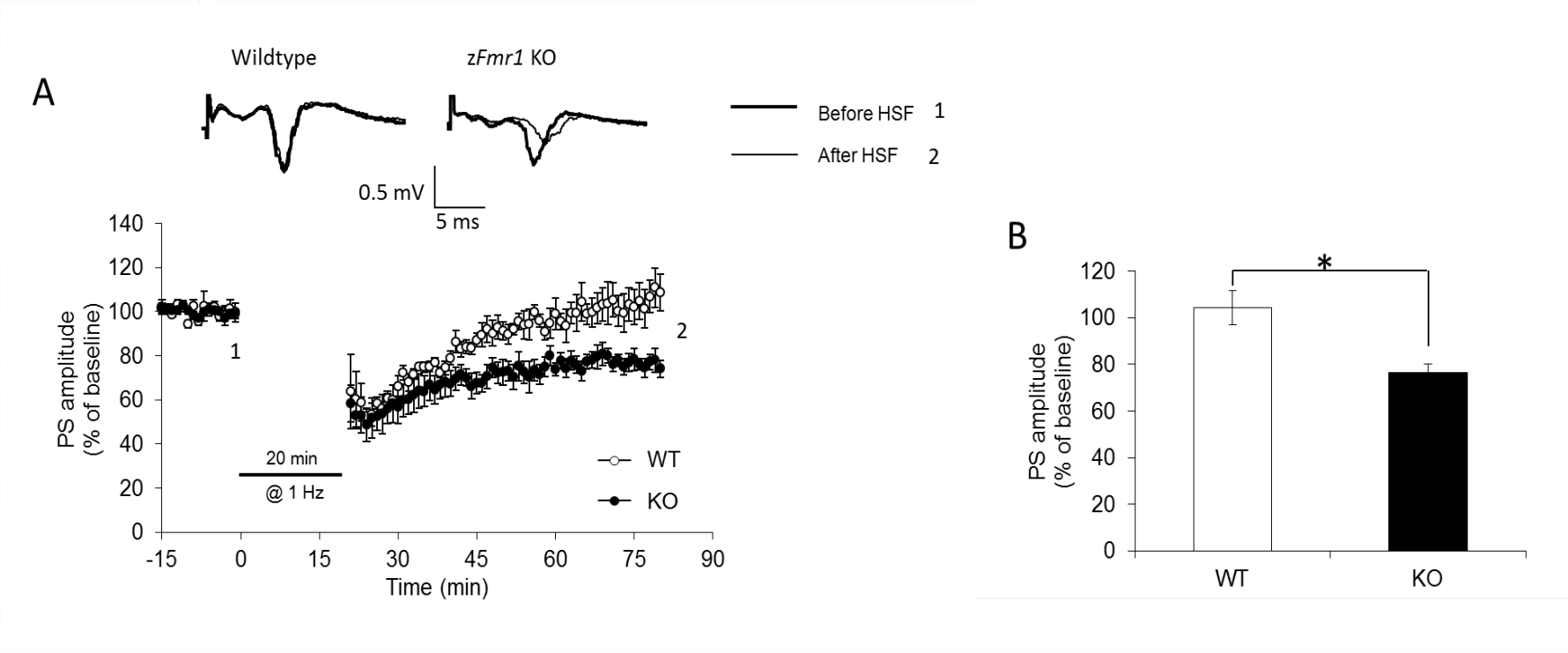




(Ng et al., 2013)

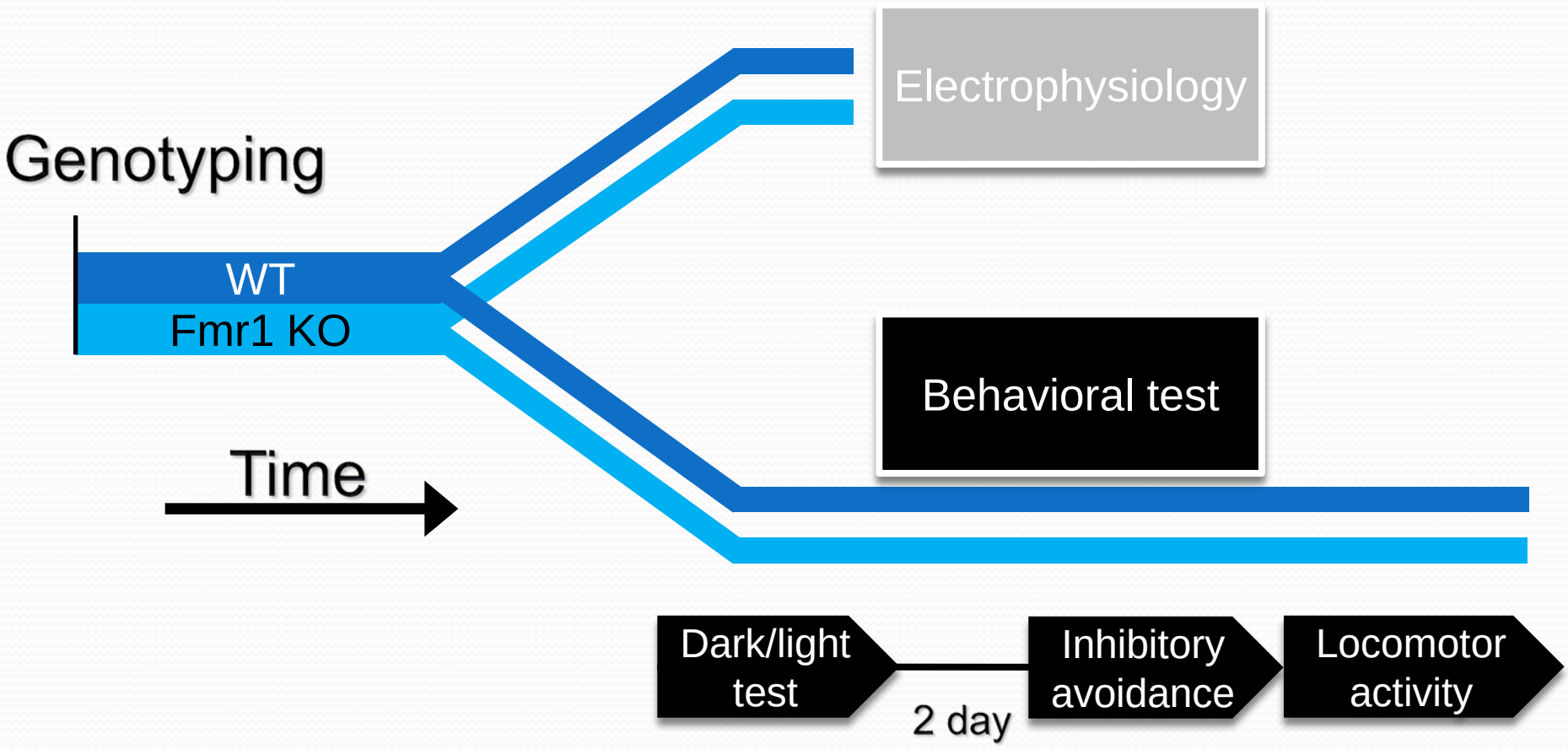
Results

Enhanced long-term depression (LTD)



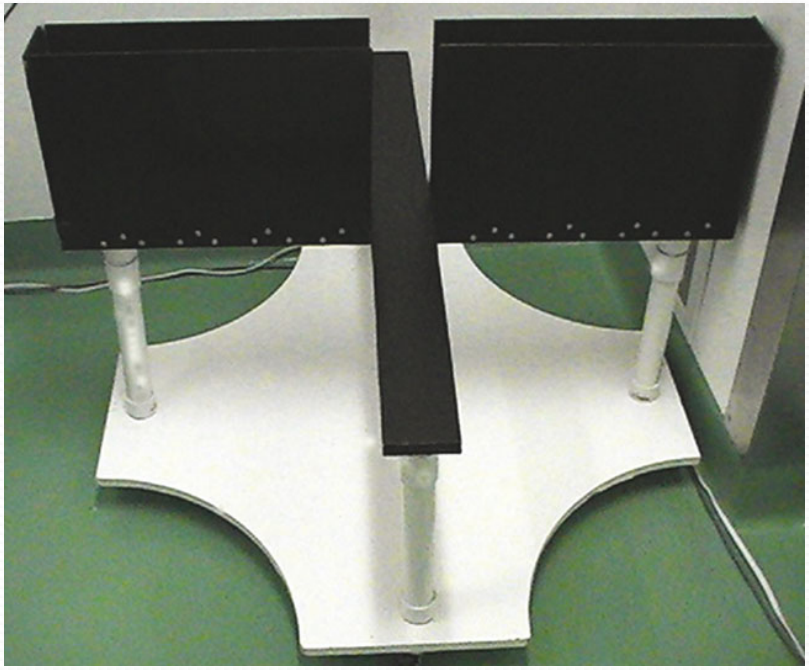
(Ng et al., 2013)

Outline of experimental procedures

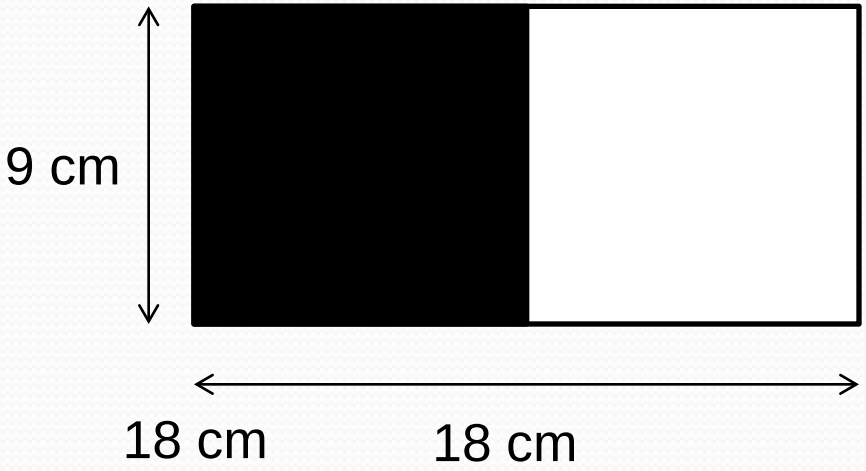


Dark/light

~ Evaluate the anxiolytic-like response in zebrafish

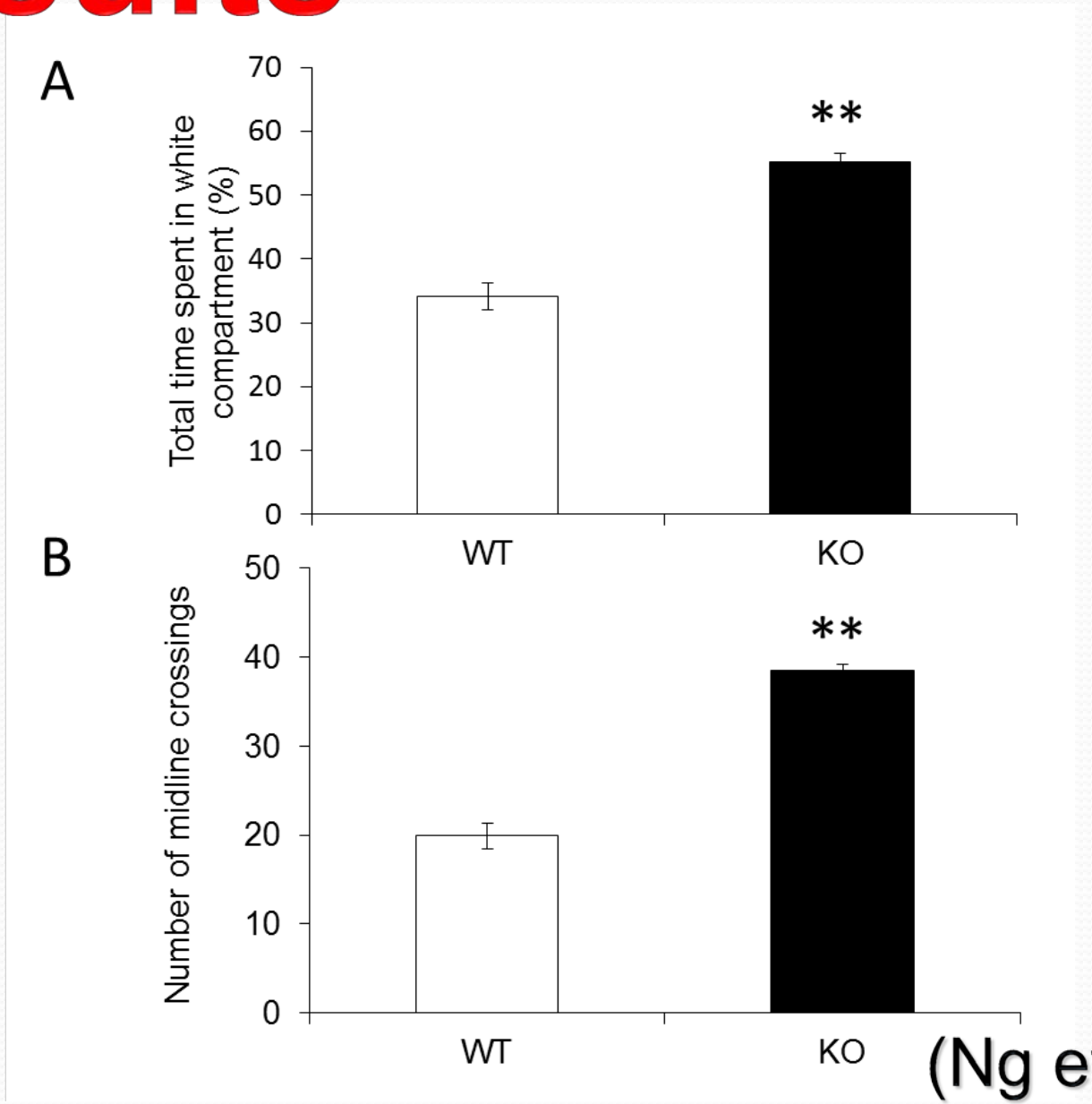


Elevated plus maze
(for rodent)



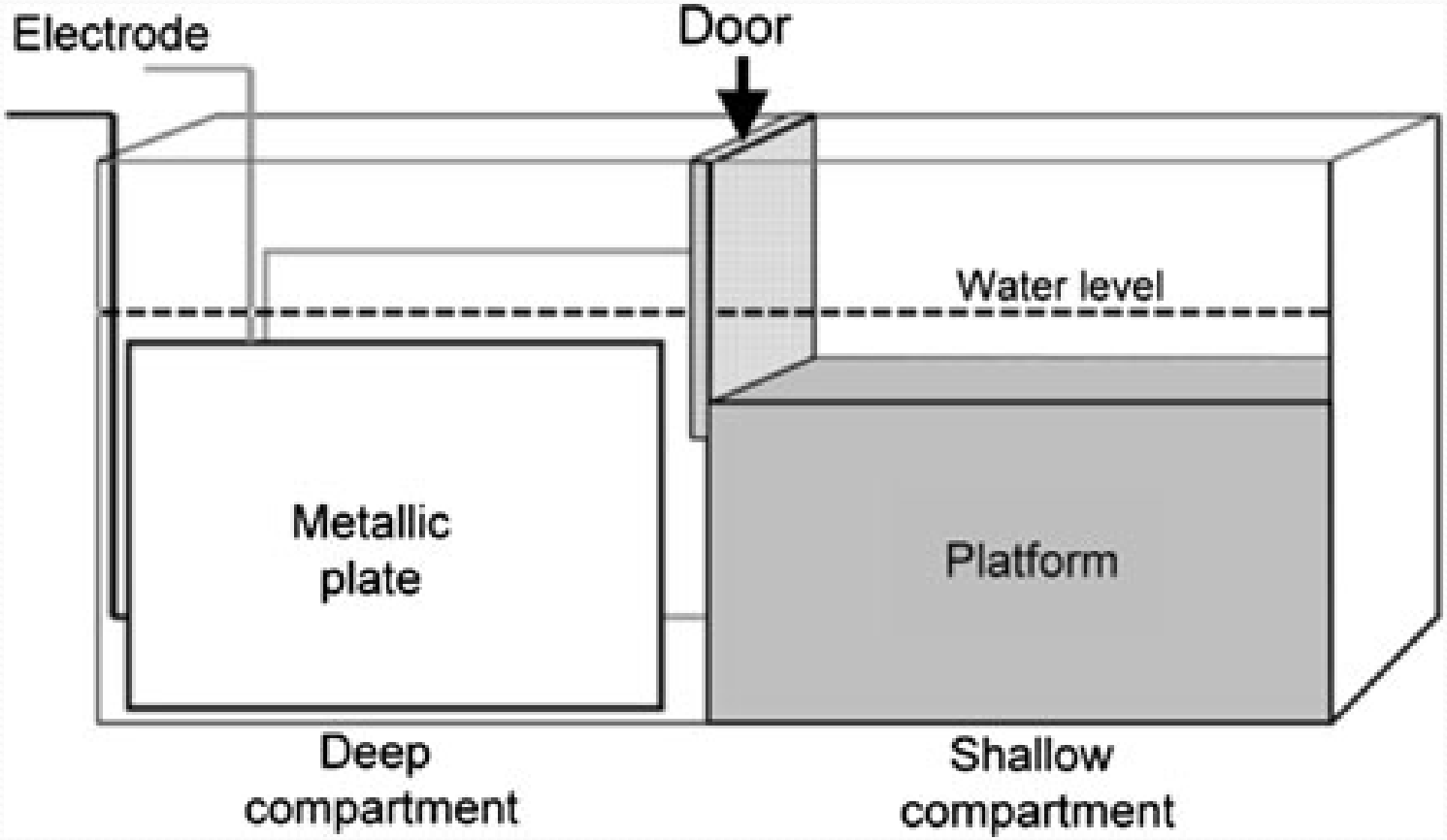
the fish were allowed to swim freely between the two compartments without sliding door for 5 min

Results

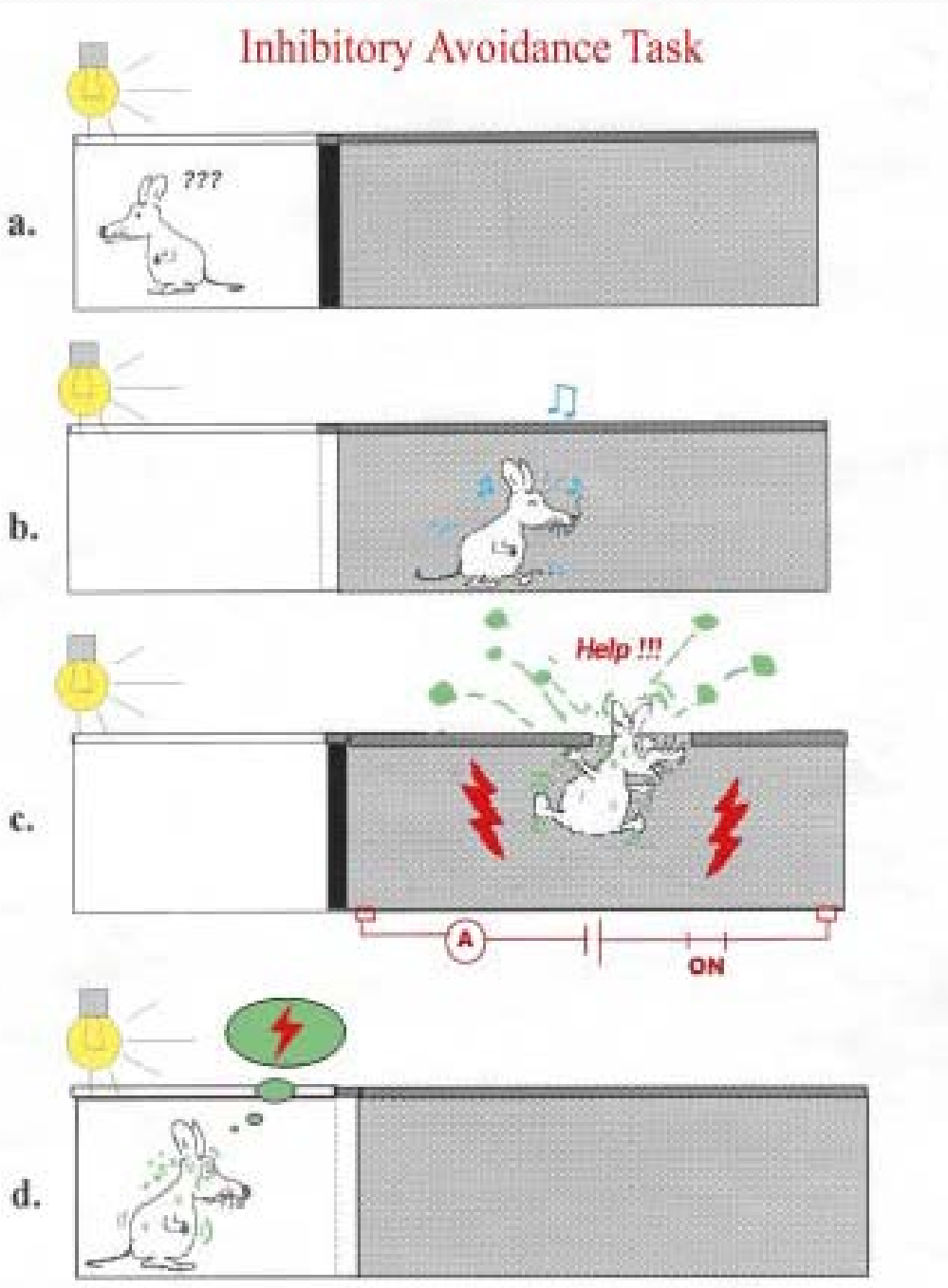


(Ng et al., 2013)

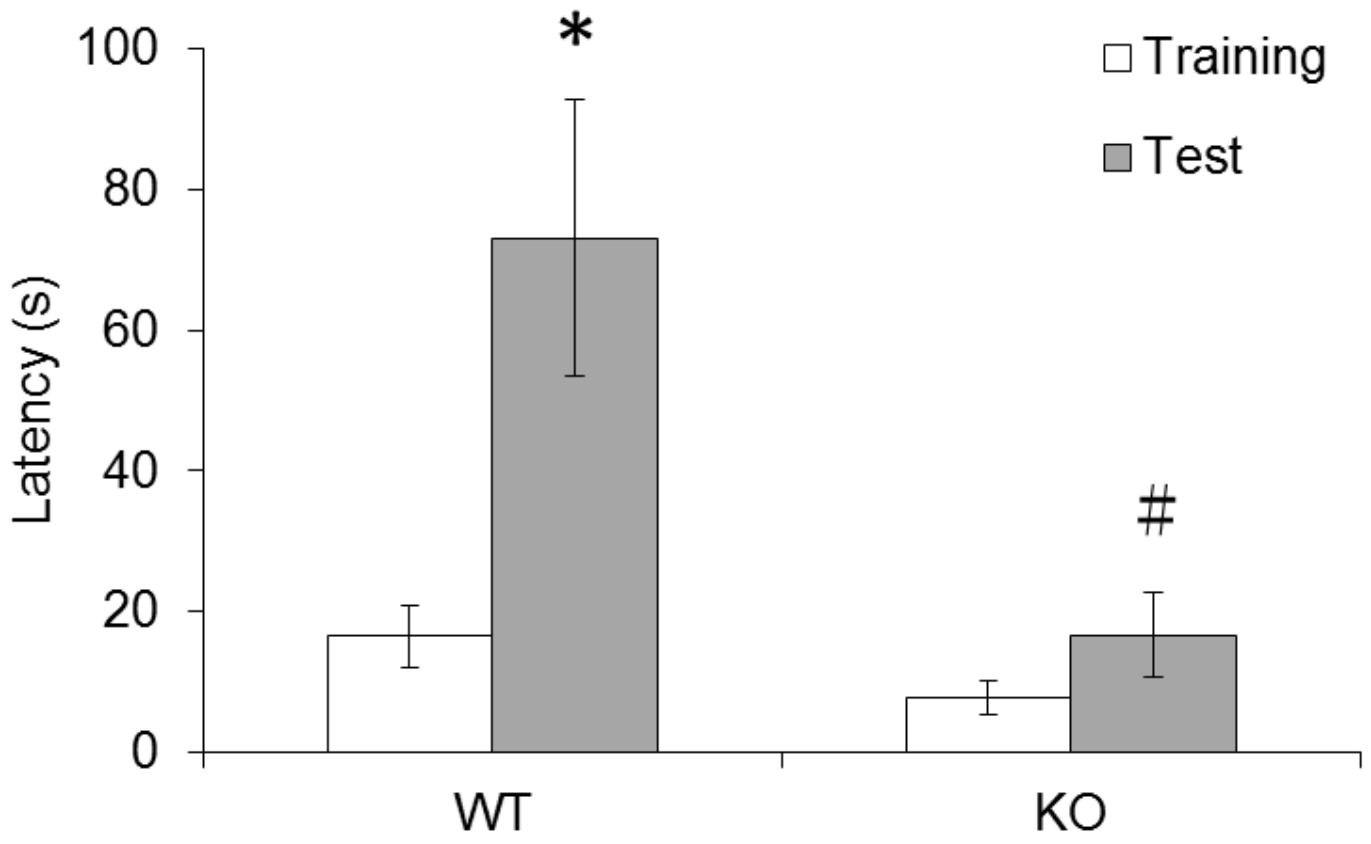
Inhibitory avoidance



(Ng et al., 2012b)

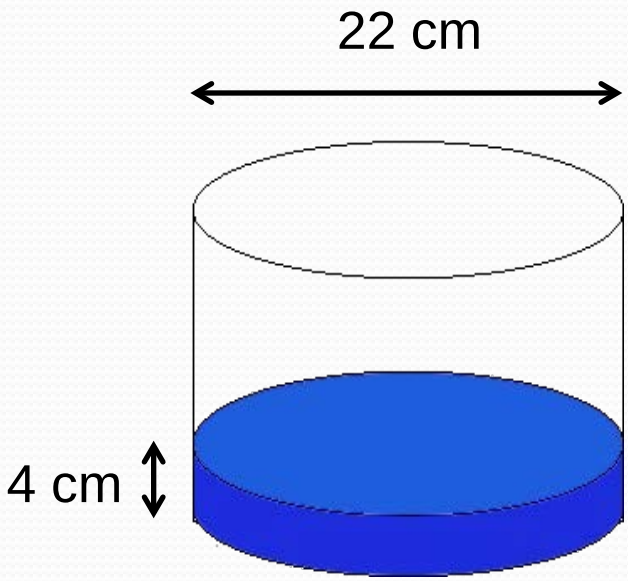

Inhibitory Avoidance Task
a.
???
b.
c.
Help !!!
A
ON
d.

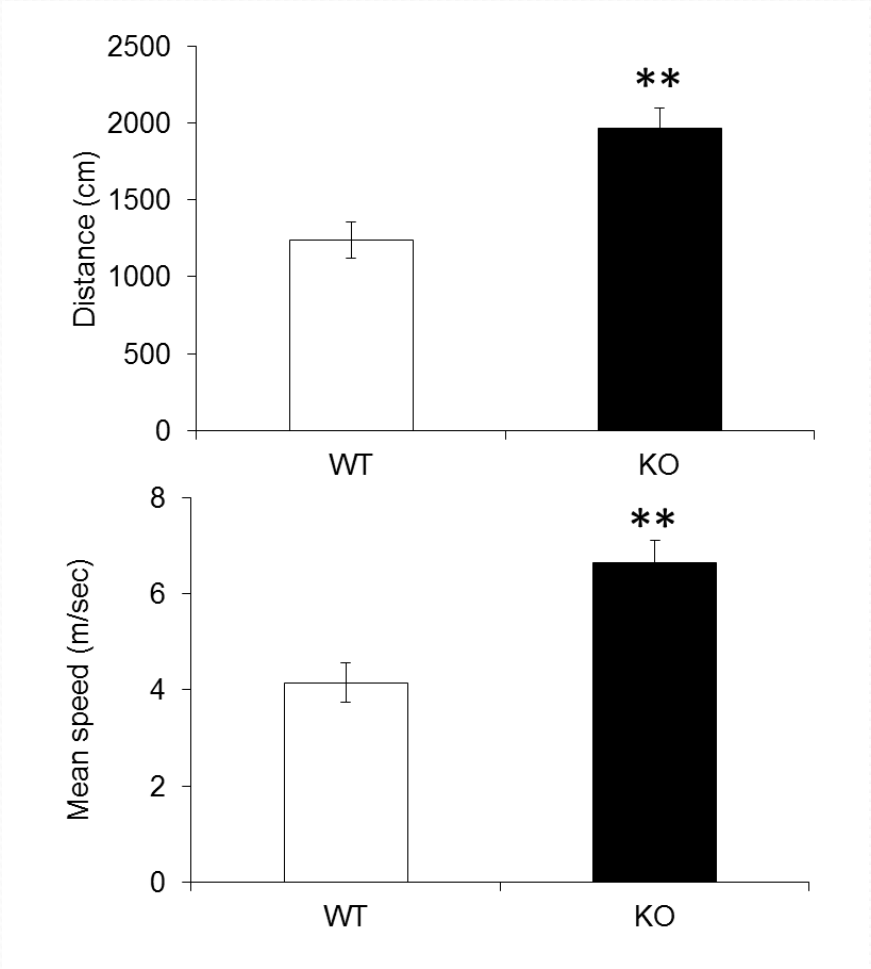
Impaired avoidance learning in Fmr1 KO zebrafishes



(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.

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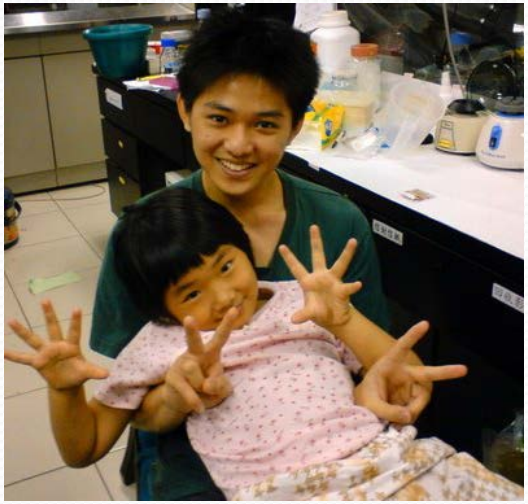
許竣博



吳民聰博士

吳世郁

Thanks for your listening