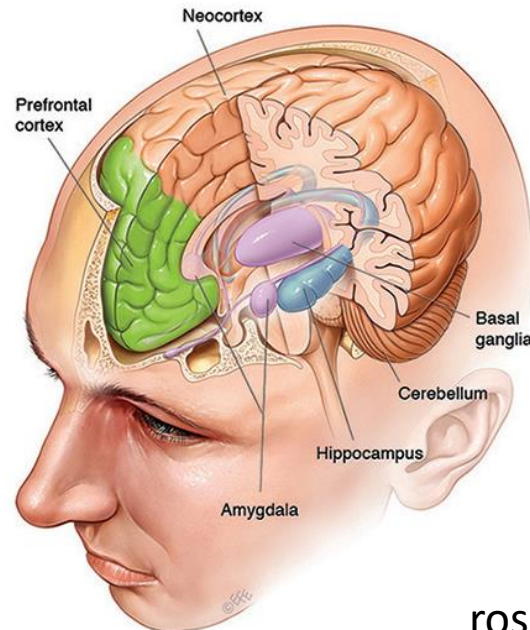


# Hippocampal adult neurogenesis in cognitive functions



Topic research :  
adult neurogenesis in  
different mouse models  
of intellectual deficiency

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# Adult Neurogenesis : a little history...



- **Ramon y Cajal, 1928**

*“The neuronal networks can be reorganized and new connections between neurons can be established, but Brain was **not able** to generate postnatal neurons”.*

Neurogenesis took place **ONLY** during the development.

End of development : fixed number of neurons. This number decreases with aging without replacement.

- **Altman & Co-workers, 1963, 1965 :**

Using tritiated thymidine radioactivity, presence of thymidine-labeled cells in the ventricular wall of the brain in rats.

 no proof new cells = neurons

This first published evidence was completely ignored by the scientific community

1928, Ramon y Cajal

Altman et al, 60's

# Adult Neurogenesis : a little history...

- Kaplan & co, 70-80's : These cells = neurons. Connections with other neurons in CA3
- Nottebohm, 1980's : Newborn neurons in adult birds.

- Rakic, 70-80's : eventually accepted in birds but no adult NG in mammals

- 1985, in *Sciences*



## Limits of Neurogenesis in Primates

Abstract. Systematic analysis of autoradiograms prepared from postpubertal rhesus monkeys given single and multiple injections of tritium-labeled thymidine and killed 3 days to 6 years later displayed a slow turnover of glial cells but failed to reveal any radiolabeled neurons. Therefore, unlike neurons of some nonprimate species, all neurons of the rhesus monkey brain are generated during prenatal and early postnatal life. A stable population of neurons in primates, including humans, may be important for the continuity of learning and memory over a lifetime.

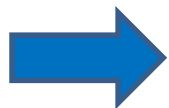
1928, Ramon y Cajal

Altman et al, 60's

Kaplan et al, 70's

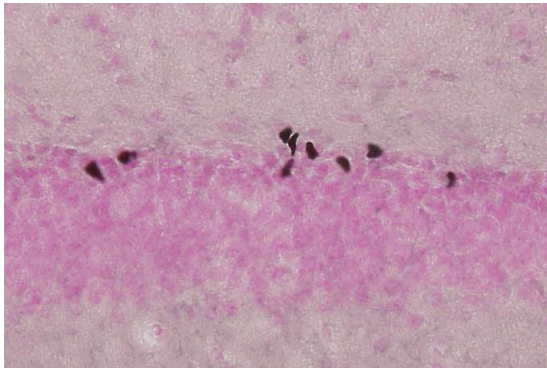
Nottebohm, 1980's

Rakic, 1985



Scientific community : Adult NG is restricted to evolutionarily "lower order" animals

# Adult Neurogenesis : a little history...



- Late 1990's: Gould, Gage, Van Praag & Kempermann & Co ....

new technology  
(BrdU immuno)

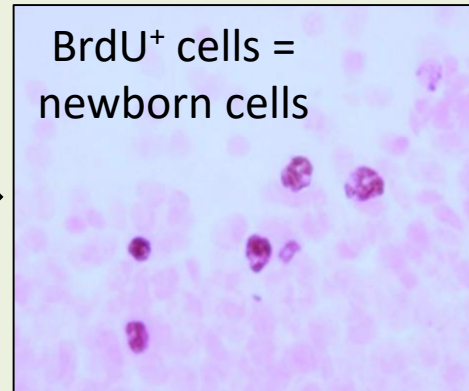
Explosion of the number of studies about neurogenesis in adult brain



BrdU injection

++ hours to ++ days

BrdU<sup>+</sup> cells = newborn cells



BrdU:(BromodeoxyUridine) = thymidine analog that incorporates DNA of dividing cells during the S phase of the cell cycle.

Since 90's : Newborn cells/ neurons can be « easily » detected in the adult brain

1928, Ramon y Cajal

Altman et al, 60's

Kaplan et al, 70's

Nottebohm, 1980's

Rakic, 1985

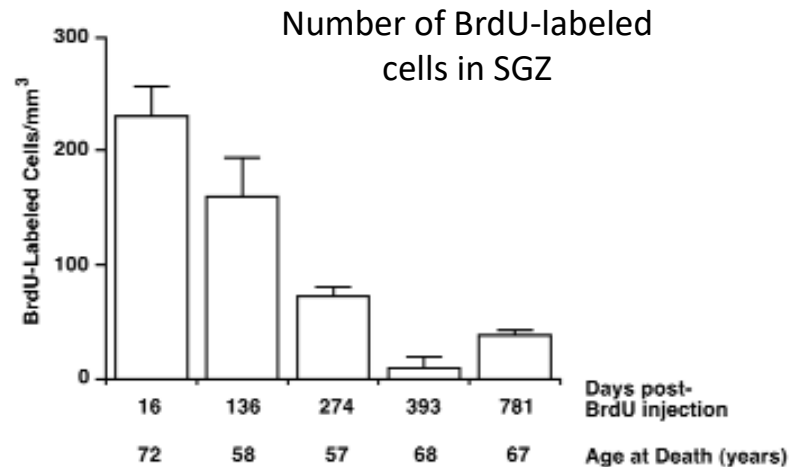
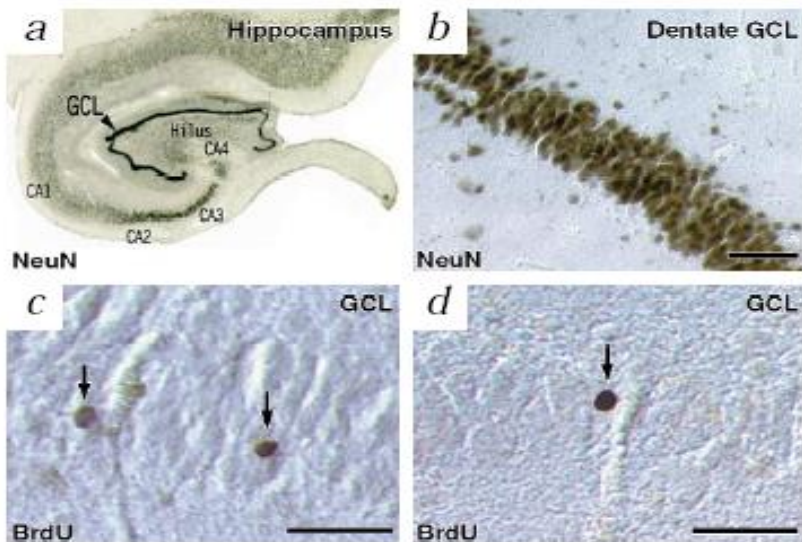
1990's, ++ studies

1998, Eriksson et al,

# Adult Neurogenesis : a little history...

- **1998 : Eriksson et al**, newly generated cells are detected in the **adult human brain** of patients previously treated with BrdU (postmortem tissue of cancer patients)

Patients : carcinomas tongue, larynx pharynx, No anti-cancer therapy



it is well accepted that adult neurogenesis is a lifetime process : new neurons are **continuously** generated in the adult brain

...in several vertebrate species : mammals, birds, reptils, fishes, and also in non-vertebrate : insects, cephalopods

1928, Ramon y Cajal

Altman et al, 60's

Kaplan et al, 70's

Nottebohm, 1980's

Rakic, 1985

1990's, ++ studies

1998, Eriksson et al,



# Adult Neurogenesis : a last debate in Humans...

## LETTER

doi:10.1016/j.stem.2018.03.001

Published  
in april  
2018

### Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults

Shawn F. Sorrells<sup>1,2\*</sup>, Mercedes F. Paredes<sup>1,3\*</sup>, Arantxa Celis<sup>1,3</sup>, David James<sup>1</sup>, Simone Mayer<sup>1,3</sup>, Julia Chang<sup>6</sup>, Kurtis I. Auger<sup>1,3</sup>, Arnold R. Kriegstein<sup>1,3</sup>, Gary W. Mathern<sup>8,9</sup>, Michael C. Oh<sup>1,3</sup>, Zhengang Yang<sup>5</sup> & Arturo Alvarez-Buylla<sup>1,2</sup>

Cell Stem Cell

Minireview

CellPress

### Human Adult Neurogenesis: Evidence and Remaining Questions

Gerd Kempermann,<sup>1,\*</sup> Fred H. Gage,<sup>2,\*</sup> Ludwig Aigner,<sup>3</sup> Hongjun Song,<sup>4</sup> Maurice A. Curtis,<sup>5</sup> Sandrine Thuret,<sup>6</sup> H. Georg Kuhn,<sup>7,8</sup> Sebastian Jessberger,<sup>9</sup> Paul W. Frankland,<sup>10</sup> Heather A. Cameron,<sup>11</sup> Elizabeth Gould,<sup>12</sup> Rene Hen,<sup>13</sup> D. Nora Abrous,<sup>14</sup> Nicolas Toni,<sup>15</sup> Alejandro F. Schinder,<sup>16</sup> Xinyu Zhao,<sup>17</sup> Paul J. Lucassen,<sup>18</sup> and Jonas Frisén<sup>19,\*</sup>

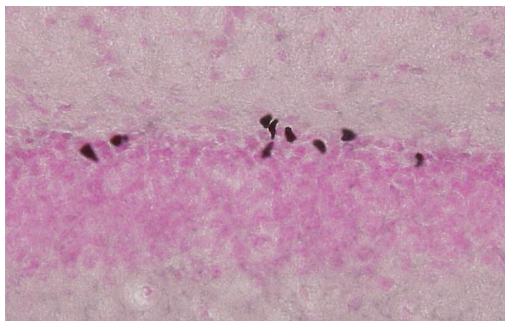
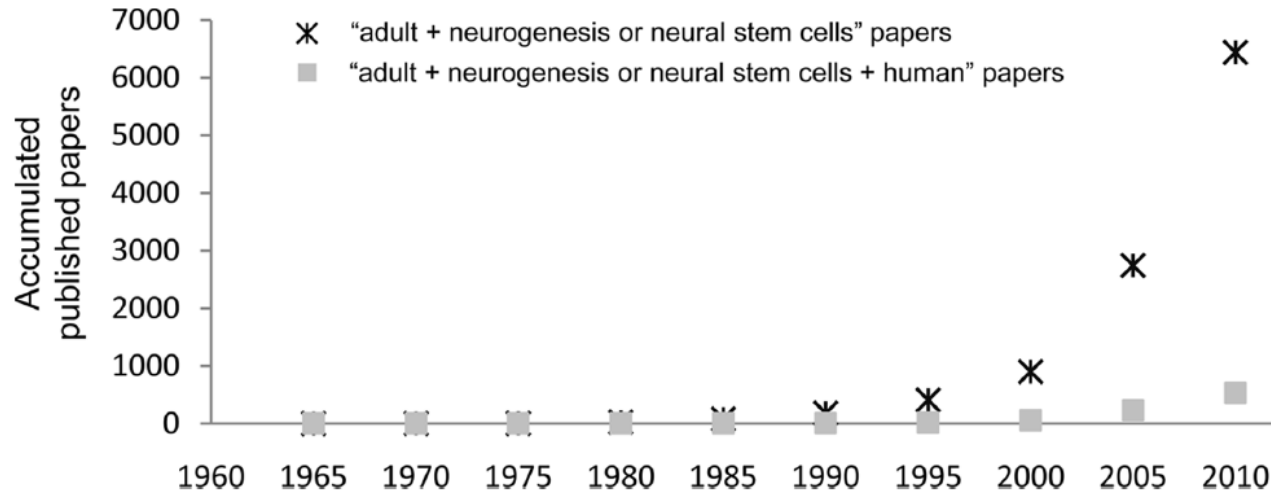
Cell Stem Cell

### Human Hippocampal Neurogenesis Persists throughout Aging

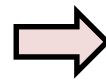
Authors

Maura Boldrini, Camille A. Fulmore, Alexandria N. Tartt, ..., Andrew J. Dwork, René Hen, J. John Mann

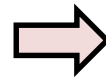
# Adult Neurogenesis : a brief history...



new neurons are continuously generated in the adult brain



function of adult neurogenesis ?



Potential Implications in the clinical treatment of neurodegenerative diseases and brain tumor formation

1928, Ramon y Cajal

Altman et al, 60's

Kaplan et al, 70's

Nottebohm, 1980's

Rakic, 1985

1990's, ++ studies

1998, Eriksson et al,

# Adult Neurogenesis : Methodology

Since 90's : Newborn neurons can be « easily » detected in the brain

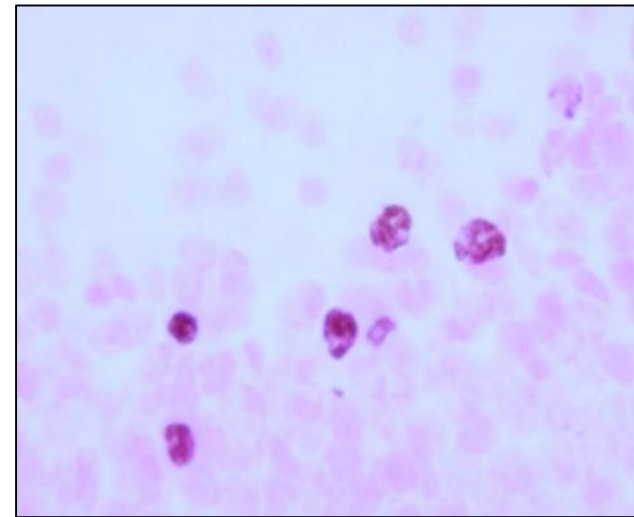


BrdU injection

++ hours to ++ days

Perfusion /  
Brain dissection / Brain sectioning/  
immunohistochemical methods  
(specific antibodies)

BrdU (BromodeoxyUridine)  
is thymidine analog that  
incorporates DNA of dividing  
cells during the S phase of  
the cell cycle.



BrdU<sup>+</sup> cells = newborn cells



# Adult Neurogenesis : Methodology

Since 90's : Newborn neurons can be « easily » detected in the brain

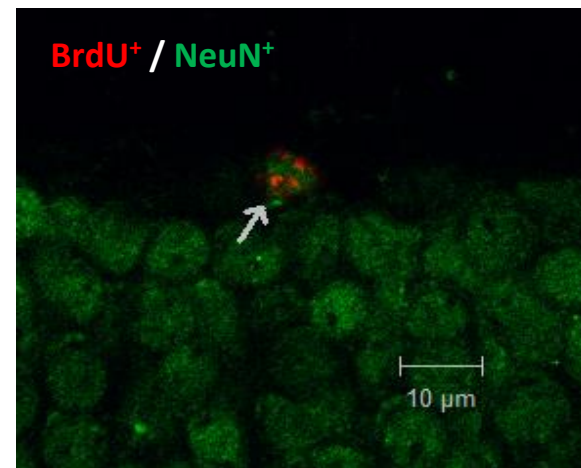


BrdU inj

++ hours to ++ days

Perfusion / Brain dissection / Brain sectioning / immunohistochemical methods **OR** / **AND** immunofluorescent methods to detect **the phenotype of the newborn cells** : neurons / astrocytes / oligodendrocytes / microglia....

✓ **Main Interest:**  
ability of multiple labellings  
with immunofluorescent  
markers



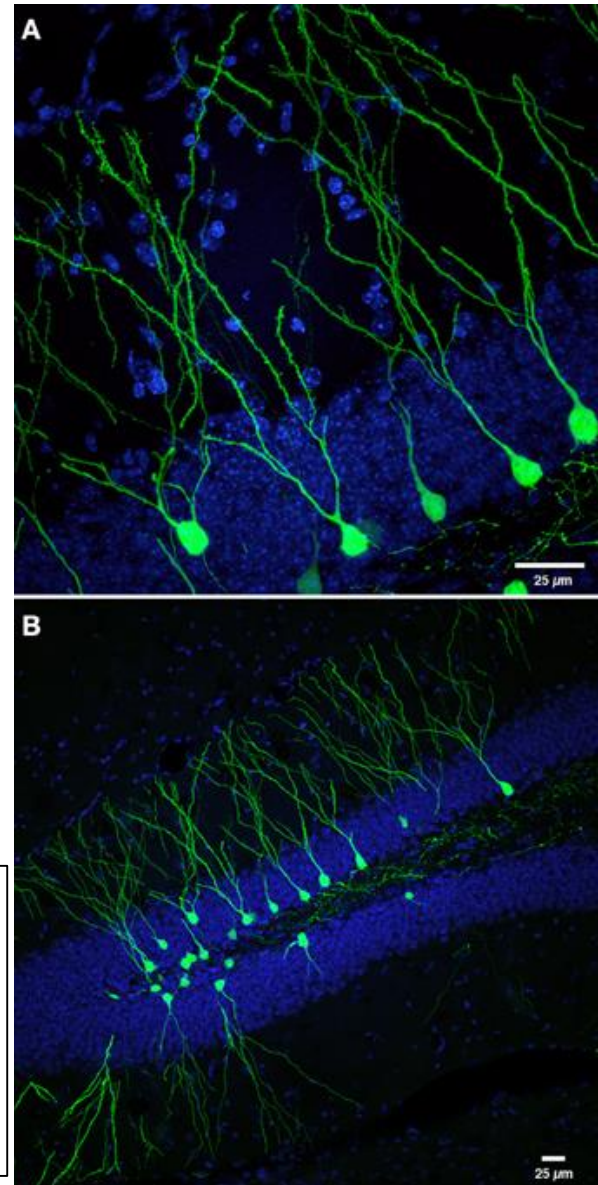
# Adult Neurogenesis : Methodology

## ➤ GFP - Retroviral labeling of newborn cells

Injection of retrovirus coupled with GFP (green fluorescent protein) that infect only cells in proliferation.

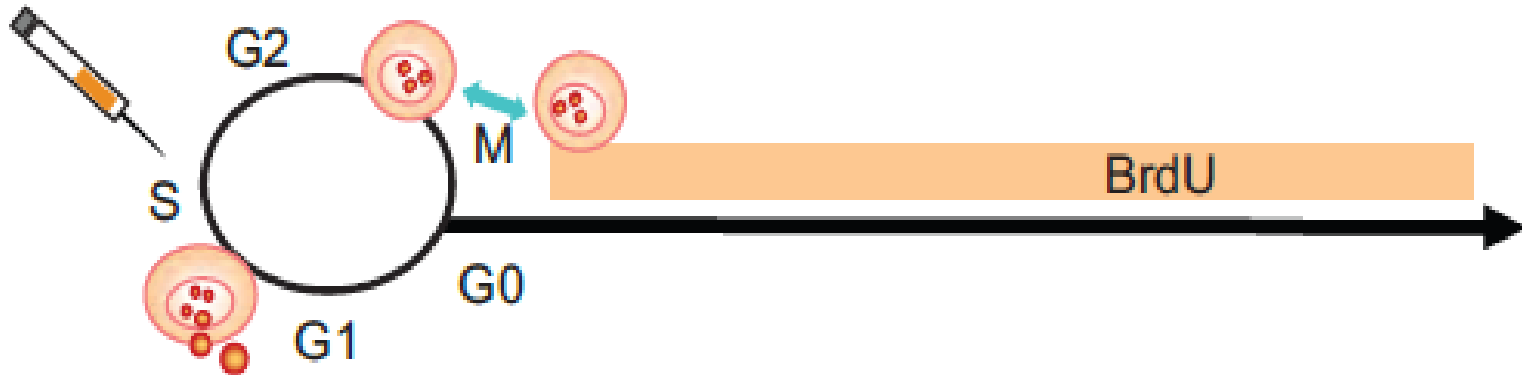
- ✓ Main interest: follow the development of dendritic arborization and dendritic spines

Photomicrograph shows retrovirally labeled newborn dentate GCs expressing green fluorescent protein (GFP) throughout the entire cell, at 42 days post-retroviral injection (dpi). Scale bar, 25  $\mu$ m.

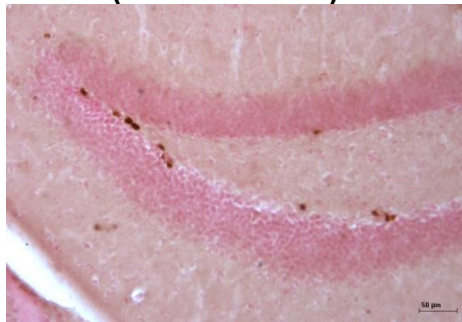


# Adult Neurogenesis : Methodology

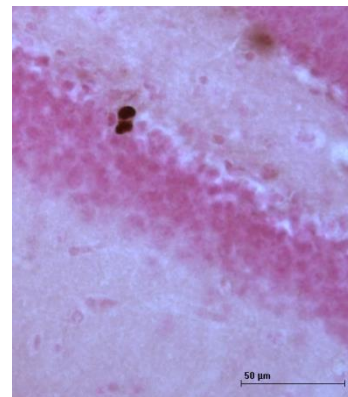
Proliferation markers: Ki67 and PH3



Ki67 in all cycling cells  
(G1 S G2 M)



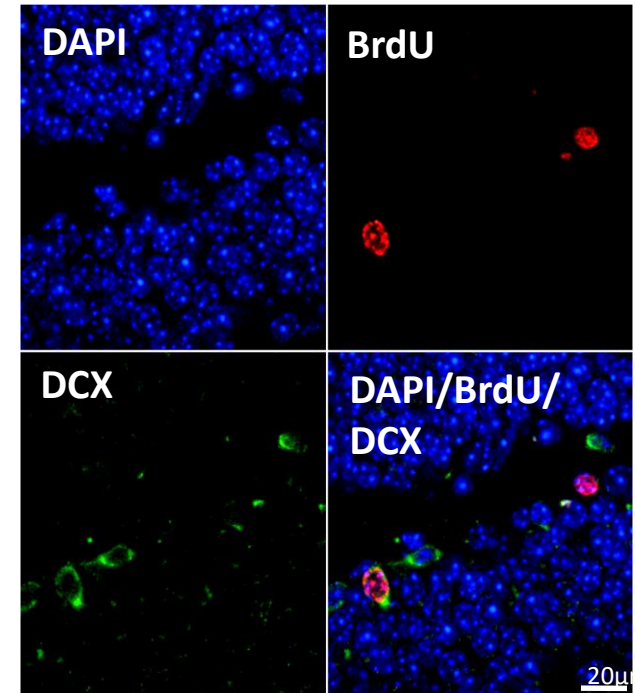
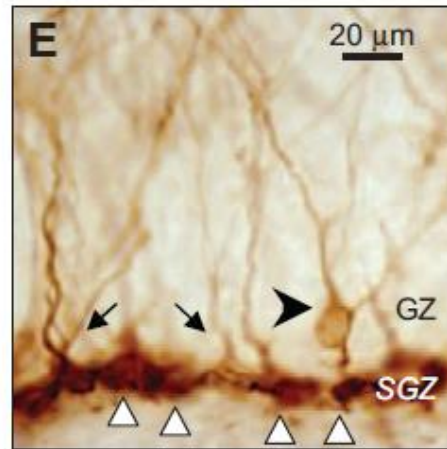
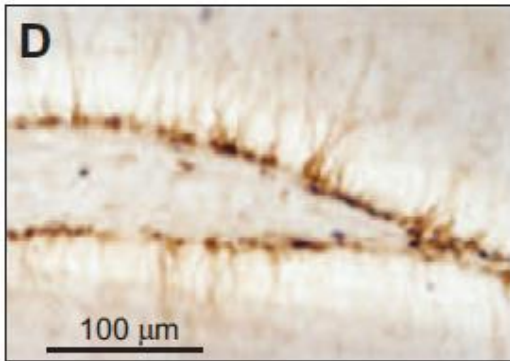
PH3 in G2 & M phases



✓ Interest : to study cell cycle duration, cell cycle exit

# Adult Neurogenesis : Methodology

Immature neuron marker : Doublecortin X-linked (DCX)

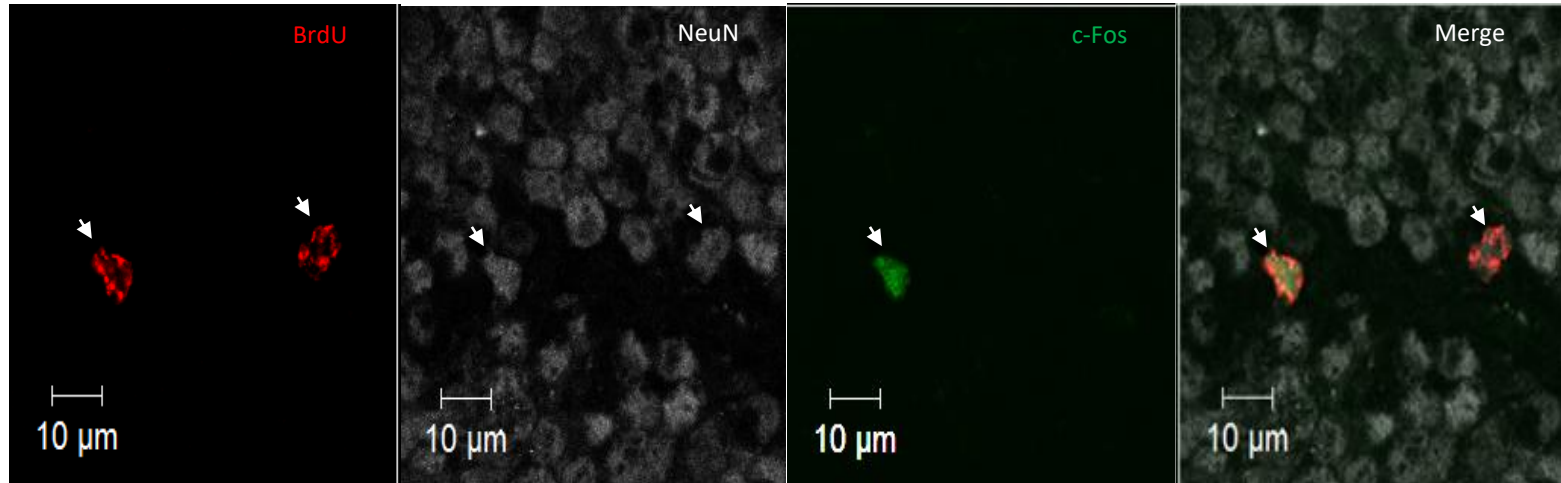


All immature neurons are labeled (from 3 days old to 4 weeks old)

- ✓ Interest : rapid estimation of neurogenesis without injection  
To track the speed of maturation

# Adult Neurogenesis : Methodology

Functionality of adult born neurons : expression of IEGs



Confocal images of BrdU (red), NeuN (grey), c-Fos (green) immunohistochemistry in the dentate gyrus. Colocalization (merge) of the three markers suggests activation of labeled newborn neurons.

✓ **Functionality & Recruitment in existing neuronal networks**  
(The activation of new neurons confirms their integration into neuronal networks)

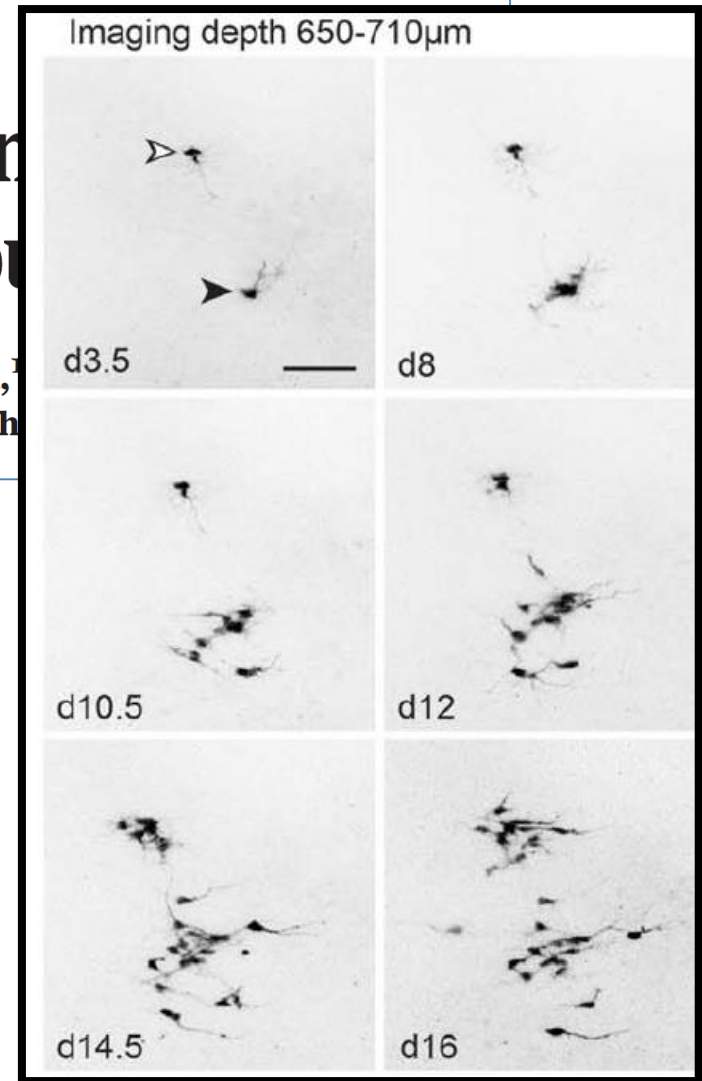
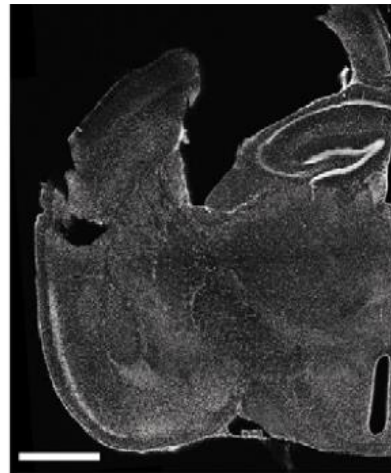
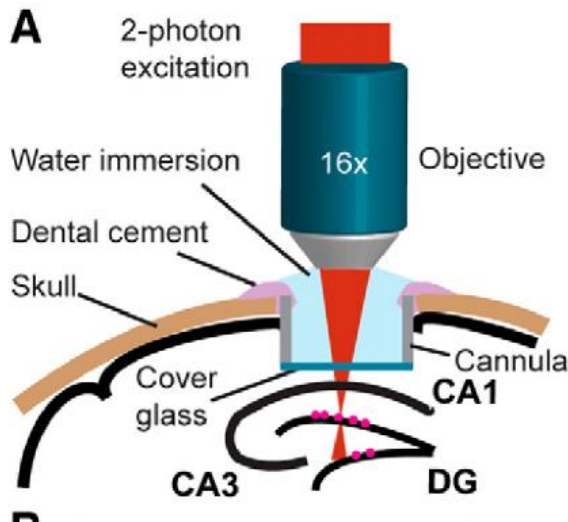


# Adult Neurogenesis : Methodology

## NEURODEVELOPMENT

# Live imaging of neurogenesis in the adult mouse hippocampus

Gregor-Alexander Pilz,<sup>1\*</sup> Sara Bottes,<sup>1\*</sup> Marion Betizeau,<sup>1</sup> Stefano Carta,<sup>1,6</sup> Benjamin D. Simons,<sup>3,4,5</sup> Fritjof Helmchen





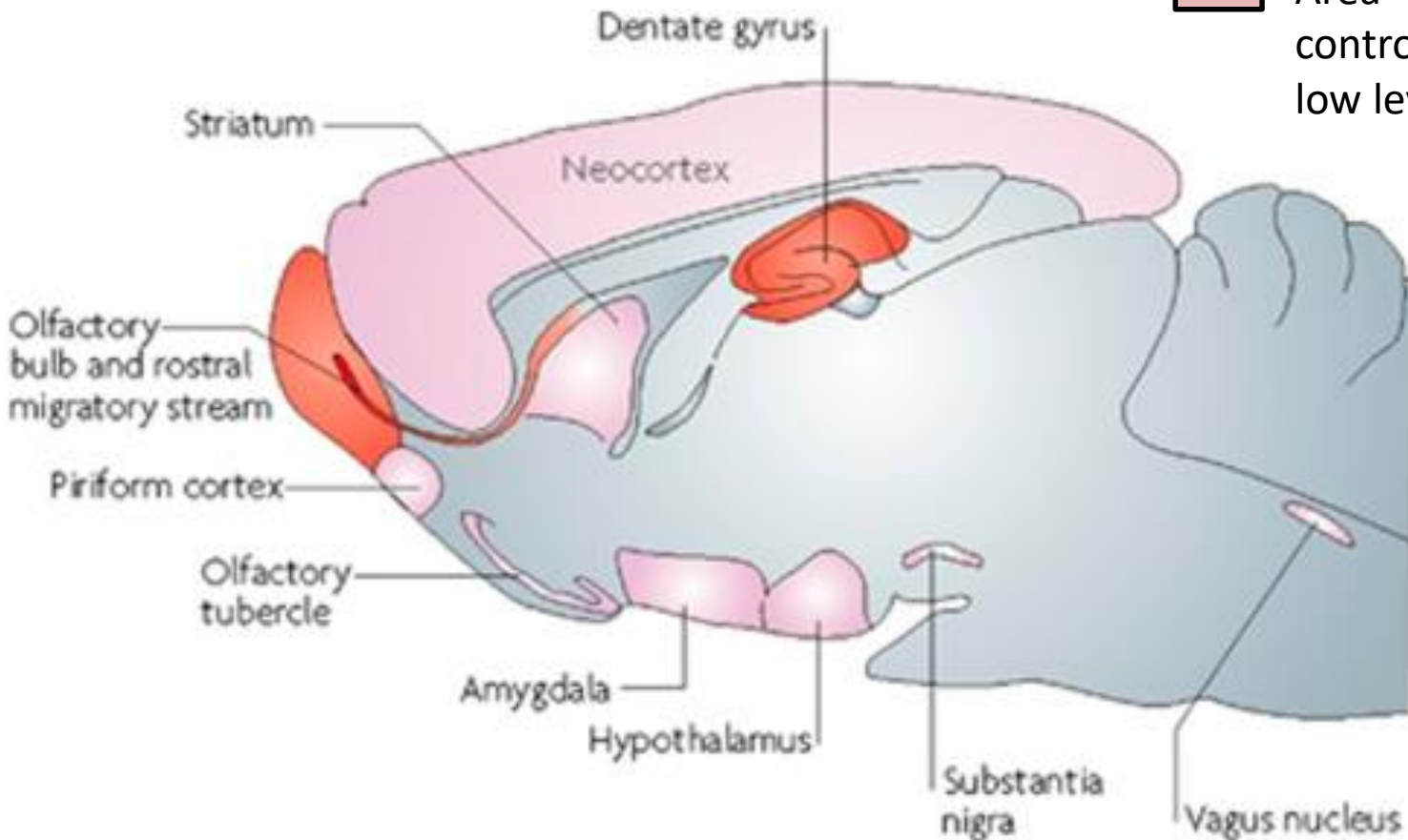
- Chronic *in vivo* imaging of NSPCs in the adult DG to track their development



# Neurogenic regions in Mammals

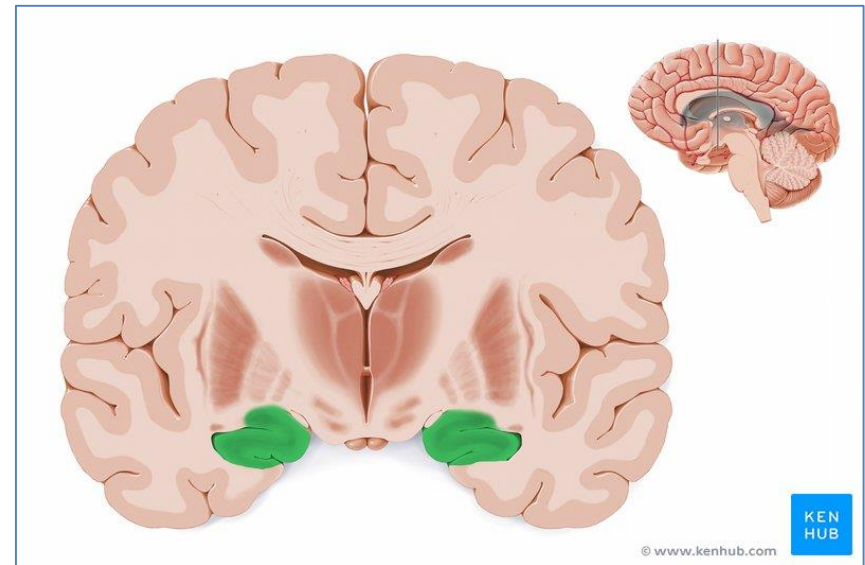
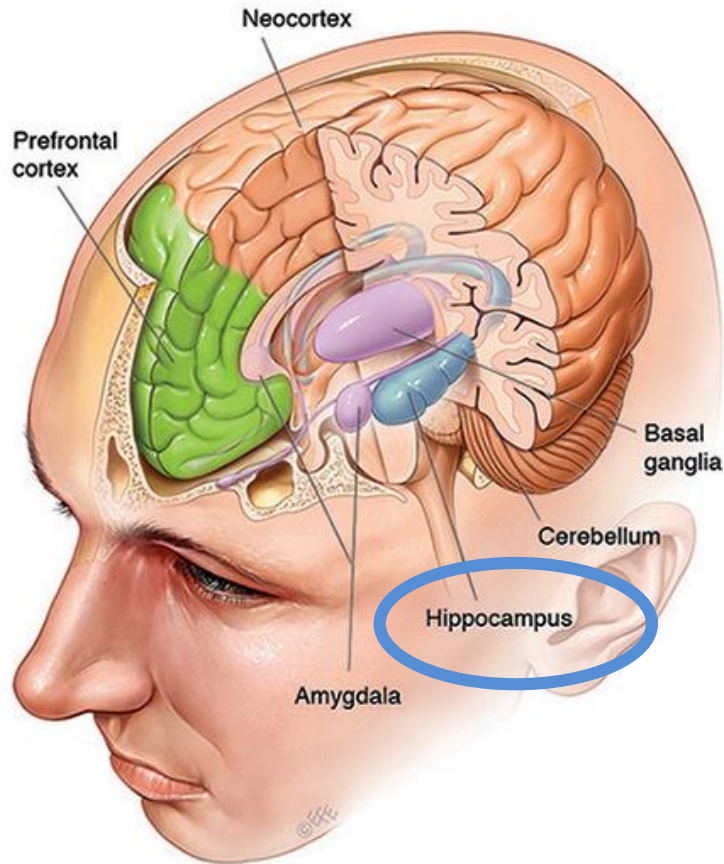
new neurons are **continuously** generated in the adult brain

-  Neurogenic regions
-  Area for which there is controversial evidence for low level of ANG.



# The hippocampal formation

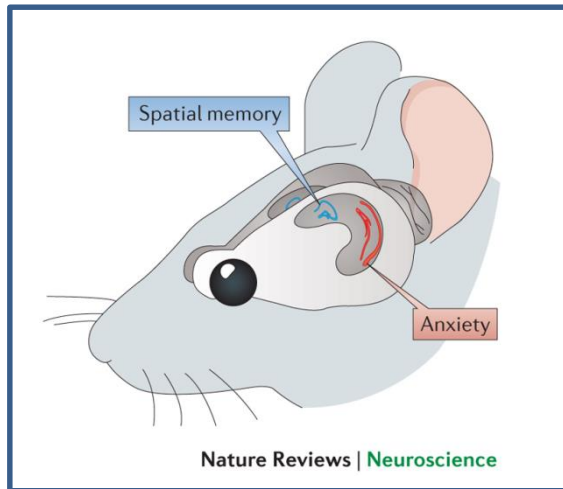
- The hippocampal formation is located bilaterally in the medial temporal lobe,
- Brain structure involved in cognitive functions (memories ...)



Frontal section

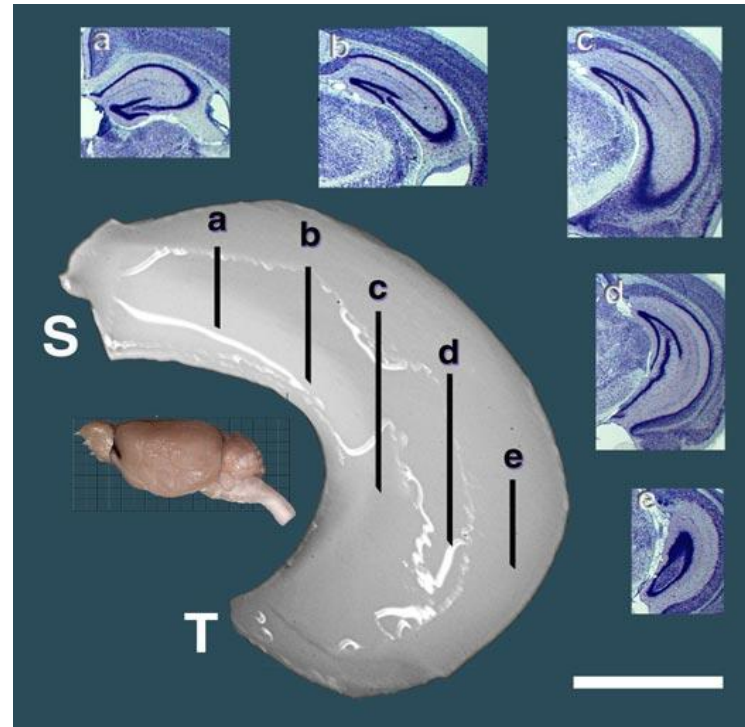
# The hippocampal function

- Main function: learning and memory processes

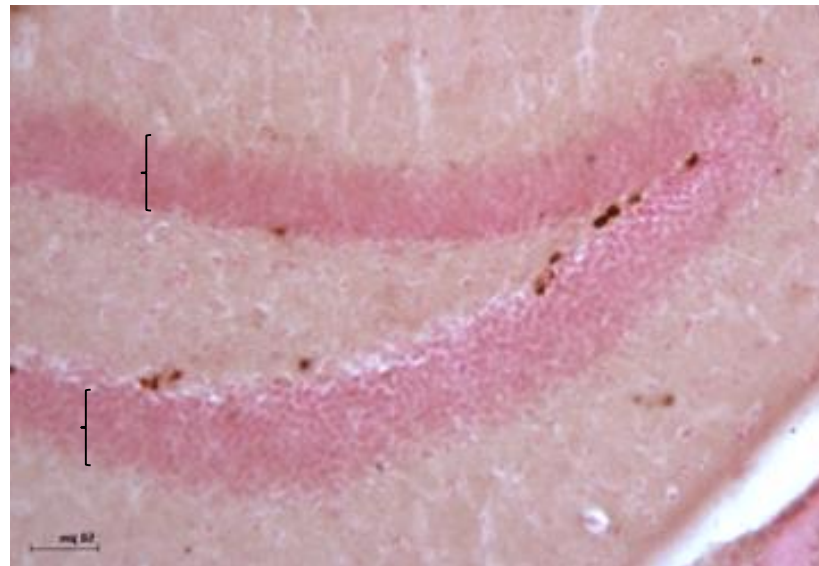
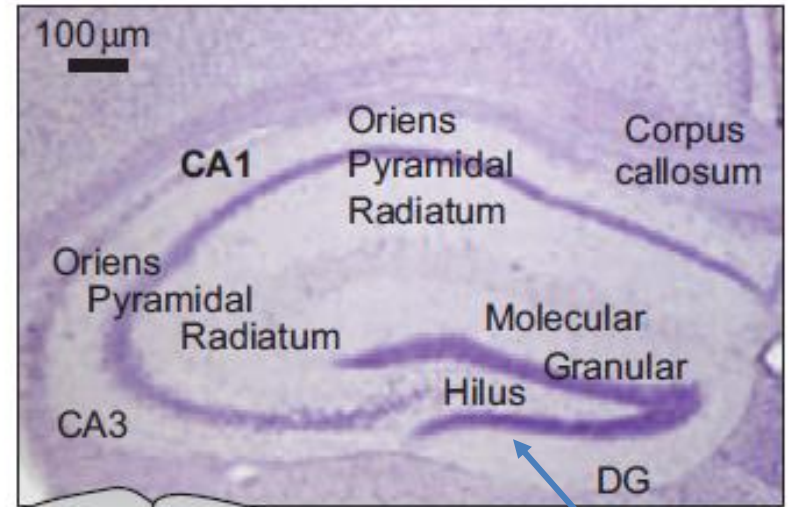
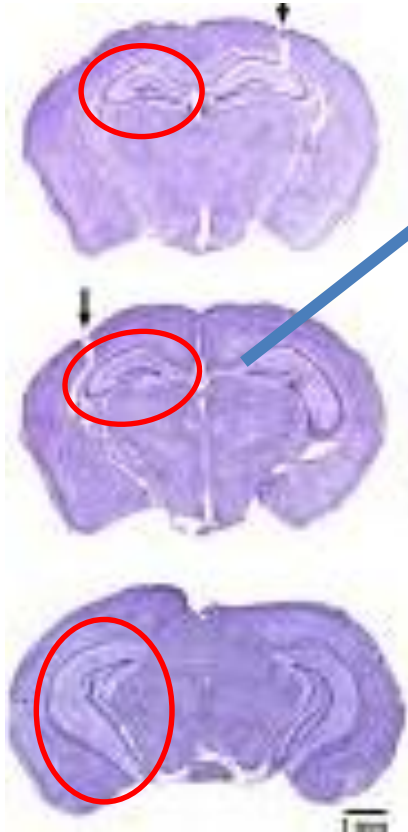
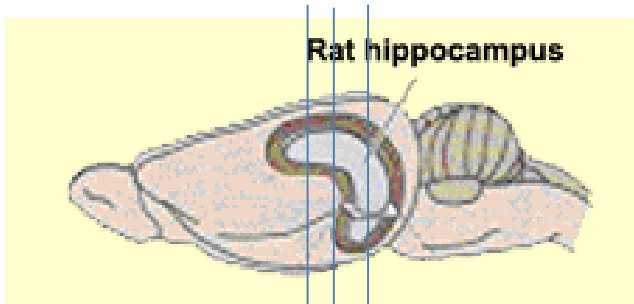


The dorsal hippocampus may have a preferential role in learning and memory,

whereas the ventral hippocampus is involved in emotional behaviors



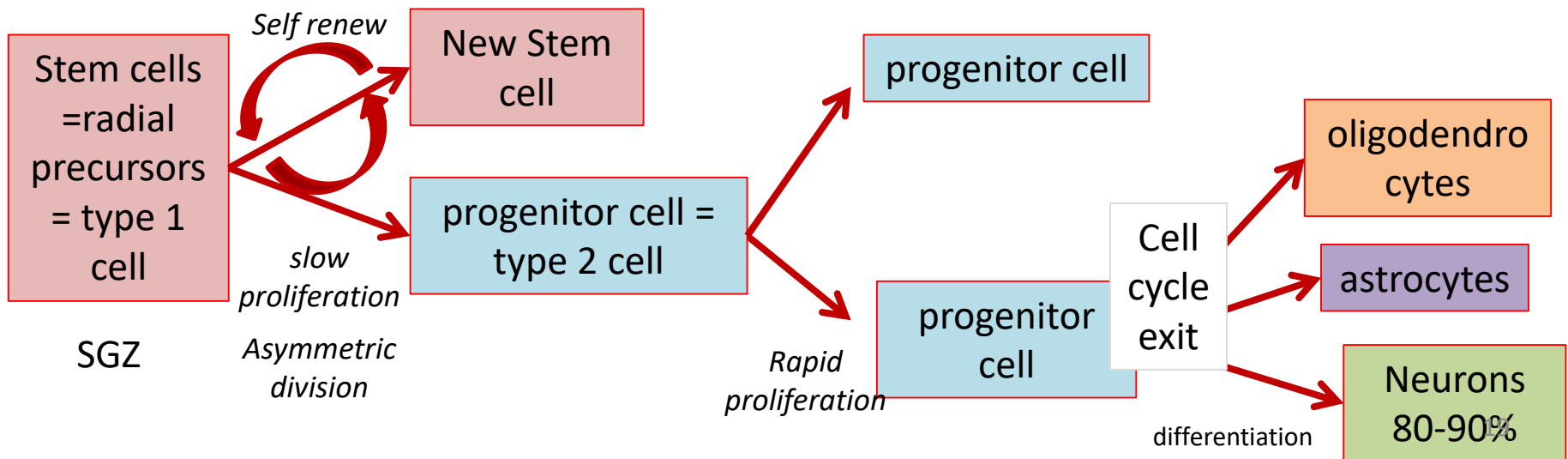
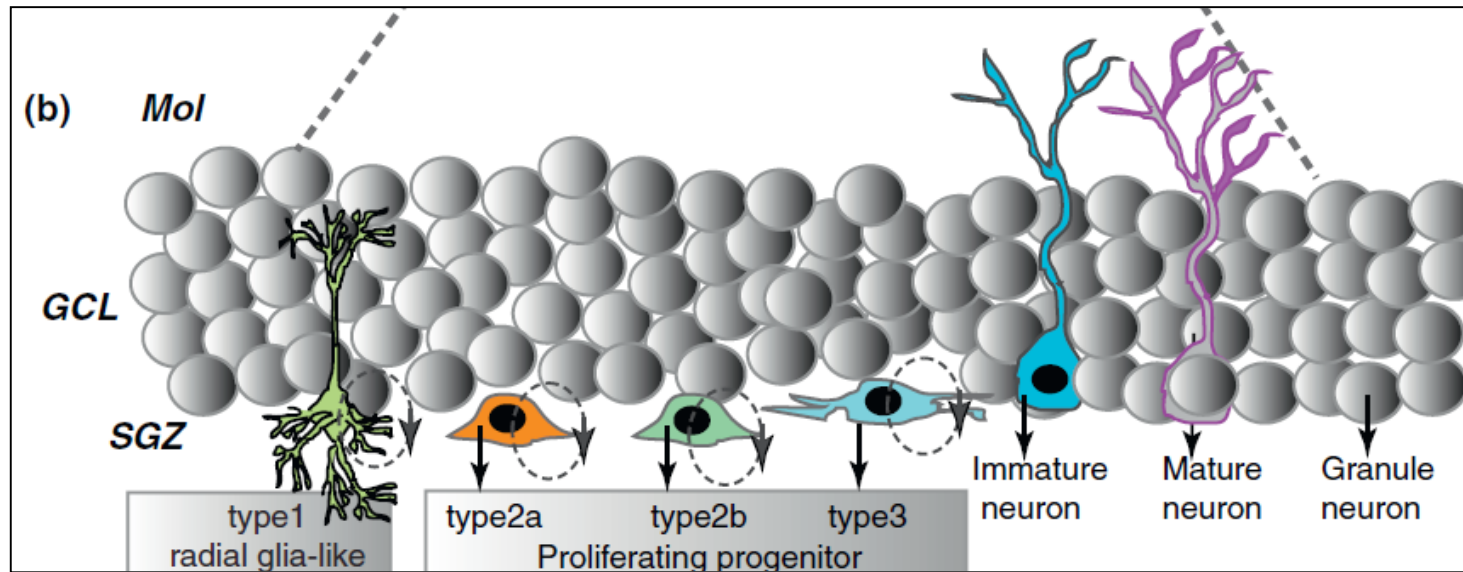
# The hippocampal function



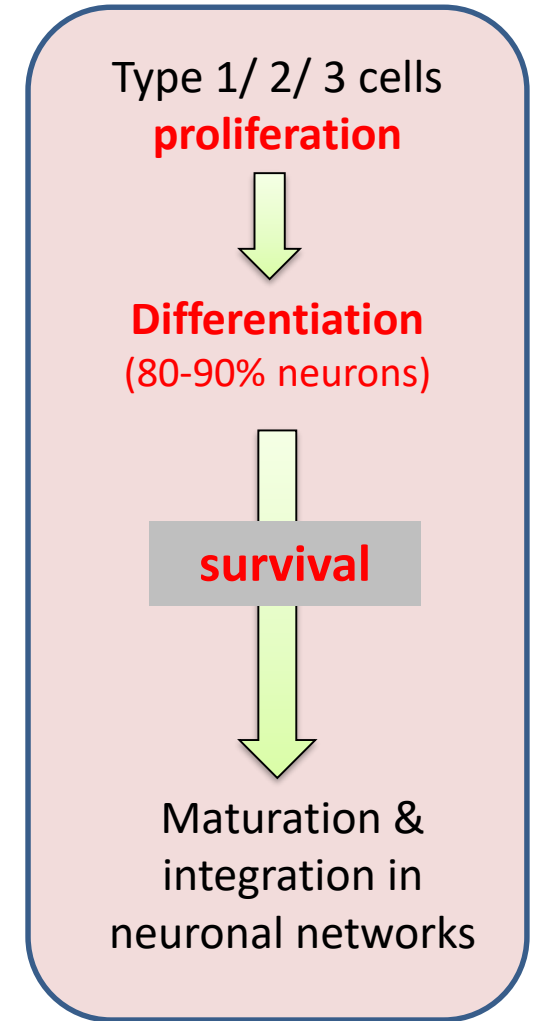
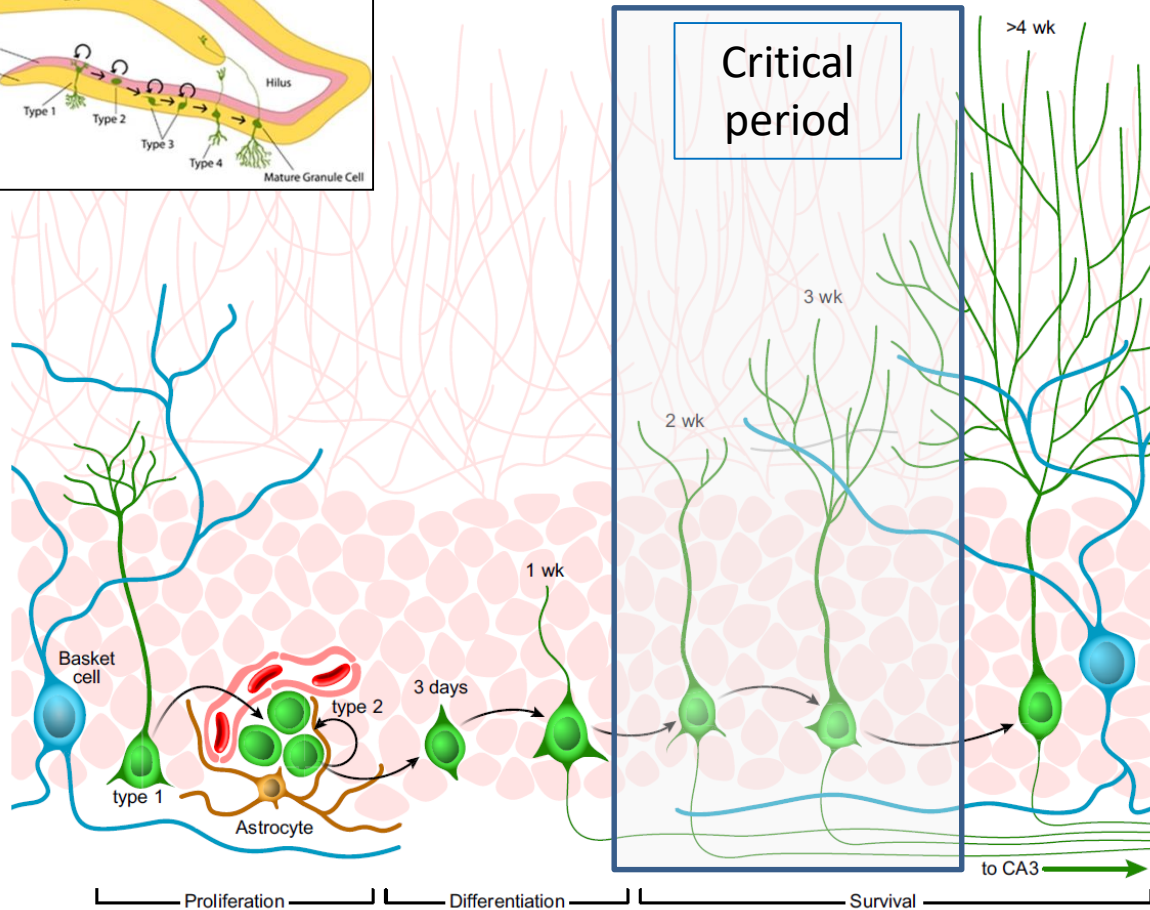
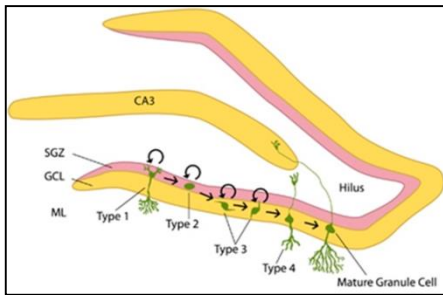
New neurons in granule cells layer of the Dentate gyrus



# Adult neurogenesis : Several steps



# Adult neurogenesis : Several steps



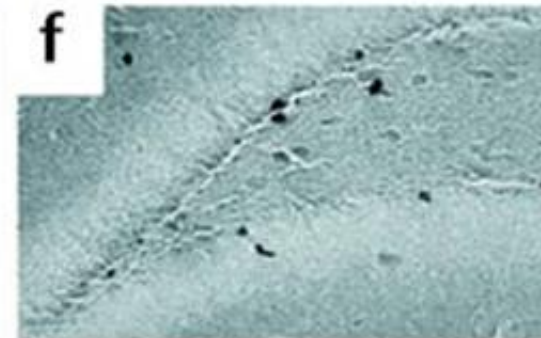
approximately 50% newborn neurons survive in rats  
Less than a third in mice (30%)

Immature neurons (1-3 weeks of age) are very sensitive and hyperexcitable

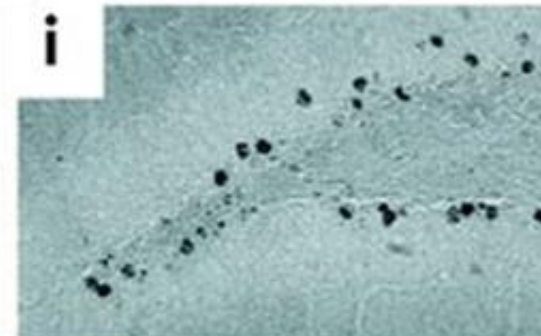


# What factors influence the adult neurogenesis ?

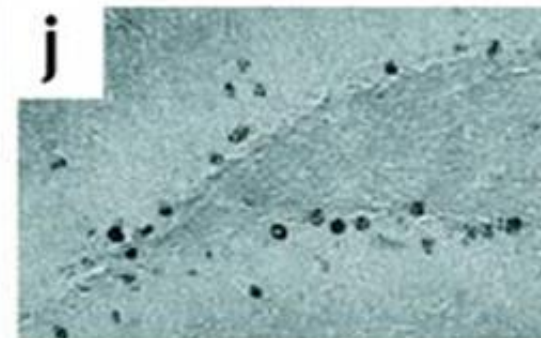
BrdU-positive Cells



control



running



Enriched conditions

# What factors influence adult neurogenesis ?

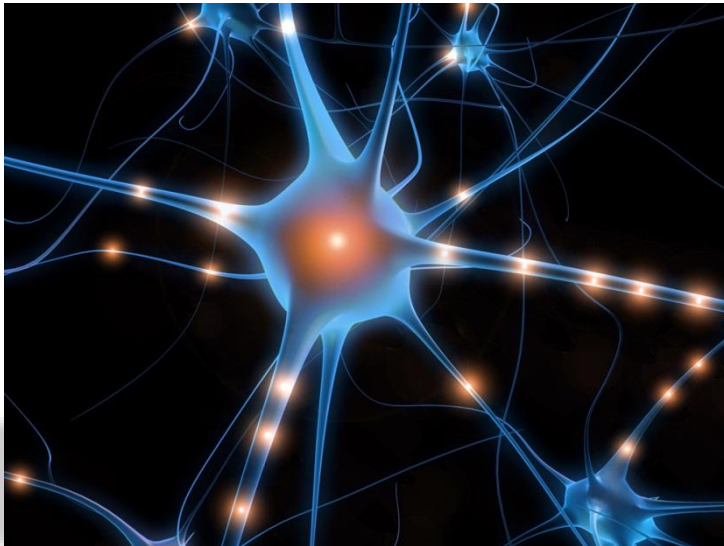


- Enriched environment
  - Exercise
  - Learning
- ADP Treatment
  - ...



- Stress
- Aging
- Depression
- Sleep deprivation
- Social isolation
- Alzheimer D./  
Parkinson D.
- Diabetes
  - ...

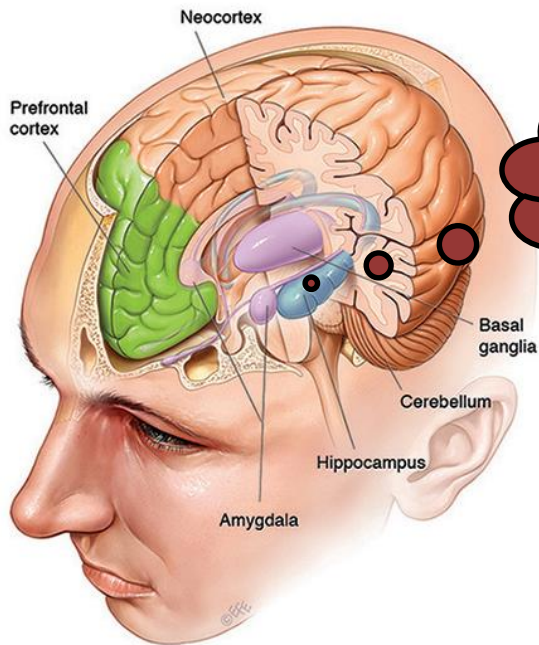
# Functional role of adult newborn neurons ?



## How many new neurons ?

- Humans : **700 adult born cells / day** (<sup>14</sup>C dating)
- **9.000 per day** – 250.000 per month in the young adult rat

# Functional role of adult newborn neurons ?



Learning & Memory

- Are newborn neurons involved in learning & memory ?

## FIRST HYPOTHESIS:

- If NG increases, does learning & memory perf. increase ?
- If NG decreases, does learning & memory perf. decrease ?

# Enrichment / neurogenesis / spatial learning performances

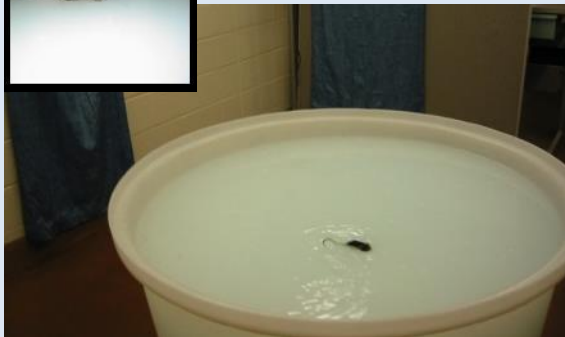
✓ Kempermann et al, 1997

Enriched Cdt°



- Effect on spatial learning ?
- Effect on ANG ?

## MORRIS WATER MAZE TASK



Find a hidden platform using spatial extra-maze cues.

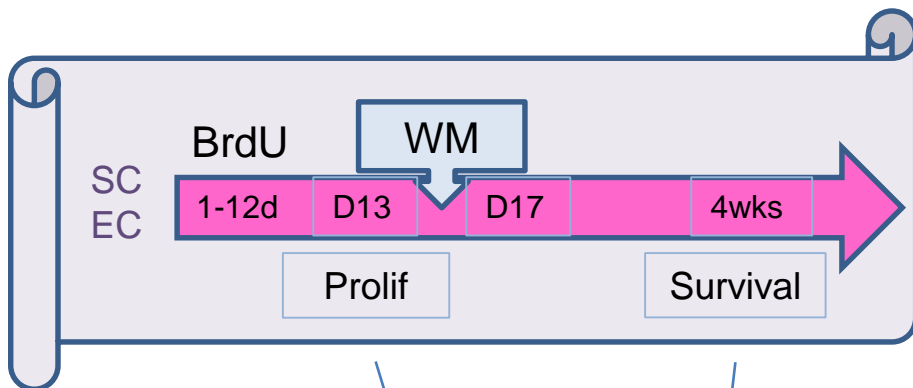


BrdU  
injections

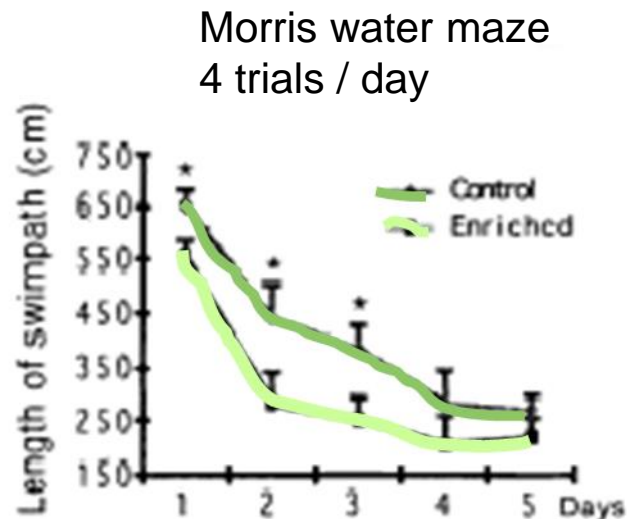
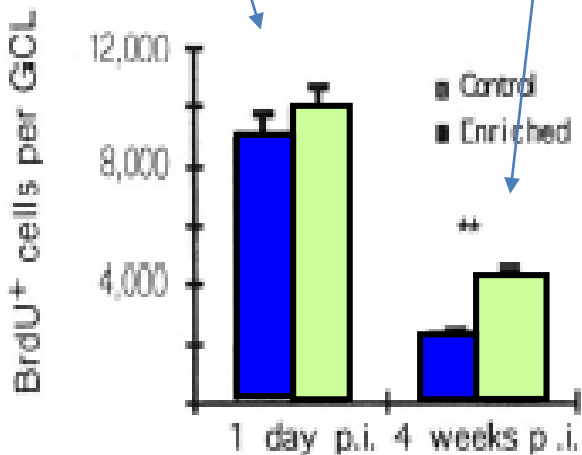


# Enrichment / neurogenesis / spatial learning performances

✓ Kempermann et al, 1997



Environmental enrichment :  
 - Increased survival of newborn cells in DG  
 - Improved spatial learning



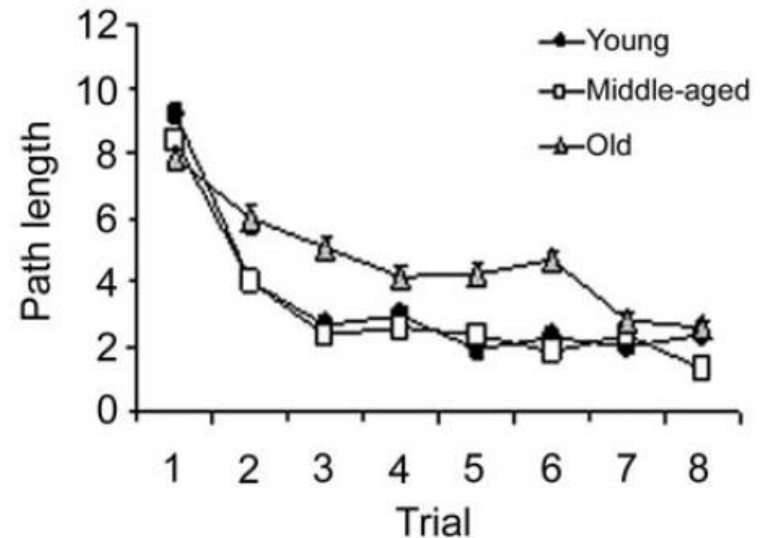
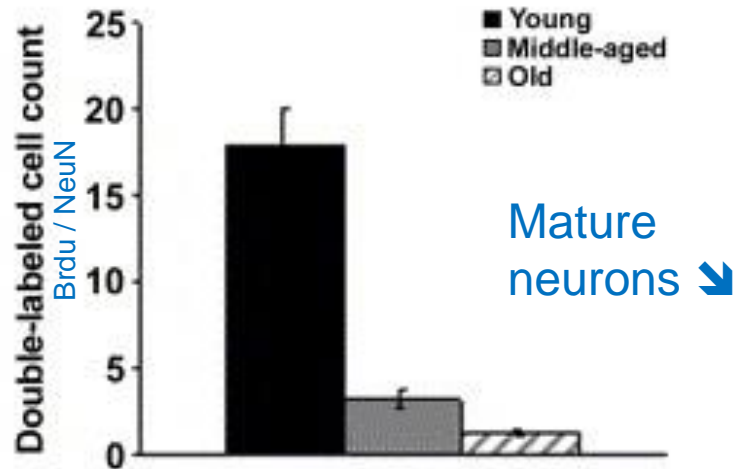


## Aging / neurogenesis / spatial learning performances

### ✓ Driscoll et al., 2006

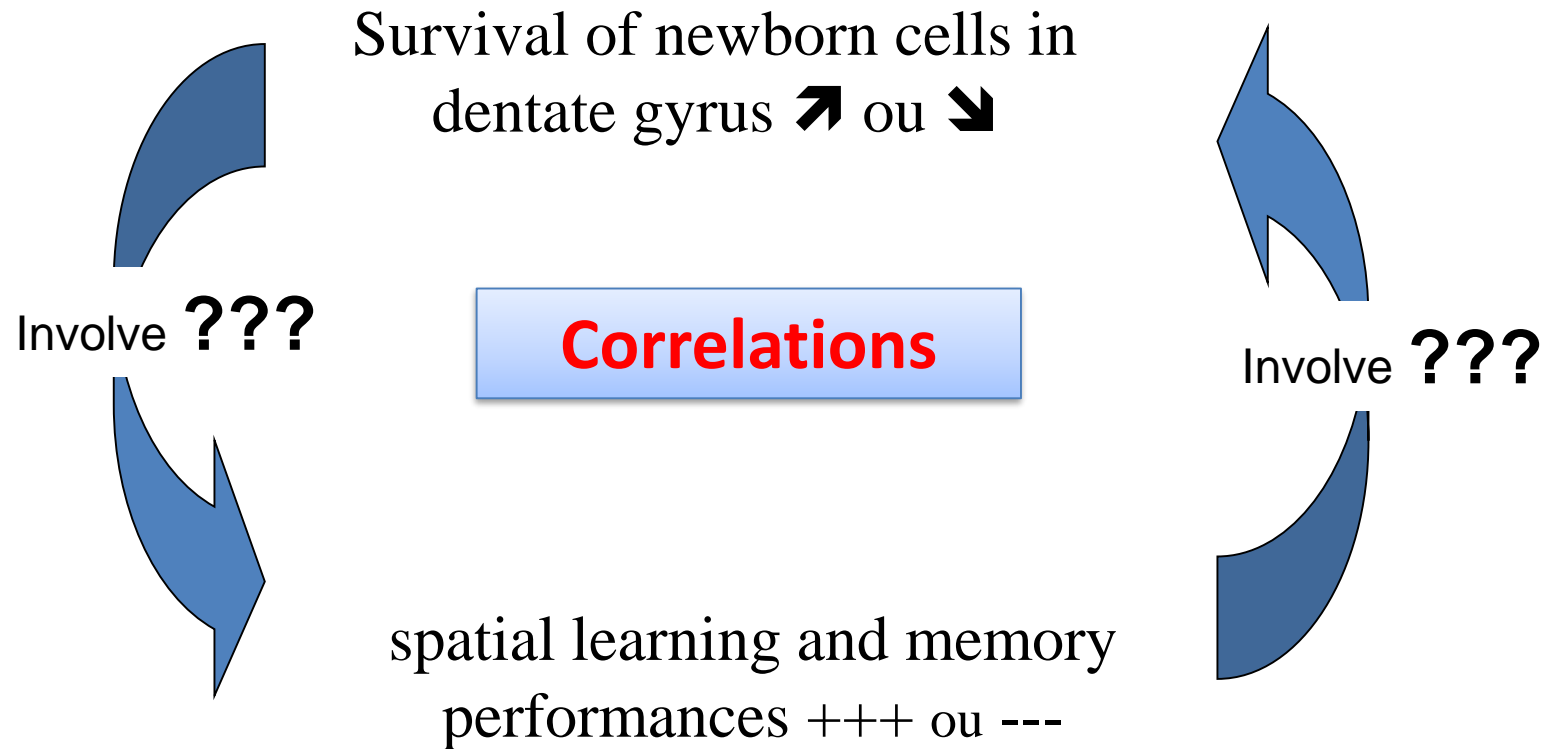
BrdU / WM / analyse 5 weeks after

- young adult (3 months),
- middle-aged (12 months),
- old (24 months)



Negative impact of aging on adult neurogenesis and spatial learning

**Enrichment – aging / neurogenesis /  
spatial learning performances**



**Effect of learning on adult neurogenesis ?  
Are the adult born neurons really involved in learning ?**

- If ablation NG, bad perf ?**

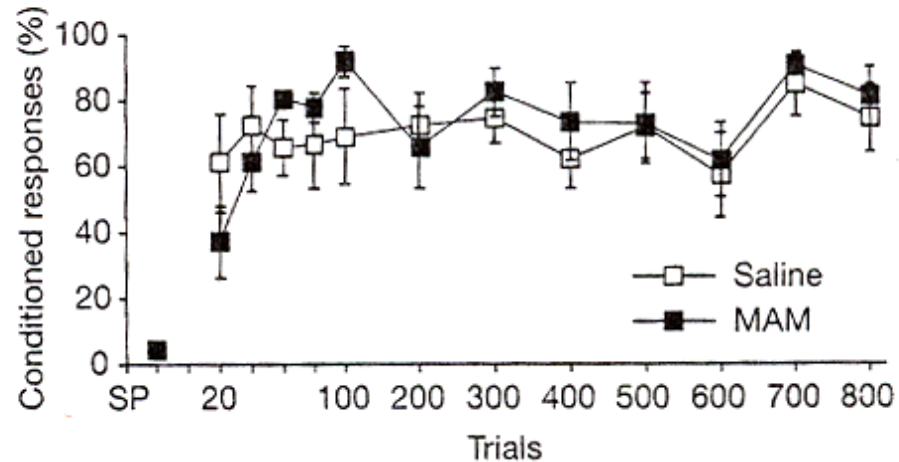
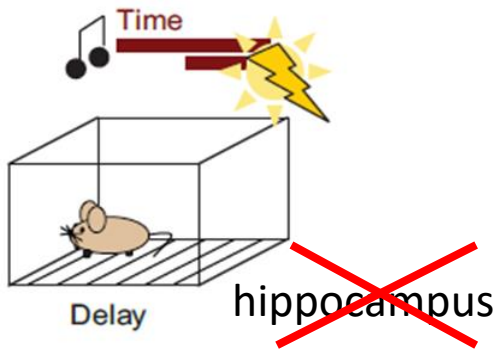
# Adult born neurons involved in learning/memory?

✓ Shors et al, 2001

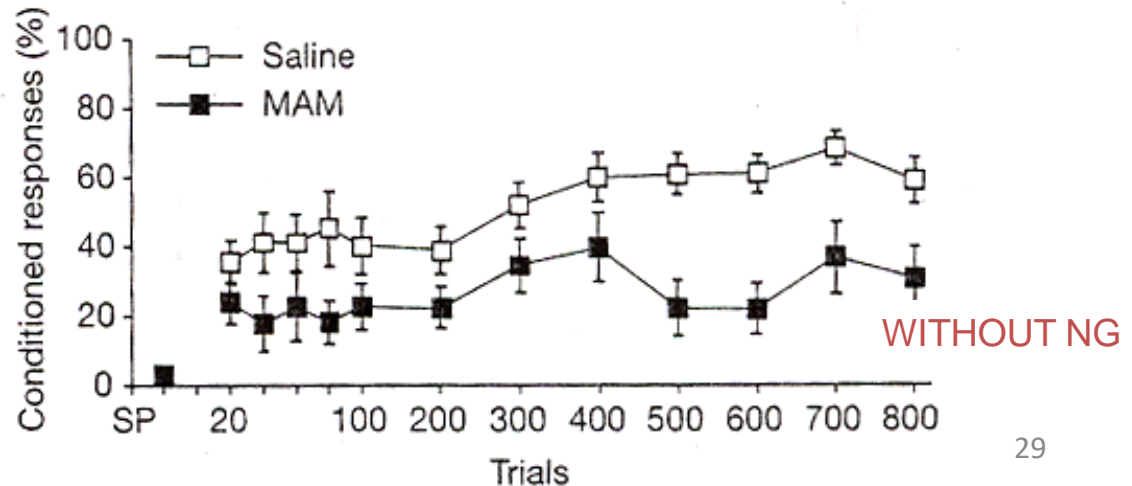
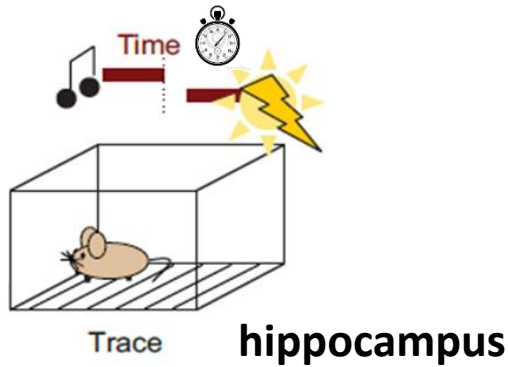
Ablation methods : MAM (Methyl-azoxy-methanol Acetate)

**Delay conditioning**

(assoc<sup>o</sup> between 2 stimuli (tone-shock))



**Trace conditioning**



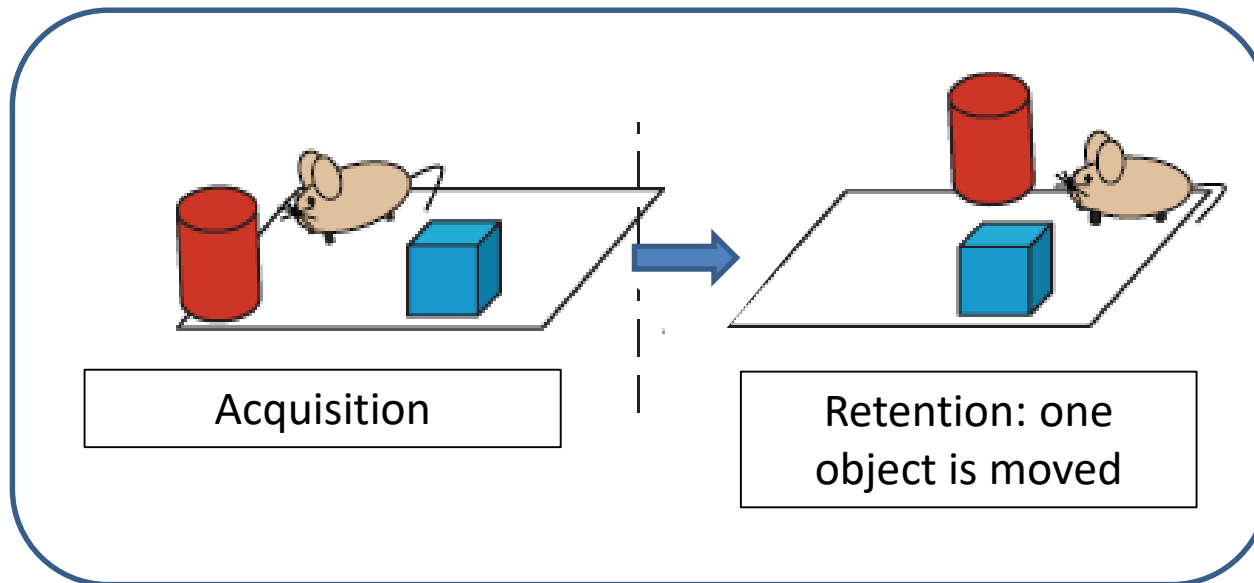


# Adult born neurons involved in learning/memory?

## ➤ Object location recognition memory



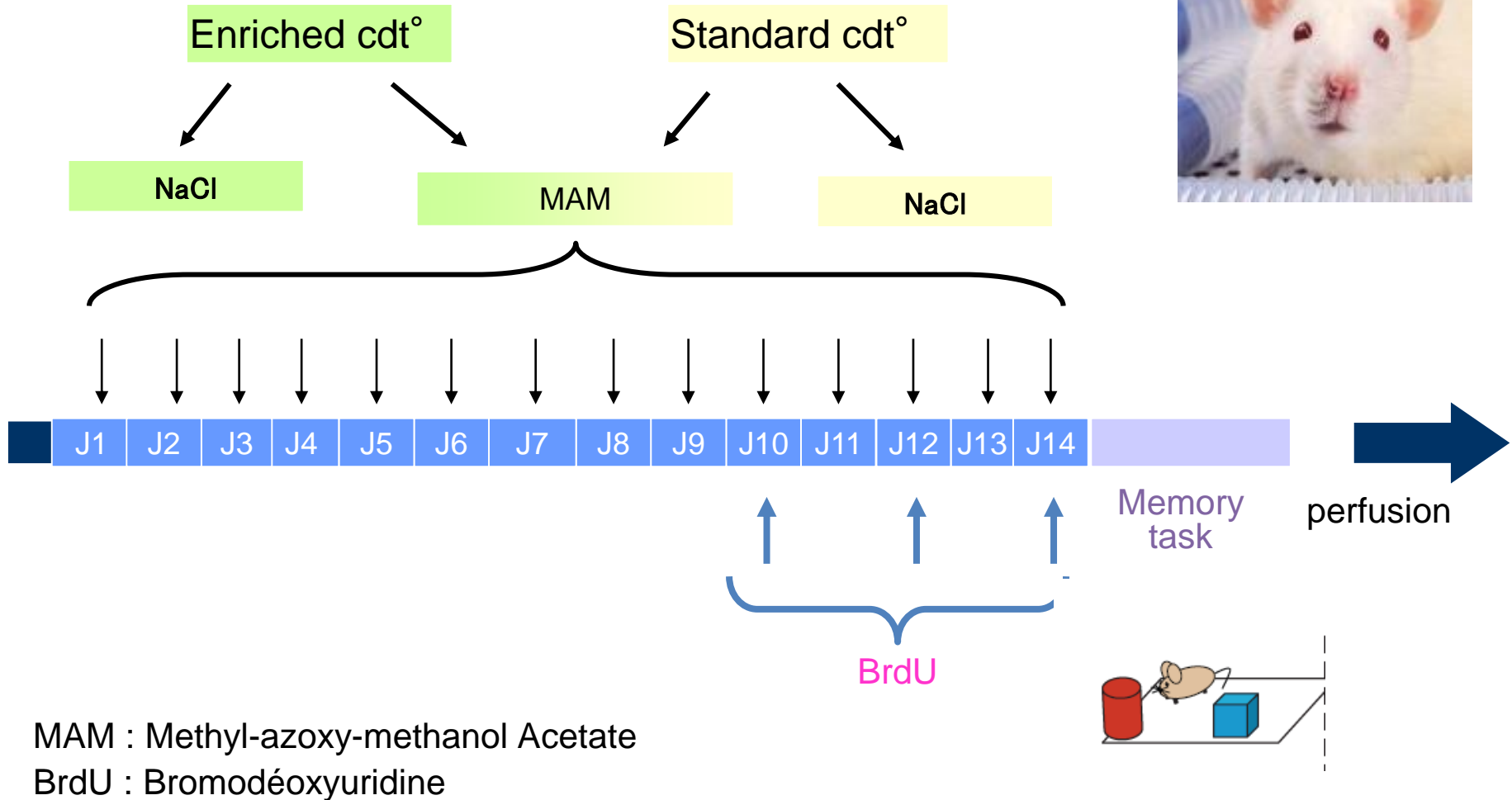
Attraction  
for  
novelty



Measure : object exploration time

# Adult born neurons involved in learning/memory?

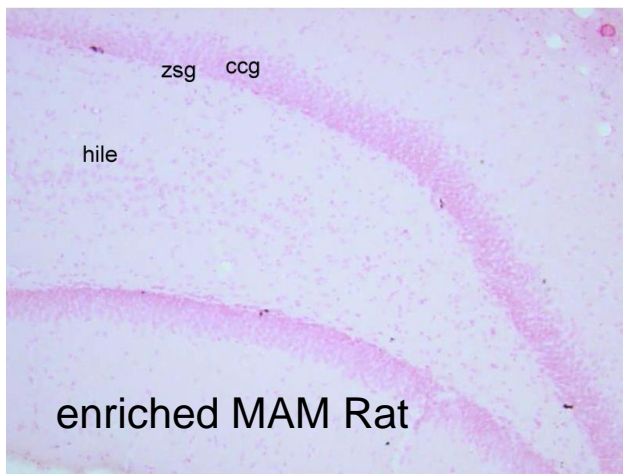
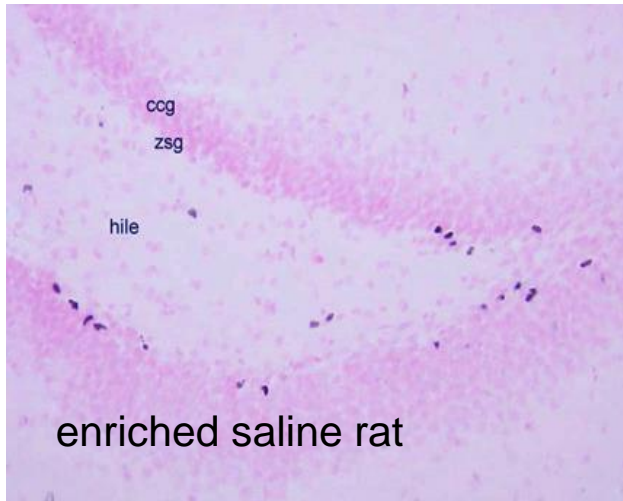
## ➤ Brüel-Jungermann et al., 2005



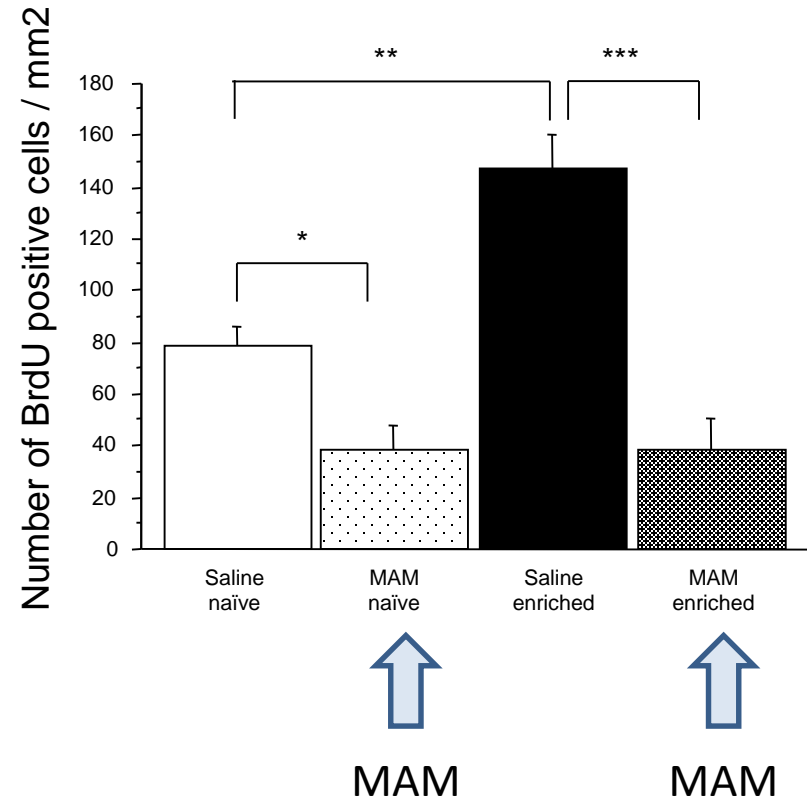


# Adult born neurons involved in learning/memory?

## ➤ Brüel-Jungermann et al., 2005



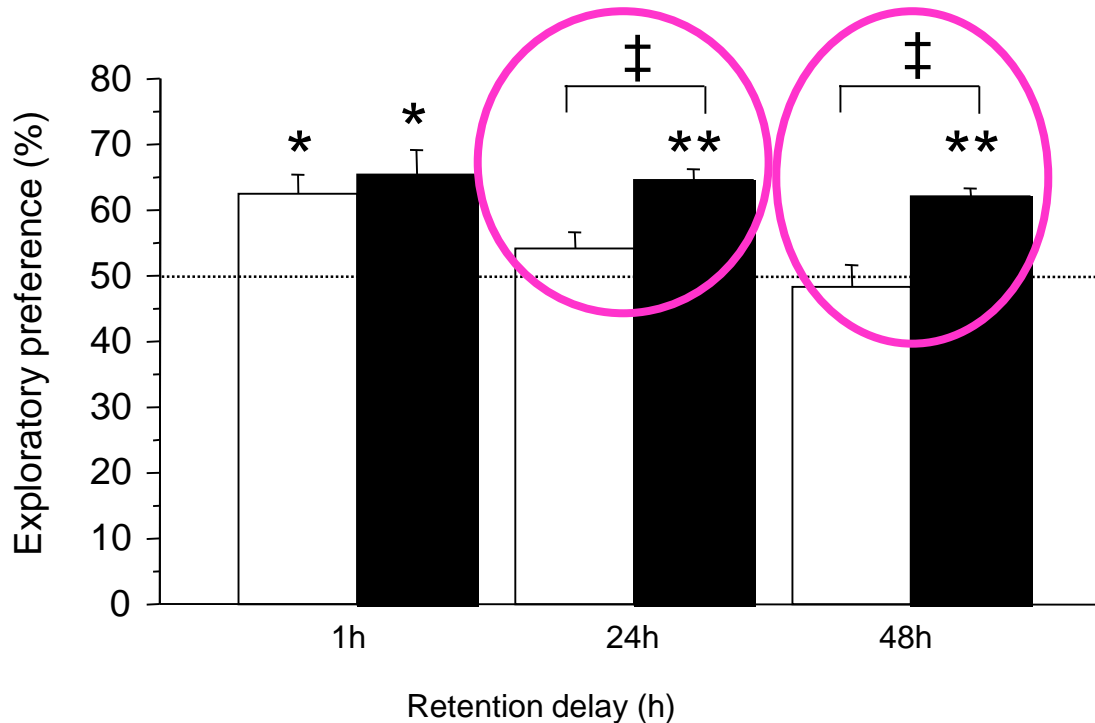
## MAM effect on neurogenesis



# Adult born neurons involved in learning/memory?

## ➤ Bruel-Jungermann et al., 2005

### Effect of enriched conditions on object recognition memory



↪ No effect of enriched environment on short-term memory

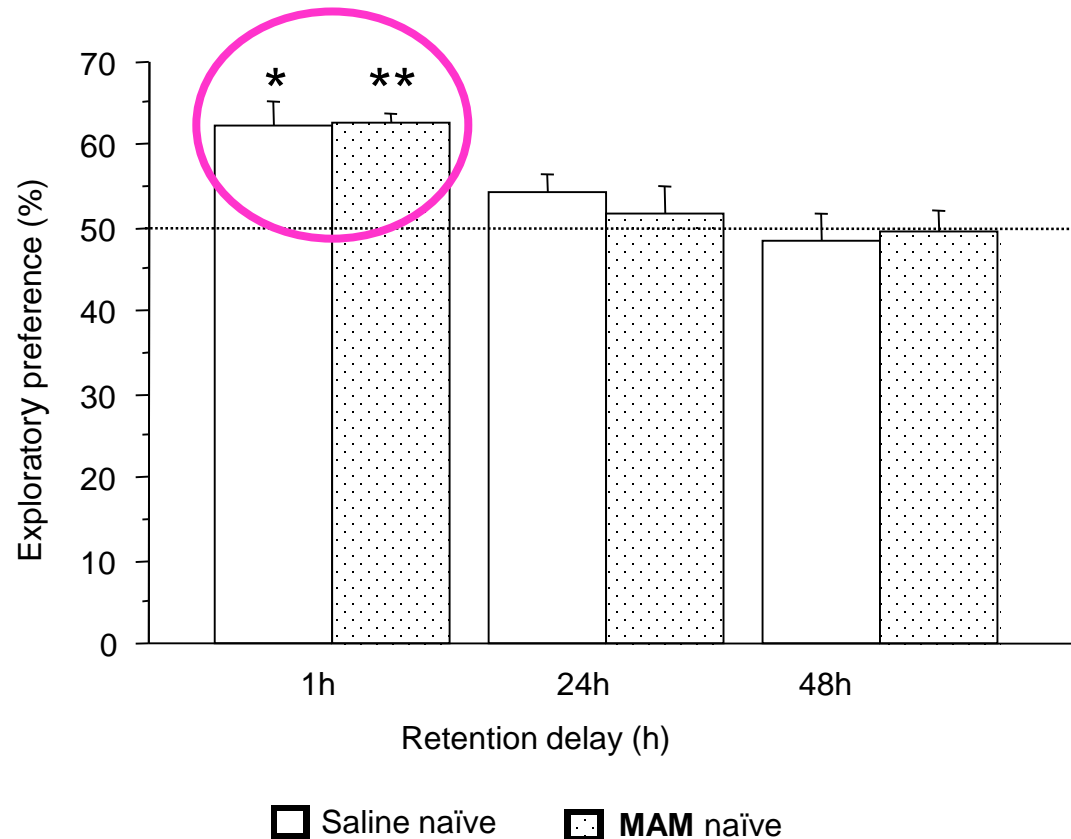
↪ Enriched environment improved long-term memory

□ Saline naïve    ■ Saline enriched

# Adult born neurons involved in learning/memory?

## ➤ Bruel-Jungermann et al., 2005

### Effect of MAM treatment on memory in standard rats

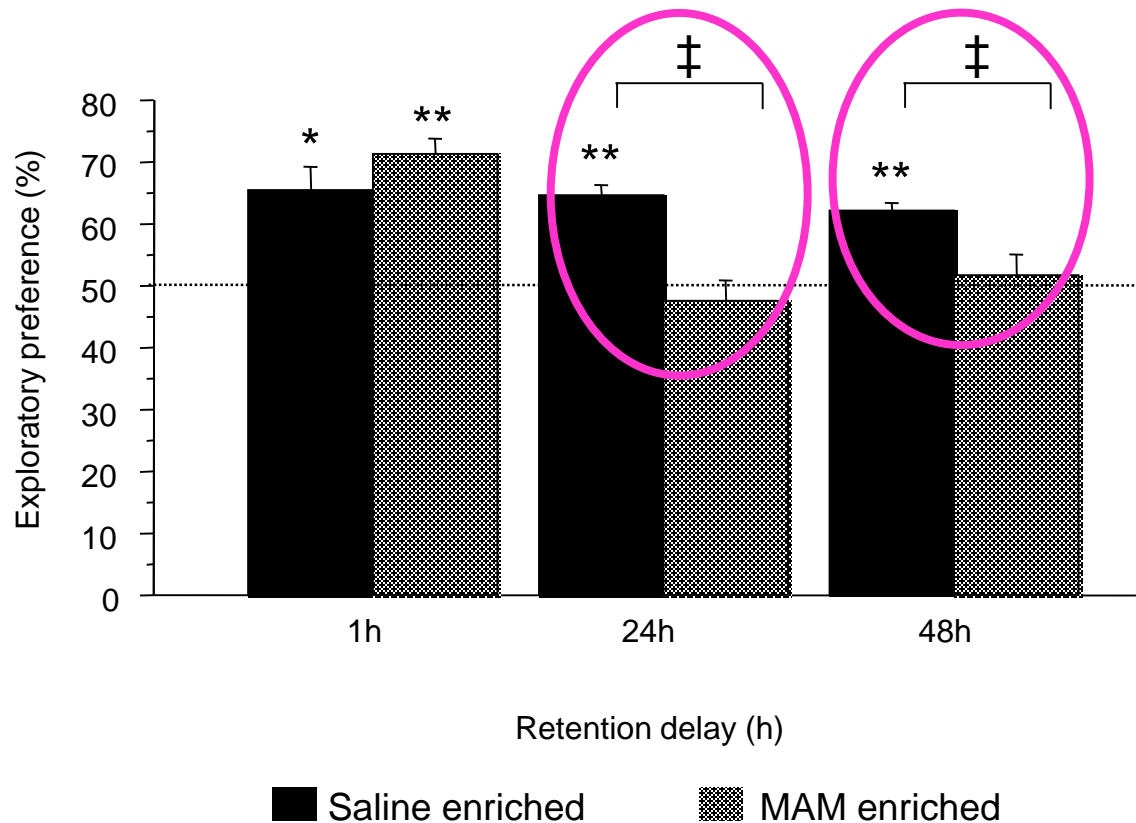


↪ No effect of MAM treatment on short-term memory

# Adult born neurons involved in learning/memory?

## ➤ Bruel-Jungermann et al., 2005

### Effect of MAM treatment on memory in ENRICHED rats

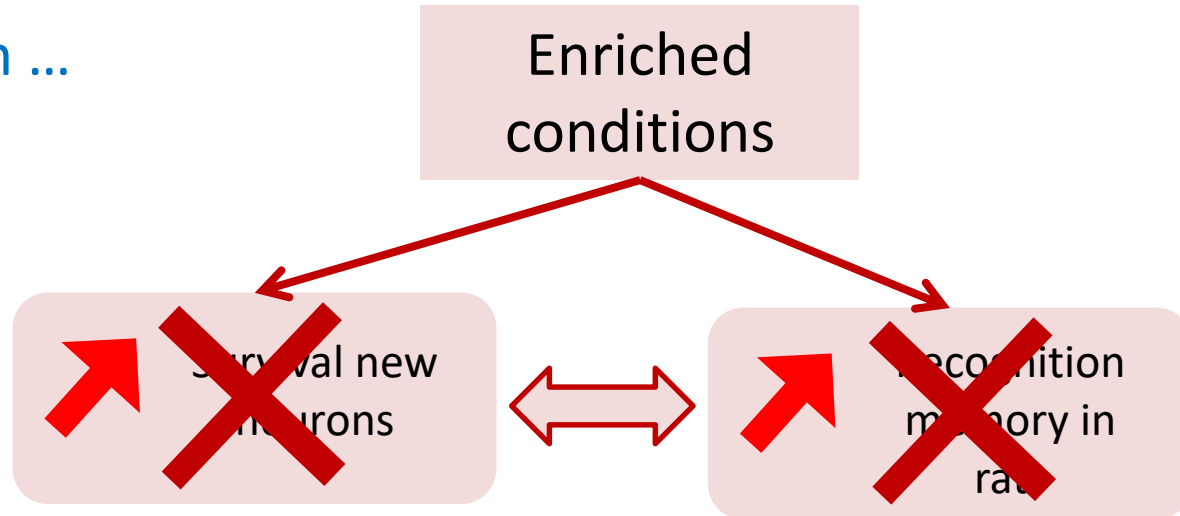


↪ MAM treatment during environmental enrichment completely prevented the enrichment-induced long-term memory improvement

# Adult born neurons involved in learning/memory?

## ➤ Bruel-Jungermann et al., 2005

Conclusion ...



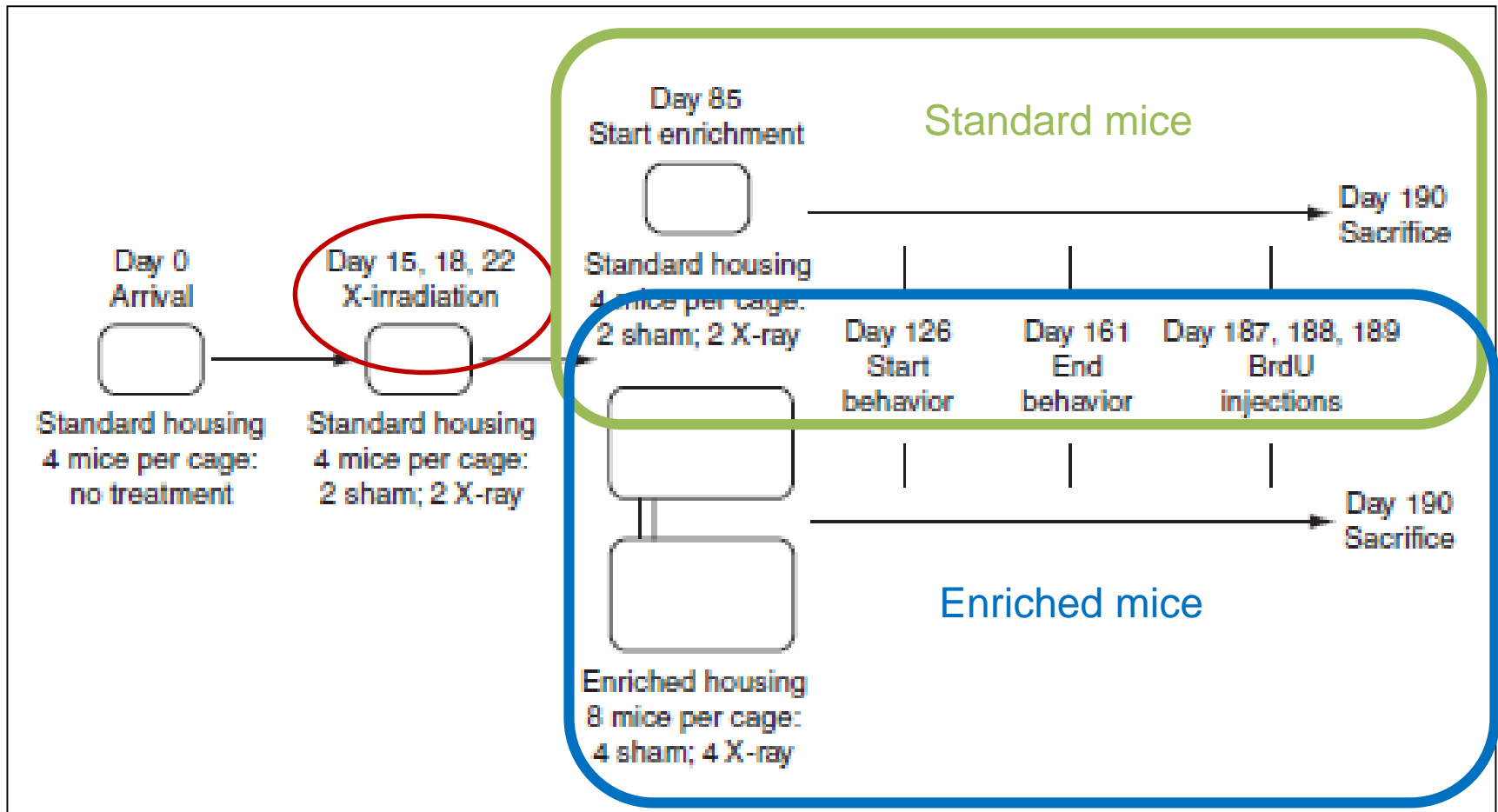
**Adult new born neurons are really involved in memory**

➡ Other memories ? / Other behavioural tasks ?

# Adult born neurons involved in learning/memory?

➤ Meshi et al., 2006

Effect of enriched conditions on spatial learning when NG is stopped (irradiation)



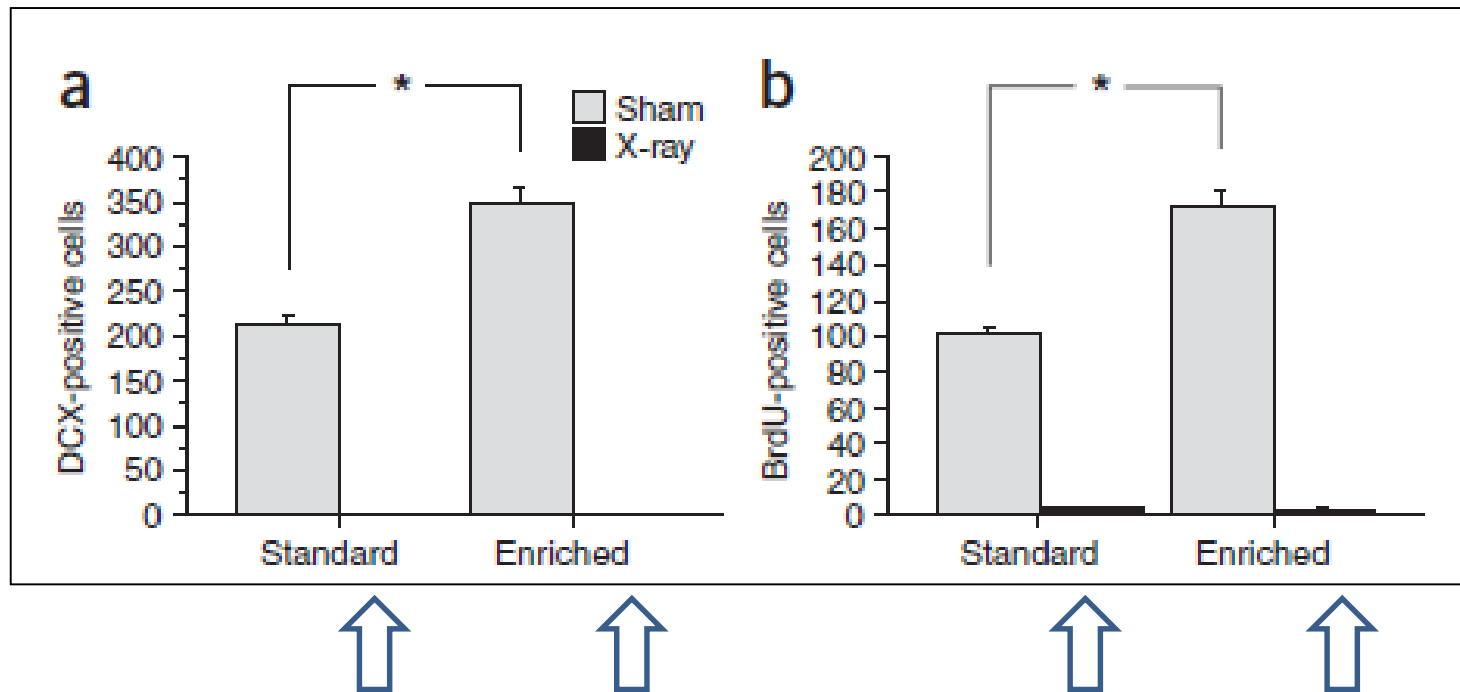


## Adult born neurons involved in learning/memory?

➤ [Meshi et al., 2006](#)

Effect of enriched conditions on spatial learning when NG is stopped

Effect of irradiation on NG ...

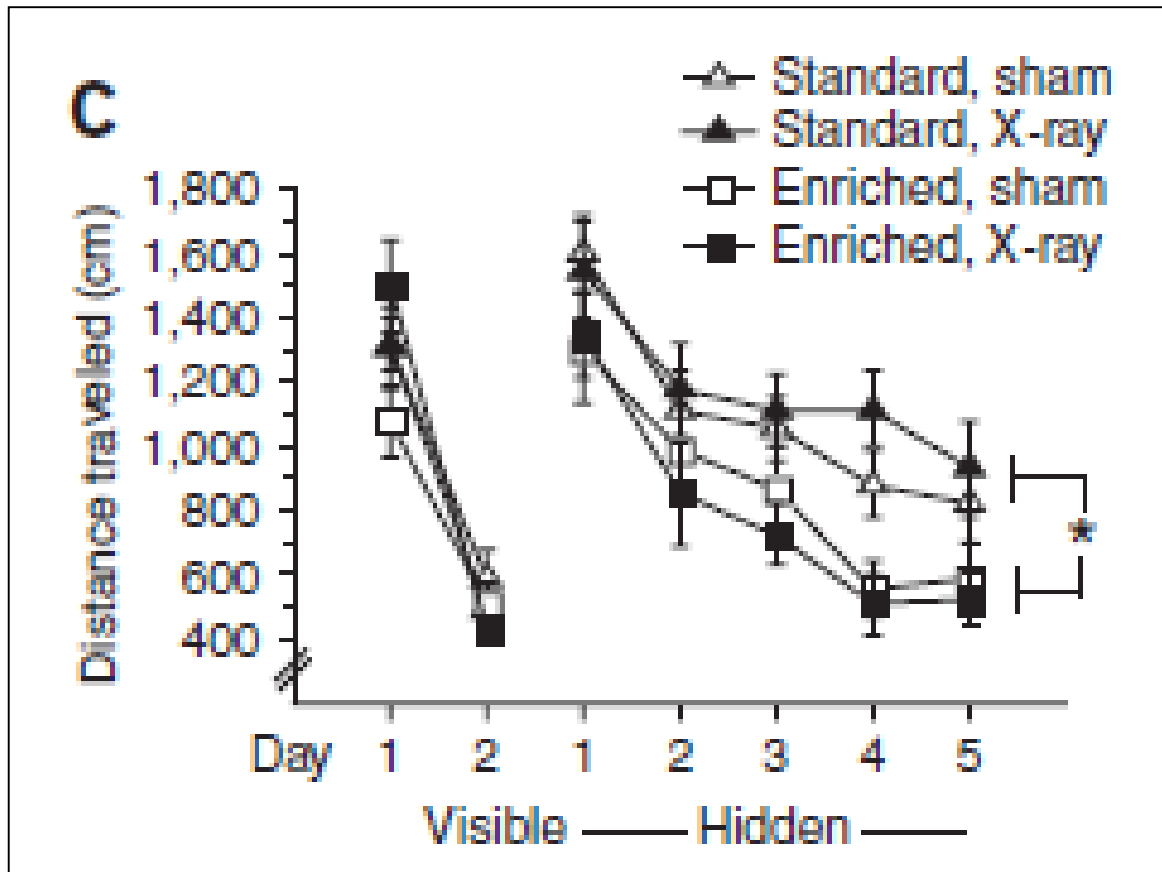


## Adult born neurons involved in learning/memory?

➤ Meshi et al., 2006

Effect of enriched conditions on spatial learning when NG is stopped

Spatial learning

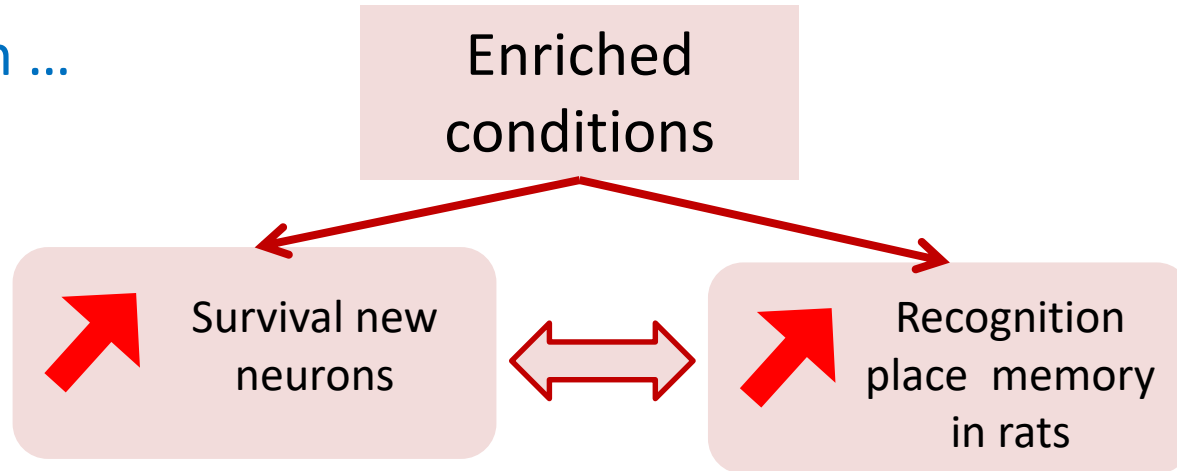


➤ Absence of adult born neurons not affected spatial learning in MWM.

## Adult born neurons involved in learning/memory?

### ➤ Bruel-Jungermann et al., 2005

Conclusion ...



**Adult new born neurons are really involved in memory**

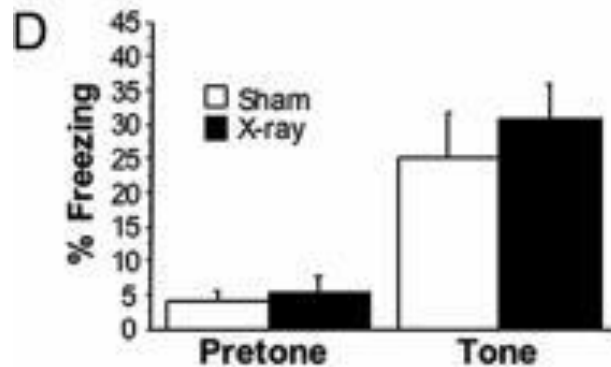
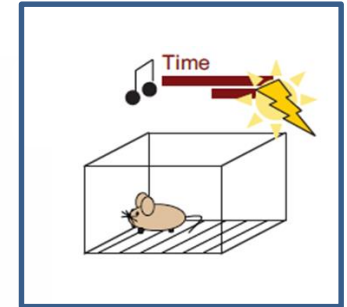
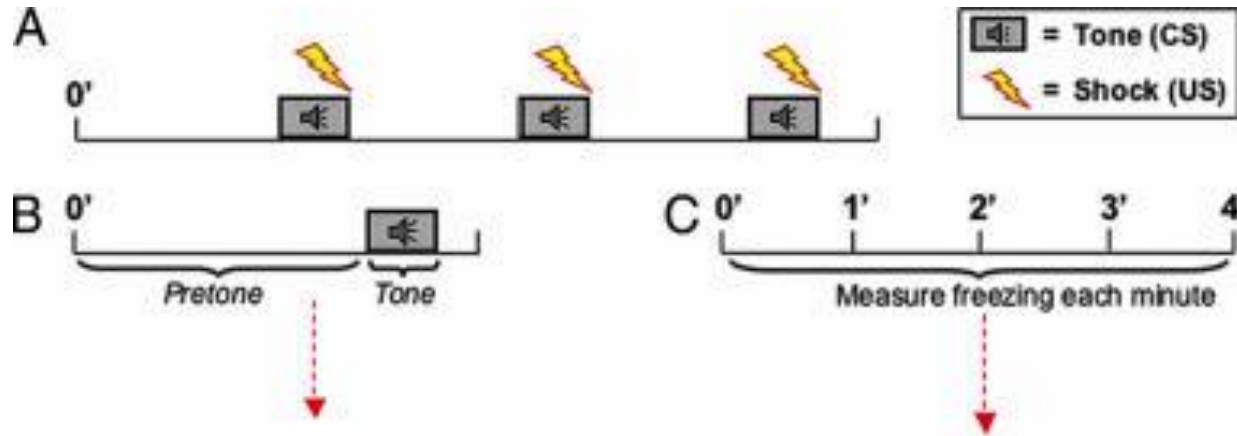
➔ Other memories ?

### ➤ Meshi et al., 2006 **Not involved in spatial memory**

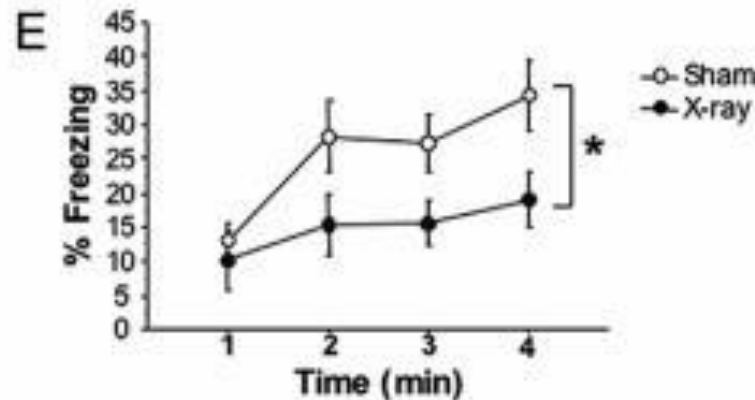
- Different species (rats / mice)
- Different ablation methods (MAM / irradiation)

# Adult born neurons involved in learning/memory?

## ➤ Saxe et al, 2006 Irradiation effect on fear conditioning (mice)



**Cued fear conditioning**  
(new context/ same tone)

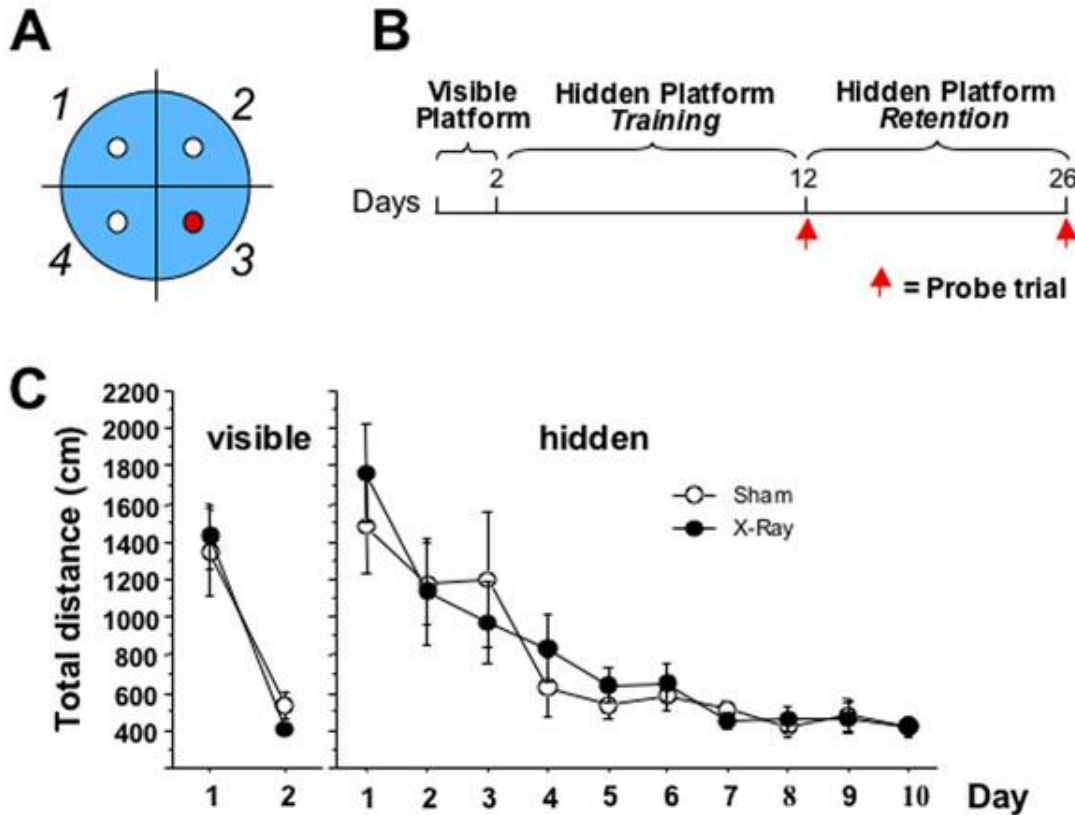


**Contextual fear conditioning**  
(same context)

Irradiation impairs contextual but not cued fear conditioning

# Adult born neurons involved in learning/memory?

## ➤ Saxe et al, 2006 Irradiation effect on MWM perf. (mice)

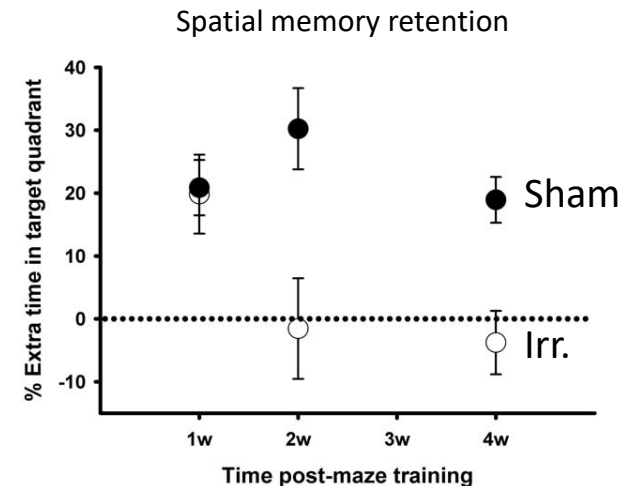
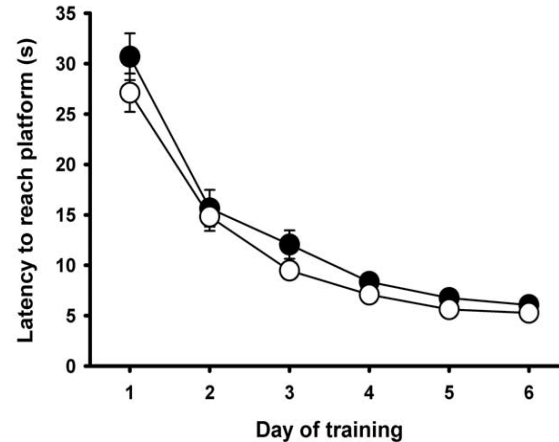
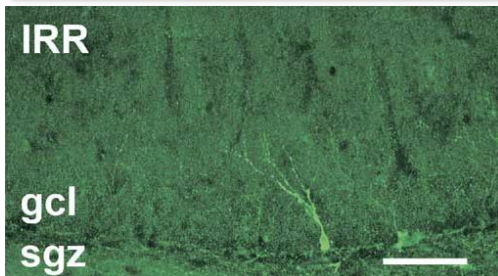
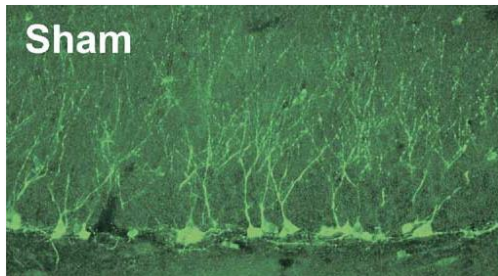
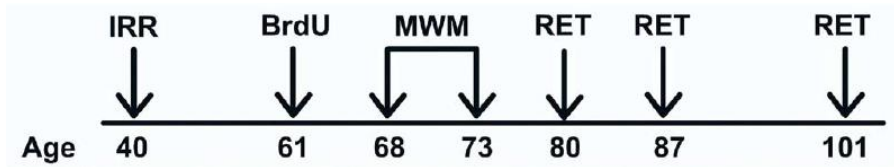


- Irradiation does not impair spatial learning in the Morris water maze.
- In agreement with Meshi et al, 2006

# Adult born neurons involved in learning/memory?

## ➤ Snyder et al, 2005 Irradiation effect on MWM perf. (rats)

rats



Role of newborn neurons in spatial LONG TERM memory



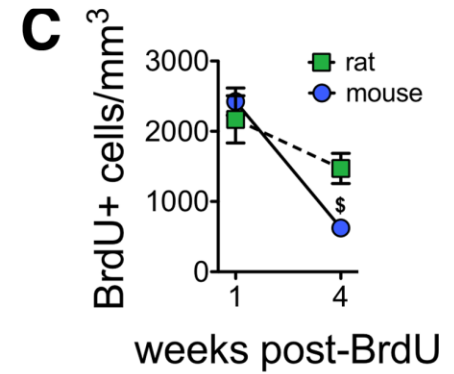
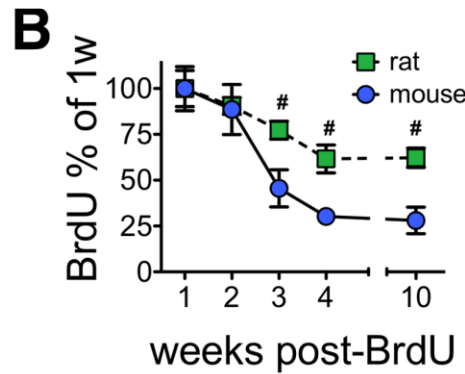
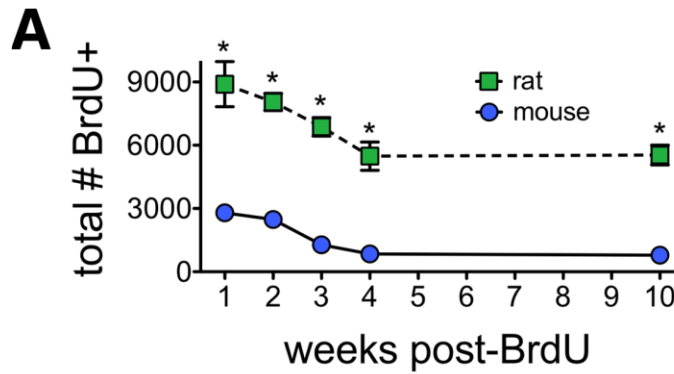
# Functional role of adult newborn neurons ?

What are the conclusions ?  
A lot of contradictions ? Why ?

- Species are different (Snyder, 2009)

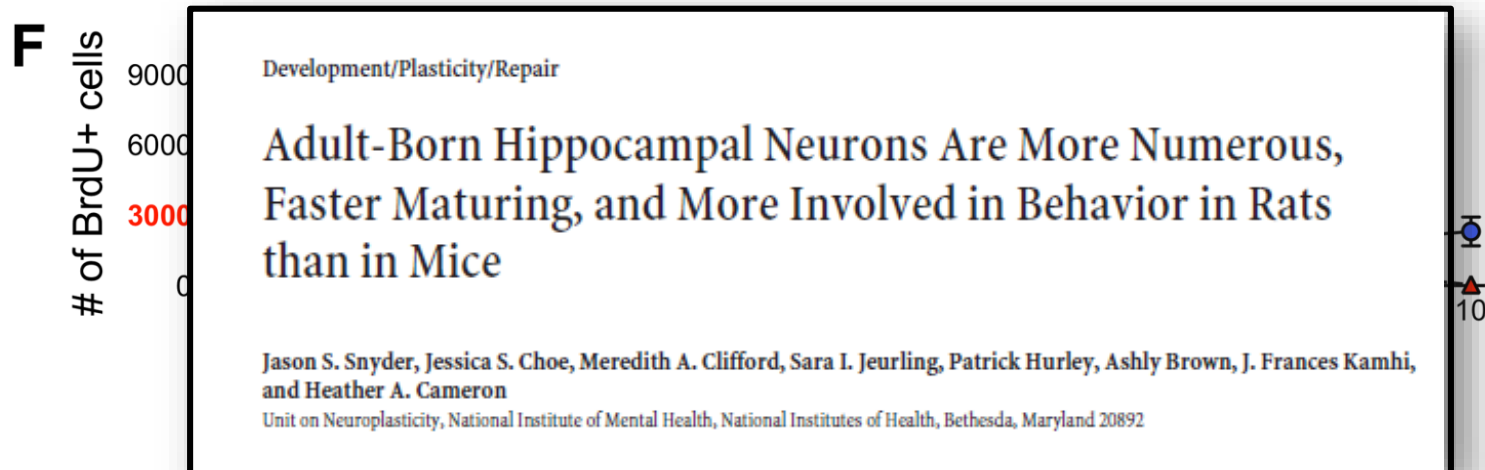
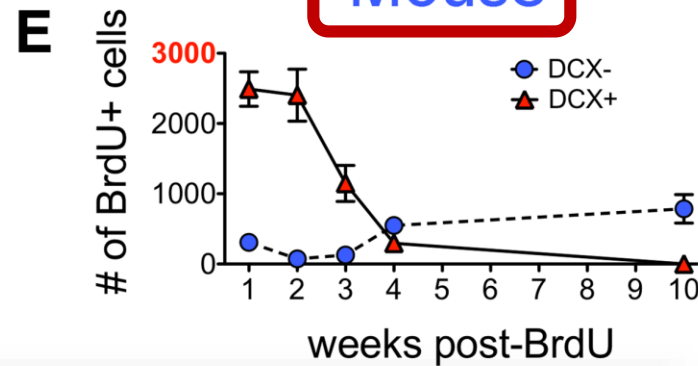
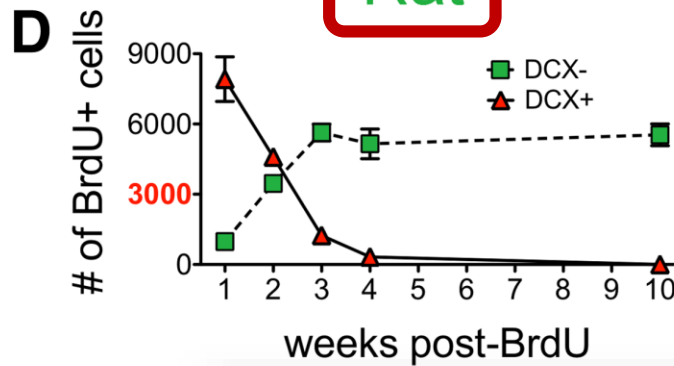


# ➤ Snyder, 2009 – NG RAT / MOUSE



**Rat**

**Mouse**



# Functional role of adult newborn neurons ?

What are the conclusions ?  
A lot of contradictions ? Why ?

- Different species
- Age effect
- Genotype effect (Kim et al, 2009)
- Different methods (ablation methods / different behavioural tasks / age of newborn cells / ...)

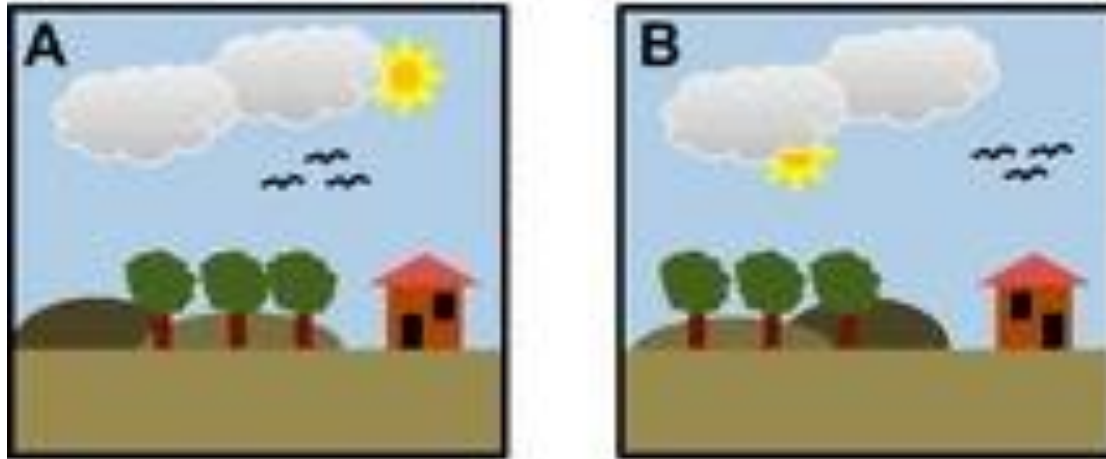
Irradiation → drastique reduction of NG

TMZ → smaller reduction but mainly immature neurons

# The hippocampus and hippocampal function

## ➤ Role in pattern separation : **SPECIFIC ROLE OF DG**

The dentate gyrus is mainly involved in **pattern separation processes**



Two events, consisting of highly similar objects and configurations, can be learned to be different

Spatial discrimination

# The hippocampus and hippocampal function

## ➤ Role in pattern separation : **SPECIFIC ROLE OF DG**

The dentate gyrus is mainly involved in **pattern separation processes**

Birthday party in 2009



Birthday party in 2010

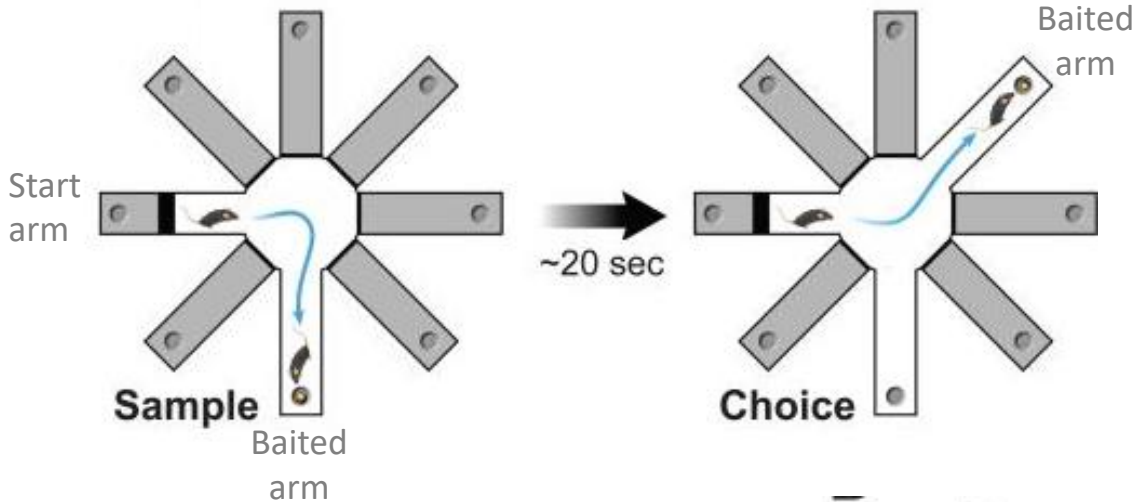
Two events, consisting of highly similar objects and configurations, can be learned to be different

Spatial discrimination **or temporal** discrimination

# Adult born neurons involved in pattern separation?

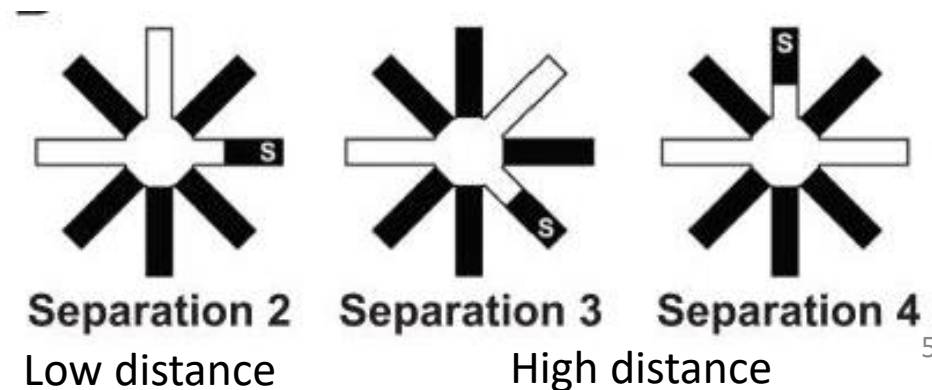
✓ Clelland et al, 2009

Protocol: pattern separation in Delayed Non Matching to Place in radial maze



At each trial, the mouse has to choose the new baited arm (...and should remember the previous arm visited).

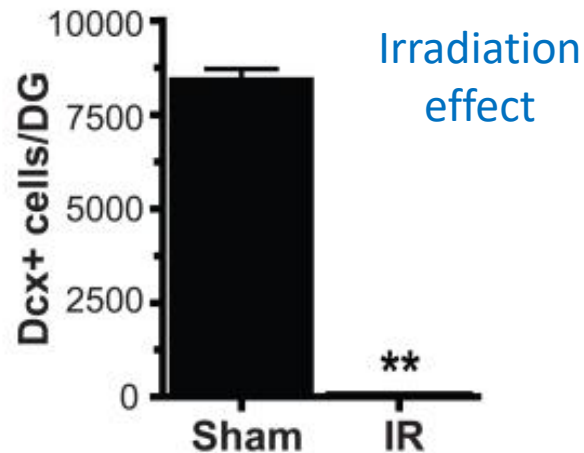
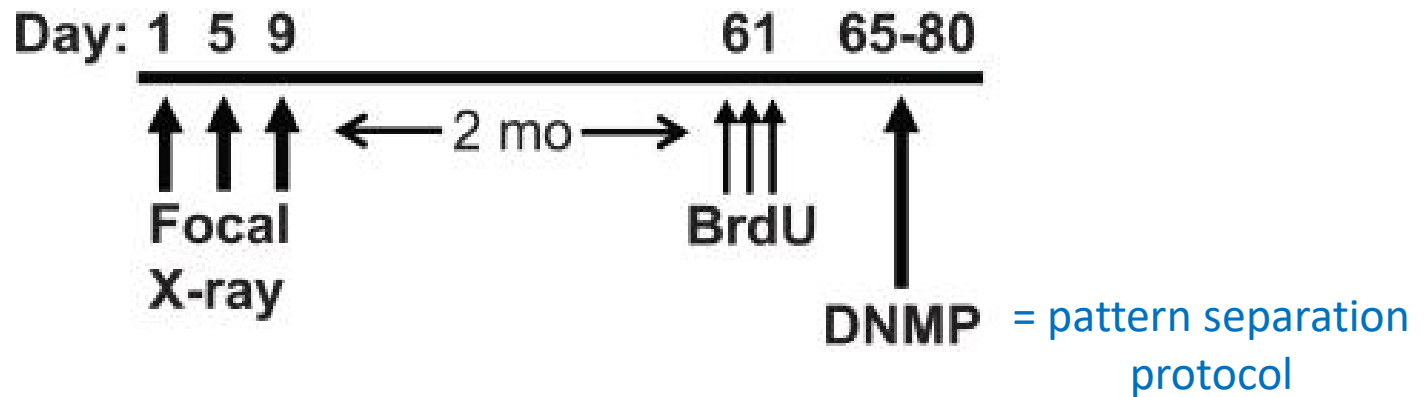
The distance between the previous baited arm and the new baited arm can be low or high.





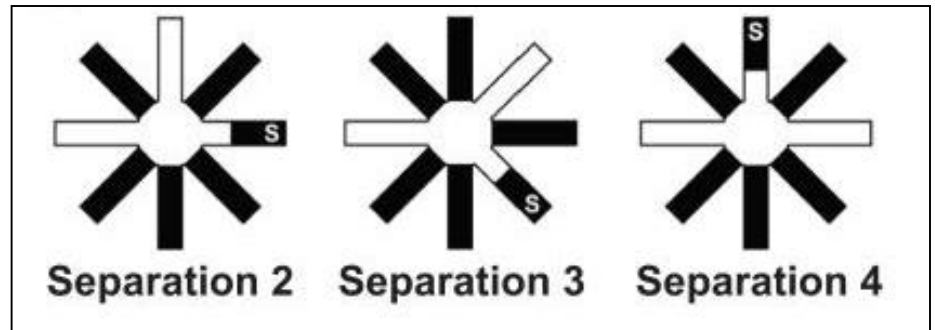
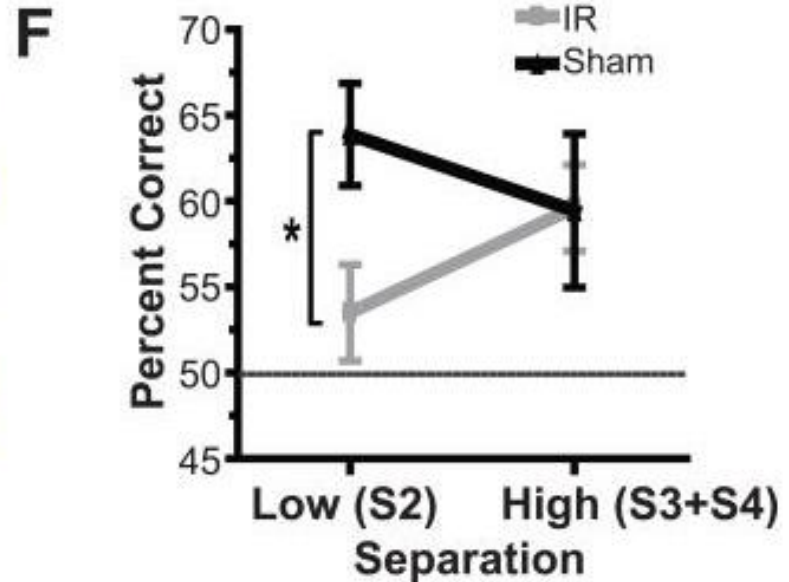
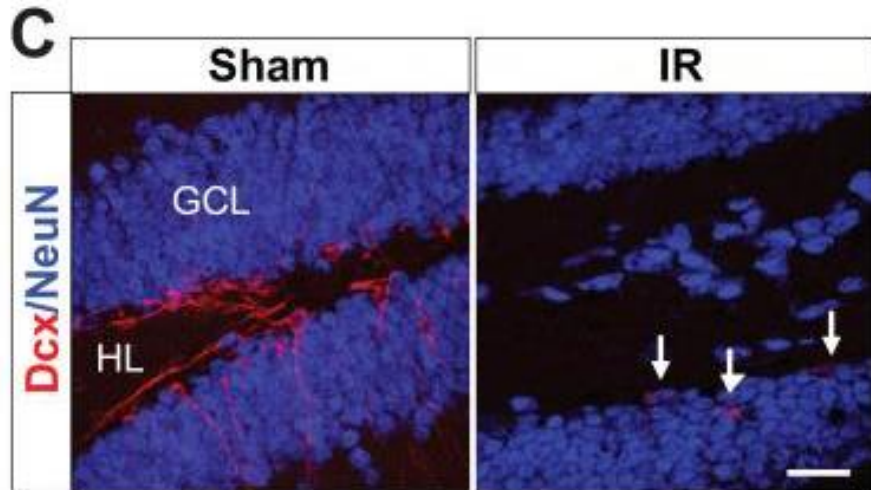
# Adult born neurons involved in pattern separation?

✓ Clelland et al, 2009

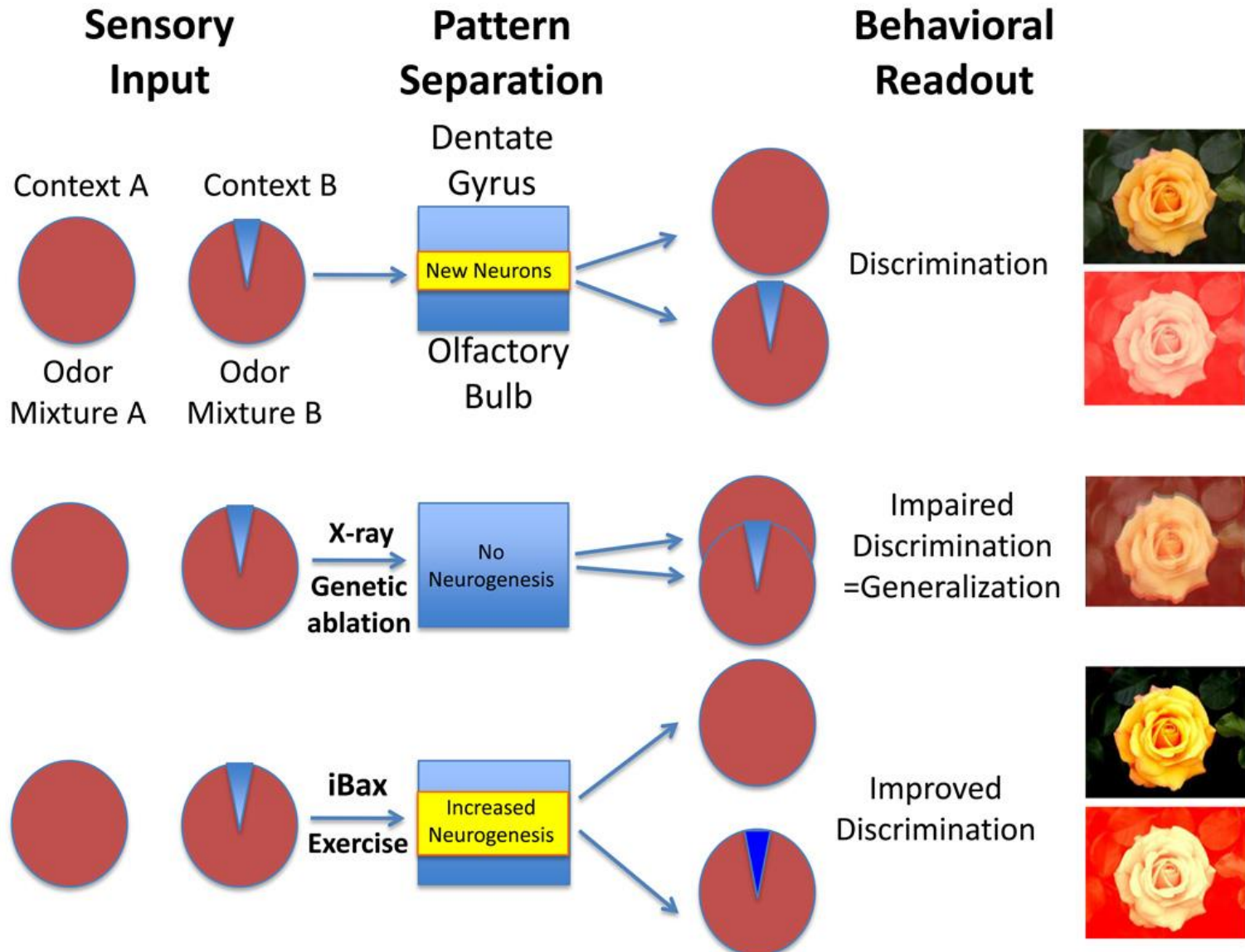


# Adult born neurons involved in pattern separation?

✓ Clelland et al, 2009



# Adult born neurons involved in pattern separation?



## Conclusions

- ✓ New born neurons in ADULT brain (mainly in DG / OB)
- ✓ Different factors can modulate (increase/ decrease) neurogenesis and memory
- ✓ Numerous studies have tried to understand the contribution of newborn neurons to hippocampus-dependent cognitive functions
- ✓ But a lot of contradictory results ...
- ✓ Adult born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice (Snyder, 2009)
- ✓ Time-dependent involvement of adult-born neurons in behavior
- ✓ Evidence for a specific role of adult neurogenesis in pattern separation