UENB013 : Hippocampus: from cells and physiology to human pathology and behavior

Cellular and synaptic morphology

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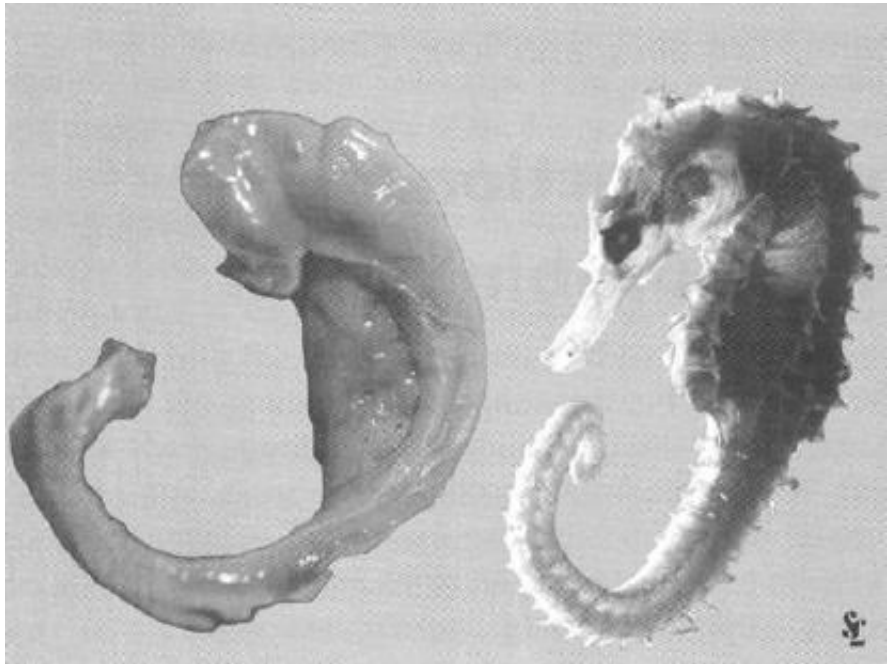


Plan

Part I

I. Structure, Afferents and Efferents of the rodent hippocampus

I. The hippocampus in rodents and primates
II. Septal-temporal (dorsal-ventral) orientation
III. Major Fiber bundles of the hippocampus: Afferents + Efferents
IV. Topographic connectivity of hippocampal Afferents + Efferents
V. Functional modules of the hippocampus?

II. The « tri » synaptic hippocampal circuit

I. Layers and Strata of the hippocampal formation
II. The Dentate Gyrus network
    I. Granule Cells
    II. Mossy Cells
    III. Inhibitory interneurons
III. Inhibitory/excitatory balance and disease
    I. Selective ablation of Mossy Cells

Part II

IV. CA3
    I. Granule cell mossy fibers projections to CA3
        I. Mossy fibers, Fliopodial extentions, *en passant* boutons
        II. Ultrastructure of mossy fibers
    II. Functional Mossy Fiber inputs to CA3
    III. Influence of spine shape and synapse location on dendritic integration
    IV. CA3 Afferents and Efferents
V. CA1
    I. CA1 Afferents
    II. Inhibitory interneurons
    III. CA1 Efferents/Subiculum
VI. The « tri » synaptic hippocampal circuit and the entorhinal cortex
VII. CA2

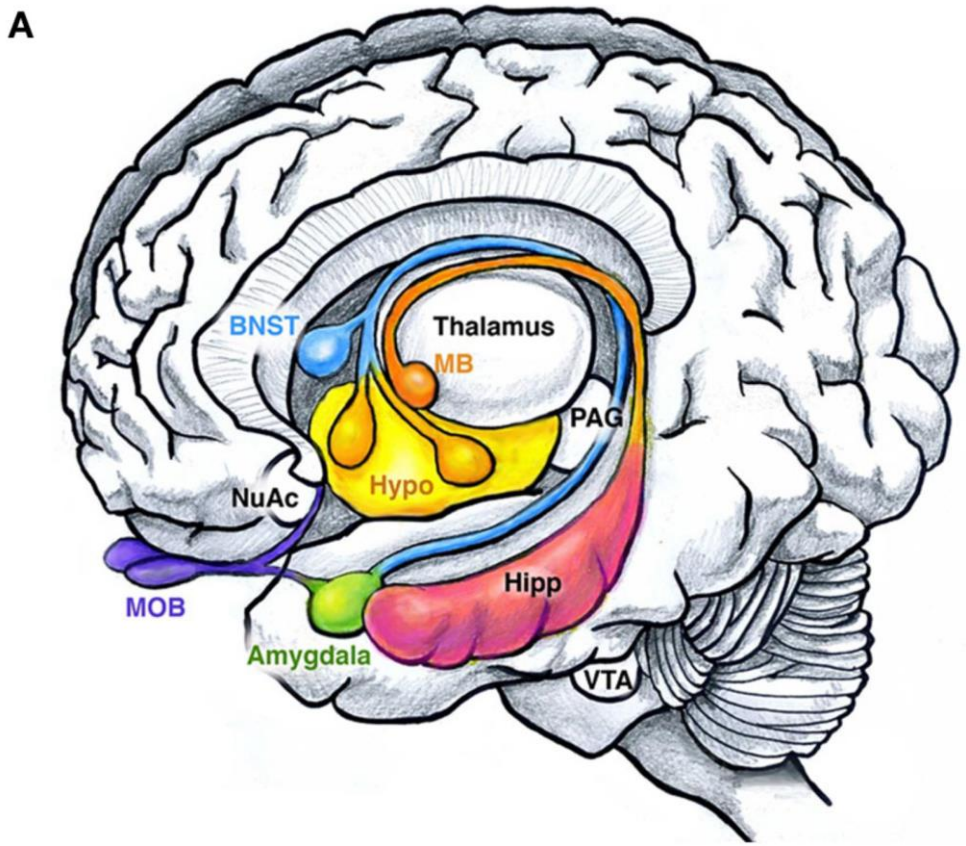
III. Modern anatomical techniques

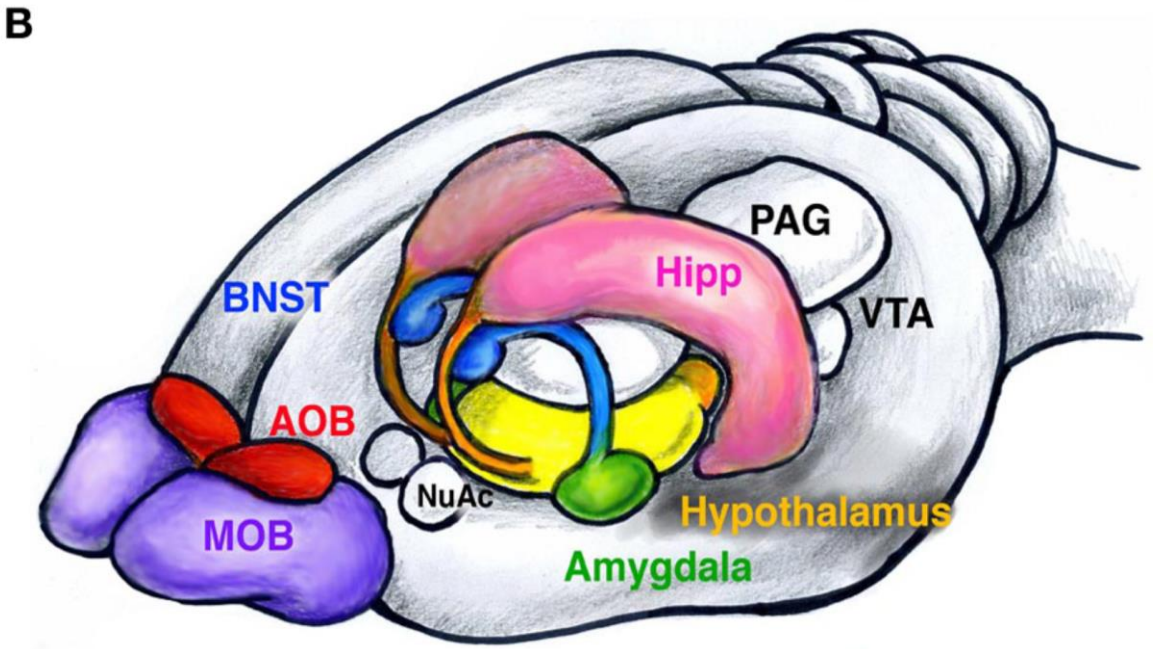
I. Monosynaptic tracing with glycoprotein–deleted rabies viruses

I. Structure, Afferents and Efferents of the rodent hippocampus

The limbic system

Limbic is latin for border or “limbo” meaning intermediate or transitional state indicating the position of limbic structures between the neocortex and subcortical structures (diencephalon). It is generally associated with regulating behavioral responses to external stimuli (motivational, emotional, social, defense, aggression). The limbic system includes the **Amygdala** (green), **bed nucleus of stria terminalis** (**BNST**, blue), **hypothalamus** (yellow), and **hippocampus** (pink), septal nuclei, cingulate cortex, entorhinal cortex, perirhinal cortex, and parahippocampal cortex. The hippocampus attaches to the **mamillary bodies** (orange) through the fimbria-fornix.



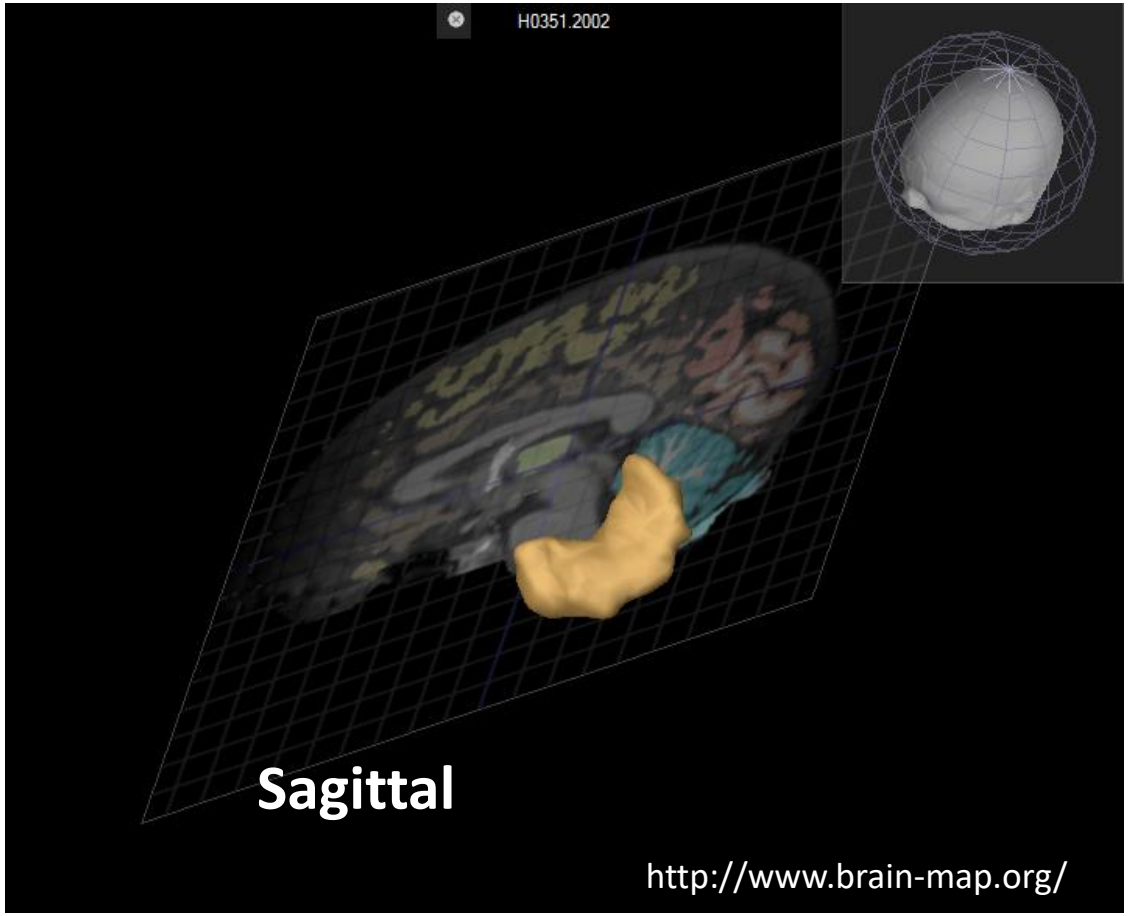


Katie Sokolowski and Joshua G. Corbin 2012, frontiers in mol. Neuro.

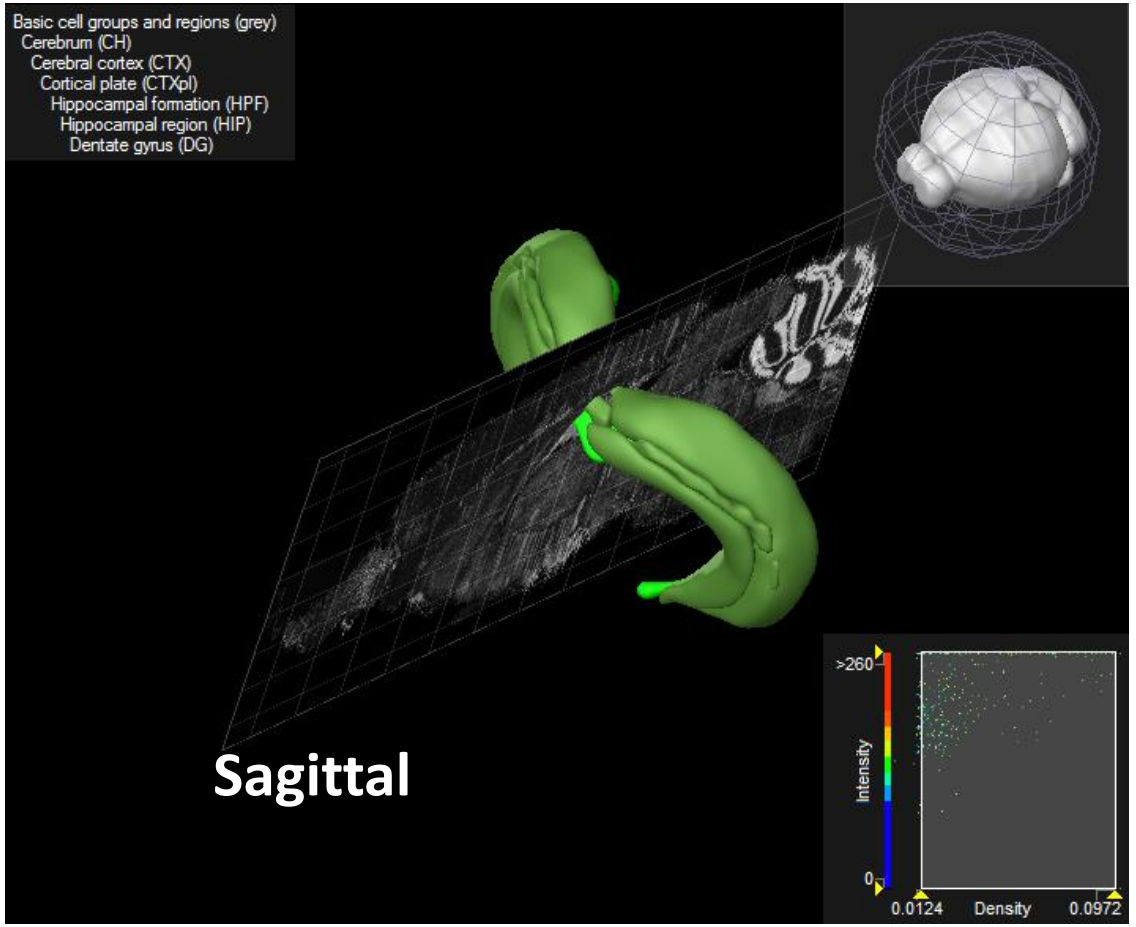
Main olfactory bulbs (MOB, purple)
Accessory olfactory bulbs (AOB, red).

The human vs mouse brain: Hippocampus

Human

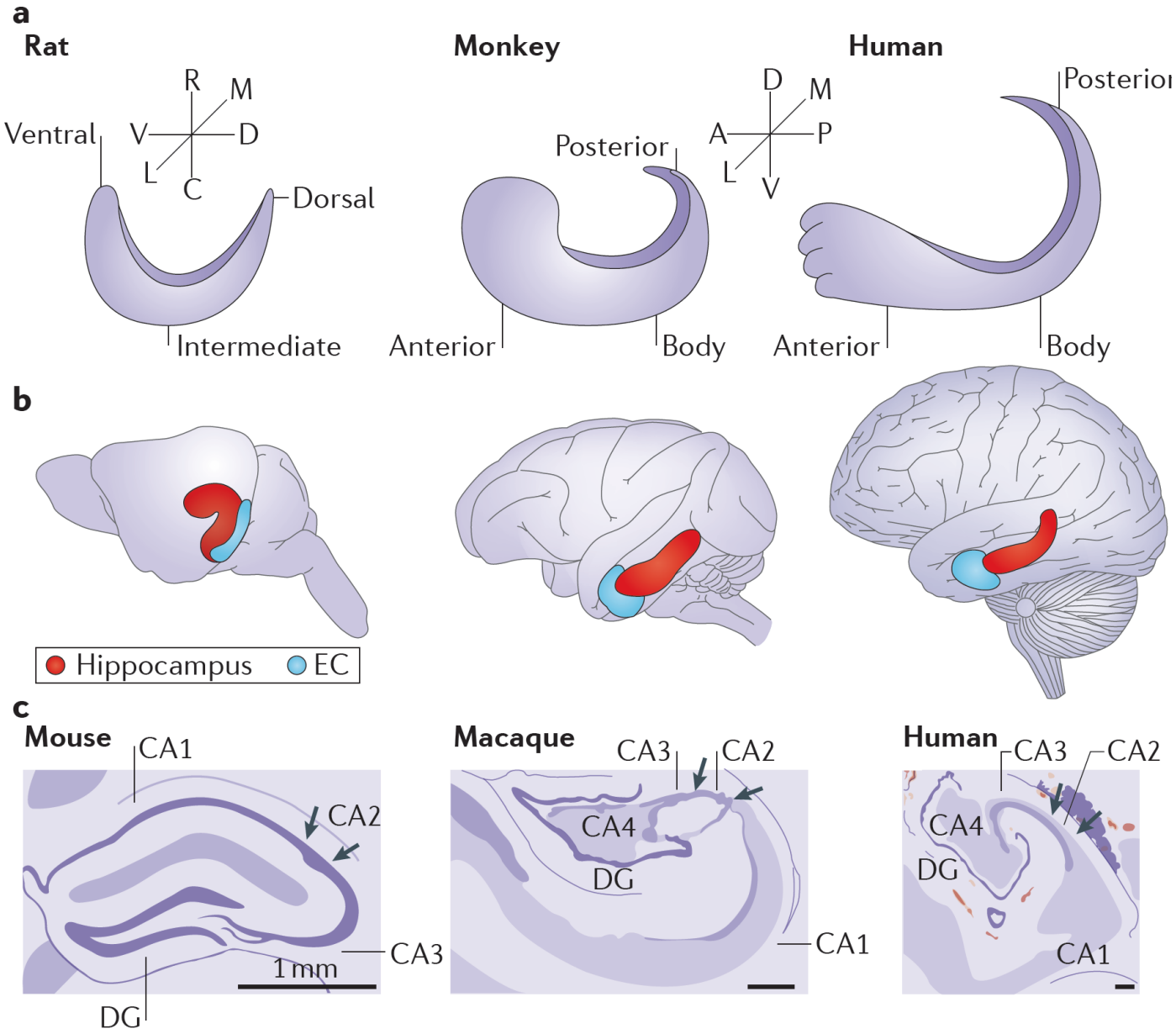


Mouse



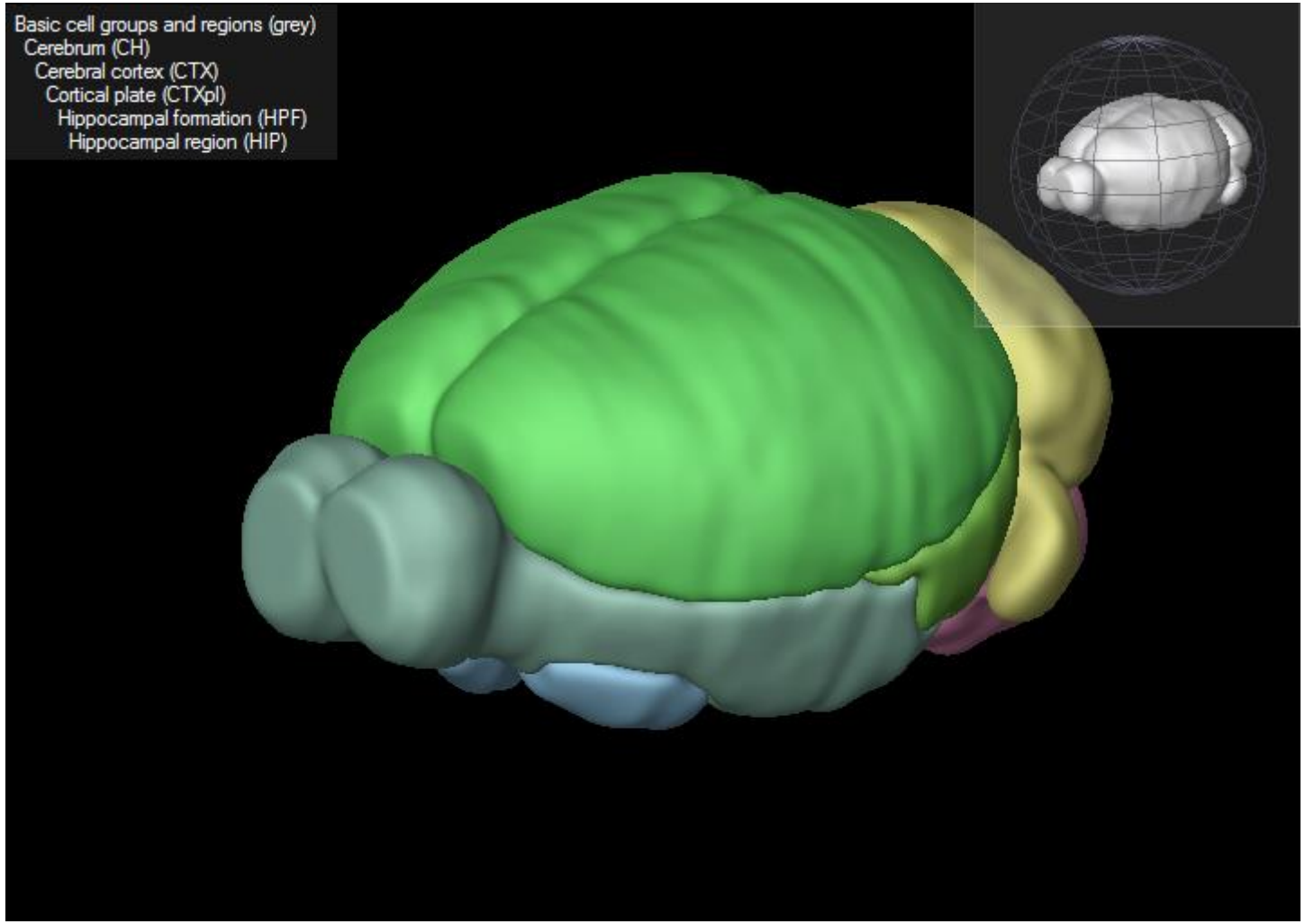
Larger and more developed cerebral cortex appears to have forced the position of the hippocampus to a more ventral position in humans and primates (left) when compared to rodents (right)

Cross-species comparison of the hippocampal anatomy



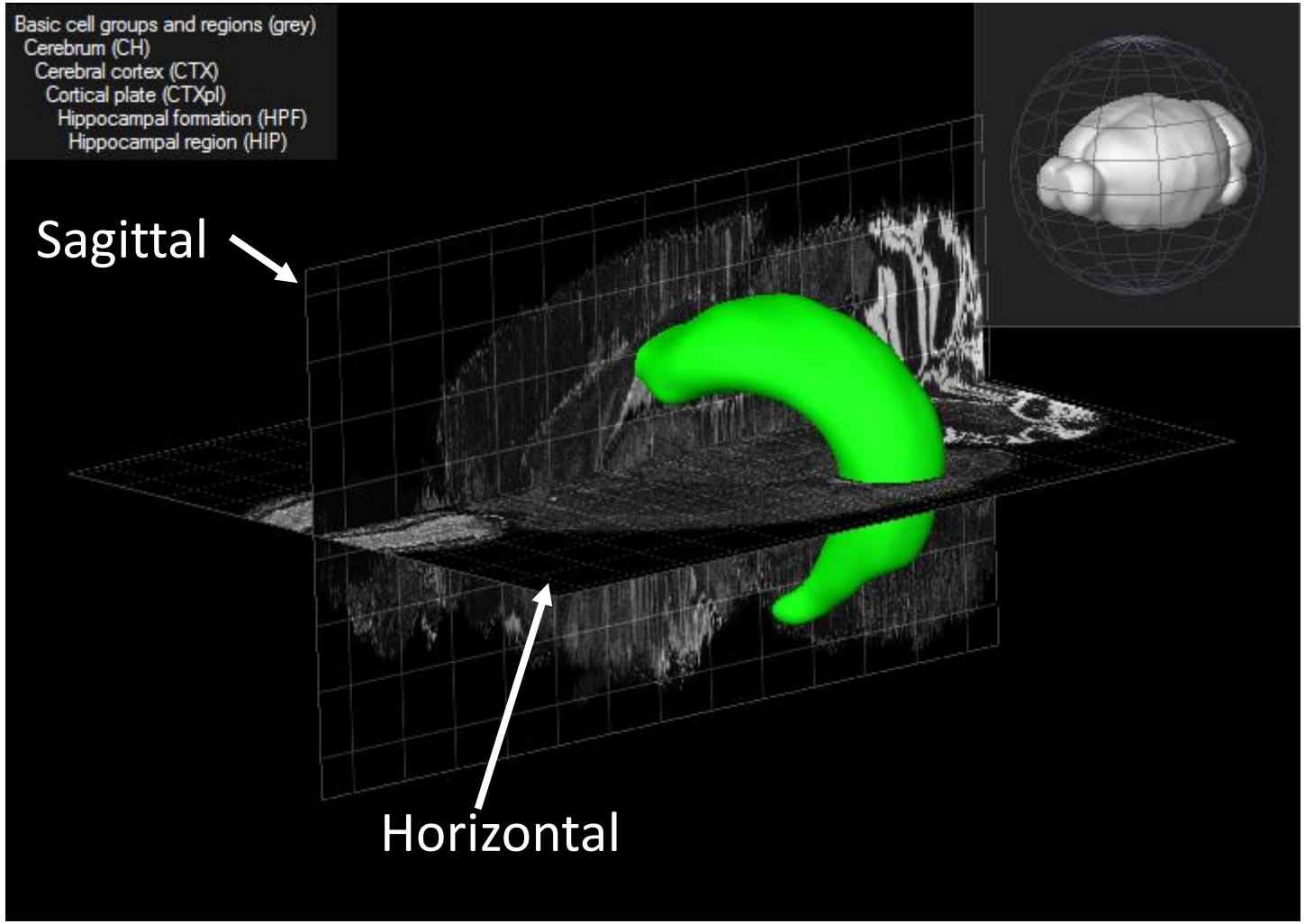
Strange et al. 2014, Nat.Neuro. reviews

Alan Mouse Brain Atlas



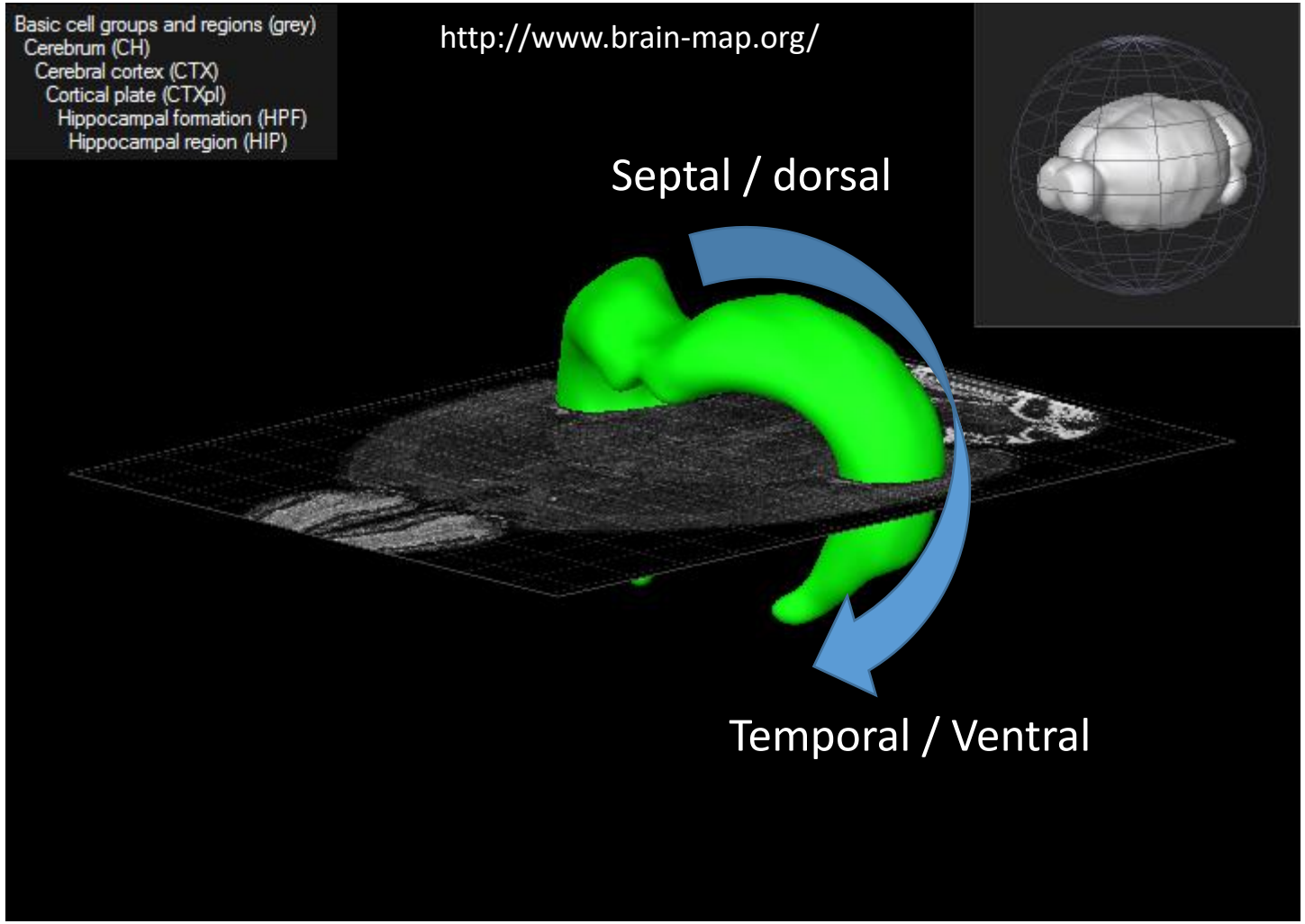
http://www.brain-map.org/

The mouse brain: Hippocampus

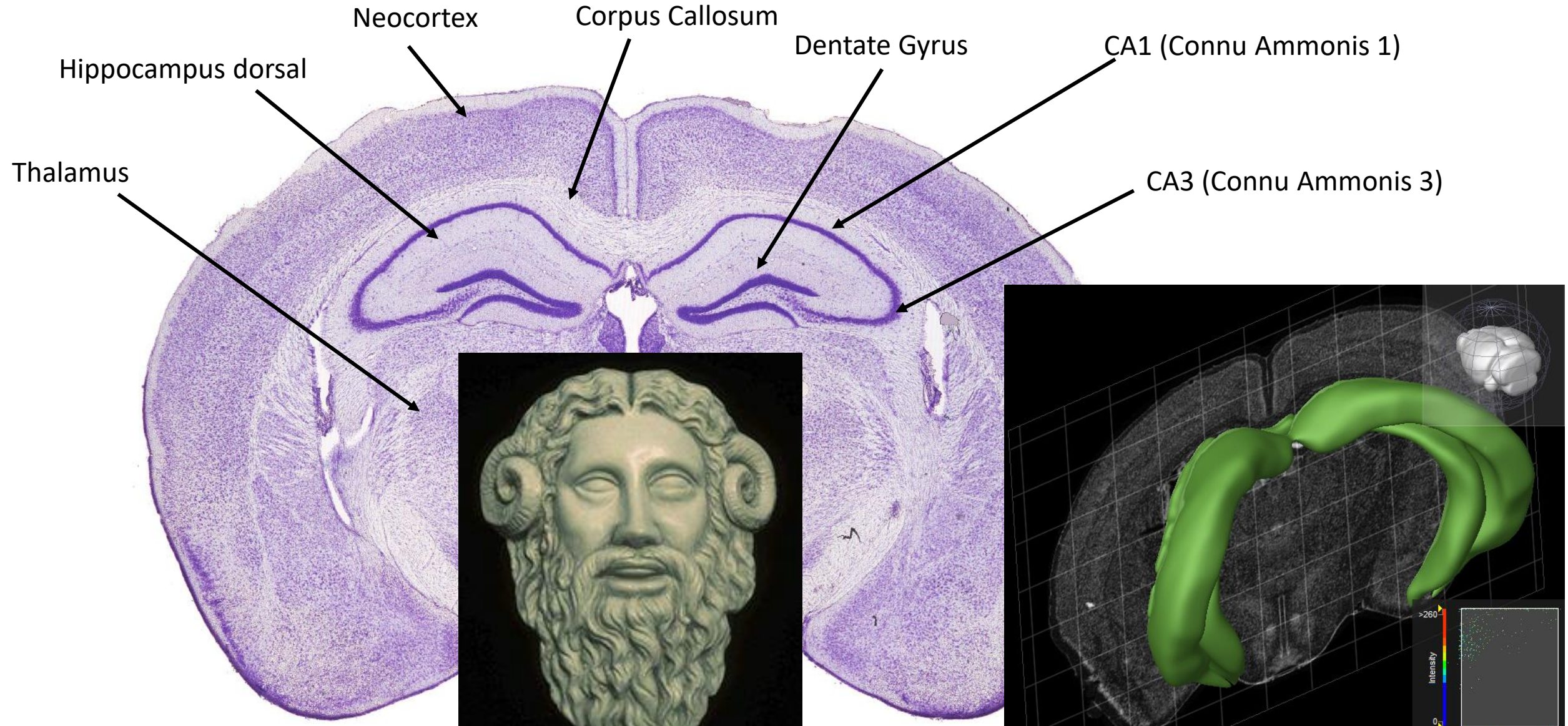


http://www.brain-map.org/

The mouse brain: The transverse axis of the Hippocampus



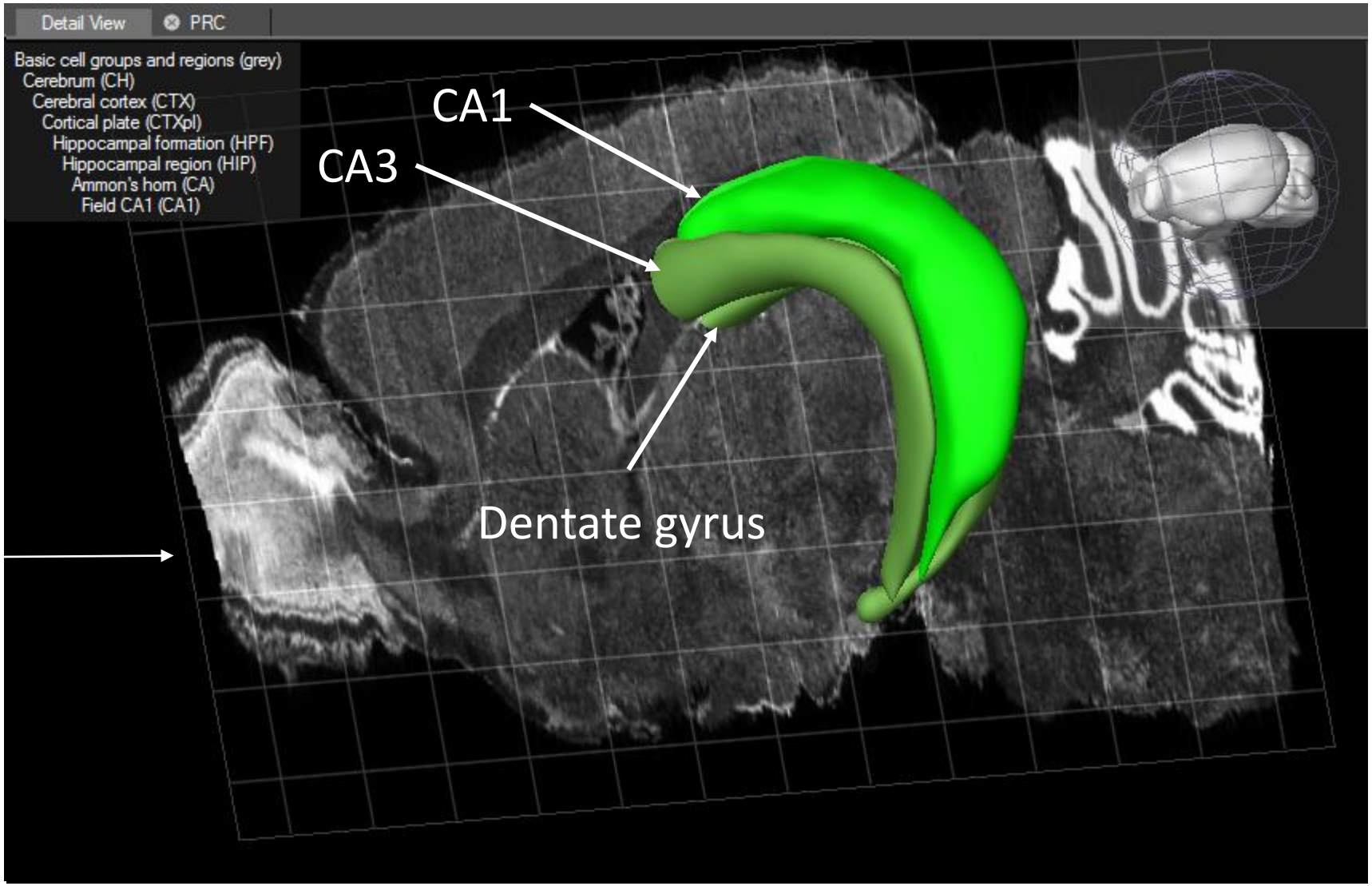
Coronal section of the mouse brain hippocampus



http://www.brain-map.org/

3d reconstruction of the « trisynaptic » hippocampal circuit

Sagittal Plane



Major fiber bundles of the hippocampal formation:

Angular bundle

-Carries fibers between the entorhinal cortex (ec) and other sub areas of the hippocampal formation (i.e. perforant path)

Fimbria-fornix

-Connect hippocampal formation with: the basal forebrain, Hypothalamus, and brain stem

Fimbria = red

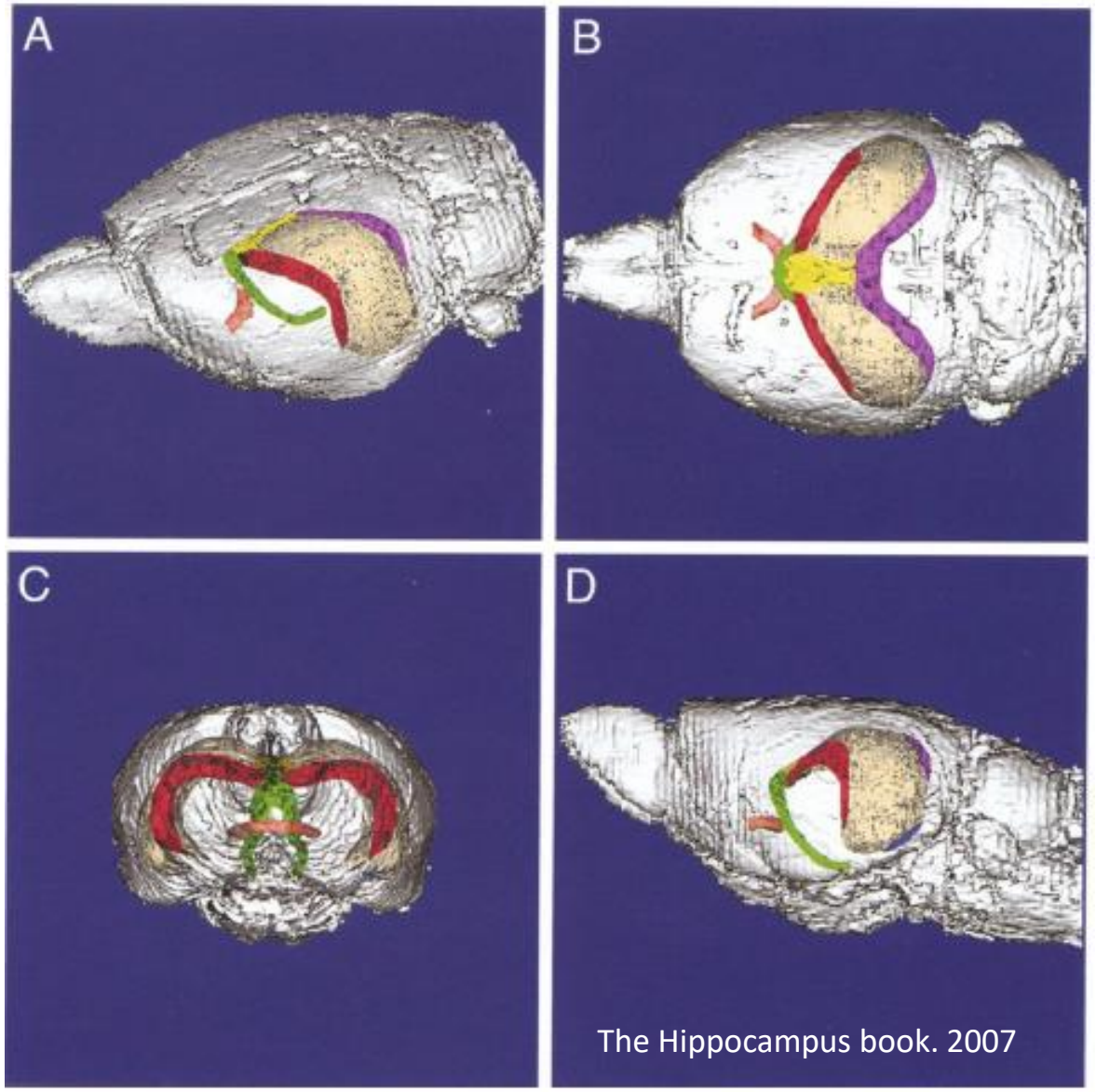
Fornix = yellow

Dorsal and ventral commissures

-connects the two hemispheres (contralateral connections)

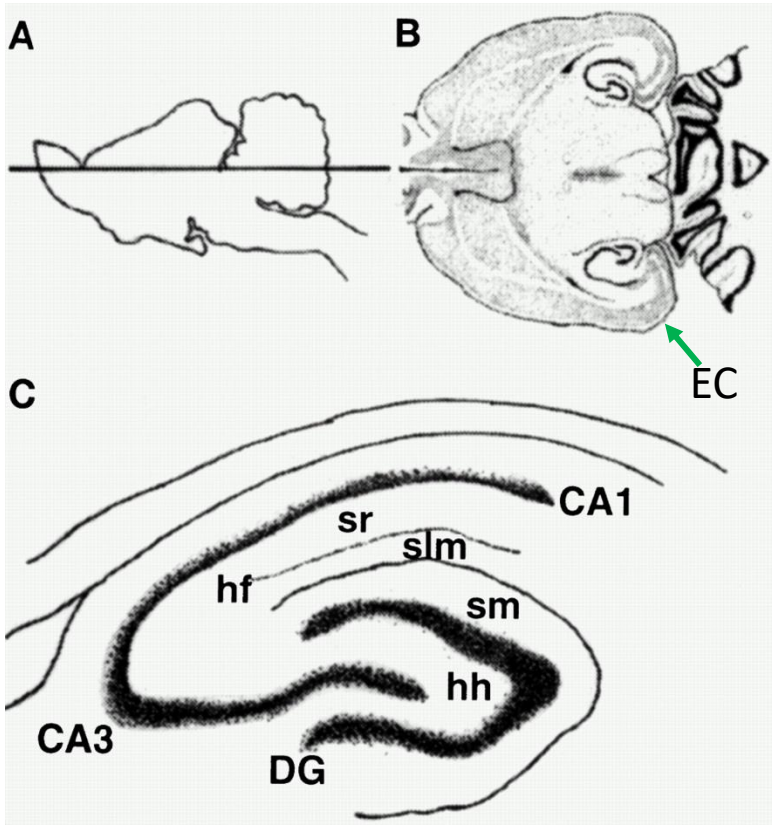
Dorsal = purple

Ventral = green



The Hippocampus book. 2007

Angular bundle in rodents is the predominant path that connects the Entorhinal cortex to the hippocampus



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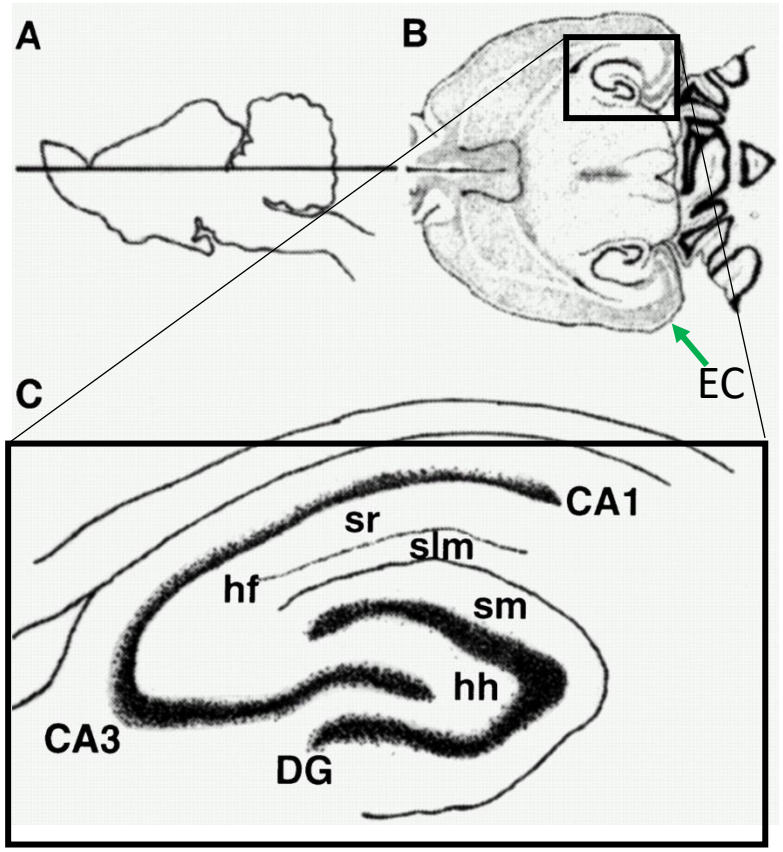
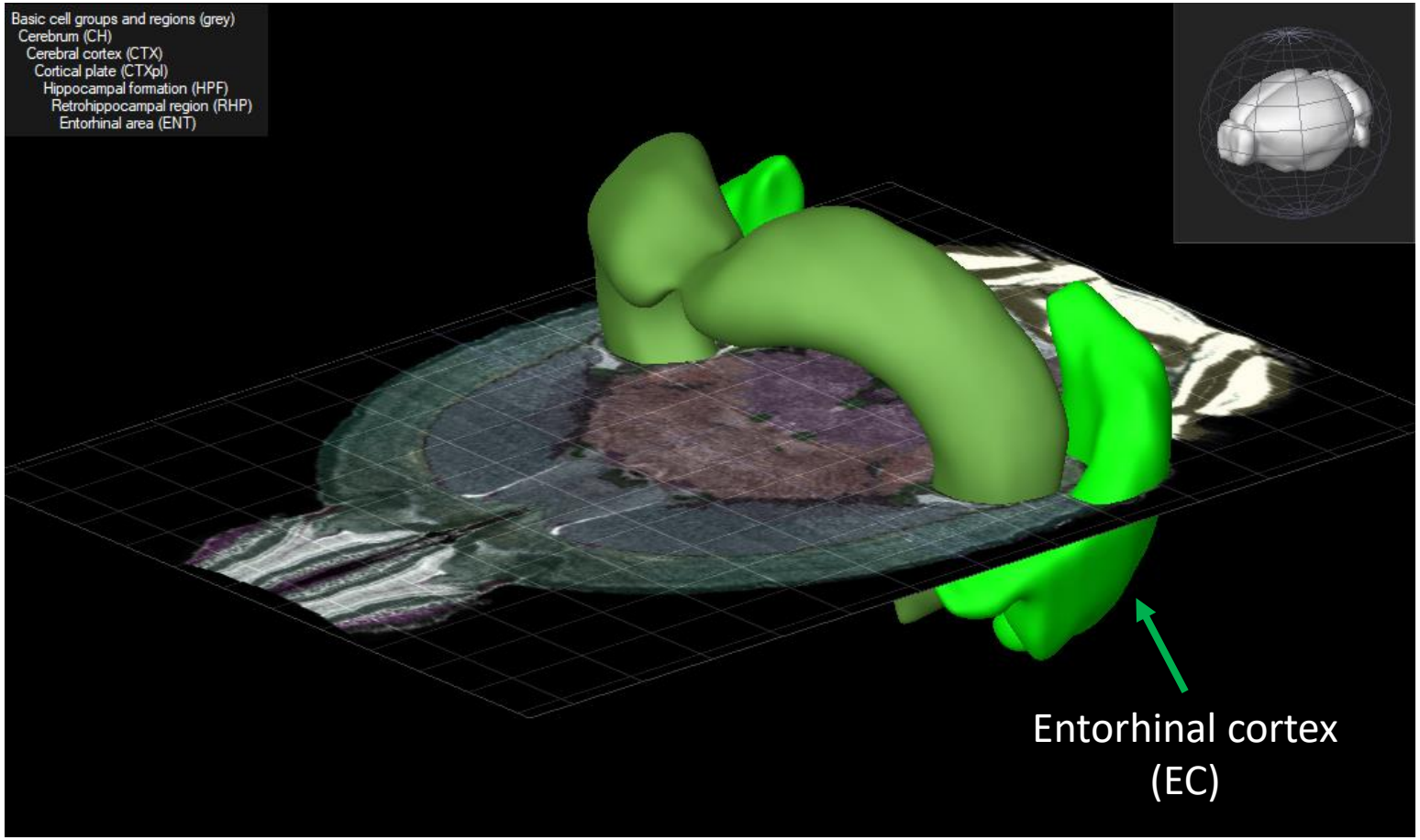
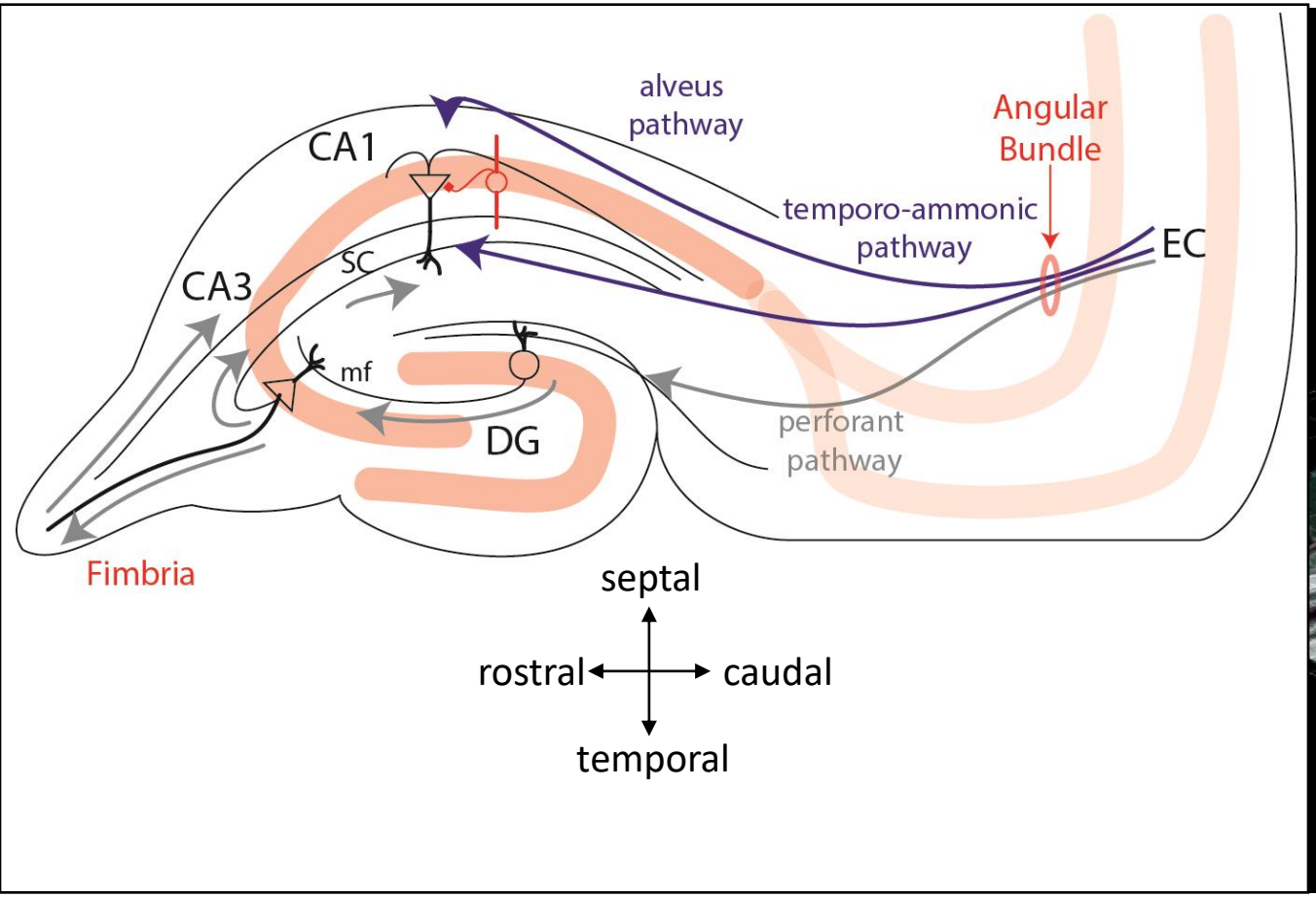
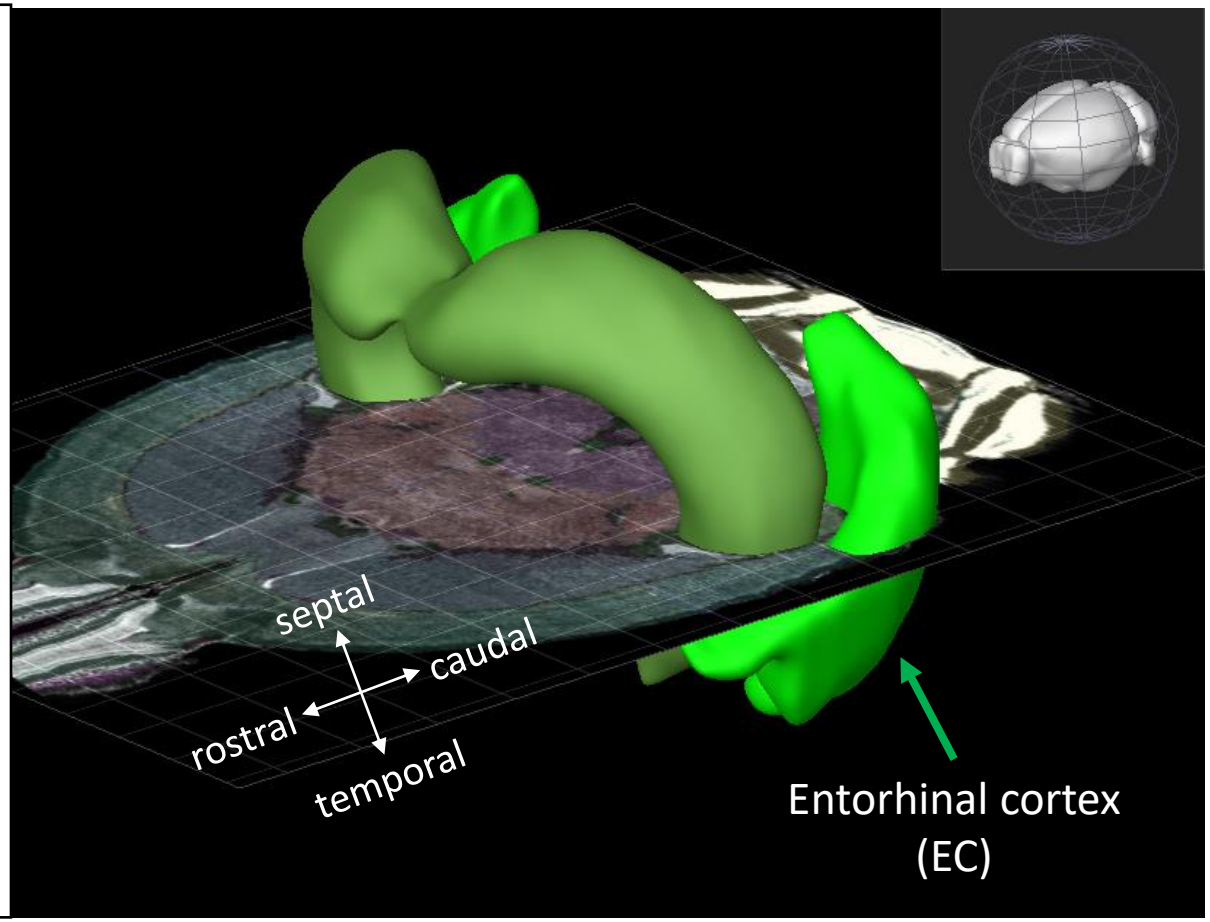




- Angular bundle fibers are interposed between the entorhinal cortex (EC) and the presubiculum and para subiculum.
- Predominant path that connects the EC to all septotemporal levels of hippocampal fields
- Also contains commissural fibers to and from a subcortical and cortical structures.

Angular bundle in rodents is the predominant path that connects the Entorhinal cortex to the hippocampus





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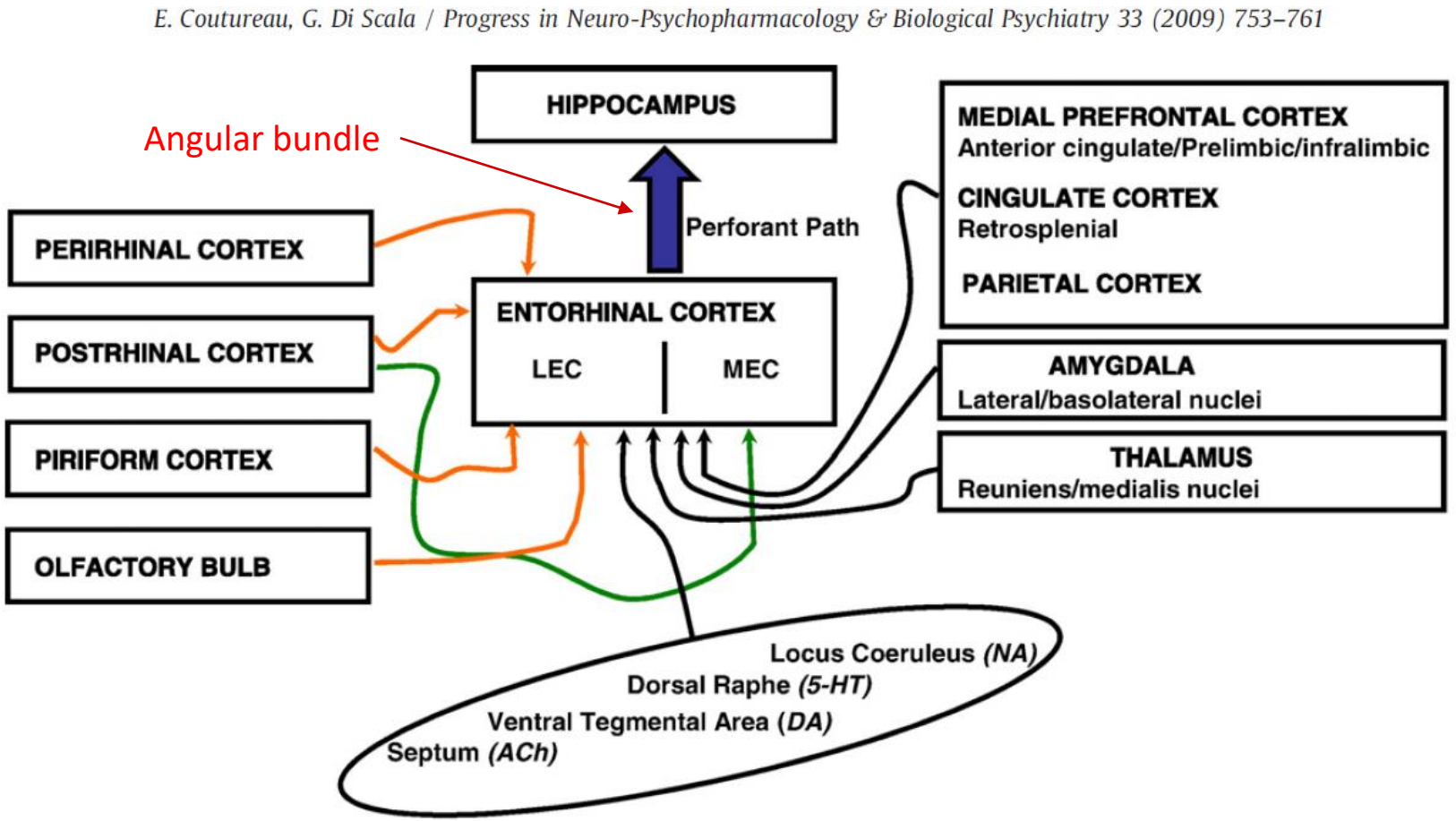
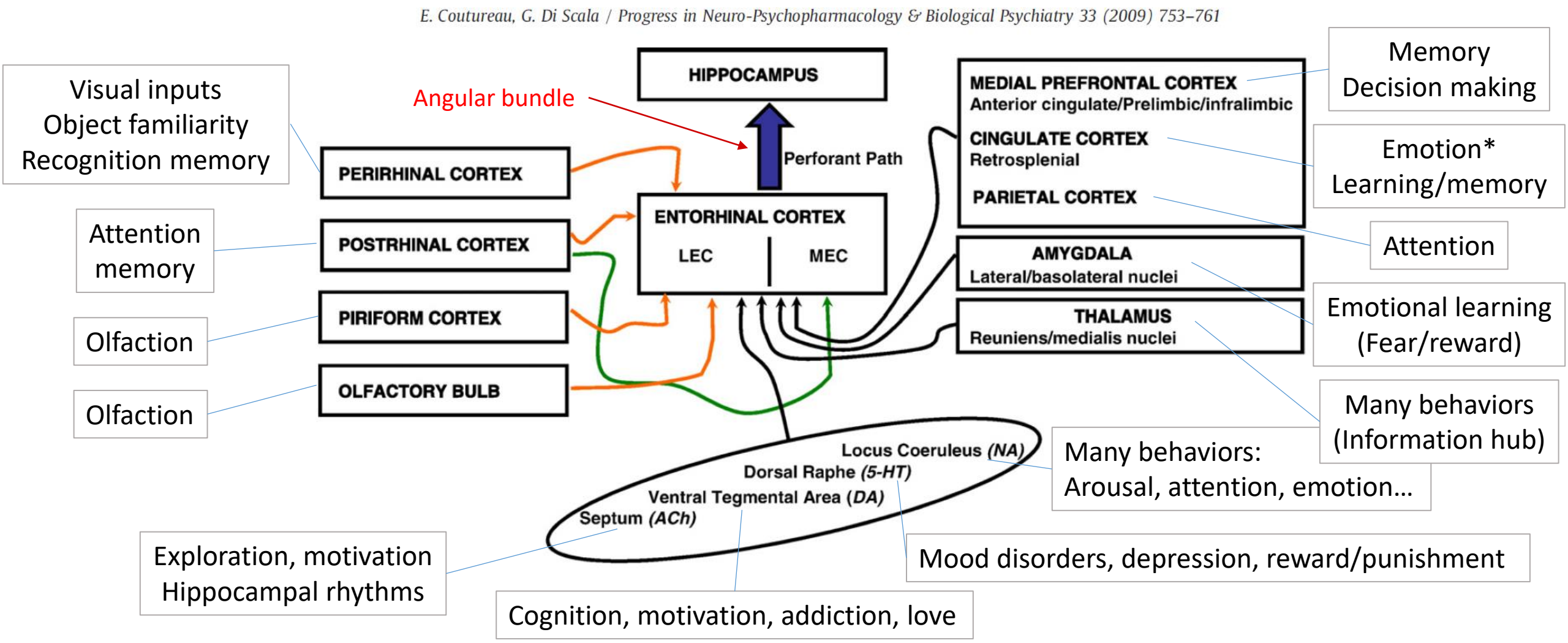
Principal paths of information flow to the Entorhinal cortex: and transmission to the Hippocampus through the Perforant path (angular bundle)



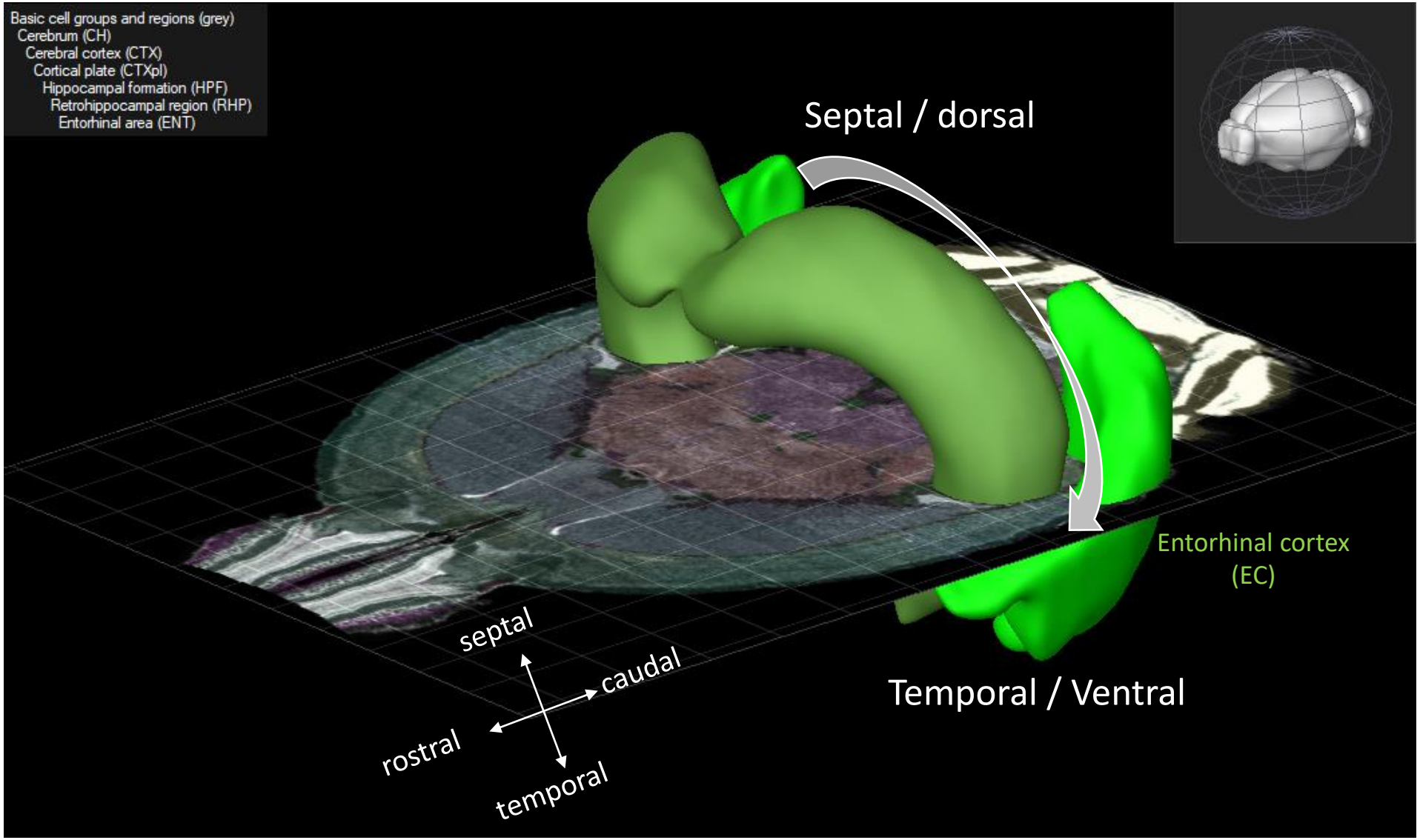
Major inputs to the hippocampus are relayed through the Perforant path from the entorhinal cortex

Behaviors associated with the entorhinal cortex to hippocampus Perforant Path (angular bundle)



The behaviors indicated represent a non-extensive list of behaviors associated with each region of the brain

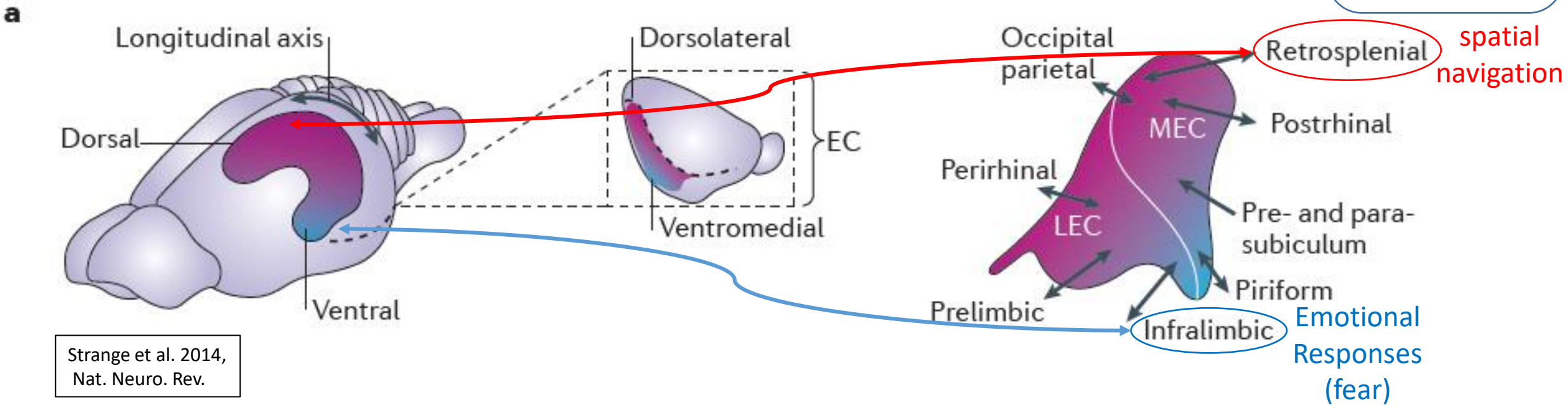
Topographic inputs to the transverse axis of the Hippocampus



http://www.brain-map.org/

Topographic connectivity between the Entorhinal cortex and the Hippocampus

Vincent Hok
Spatial navigation



Strange et al. 2014, Nat. Neuro. Rev.

The dorsal (septal) hippocampus (purple) is preferentially connected to the dorsal-lateral entorhinal cortex

The ventral (temporal) hippocampus (blue) is preferentially connected to the ventral medial entorhinal cortex

Major fiber bundles of the hippocampal formation:

Angular bundle
-Carries fibers between the entorhinal cortex (ec) and other sub areas of the hippocampal formation (i.e. perforant path)

Fimbria-fornix
-Connect hippocampal formation with: the basal forebrain, Hypothalamus, and brain stem
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Dorsal and ventral commissures
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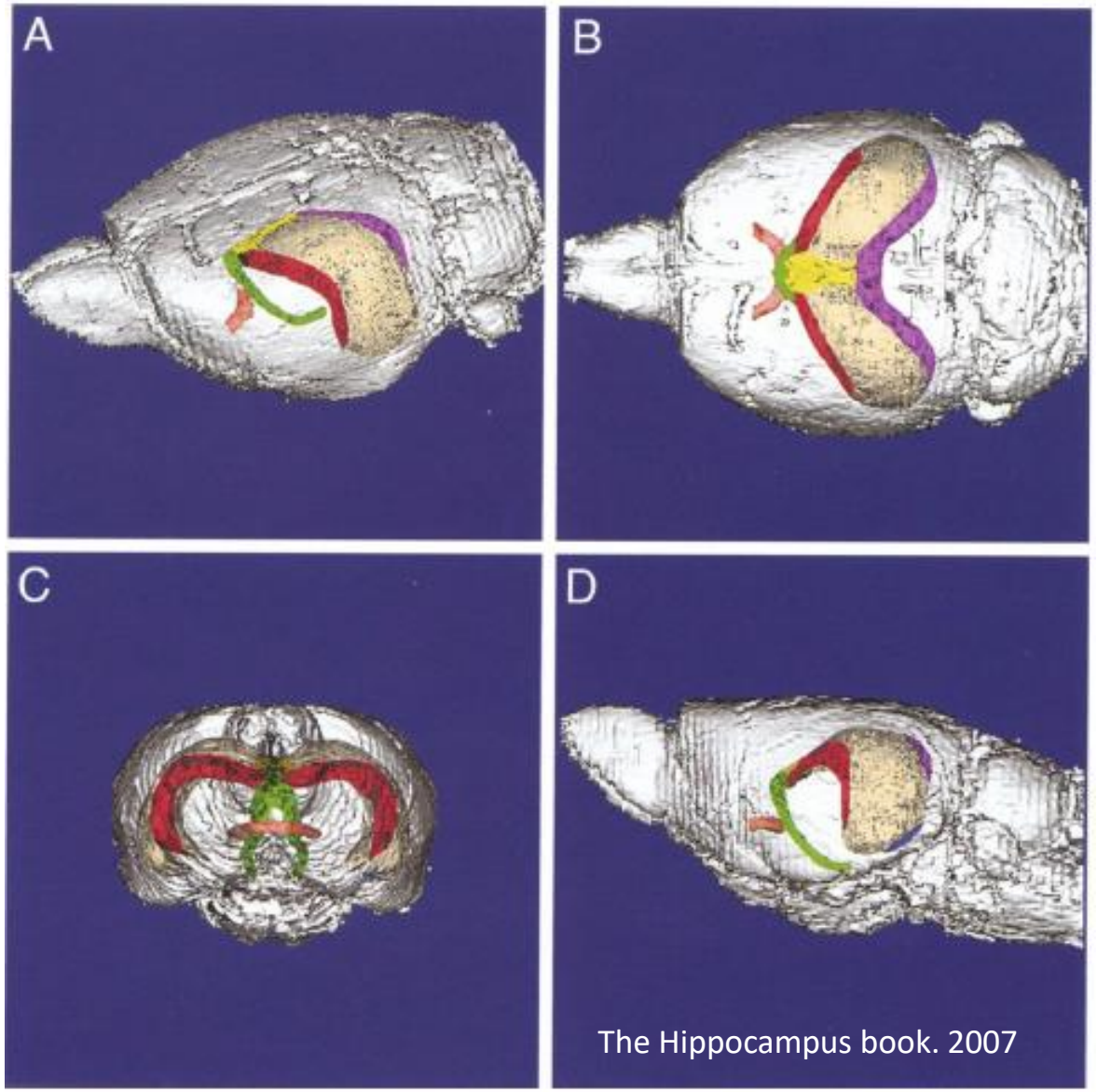


The Hippocampus book. 2007

Fimbria fornix in rodents

Fimbria / Fornix

The **fimbria** is a prominent band of white matter along the edge of the hippocampus. It is an accumulation of myelinated axons (**mostly efferent**) that first collect on the ventricular surface of the hippocampus.

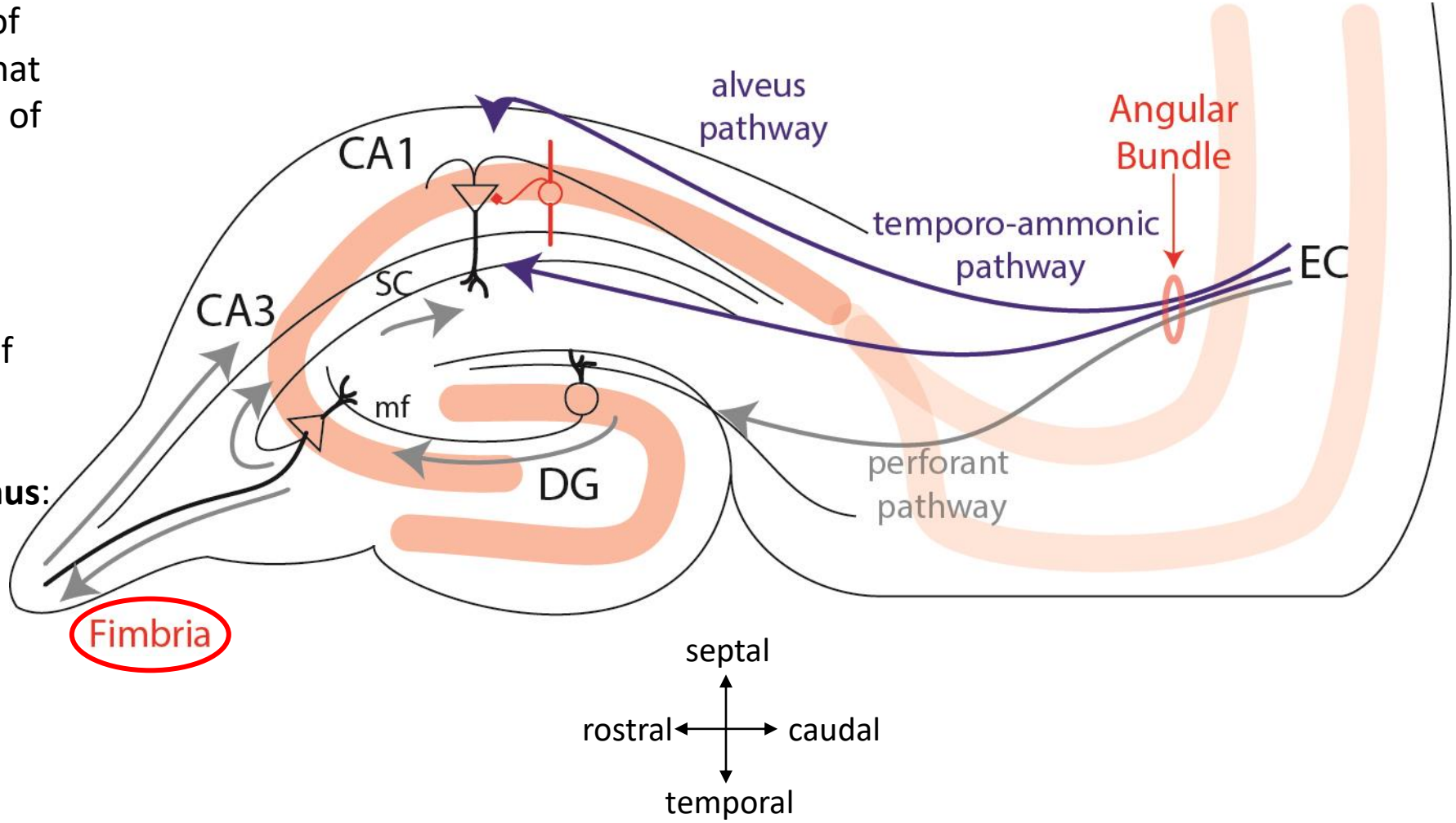
Projections:

Basal forebrain:
Nucleus accumbens, Diagonal band of broca, Septal nuclei (cholinergic)

Diencephalon thalamus/hypothalamus:
Supramammillary nucleus, nucleus reuniens, paraventricular nucleus

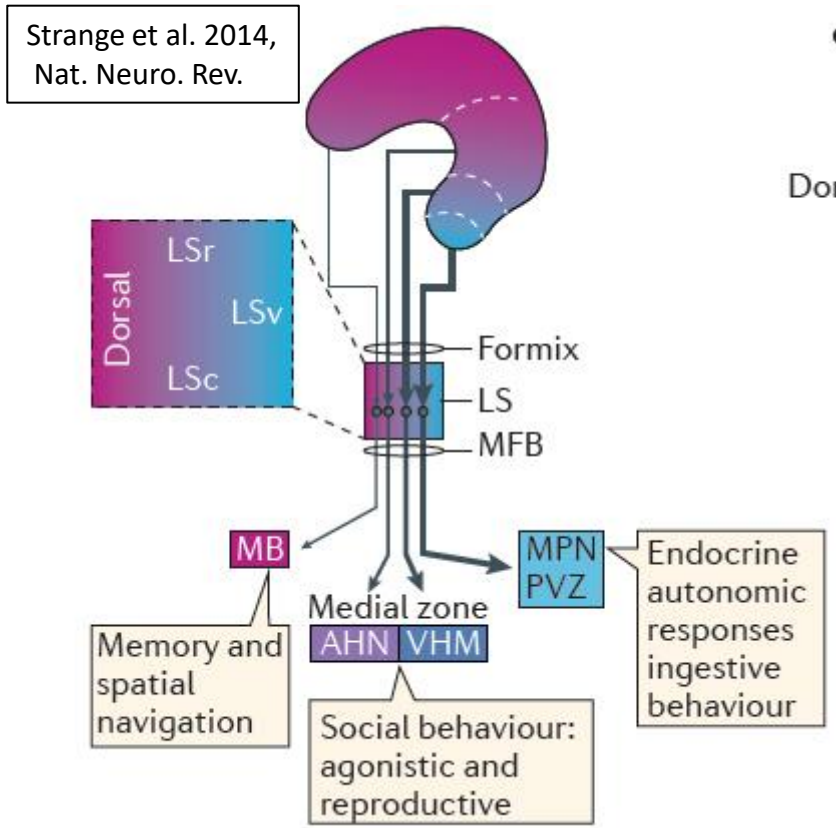
Midbrain:
Ventral tegmental area, locus coeruleus, raphe nuclei (5-HT)

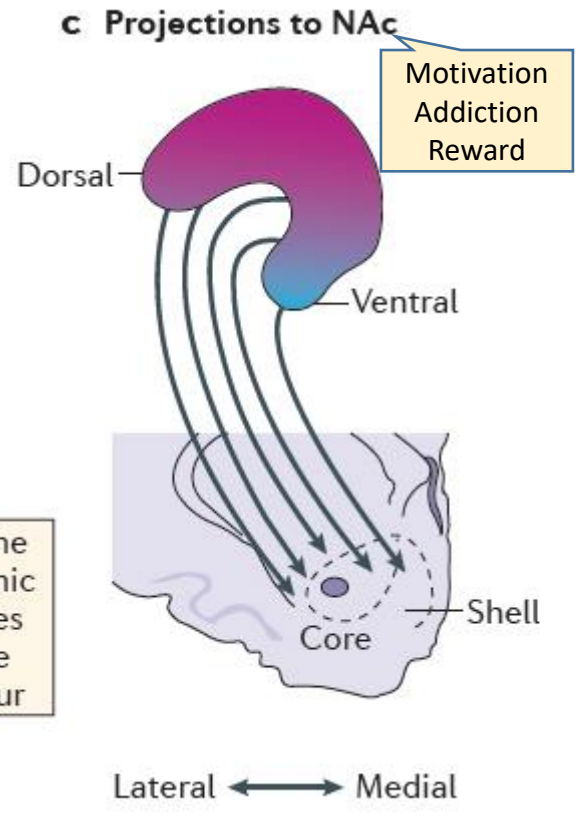
Scheme of connections to the hippocampus a perceived for a horizontal cut of the temporal (or ventral) hippocampus

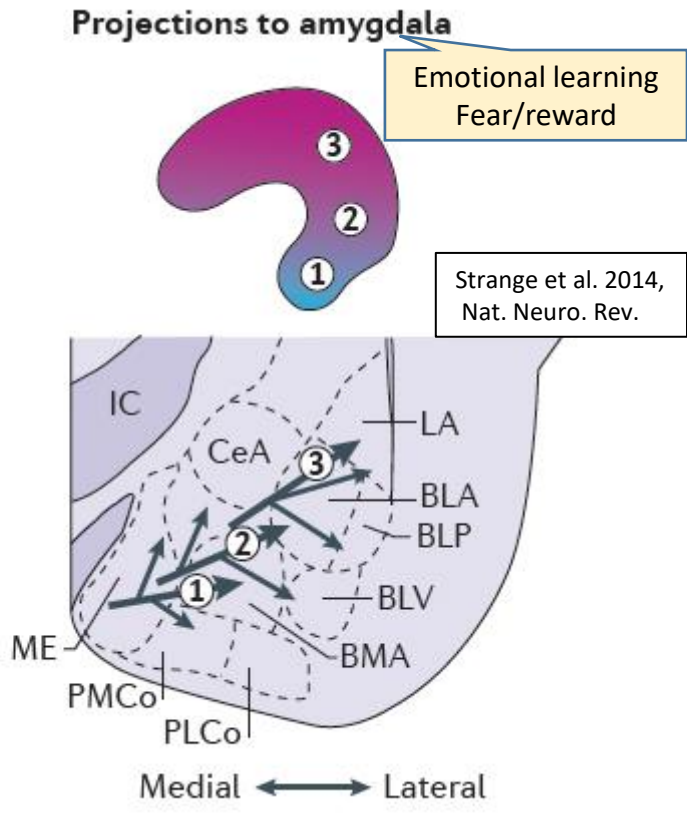


Topographic hippocampal efferent projections to subcortical structures

LS-lateral septum
MB-Mammillary body
MPN-Medial preoptic nucleus
PVZ- hypothalamic periventricular zone
AHN-Anterior Hypothalamic N.
VHN-Ventralmedial Hypothalamic N.
NAc- N. Accumbens
MFB- medial forebrain bundle







Topographic connectivity from the hippocampus to the lateral septum, NAc, and the Amygdala is conserved.

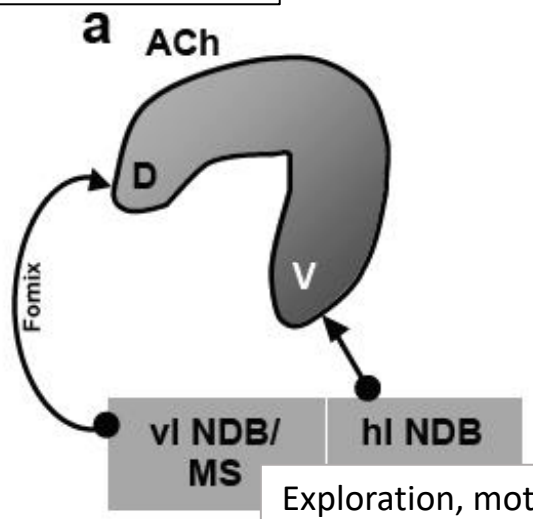
The Dorsal Hippocampus sends projections to the dorsal LS and the to the MB. Dorsal lateral Septum projects to MB the then to hypothalamic structures AHN et VHN. Ventral hippocampus projects to the ventral LS and then MPN and PVZ. Hippocampal projections to the NAc core and shell are topographically separated as are projections to the Amygdala.

BLA, basolateral amygdala; BLP, posterior basolateral nucleus of the amygdala; BLV, ventral basolateral nucleus of the amygdala; BMA, basomedial nucleus of the amygdala; IC, internal capsule; LA, lateral amygdala; ME, medial nucleus of the amygdala; edial (shell)-to-lateral (core) portions of the NAc

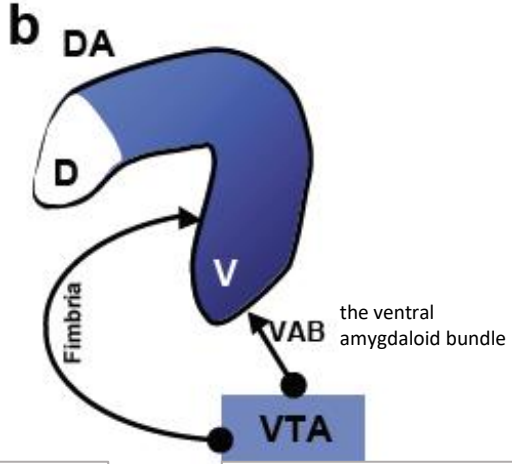
Neuromodulator and neuropeptide afferents display topographic innervation across the hippocampal longitudinal axis

D = dorsal / septal hippo
V = vental / temporal hippo

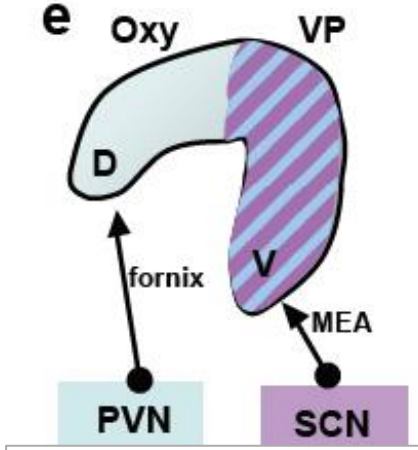
Strange et al. 2014, Nat. Neuro. Rev.



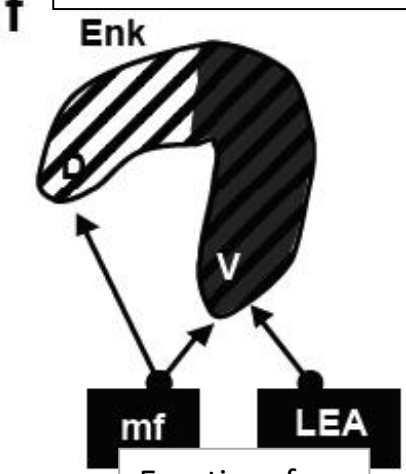
Exploration, motivation
Hippocampal rhythms



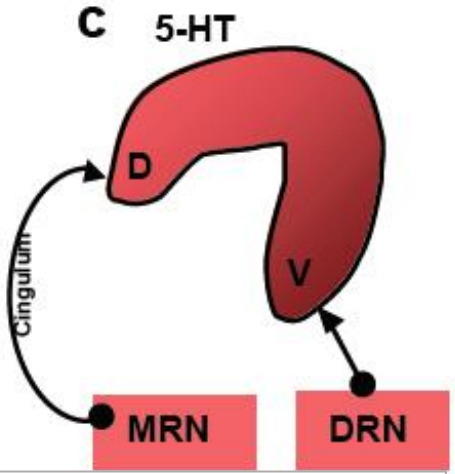
Cognition, motivation, addiction, love



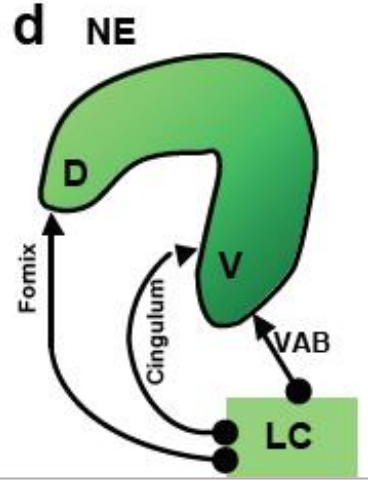
Stress, apatite, autonomic behaviors (digestion, erection)



Emotion, fear



Mood disorders, depression, reward/punishment



Many behaviors: Arousal, attention, emotion

a. The medial septum (MS), Diagonal band (DB): Acetylcholine (Ach)
b. Ventral tegmental Area (VTA): Dopamine (DA),
c. Median/Dorsal Raphe Nucleus (MRN, DRN): serotonin (5-HT)
d. Locus Coeruleus (LC): Norepinephrine (NE)
e. Suprachiasmatic nucleus (SCN): Vasopressin (VP)
e. Paraventricular nucleus (PVN): Oxytocin (Oxy)
f. Mossy fibres (mf), lateral entorhinal cortex (LEA): Enkephalin (Enk)
Ventral amygdaloid bundle (VAB), Medial amygdala nucleus (MEA)

The behaviors indicated represent a non-extensive list of behaviors associated with each region of the brain

Summary of afferent and efferent projections to and from the hippocampus

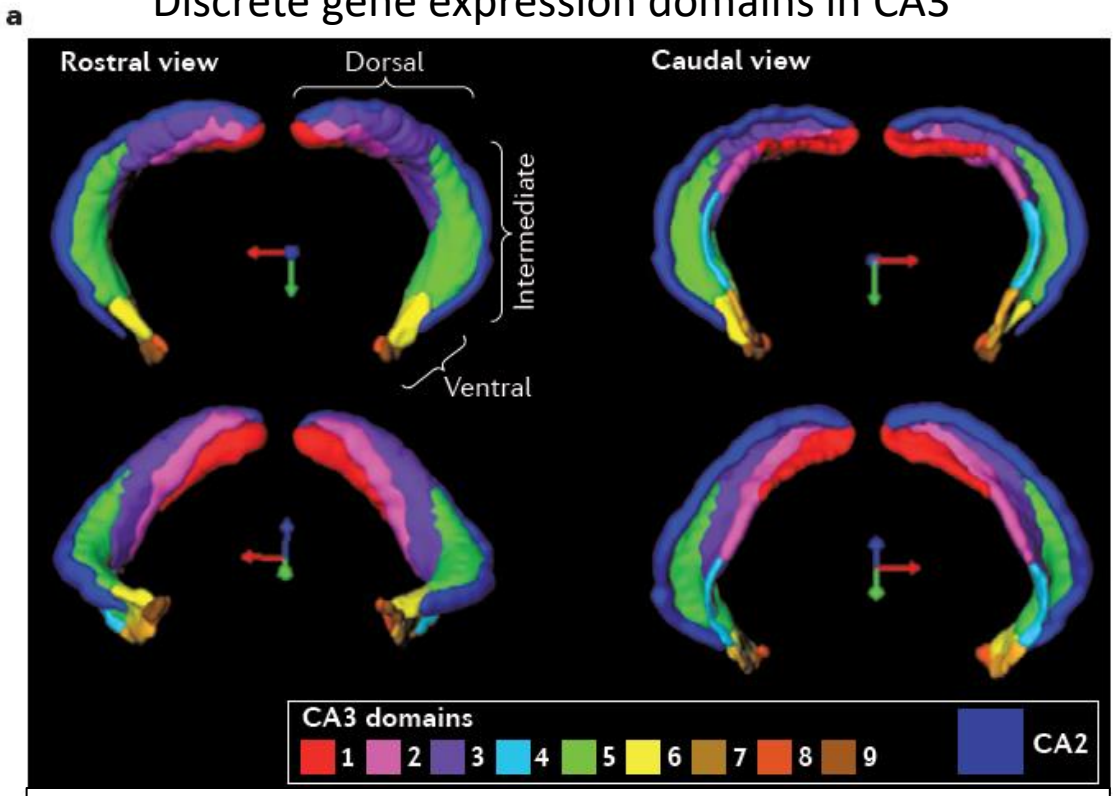
- Afferents and efferents arising from various regions of the brain implicated in determining appropriate behavioral responses to external stimuli (motivational, emotional, social, defense, aggression) topographically project to and from the hippocampus

- Neuromodulator and neuropeptide afferents also display topographic innervation of the hippocampus along the transverse (longitudinal axis).

- As different regions in the brain are associated with different behaviors it seems that different sub-regions of the hippocampus may be specific for different behaviors.

Discrete gene expression in CA3 suggests distinct functional modules exist along the septal-temporal axis

Discrete gene expression domains in CA3



Strange et al. 2014, Nat. Neuro. Rev.; Thompson et al. 2008. Neuron

Along the transverse axis of the Hippocampus multiple sub-domains of the CA3 region have been identified by the expression of specific molecular markers. These domains are proposed to represent distinct functional modules.

And just recently…

Distinct transcriptomic cell types and neural circuits of the subiculum and prosubiculum along the dorsal-ventral axis

Song-Lin Ding[1,2,*], Zizhen Yao[1], Karla E. Hirokawa[1], Thuc Nghi Nguyen[1], Lucas T. Graybuck[1], Olivia Fong[1], Phillip Bohn[1], Kiet Ngo[1], Kimberly A. Smith[1], Christof Koch[1], John W. Phillips[1], Ed S. Lein[1], Julie A. Harris[1], Bosiljka Tasic[1], Hongkui Zeng[1]

were mainly attributed to subiculum (Sub) rather than prosubiculum (PS). Using single-cell RNA-sequencing (scRNA-seq) technique we have identified up to 27 distinct transcriptomic clusters/cell types, which were registered to anatomical sub-domains in Sub and PS. Based on reliable molecular

Highlights

1. 27 transcriptomic cell types identified in and spatially registered to "subicular" regions.
2. Anatomic borders of "subicular" regions reliably determined along dorsal-ventral axis.
3. Distinct cell types and circuits of full-length subiculum (Sub) and prosubiculum (PS).
4. Brain-wide cell-type specific projections of Sub and PS revealed with specific Cre-lines.

Sub and PS have been consistently defined along the dorsoventral (DV) axis. Using these borders to evaluate Cre-line specificity and tracer injections, we have found bona fide Sub projections topographically to structures important for spatial processing and navigation. In contrast, PS along DV axis sends its outputs to widespread brain regions crucial for motivation, emotion, reward, stress, anxiety and fear. Brain-wide cell-type specific projections of Sub and PS have also been revealed using

Subiculum = Sub
Prosubiculum = PS

Summary

Subicular region plays important roles in spatial processing and many cognitive functions and these were mainly attributed to subiculum (Sub) rather than prosubiculum (PS). Using single-cell RNA-sequencing (scRNA-seq) technique we have identified up to 27 distinct transcriptomic clusters/cell types, which were registered to anatomical sub-domains in Sub and PS. Based on reliable molecular markers derived from transcriptomic clustering and *in situ* hybridization data, the precise boundaries of Sub and PS have been consistently defined along the dorsoventral (DV) axis. Using these borders to evaluate Cre-line specificity and tracer injections, we have found bona fide Sub projections topographically to structures important for spatial processing and navigation. In contrast, PS along DV axis sends its outputs to widespread brain regions crucial for motivation, emotion, reward, stress, anxiety and fear. Brain-wide cell-type specific projections of Sub and PS have also been revealed using specific Cre-lines. These results reveal two molecularly and anatomically distinct circuits centered in Sub and PS, respectively, providing a consistent explanation to historical data and a clearer foundation for future functional studies.

Major fiber bundles of the hippocampal formation:

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-Connect hippocampal formation with: the basal forebrain, Hypothalamus, and brain stem
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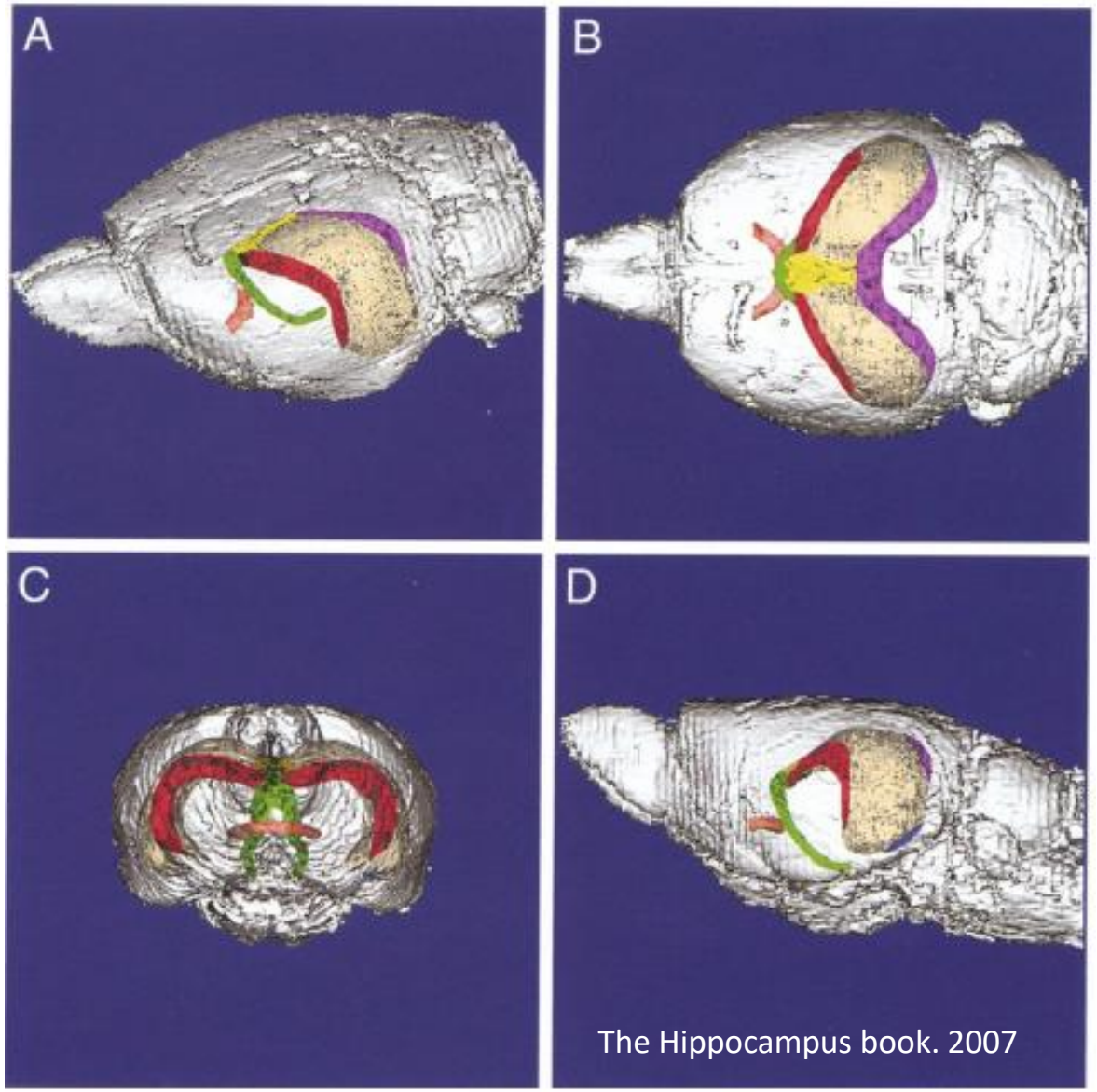


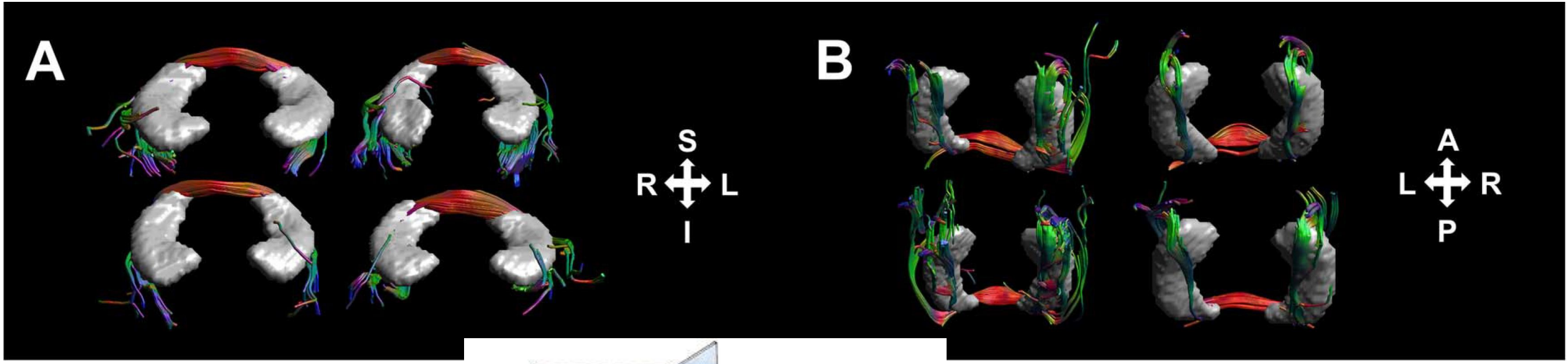
The Hippocampus book. 2007

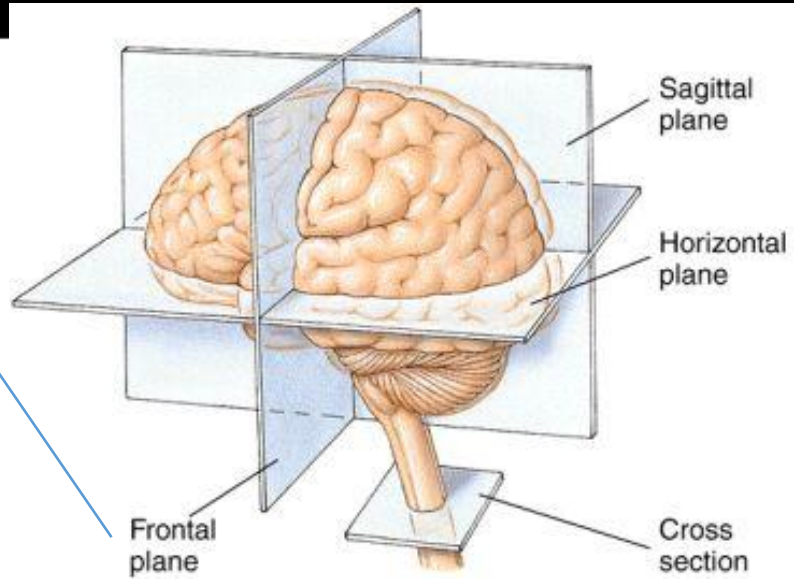
Dorsal and ventral commissures of the hippocampus

Frontal (coronal) image

Dorsal hippocampal commissures

Horizontal (transverse) image





Postans et al. 2019. Cerebral Cortex

Hippocampus quiz 1

qys.fr

3- Le code d'accès est le : 6k9mnh98

II. The « tri » synaptic hippocampal circuit

Plan

Part I

I. Structure, Afferents and Efferents of the rodent hippocampus

I. The hippocampus in rodents and primates
II. Septal-temporal (dorsal-ventral) orientation
III. Major Fiber bundles of the hippocampus: Afferents + Efferents
IV. Topographic connectivity of hippocampal Afferents and Efferents
V. Functional modules of the hippocampus?

II. The « tri » synaptic hippocampal circuit

I. Layers and Strata of the hippocampal formation
II. The Dentate Gyrus network
    I. Granule Cells
    II. Mossy Cells
    III. Inhibitory interneurons
III. Inhibitory/excitatory balance and disease
    I. Selective ablation of Mossy Cells

Part II

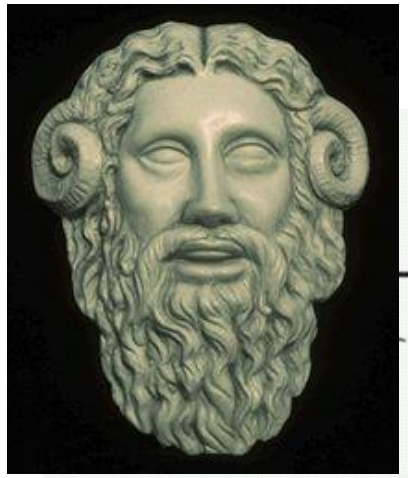
IV. CA3
    I. Granule cell mossy fibers projections to CA3
        I. Mossy fibers, Fliopodial extentions, *en passant* boutons
        II. Ultrastructure of mossy fibers
    II. Functional Mossy Fiber inputs to CA3
    III. Influence of spine shape and synapse location on dendritic integration
    IV. CA3 Afferents and Efferents
V. CA1
    I. CA1 Afferents
    II. Inhibitory interneurons
    III. CA1 Efferents/Subiculum
VI. The « tri » synaptic hippocampal circuit and the entorhinal cortex
VII. CA2

III. Modern anatomical techniques

I. Monosynaptic tracing with glycoprotein–deleted rabies viruses

The « trisynaptic » hippocampal circuit

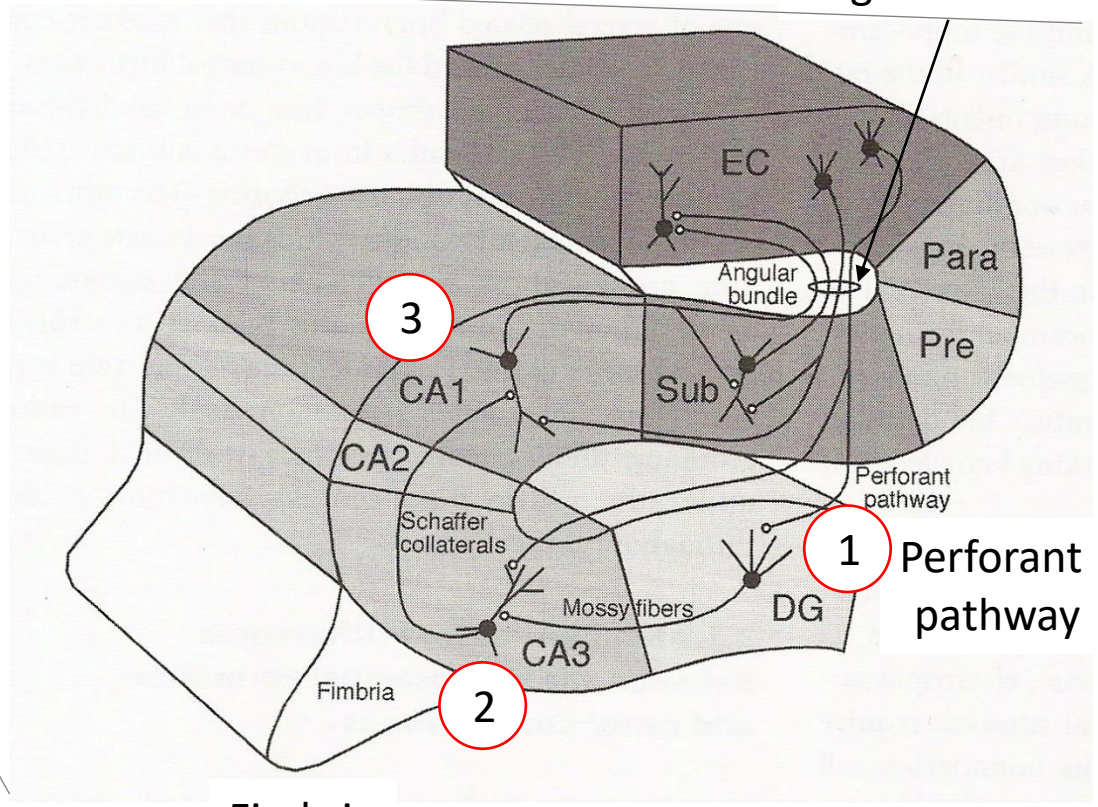
Cornu Ammonis or Ammon's horn



CA3
(Cornu Ammonis 3)

DG = dente gyrus
(named after toothed appearance of this structure)

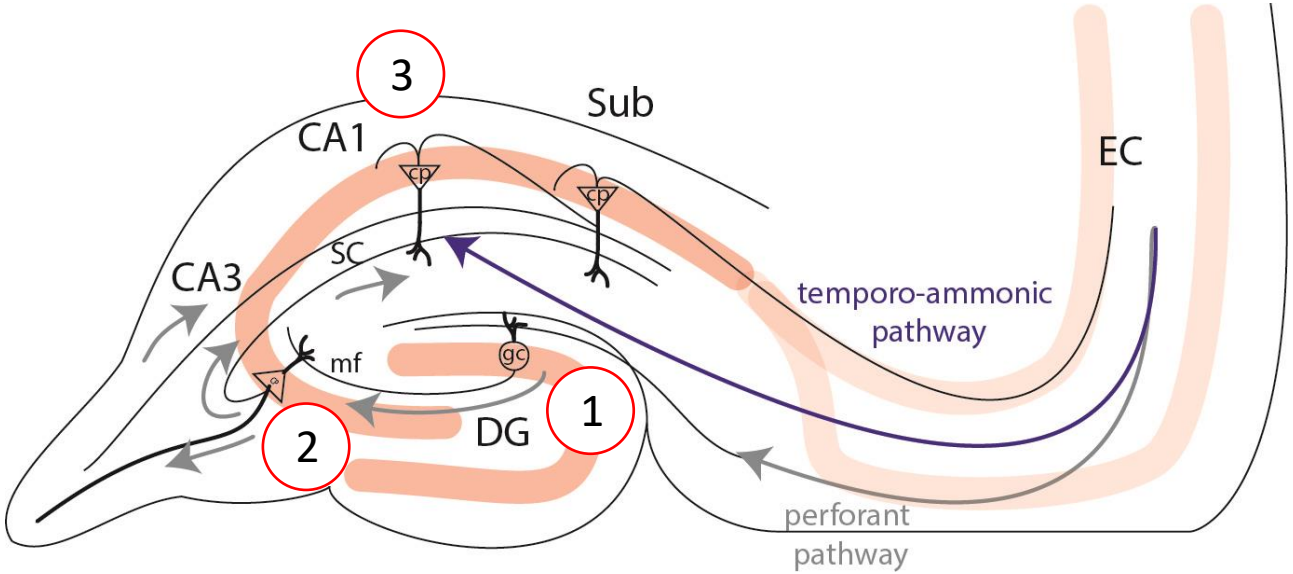
Angular bundle

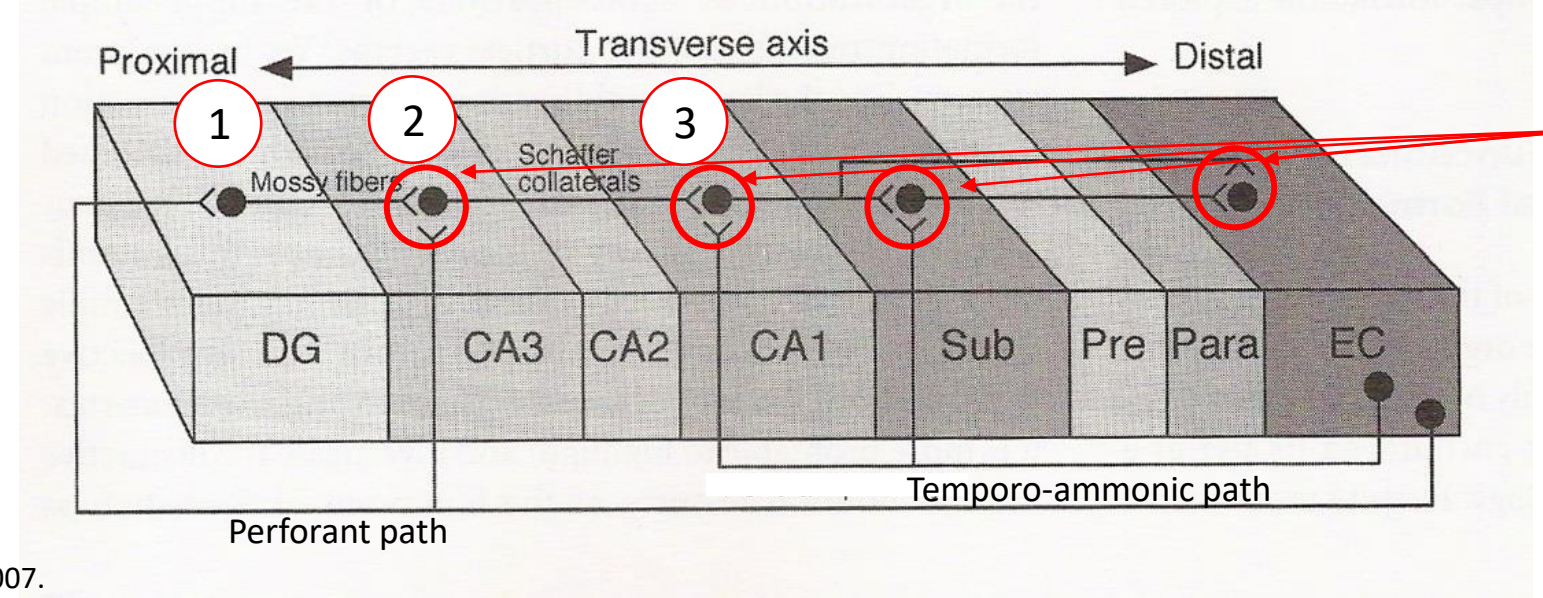


Perforant pathway

Fimbria

Convergence of inputs in the « trisynaptic » hippocampal circuit



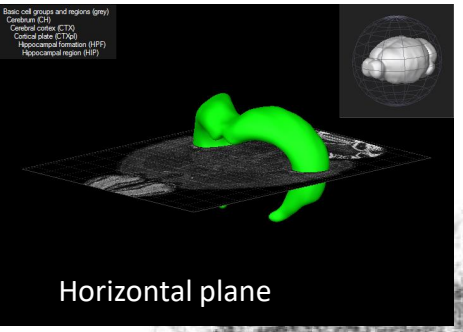


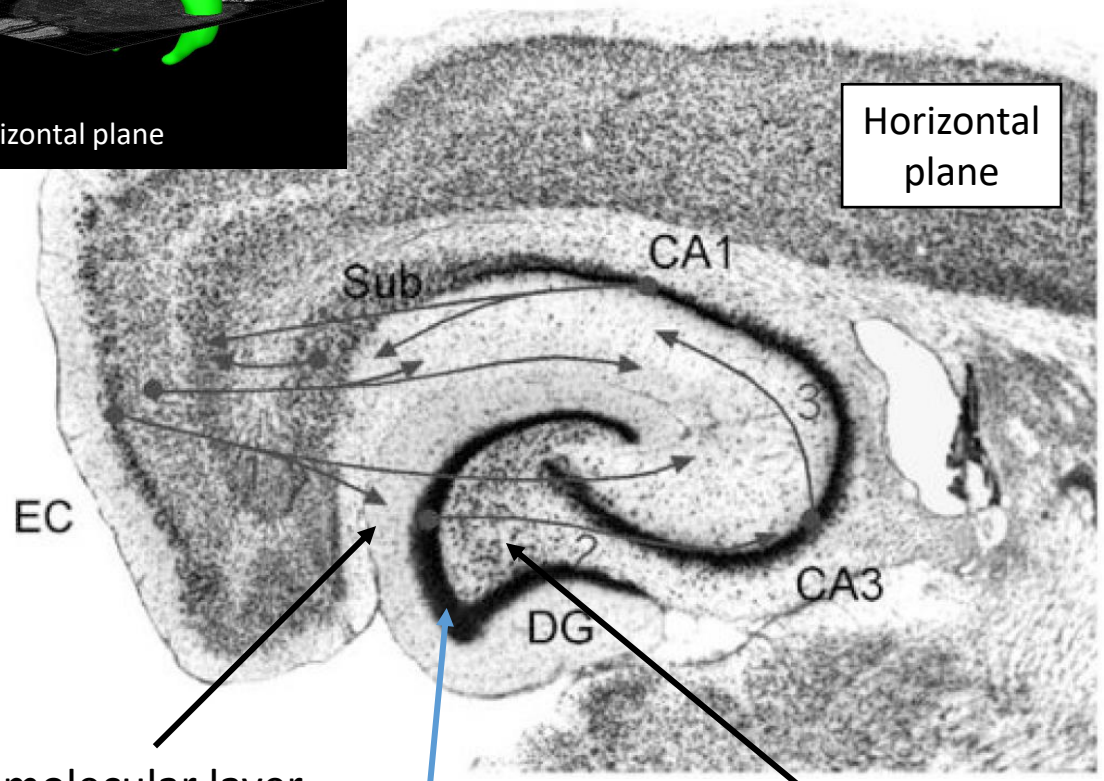
Divergence and convergence of information at several points in the trisynaptic circuit.

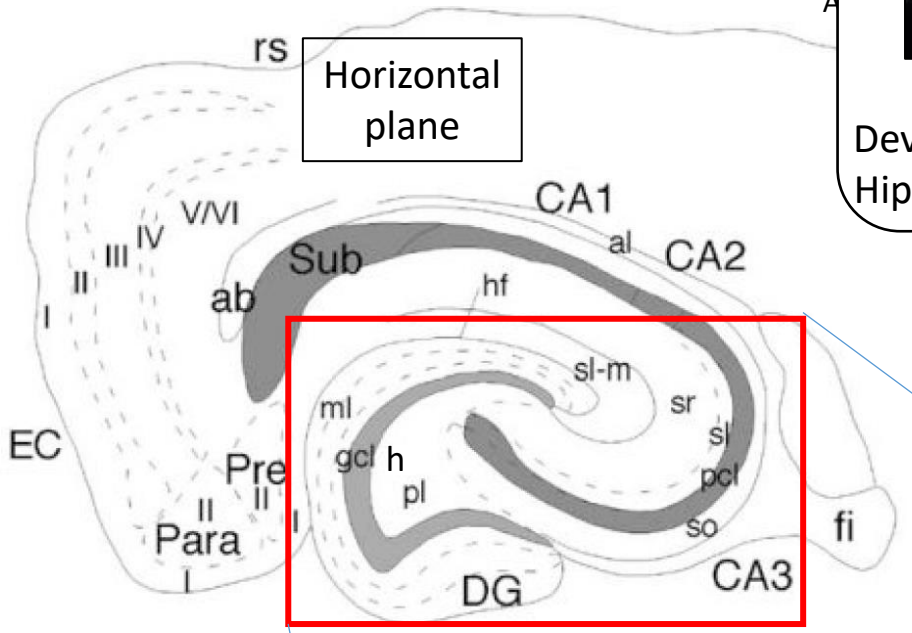
EC = Entorhinal Cortex
Sub = subiculum
Pre = Presubiculum
Para = Parasubiculum

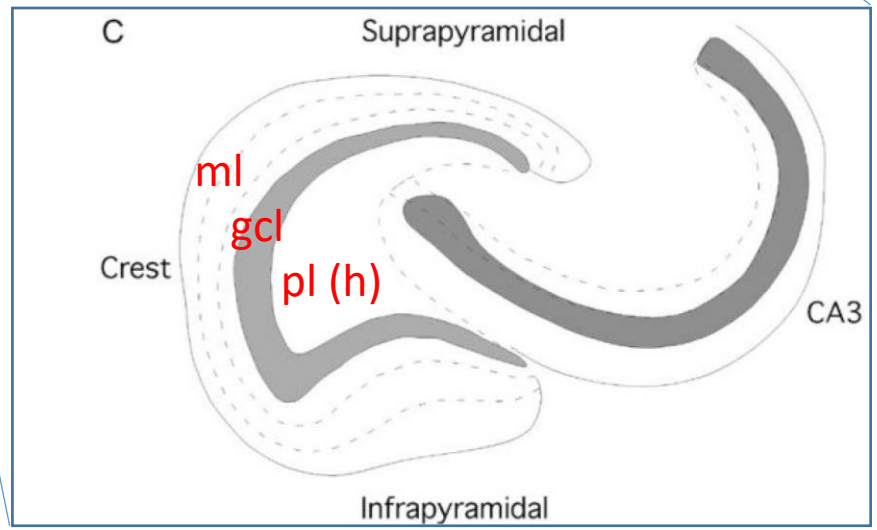
The hippocampus book, 2007.

The layers of the hippocampal formation





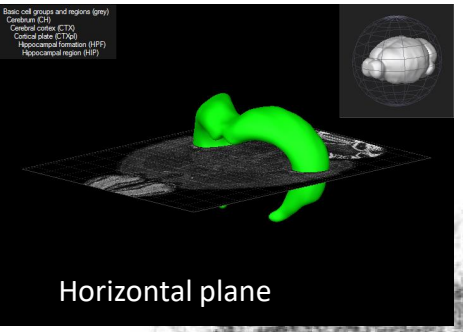


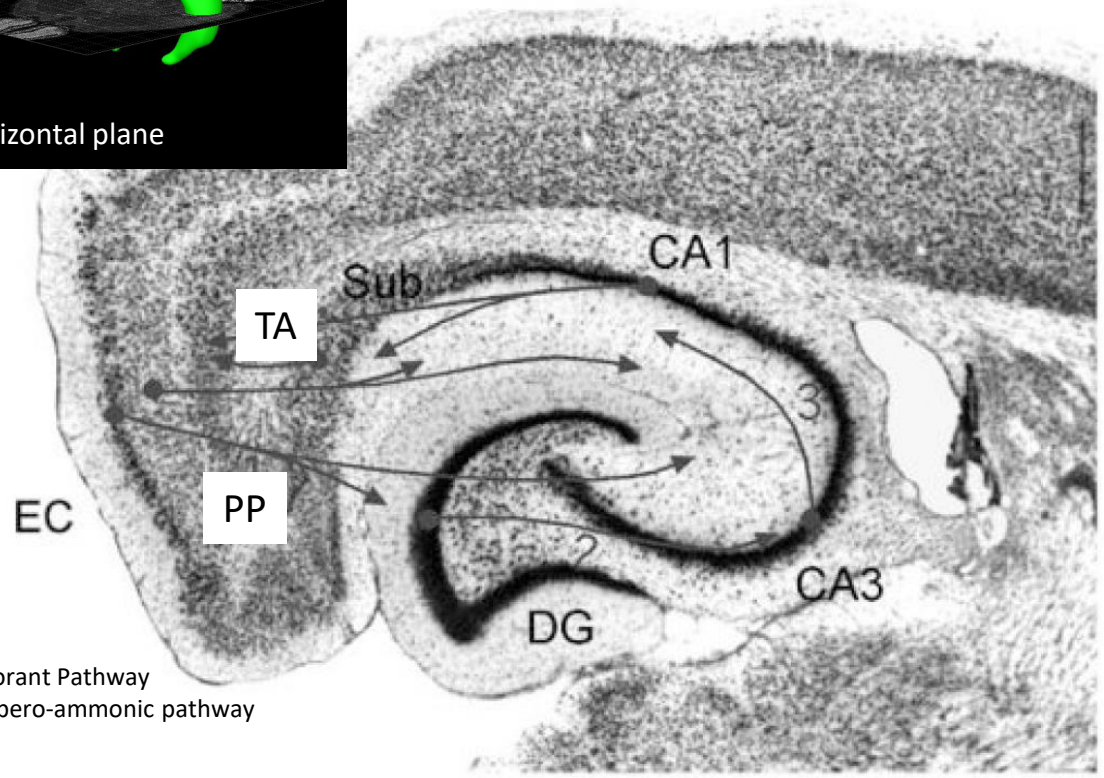


Fiona Francis
Development of the Hippocampal cortex

The hippocampus and dente gyrus form a **3 layered cortex**, as opposed to the 6 layer neocortex. For this reason the hippocampus is referred to as a paleocortex or archicortex (meaning “old cortex”) or allocortex.

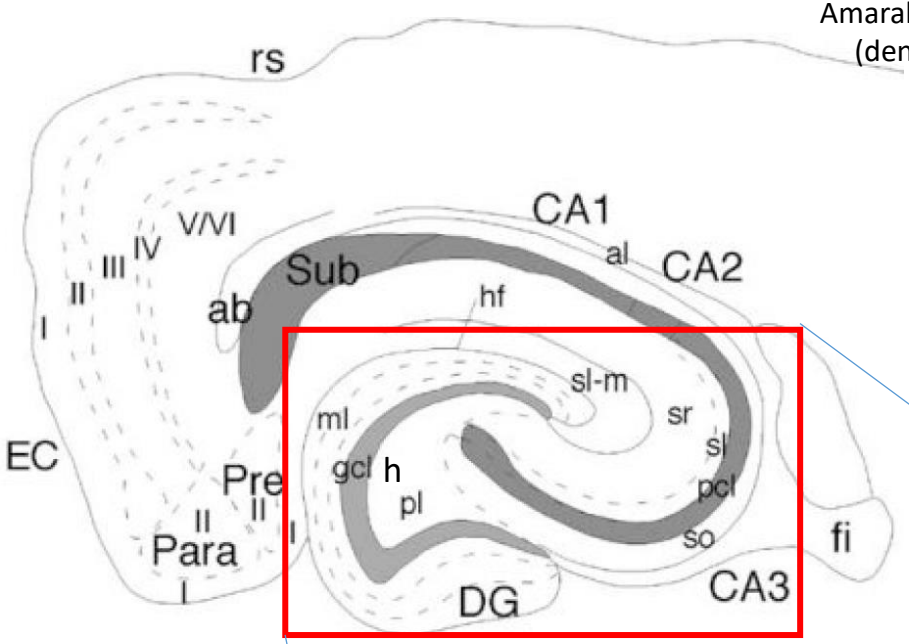
Stratum (layers) of the hippocampal formation

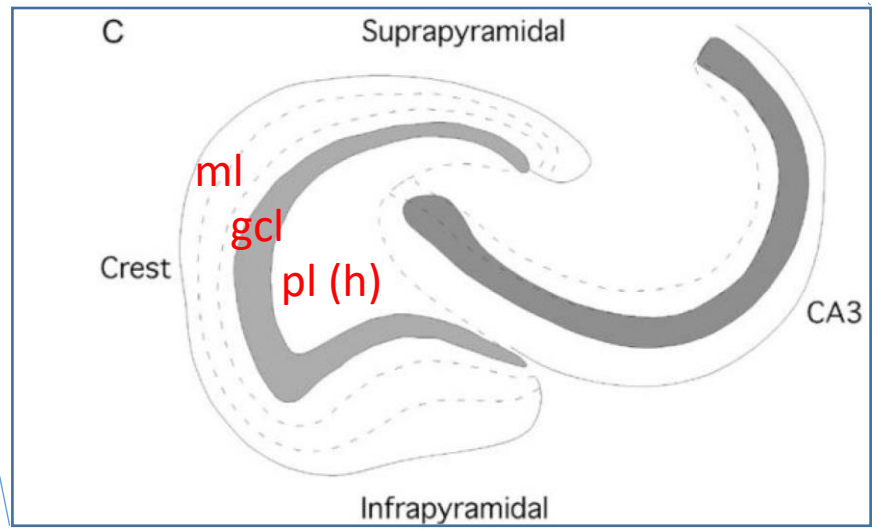




PP-Perforant Pathway
TA-Tempero-ammonic pathway

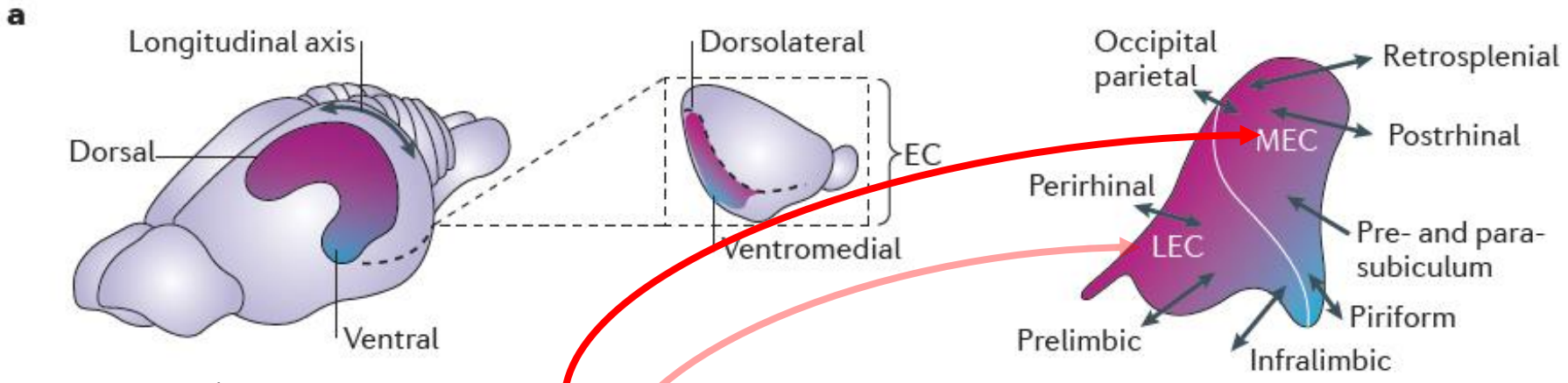
Amaral et al. Prog Brain Res. 2007
(dentate gyrus for dummies)



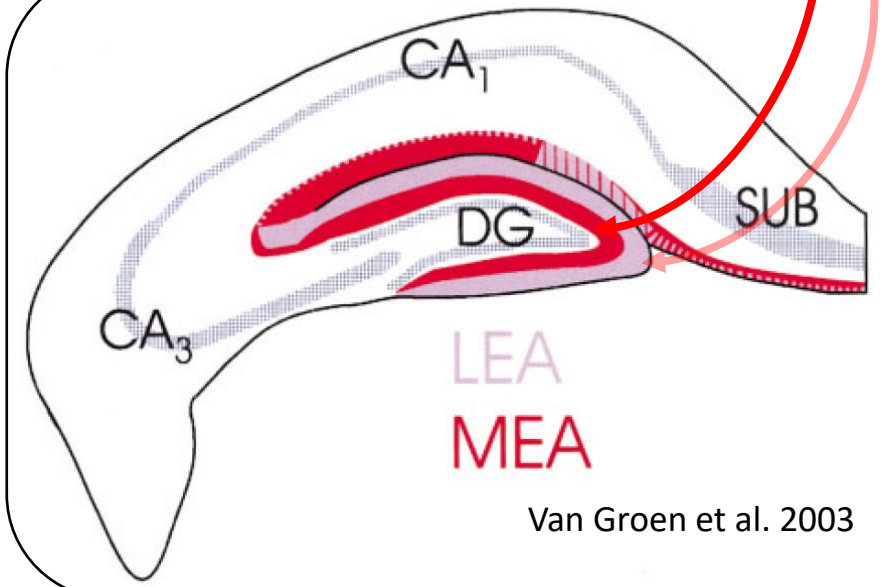


layers	**Denta gyrus layers**	**CA3 cell layers**	**CA1 cell layers**
I	ml = molecular	Al = alveus	Al
II	gcl = granule cell	So = stratum oriens	so
III	pl = polymorphic (hilus)	Pcl =pyramidal cell	pcl
IV		Sl =stratum lucidum	sr
V		Sr = stratum radiatum	Sl.m.
VI		Sl.m. = lacunosum-moleculare	

Topographic projections from Entorhinal cortex to the Hippocampus project to inner- and outer-molecular layers of the dentate gyrus



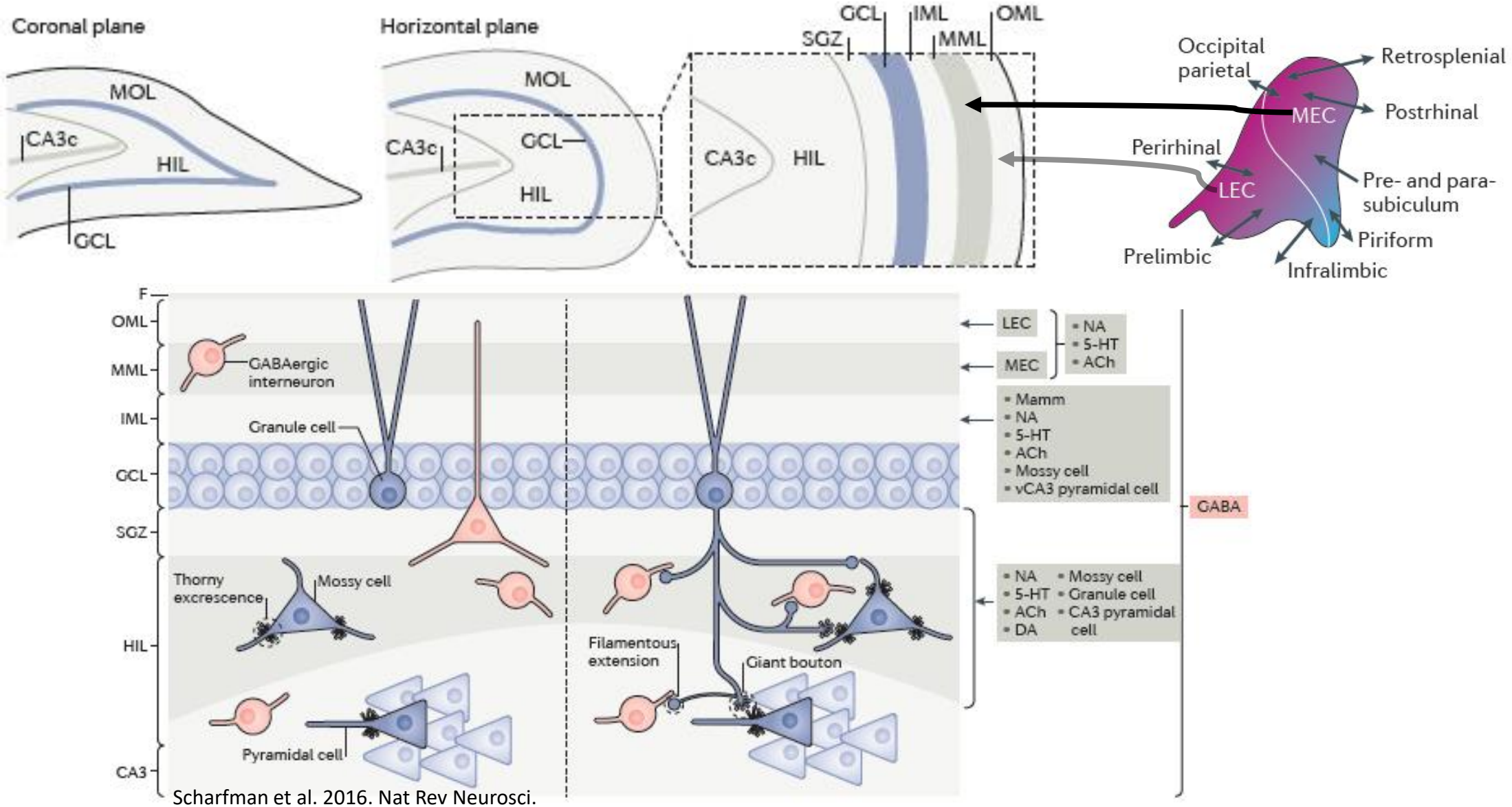
Strange et al. 2014, Nat. Neuro. Rev.



Van Groen et al. 2003

Schematic drawing of a coronal section through the septal part of the hippocampal formation to demonstrate the position of labeled axons following injections of anterogradely transported tracers injected into the entorhinal cortex. LEA (lateral entorhinal area) terminals are indicated in purple, whereas MEA (medial entorhinal area) terminals are indicated in red. Note that in the DG projections from the EC are restricted to the molecular layer.

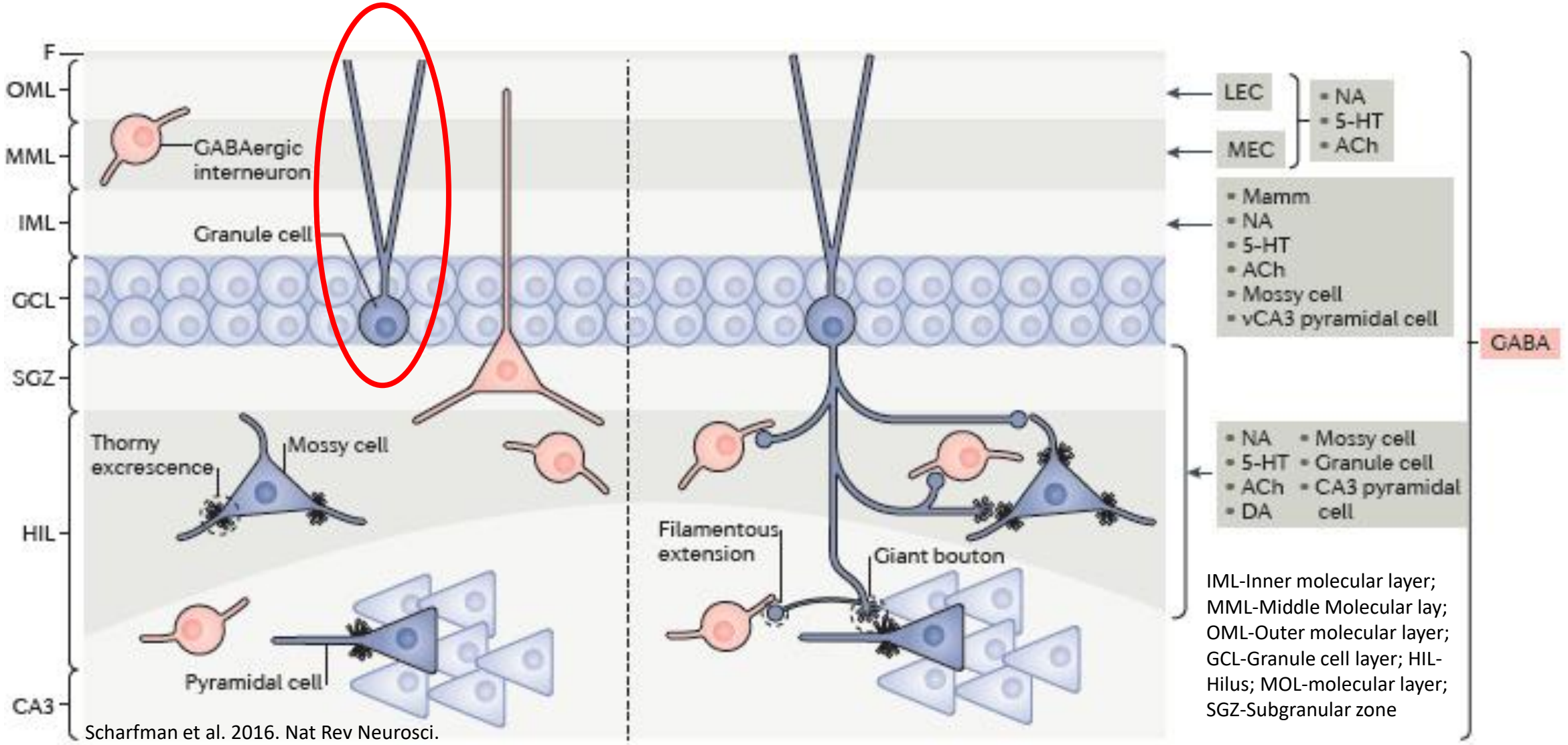
Dentate gyrus neuronal network



Scharfman et al. 2016. Nat Rev Neurosci.

IML-Inner molecular layer; MML-Middle Molecular layer; OML-Outer molecular layer; GCL-Granule cell layer; HIL-Hilus; MOL-molecular layer; SGZ-Subgranular zone

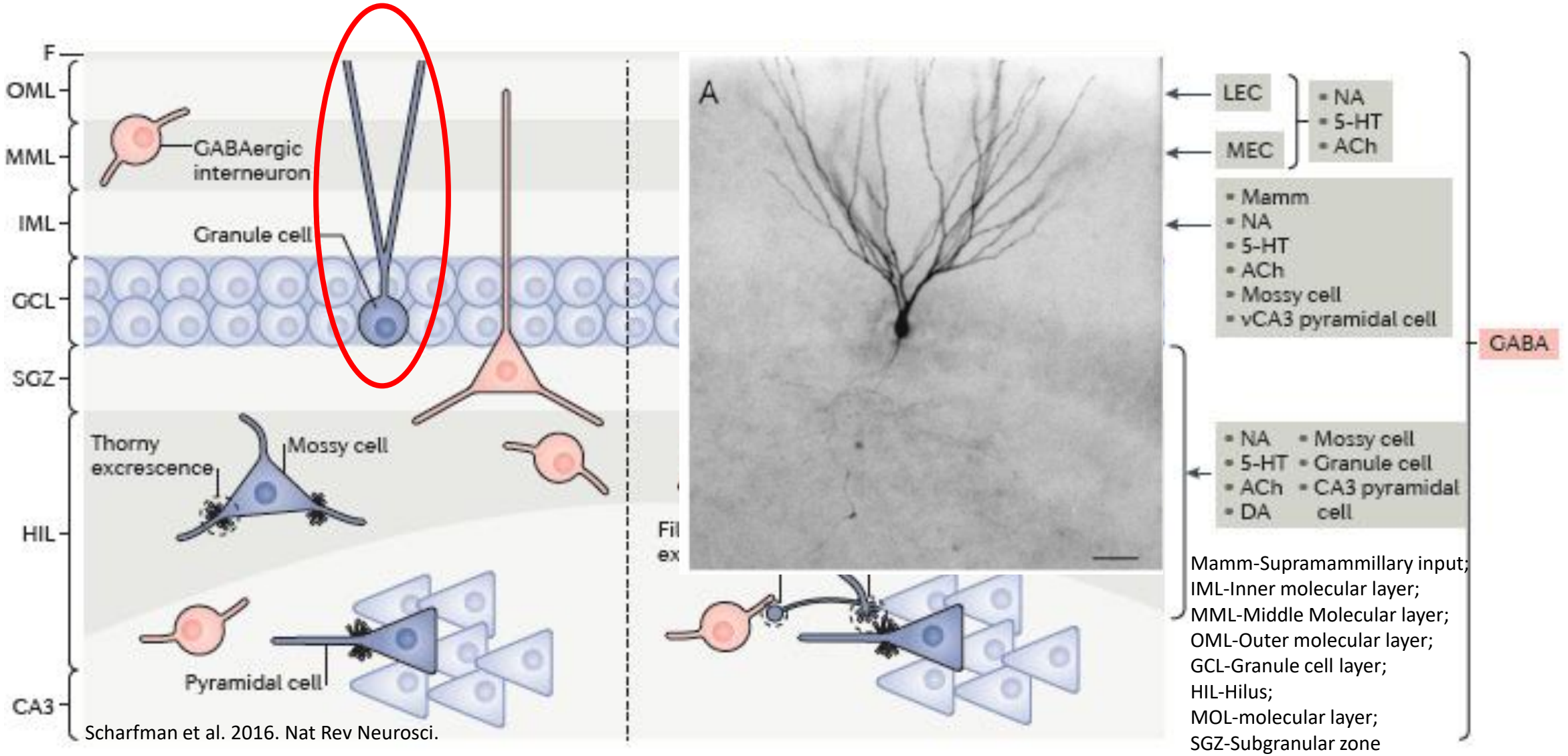
Glutamatergic neurons of the dentate gyrus : Granule cells



Scharfman et al. 2016. Nat Rev Neurosci.

The dentate gyrus network contains 3 main cell types: **Granule Cells**, **Mossy Cells**, and **Inhibitory interneurons**

Glutamatergic neurons of the dentate gyrus : Granule cells



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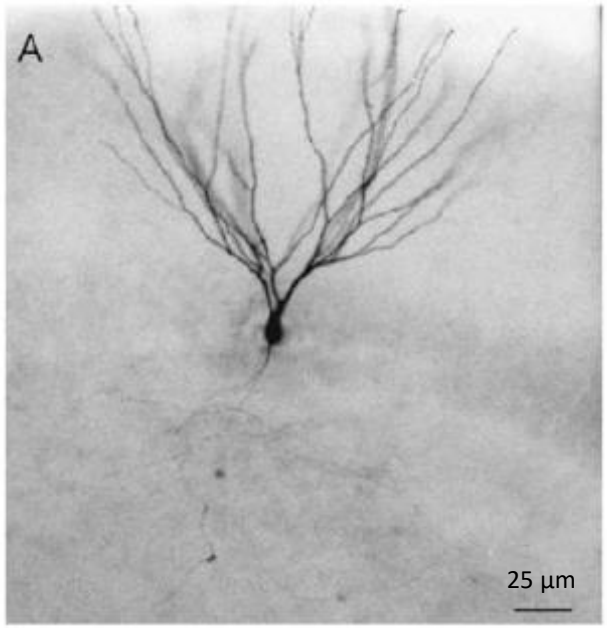
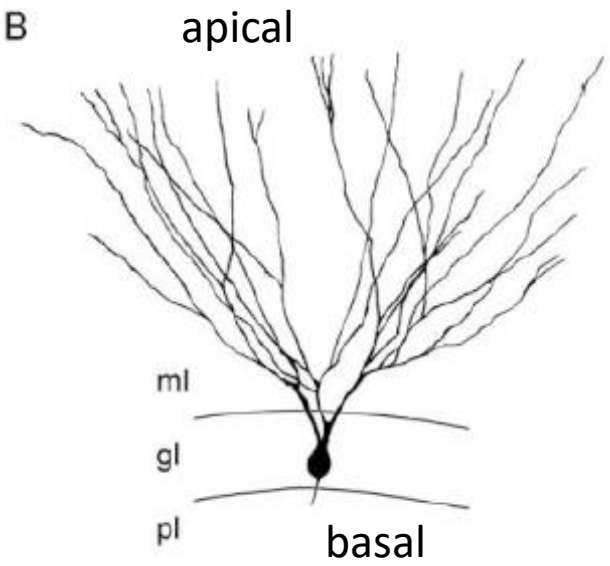
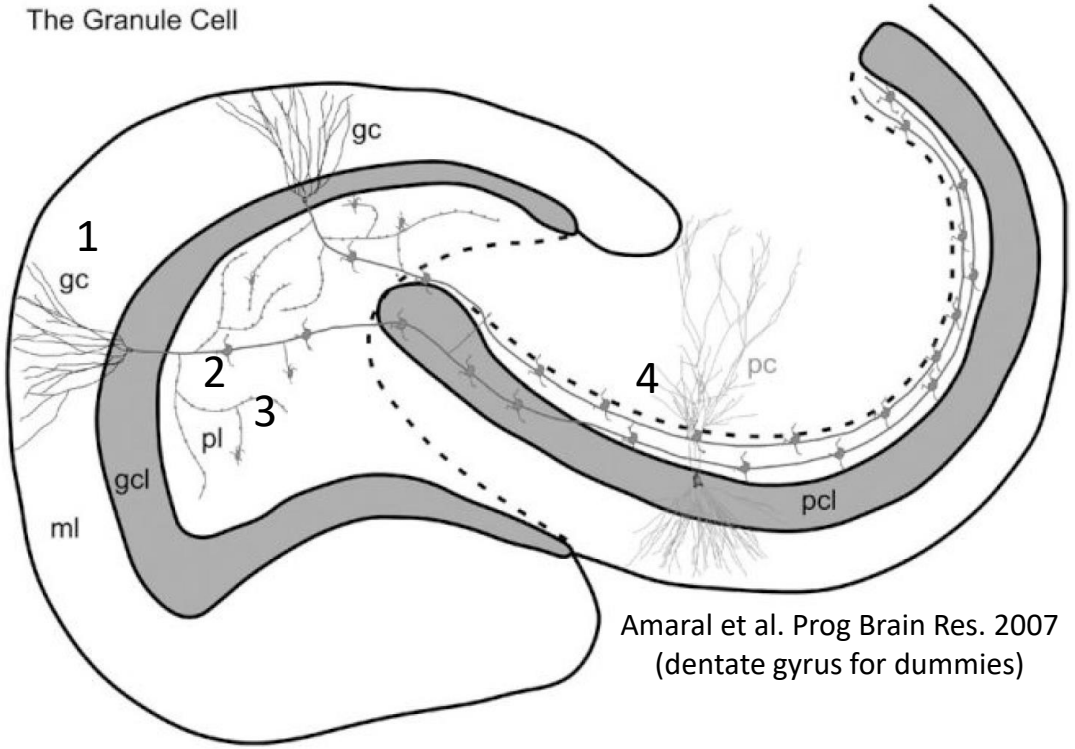


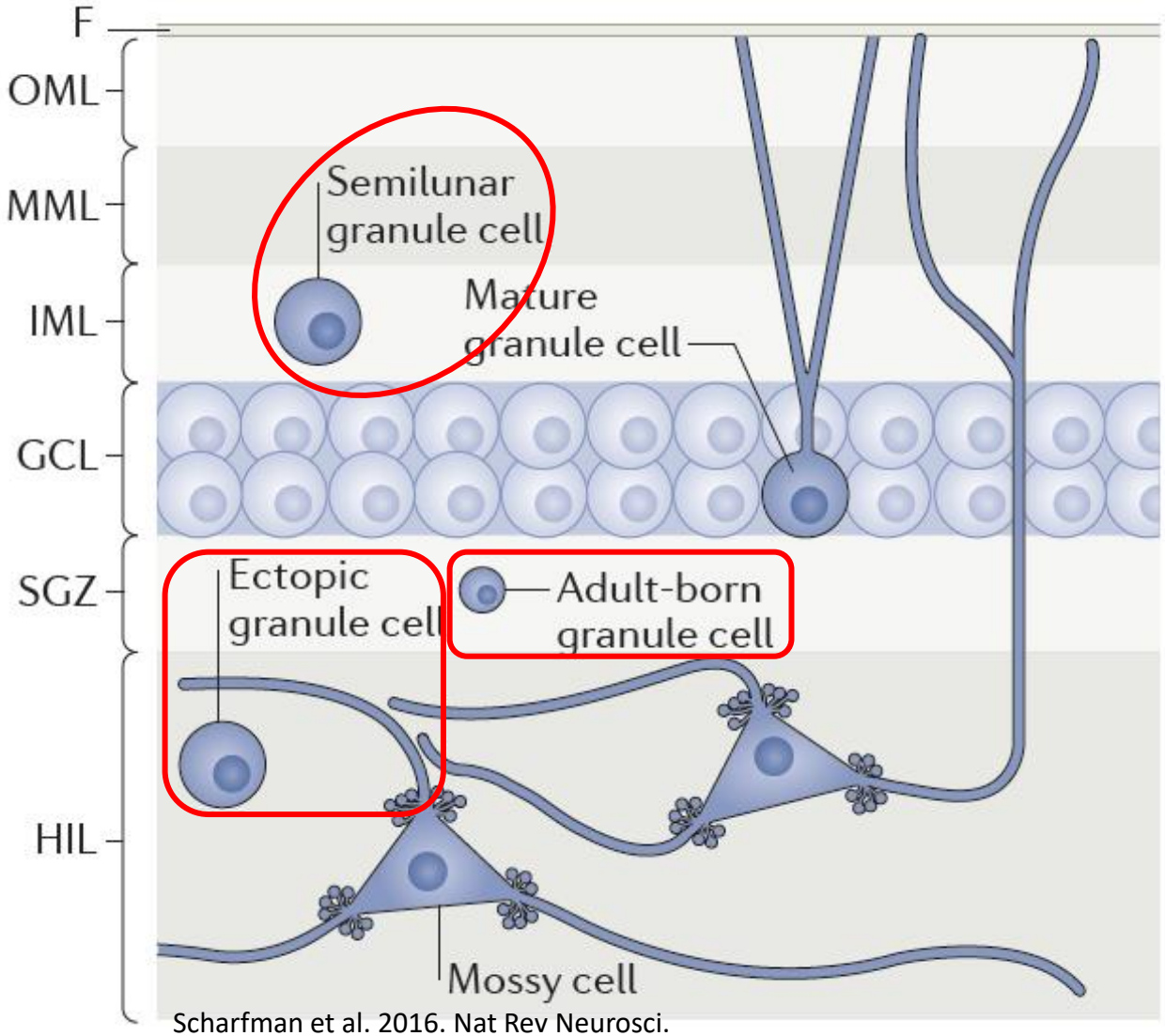




Amaral et al. Prog Brain Res. 2007
(dentate gyrus for dummies)

1. The dendrites of GCs project into the molecular cell layer from the apical surface of the cell body and receives extensive excitatory glutamatergic inputs from the entorhinal cortex **Perforant path** projections. GCs axons are referred to as "**mossy fibers**" as they often display a unique morphology in which axon terminals form multiple synaptic contacts with complex dendritic spines of **mossy cells** in the **DG** and **pyramidal neurons** in **CA3**.
2. Some of the larger mossy fibers in the polymorphic cell layer (pl) synapse onto the proximal dendrites of **mossy cells**.
3. The GC axon branches profusely in the hilus (polymorphic cell layer) mostly onto **inhibitory interneurons**
4. Mossy fibers extend into the stratum lucidum of CA3 where they form synapses on the proximal dendrites of **CA3 pyramidal neurons** and **inhibitory interneurons**

Glutamatergic neurons of the dentate gyrus : Atypical granule cells



Scharfman et al. 2016. Nat Rev Neurosci.

Semilunar granule cells:

-Receive glutamatergic inputs from the EC

-Innervate (glutamtergic) GCs and mossy cells

-Receive glutamatergic inputs from mossy cells, which may create "reverberatory circuits" through reciprocal excitation.

Ectopic granule cells:

A small percentage of GCs are located in the hilus, their function and connectivity is poorly understood.

Adult born granule cells:

"Adult neurogenesis". Stem cells located in the sub granular zone give rise to progenitors cells that migrate to the GC layer and differentiate into GC neurons and glia throughout the adult stages of life.

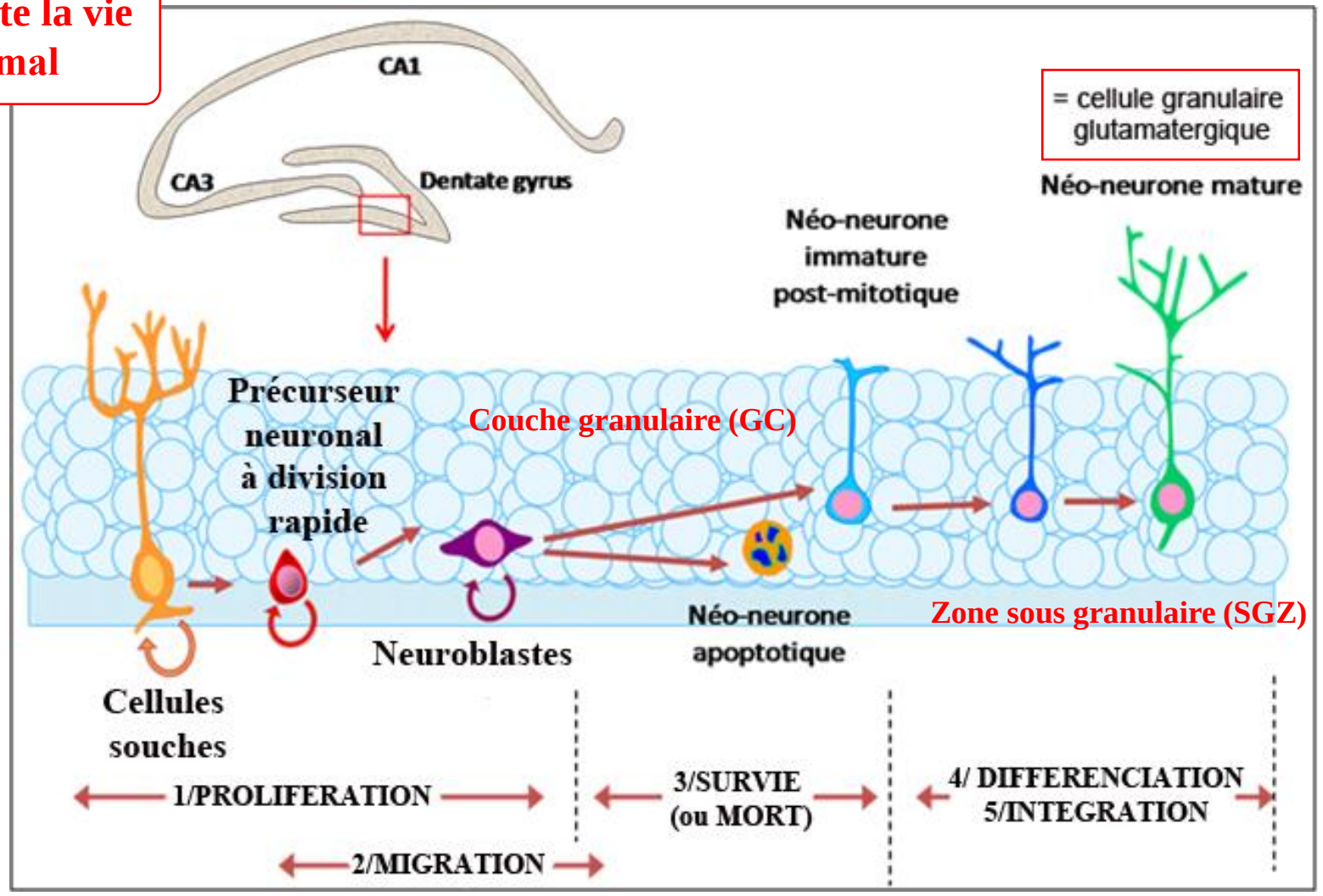
Neurogenesis in the adult hippocampus

Phénomène continu pendant toute la vie de l'animal

Serotonin increases neurogenesis

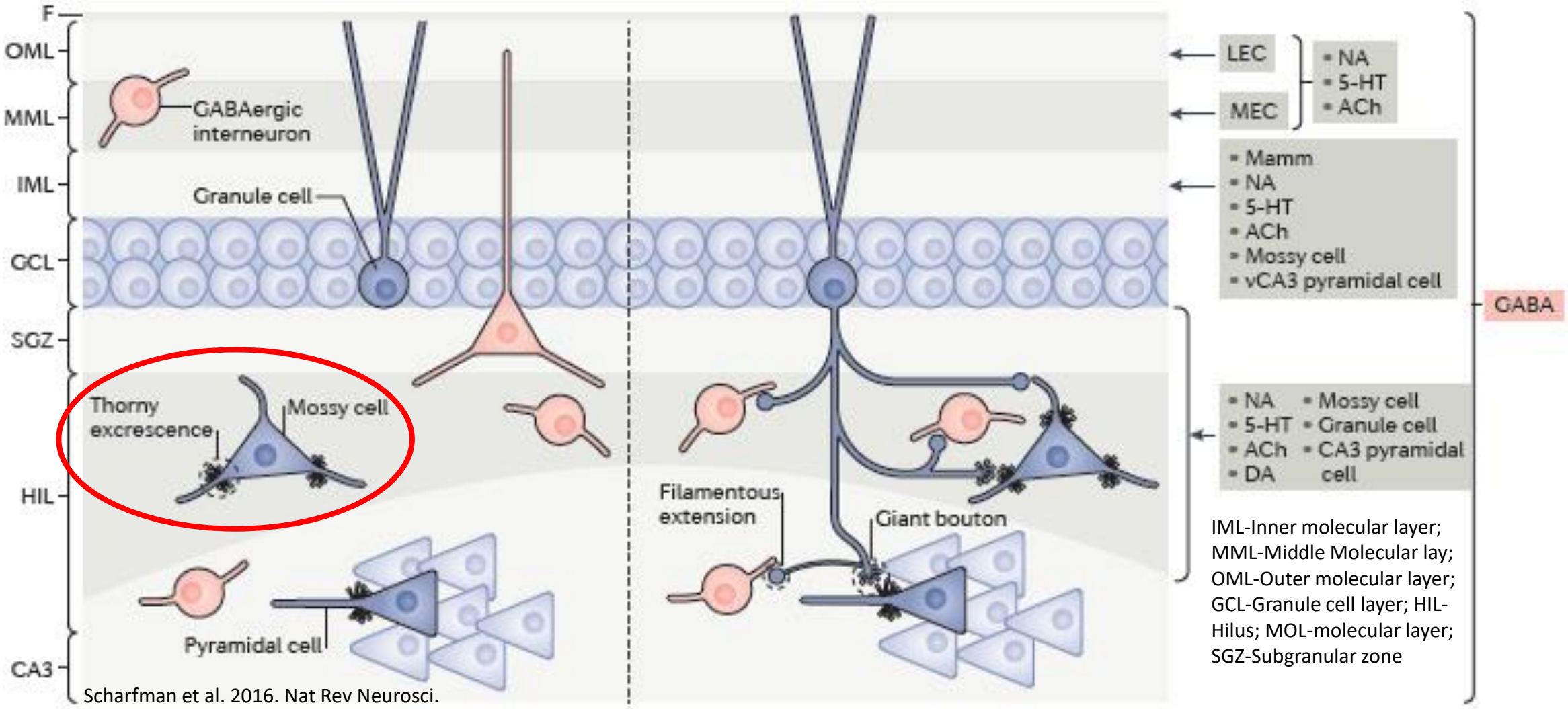
5000 à 10000 nouveaux neurones / jour.

60 % disparaissent dans les 1ères semaines



Période critique au cours de laquelle la survie peut être favorisée par l'apprentissage, l'enrichissement sensoriel, etc…

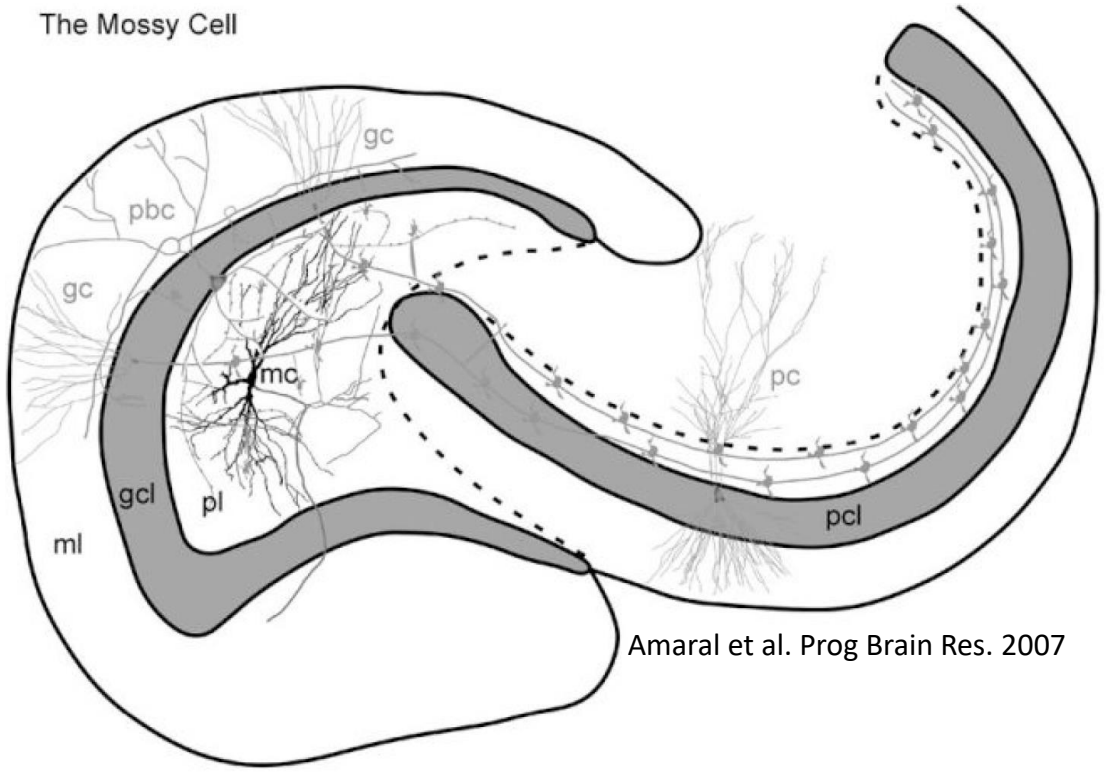
Glutamatergic neurons of the dentate gyrus : Mossy cells



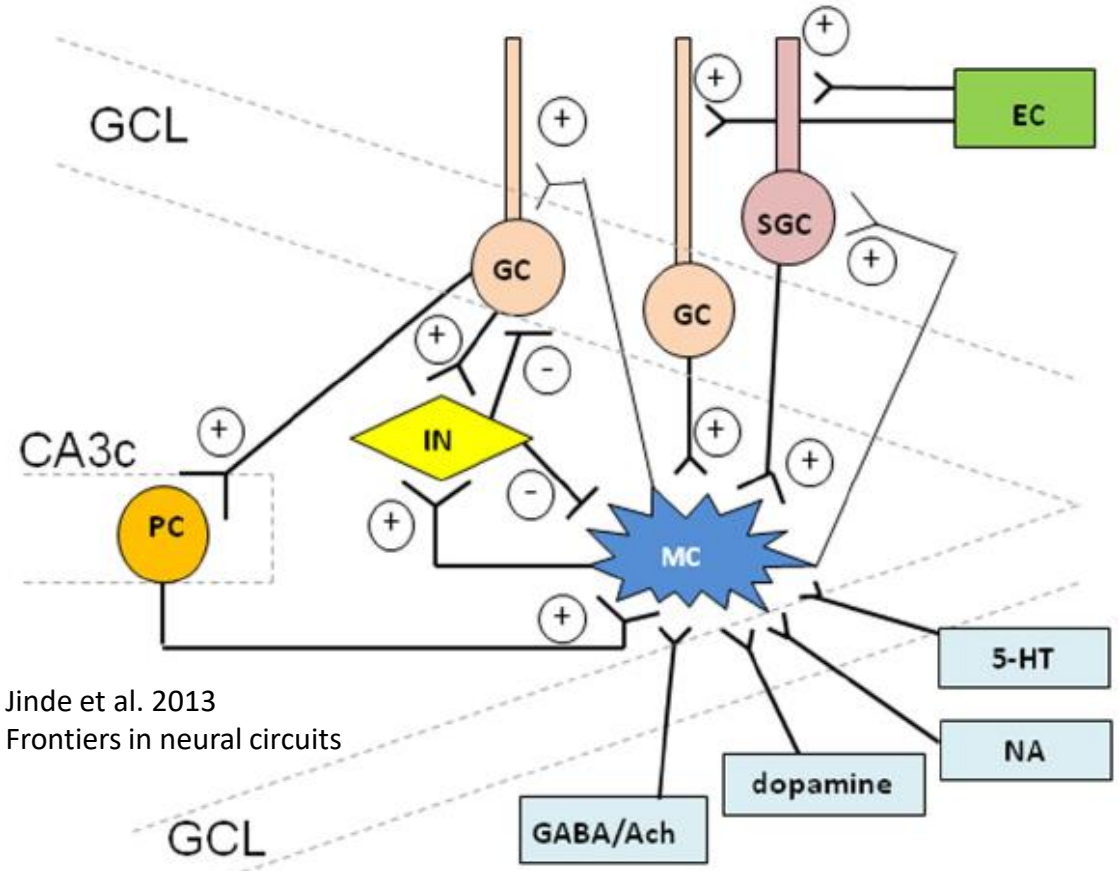
Scharfman et al. 2016. Nat Rev Neurosci.

Mossy Cells are located in the *hilus (polymorphic cell layer)* and display large spine complexes on the proximal dendrites called **thorny excrescences.** Granule cell axons form large complex presynaptic glutamatergic terminals, **called Mossy fibers**, with Mossy cell thorny excrescences. The degree to which mossy cells have thorny excrescences varies across species.

Glutamatergic neurons of the dentate gyrus : Mossy cells



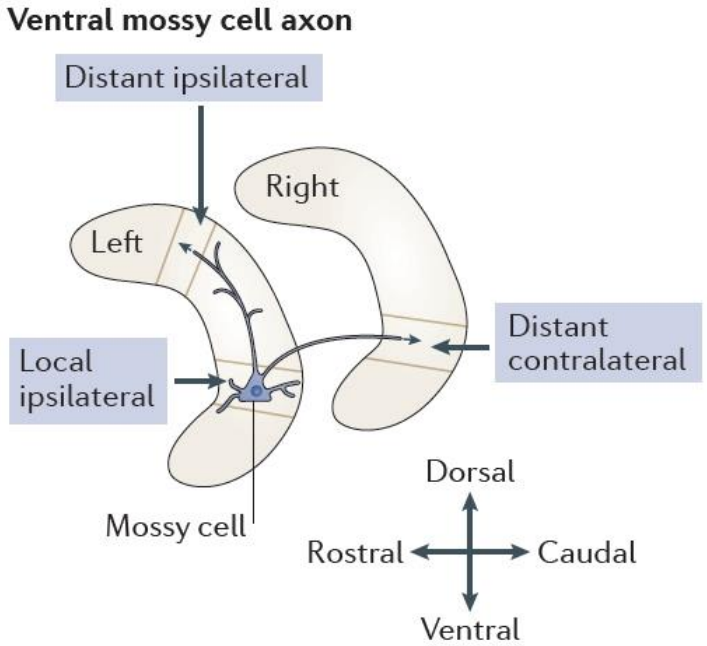
Amaral et al. Prog Brain Res. 2007

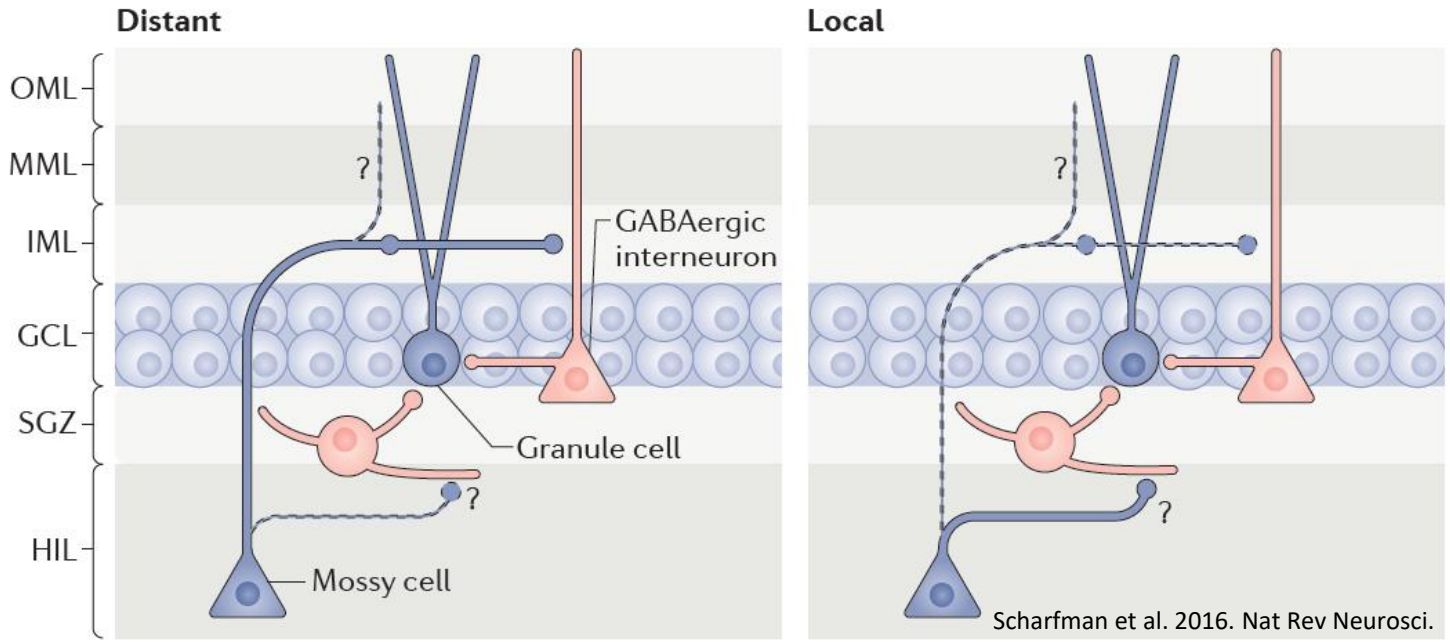


Jinde et al. 2013
Frontiers in neural circuits

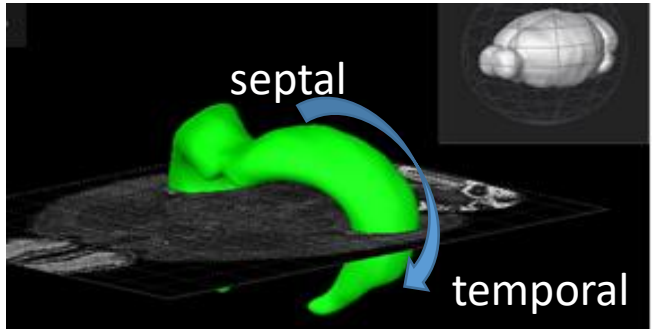
- From the cell body arises 3 or more thick dendrites covered in clusters of large complex spines (**thorny excrescences**), with 1 or 2 bifurcations. The dendrites branch a few times remaining mostly in the polymorphic cell layer (hilus).
- **Mossy cells** receive excitatory inputs from granule cell **mossy fibers** on thorny excrescences.
- **Mossy cells also** receive excitatory inputs axon collaterals of CA3 pyramidal neurons **“back projection”.**
- The axon plexus branches in the polymorphic cell layer (hilus) with ipsilateral (associational pathway) and contralateral (commissural pathway) glutamatergic projections to the inner molecular cell layer (mcl).

Mossy cells innervate granule cells and inhibitory interneurons in the septal-temporal axis of the hippocampus





Scharfman et al. 2016. Nat Rev Neurosci.



- 'Distal' or 'intralamellar' mossy cell axonal projections terminate in the IML of both the ipsilateral and contralateral DG.
- Ipsilateral projections innervate up 75 % of the septo-temporal (dorsal-ventral) axis and may therefore relay inputs along the transverse axis of the hippocampus. In rodents, Mossy Cell axons project contralaterally (commissural).
- Distal axonal projections provide glutamatergic inputs to granule cells and inhibitory interneurons.

- -Local Mossy cell collaterals are found in the hilus, MML and OML, where they appear to predominately innervate inhibitory interneurons.

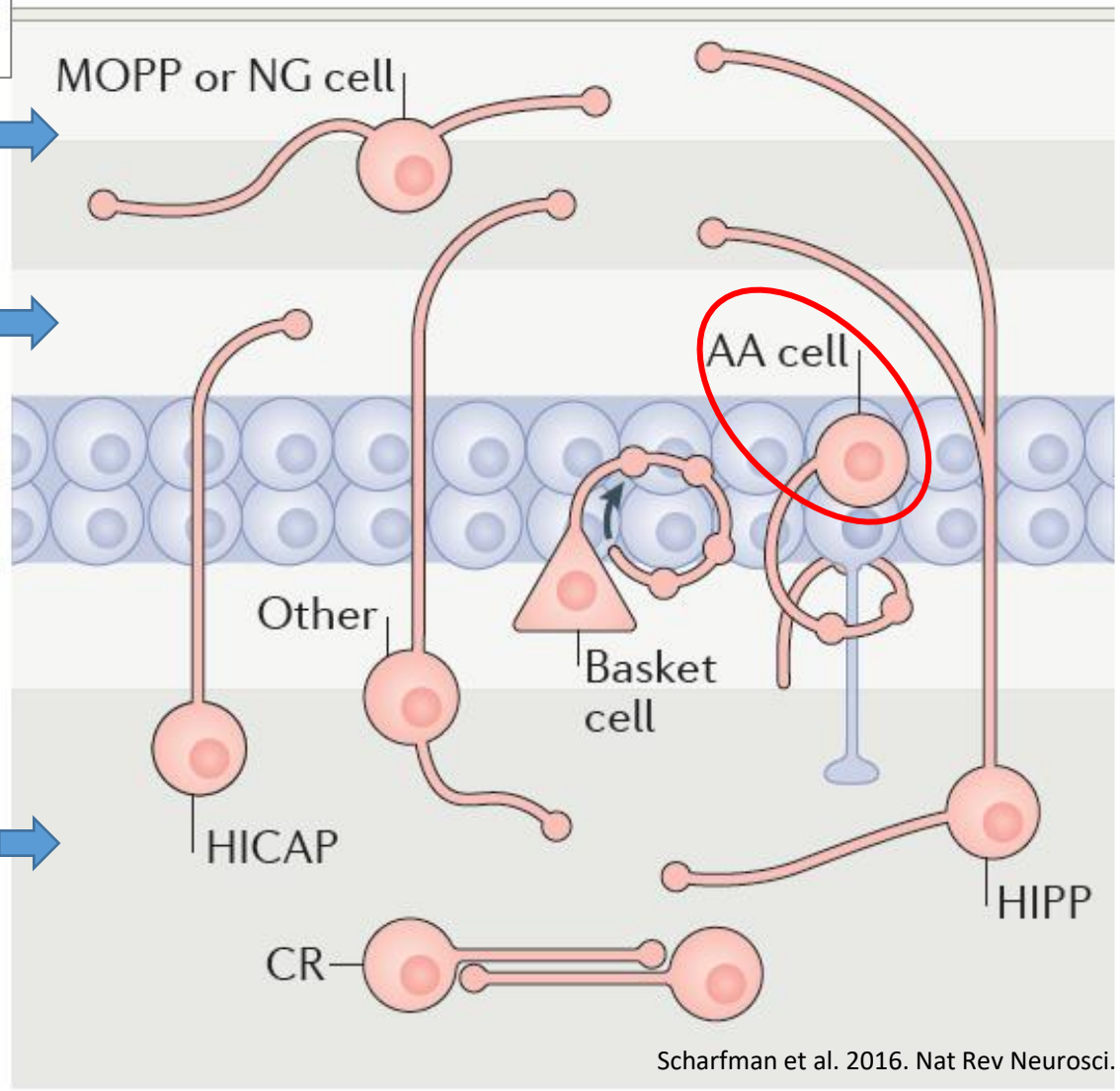
The Dentate Gyrus: GABAergic interneurons

Layer specific inputs

Entorhinal Cortex

Commisural/ Associational afferents

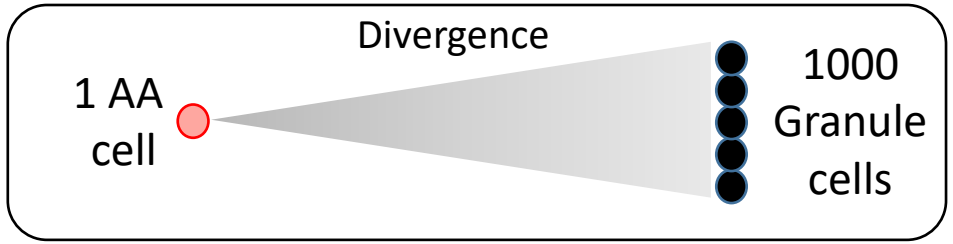
Mossy cell axons



Scharfman et al. 2016. Nat Rev Neurosci.

AA cell - Chandelier or Axo-Axonic cell

- Dendrites in the ml and hilus
- Axons descend from MCL to GCL where they branch profusely and form symmetric (inhibitory) synapses onto the axon initial segments of GCs and may therefore tightly control granule cell firing.
- 1 axon may innervate 1000 granule cells and thus may synchronize granule cell firing



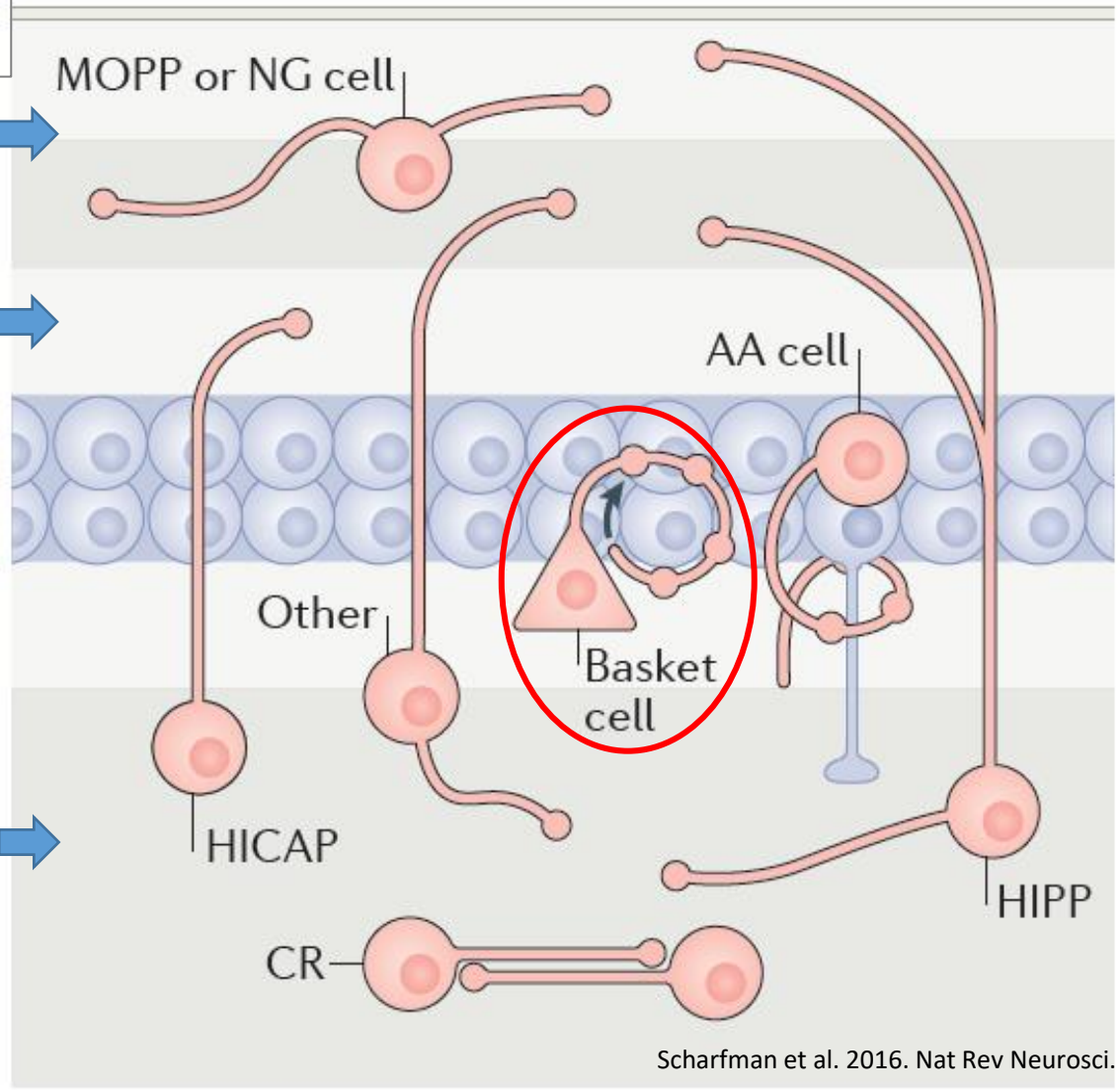
The Dentate Gyrus: GABAergic interneurons

Layer specific inputs

Entorhinal Cortex

Commisural/ Associational afferents

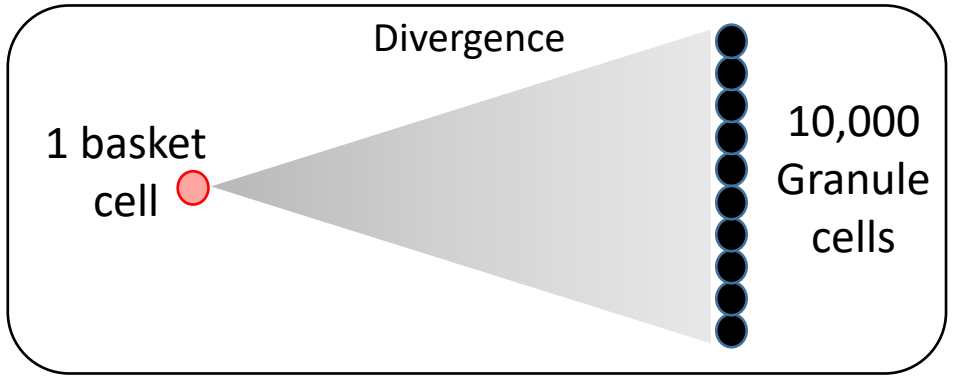
Mossy cell axons



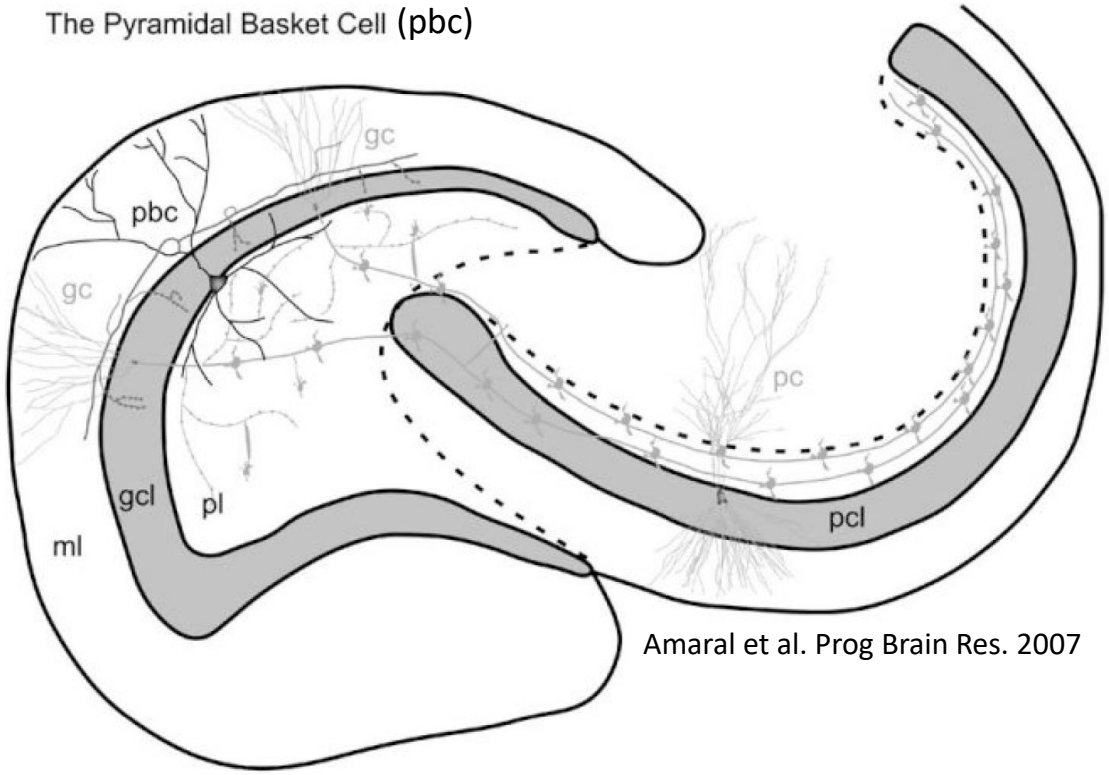
Scharfman et al. 2016. Nat Rev Neurosci.

Pyramidal basket Cell

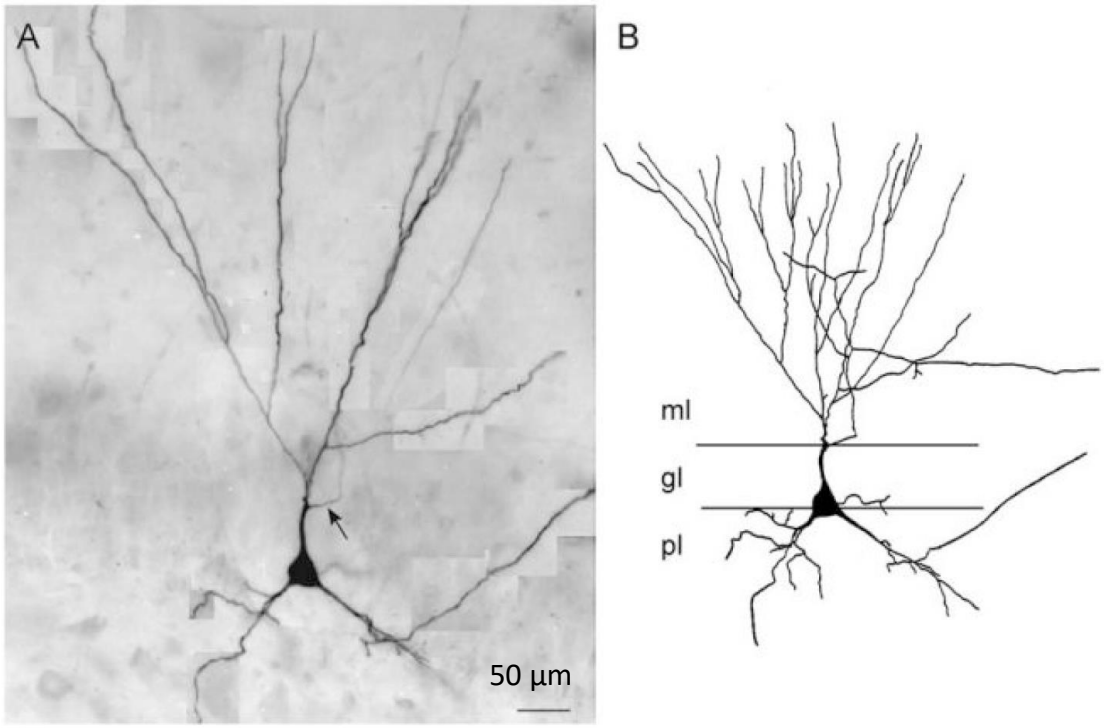
- Pyramidal cell bodies (25-35 uM) are located on the deep surface of the GC layer. Apical dendrites extend into the MC layer and basal dendrites into the hilus (polymorphic layer).
- Axons form a peri-cellular plexus or « basket » on the cell bodies of granule cells.
- The somatic location allows for a powerful inhibitory control of granule cells activity.
- Axons project 900 µM in the transverse axis and 1,500 µM in the septo-temporal axis (innervating 10,000 or 1% of GCs) and therefore may coordinated and synchronize septo-temporal activity.
- In the DG, Parvalbumin expressing basket cells are particularly resistant to excitotoxic events.



The Dentate Gyrus: pyramidal basket cells



Amaral et al. Prog Brain Res. 2007



1. Pyramidal shaped cell bodies (25-35 uM) located on the deep surface of the gcl
2. Apical dendrites extend into the ml and basal dendrites into the hilus (polymorphic layer)
3. GABAergic synapses (symmetric)
4. Axons form a peri-cellular plexus or « basket » on the cell bodies of granule cells. The somatic location allows for a powerful inhibitory control of granule cells activity
5. PBC axons are long. 900 uM in the transverse axis and 1,500 uM in the septo-temporal axis (innervating 10,000 or 1% of gcs)

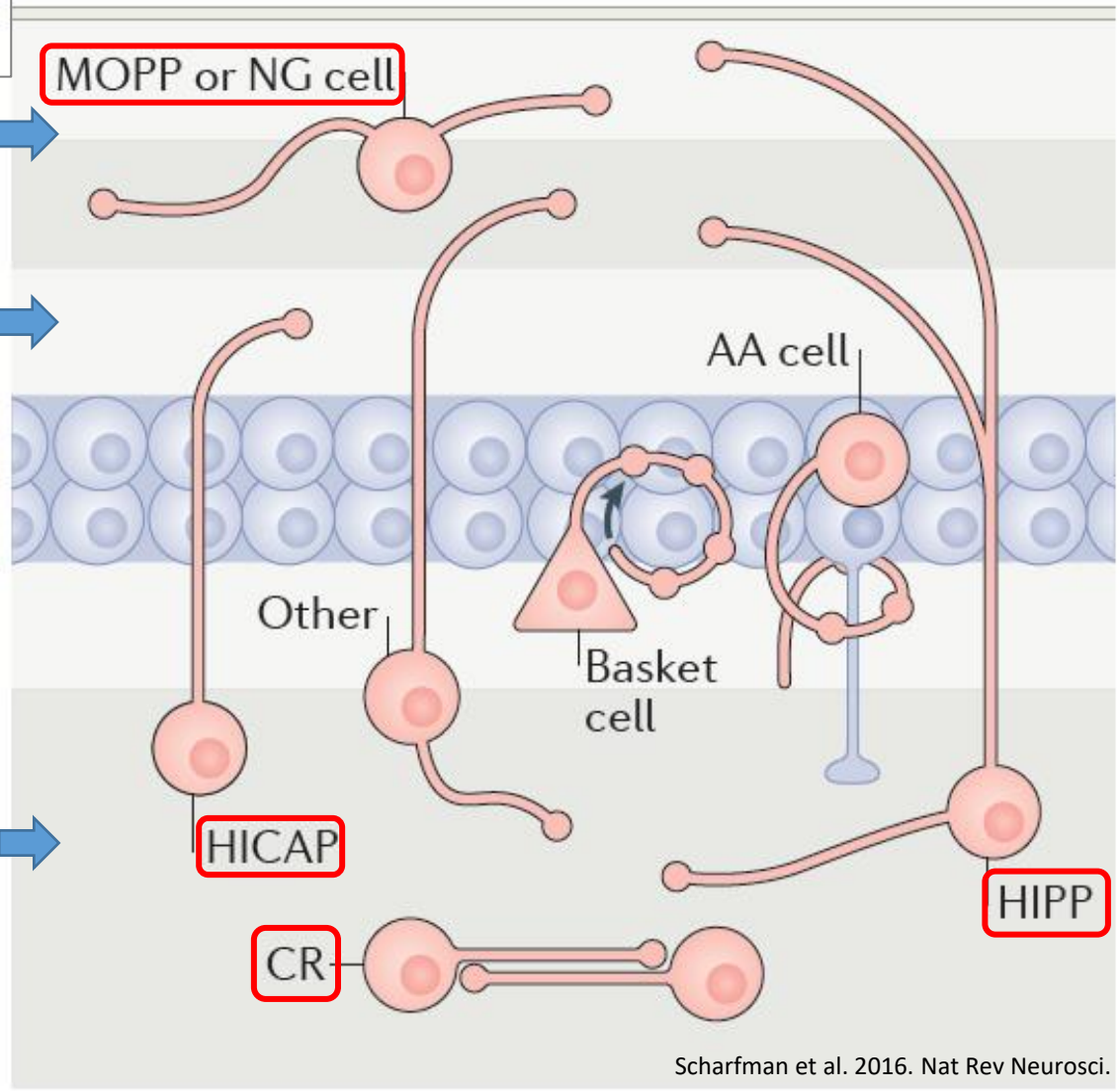
The Dentate Gyrus: GABAergic interneurons

Layer specific inputs

Entorhinal Cortex

Commisural/ Associational afferents

Mossy cell axons



Scharfman et al. 2016. Nat Rev Neurosci.

HICAP (hilar commissural-associatal pathaway)
- Thin aspiny dendrites in the hilus and ml.
- Axons extend through GCL and branch profusely in ML

MOPP (molecular layer perforant path-associated cell)
- NG = neurogliaform cell
- Dendrites remain in the ML.
- Axons remain largely in the outer 2/3 of ml

HIPP (Hilar perforant path-associated cell)
- 2 or 3 principal dendrites running parallel to GC layer.
- Long branched spines on cell body and dendrites
- Axons project to outer 2/3 of ml
- Axons and dendrites extend throughout transversal (septo-temporal) plane of the hippocampus

CR (Calretinin)
- Interneurons that exhibit reciprocal innervation.

Quiz 2:

1) Within the Dentate Gyrus of the Hippocampus perforant pathway efferents from the entorhinal cortex terminate in:

A. The molecular layer
B. The granule cell layer
C. The Hilus
D. The polymorphic cell layer
E. The Hilus and the polymorphic cell layer are the same thing idiot.

2) Mossy cells are:

A. Glutamatergic
B. GABAergic
C. Receive glutamatergic inputs from granule cell mossy fibers onto spines on their proximal dendrites called “thorny excrescences”
D. Project axons that innervate the hippocampus alone the septal/temporal axis of the hippocampus.
E. Project commissural axons that innervate the contralateral hippocampus.

Quiz 2 (page 2):

1) Granule cells of the dentate gyrus:

A. Are glutamatergic

B. Are GABAergic

C. Project axons, referred to as “mossy fibers”, that form giant synapses on the proximal dendrites of mossy cells in the hilus of the dentate gyrus and onto the proximal dendrites of pyramidal neurons in the CA3 layer.

D. Project axons that form synapses with GABAergic basket cells, but no other types on interneurons.

E. In rodents, are generated from stem cells in the sub-granule cell layer throughout the life of the animal.

2) Interneurons of the dentate gyrus are:

A. Glutamatergic

B. GABAergic

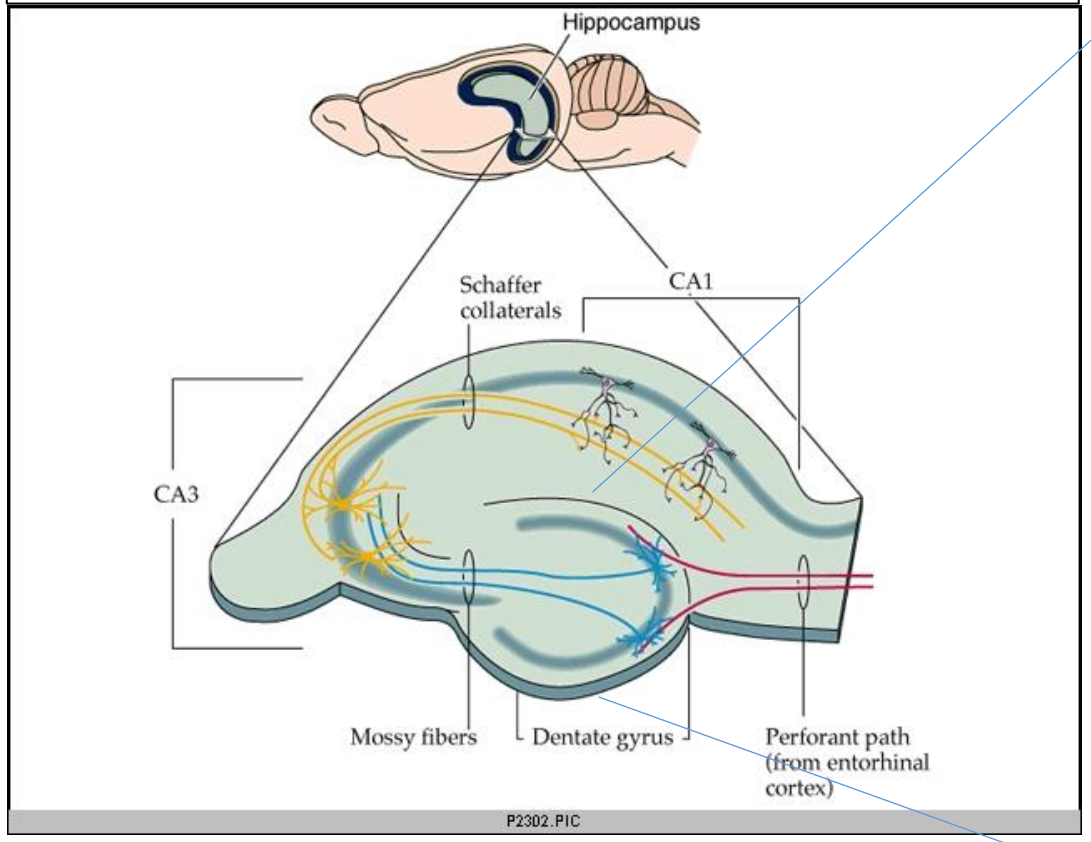
C. Are implicated in the synchronization of activity of the dentate gyrus due to their converging efferent inputs onto granule cells.

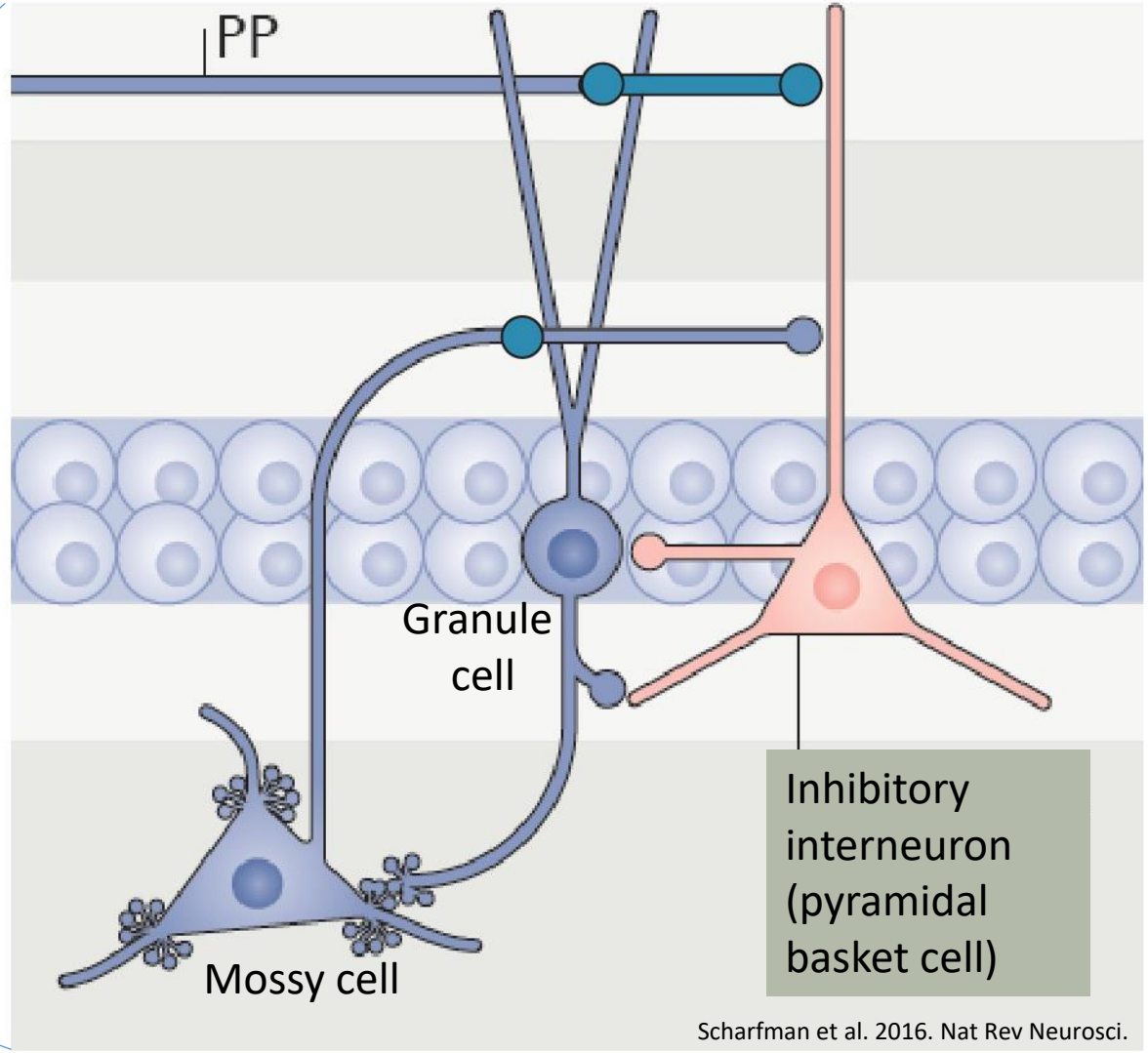
D. Project efferents that inhibit mossy cells.

E. The various subtypes exhibit more or less that same anatomical connections

Functional neuronal circuitry of the Dentate Gyrus

Classical tri-synaptic excitatory circuit

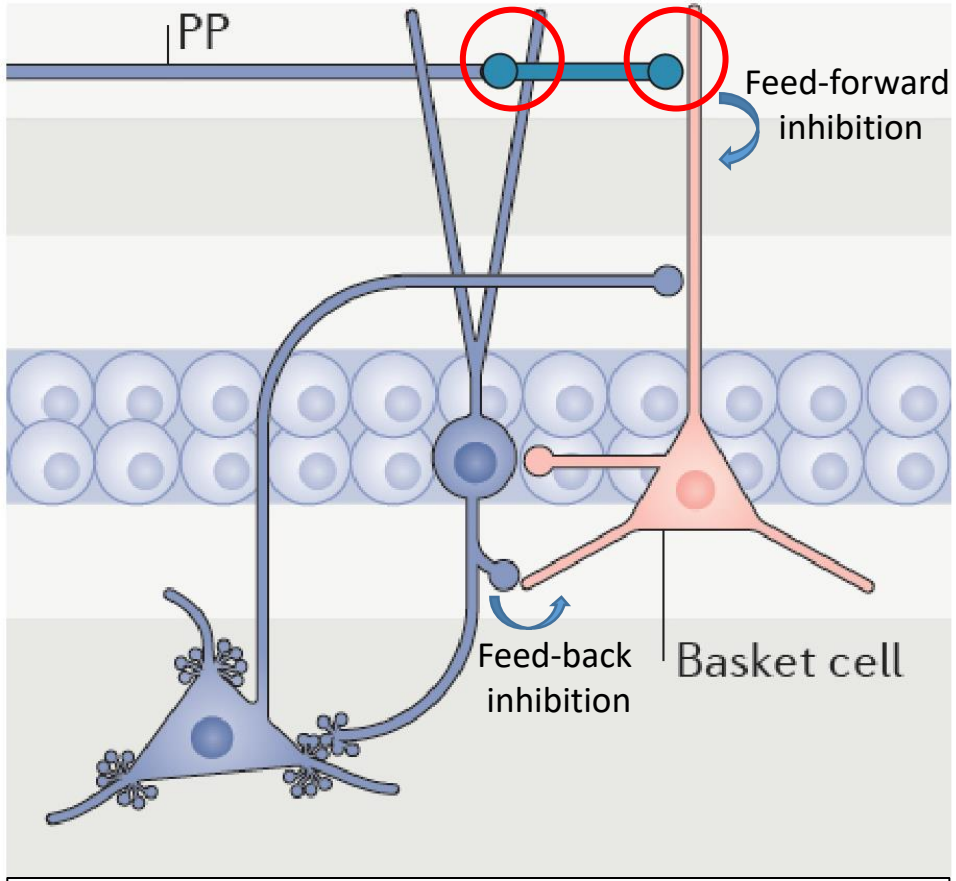




Scharfman et al. 2016. Nat Rev Neurosci.

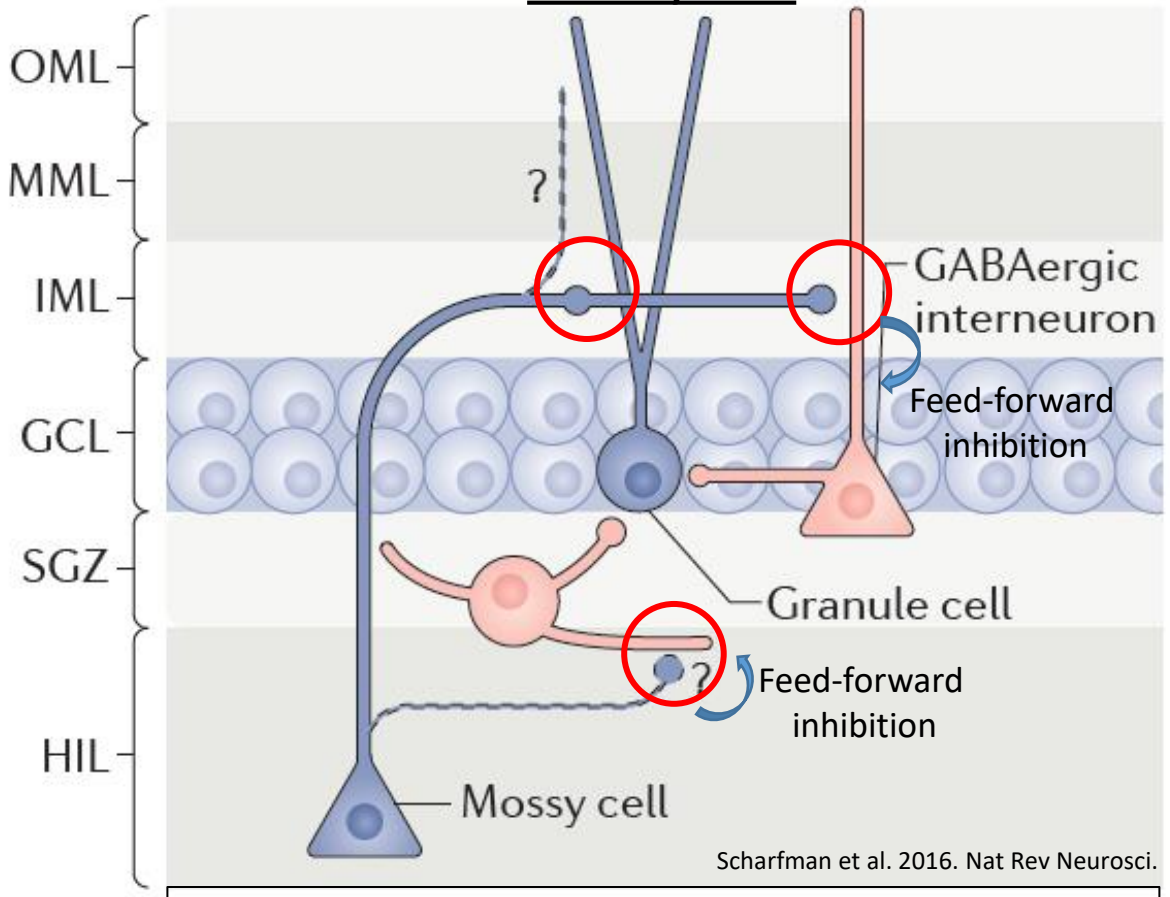
Two principal sources of excitation in the dentate gyrus neuronal circuit: Perforant path and Mossy cells

Perforant path



Do **Perforant path (PP)** inputs produce a net excitatory or inhibitory response in granule cells?

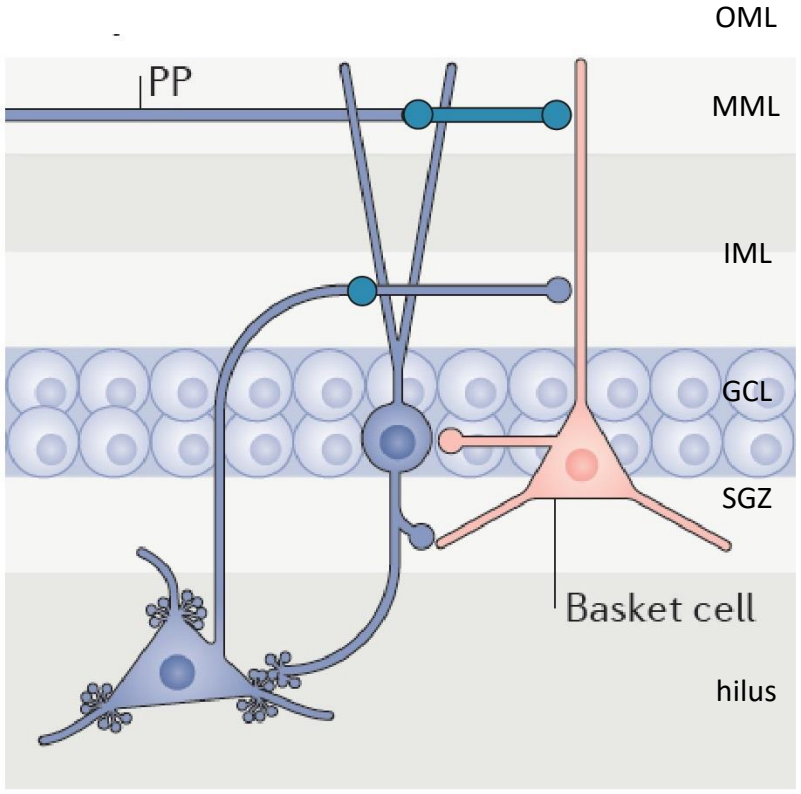
Mossy cell



Do contralateral **Mossy cell** inputs produce a net excitatory or inhibitory response in granules cells?

Inhibitory/excitatory (I/E) balance and disease

Hippocampus is particularly susceptible to damage as a consequence of ischemia/hypoxia, trauma or hypoglycemia (necrosis predominately in DG Cells). Many disease states associated with the hippocampus are proposed to arise from a disequilibrium of the inhibitory/excitatory balance:



- **Alzheimer's disease**:
  - May be associated with **a loss of inhibitory interneuron excitability**

- **Schizophrenia**:
  - Typically associated with the pre-frontal cortex, but may be a result of dysfunction in prefrontal-hippocampal integration.
  - Decrease in the volume of the hippocampus, (CA2 interneurons particularly effected) and **decrease in the number of inhibitory interneurons.**

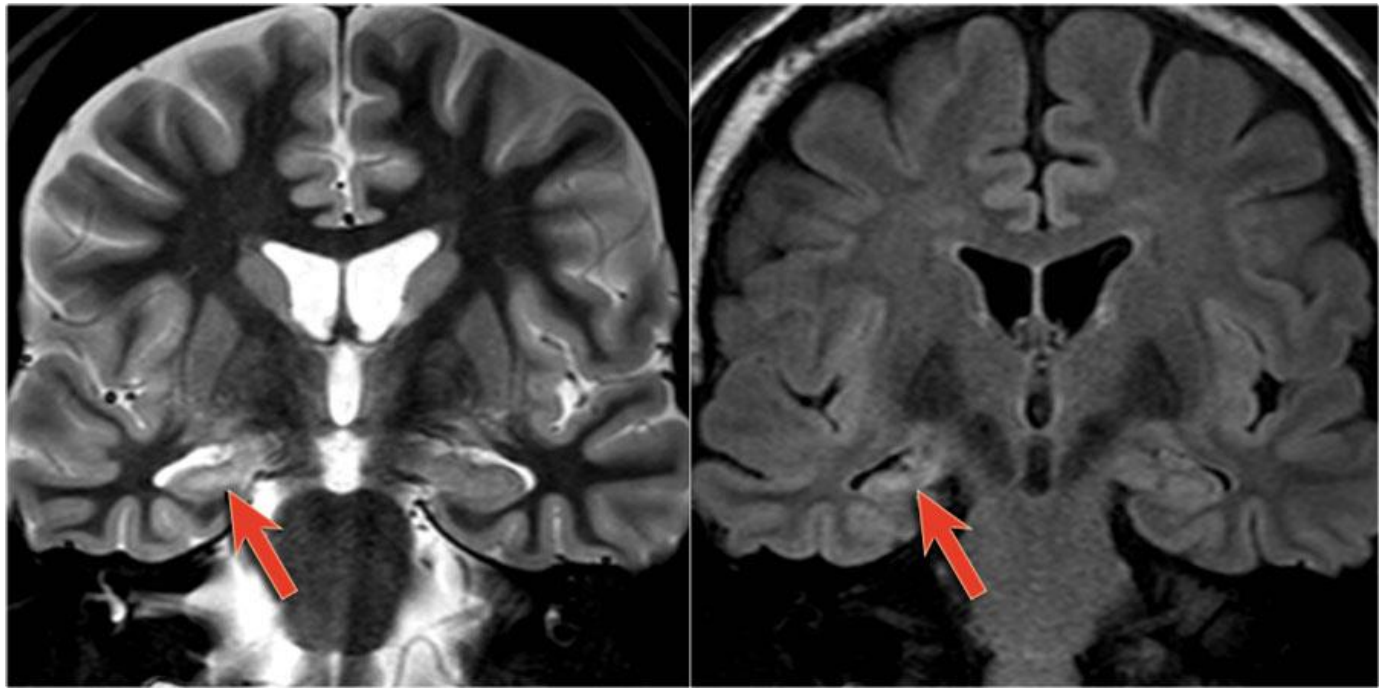
- **Temporal Lobe Epilepsy** (TLE, 60% of all epilepsies):
  - The "GABA" hypothesis suggest **dysfunction in inhibitory GABAergic synaptic transmission** may lead to the hyper-excitation observed in temporal lobe epilepsy .

Hippocampus: Temporal Lobe Epilepsy

http://www.radiologyassistant.nl/en/p4f53597deae16/role-of-mri-in-epilepsy.html

Giles Huberfeld Temporal Lobe Epilepsy

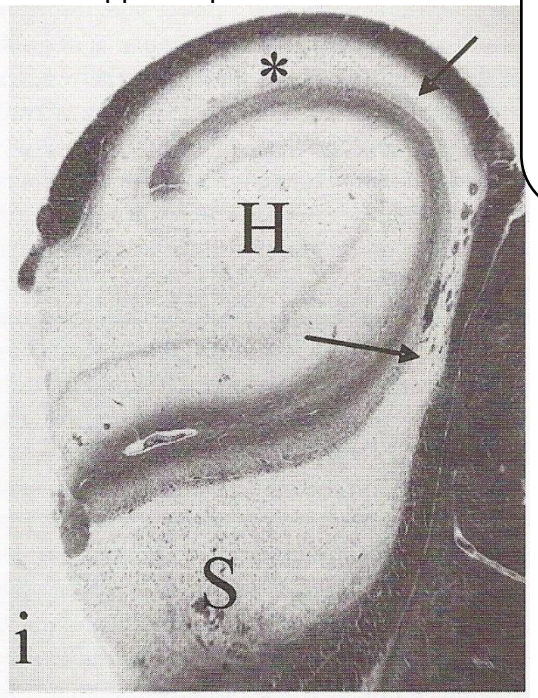
Coronal T2WI and FLAIR images show right-sided mesial temporal sclerosis.



The high signal in the hippocampus reflects gliosis

Decreased volume of the hippocampus

The Hippocampus Book. 2007



Decreased volume of the hilus and CA2 regions

- In a rat model of Temporal Lobe Epilepsy a decrease in neuron density was observe in the hilus. **Mossy cells and Hilar interneurons are particularly susceptible to cell death in comparison with granule cells**.
- Mossy cells die after many different insults to the hippocampus that increase the risk of TLEs included: ischemia status epilepticus and traumatic brain injury. **The loss of mossy cells has thus been proposed to be a source of hyper-excitability and may contribute to temporal lobe epilepsy**.

Is the selective ablation of Mossy cells sufficient to induce granule cell hyper excitability? Epilepsy?

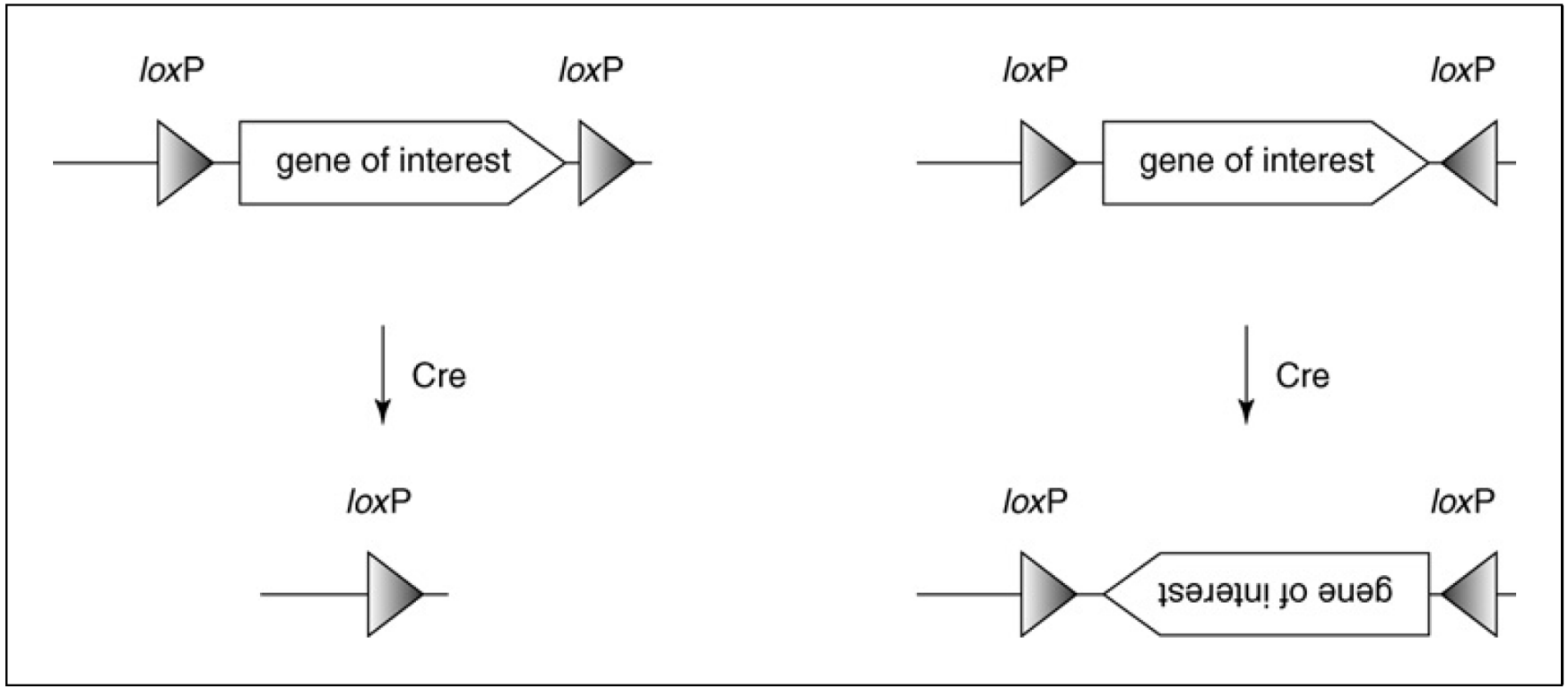
Hilar Mossy Cell Degeneration Causes Transient Dentate Granule Cell Hyperexcitability and Impaired Pattern Separation

Seiichiro Jinde,[1,3,5] Veronika Zsiros,[1,5] Zhihong Jiang,[1] Kazuhito Nakao,[1] James Pickel,[2] Kenji Kohno,[4] Juan E. Belforte,[1,6] and Kazu Nakazawa[1,*]

Example of using a genetic ablation to explore the role of dentate gyrus mossy cells

Jinde et al. 2012, Neuron.

Cre-mediated recombination



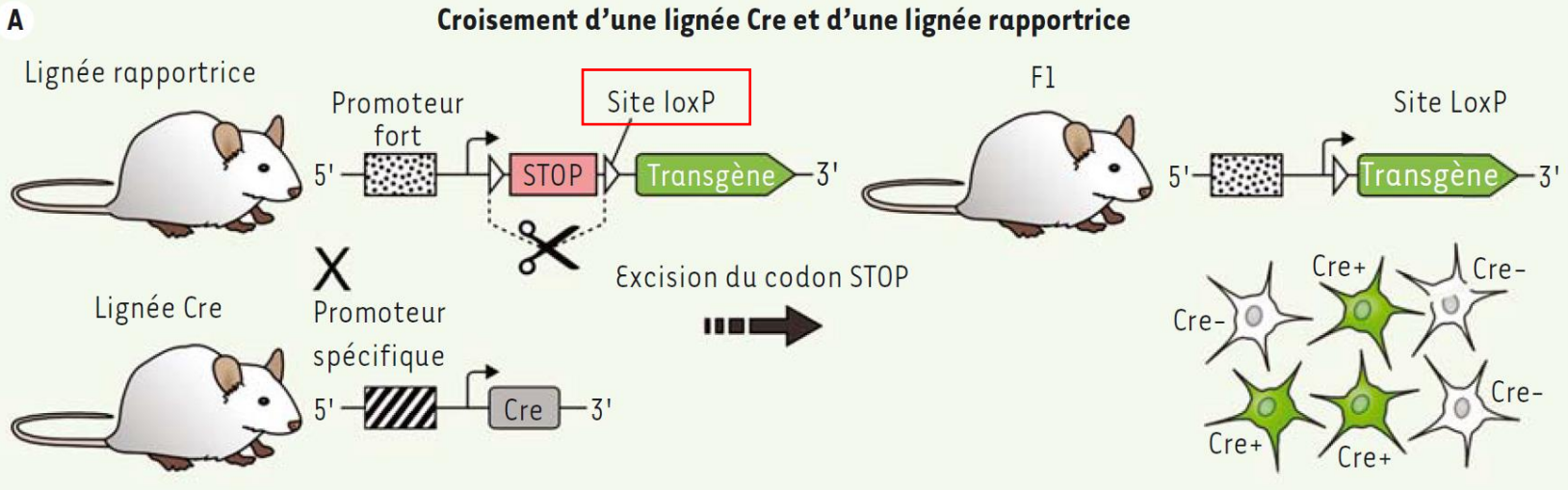
DNA sequences that are flanked by *loxP* sites in the same orientation are excised (left).

DNA sequences that are flanked by *loxP* sites are in the opposite orientation are inversed (right).

The restricted expression of cre-recombinase in distinct types of neurons allows the allows the selective expression of proteins that permit “gain of function” or “loss of function” phenotypes in these neurons

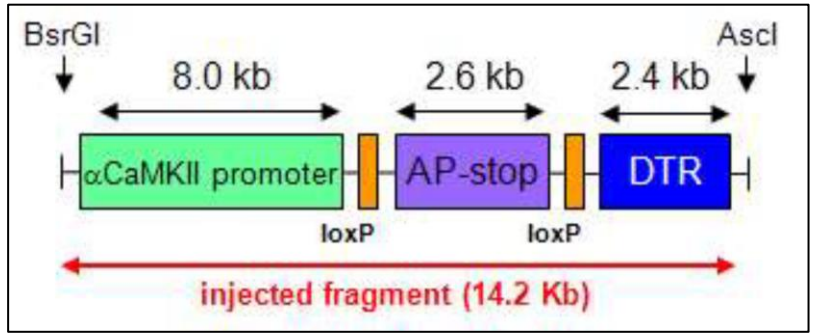
Morozov 2008. Current protocols in Neuroscience

Selective expression of genes in specific types of neurons using cre-mediated recombination

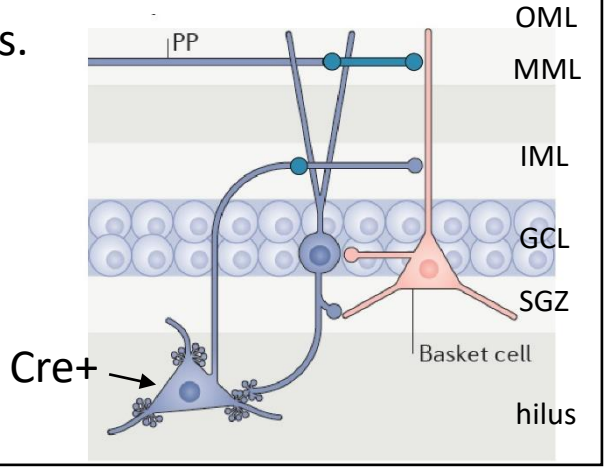


Dugue et Tricoire 2015

A transgenic "reporter" mouse line containing the Diptheria Toxin Receptor (DTR) gene downstream of a Stop codon flanked by two loxP sites is crossed with a second transgenic "Cre line" mouse that expresses the Cre-recombinase selectively in mossy cells.



Jinde et al. 2012



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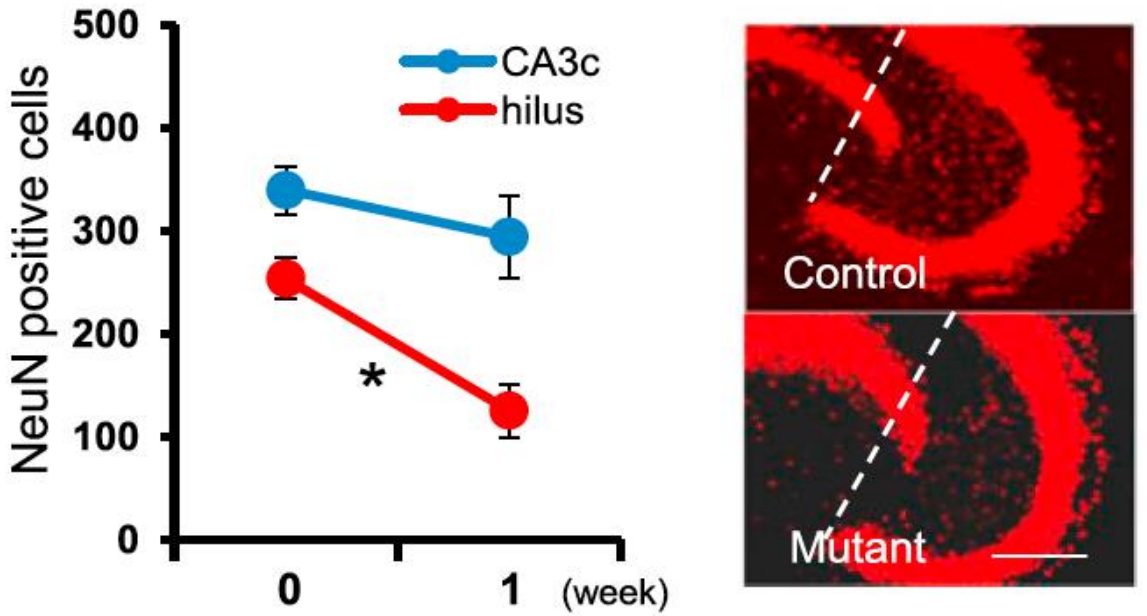
- Strategy:
  - Selective ablation of mossy cells in the dentate gyrus
- Methods:
  - Using the Cre recombinase/ loxP system the researchers induced selective expression of the **Diptheria Toxin Receptor (DTR)** in mossy cells.
    - A transgenic mouse line containing the DTR gene downstream of a Stop codon flanked by two loxP sites is crossed with a second transgenic mouse line that expresses the Cre-recombinase selectively in mossy cells.
    - The presence of Cre-recombinase in mossy cells leads to the excision of the stop codon and the selective expression of the DTR in mossy cells
  - Injecting diptheria toxin in these mice leads to the selective ablation of cells expressing the DTR receptor, *i.e.* mossy cells.

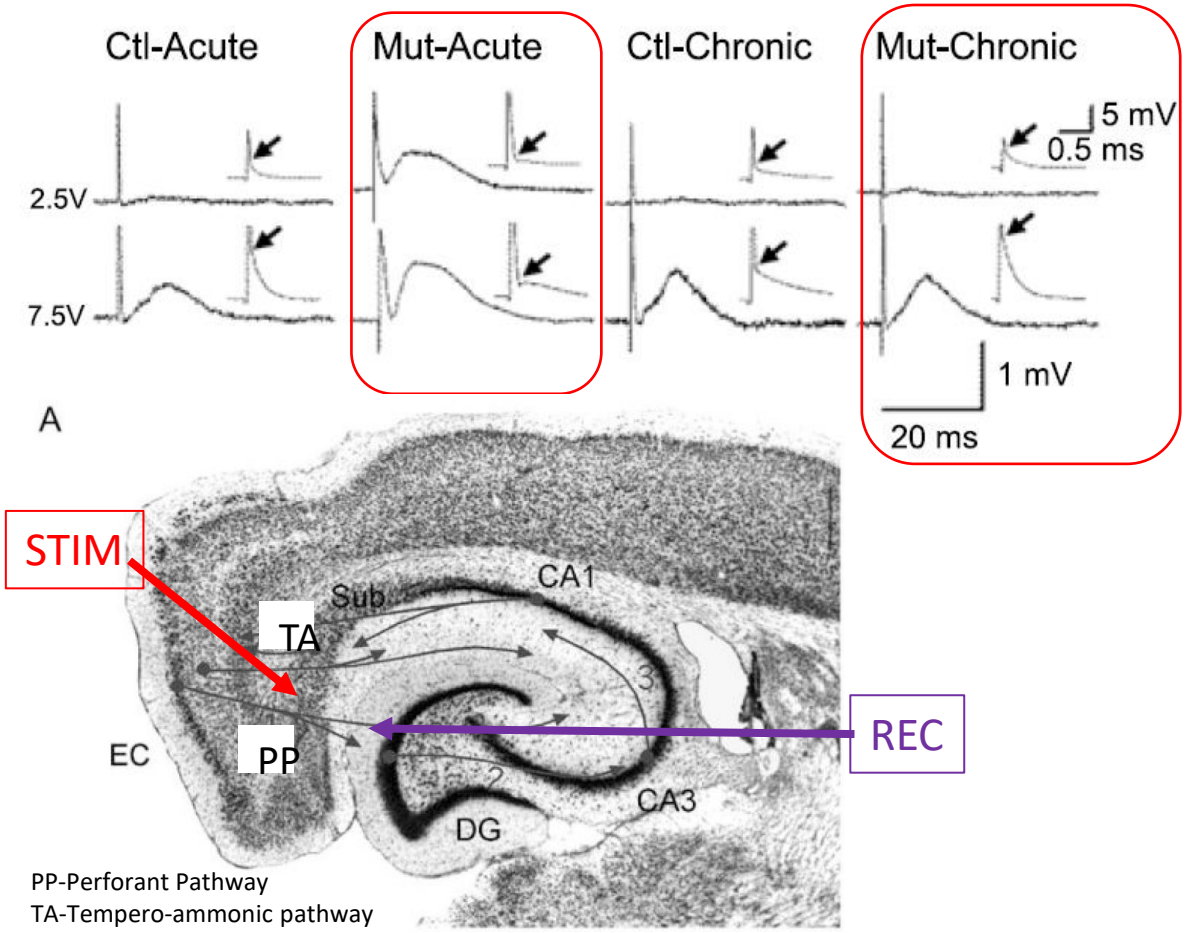
Ablation of Mossy Cells in Cre/fDTR Mutants after Diptheria toxin treament



Jinde et al. 2012, Neuron.

Perforant path stimulation reveals a transient increase in dentate gyrus excitability after selective ablation of Mossy Cells

Electrical stimulation of the perforant path (PP) at two different stimulation intensities (2.5 V and 7.5 V) induces field excitatory post-synaptic potentials recorded in the dentate gyrus.



PP-Perforant Pathway
TA-Tempero-ammonic pathway

Acute = 1 week after treatment
Chronic = 4 weeks after treatment

Jinde et al. 2012, Neuron.

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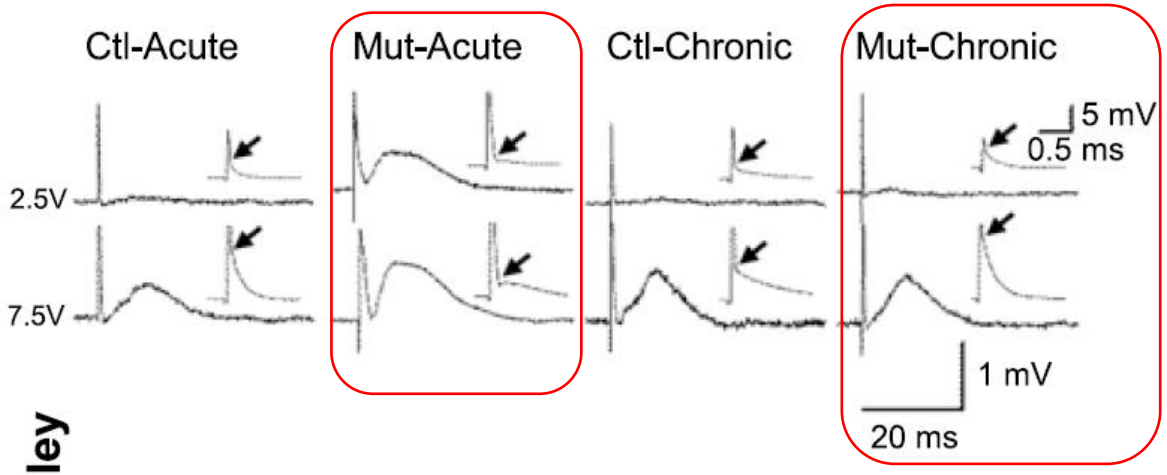
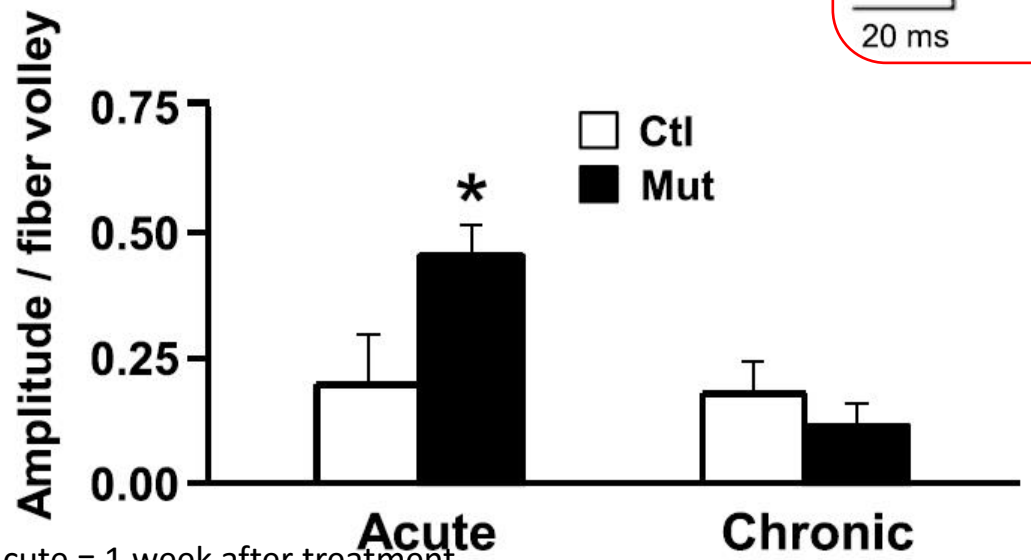




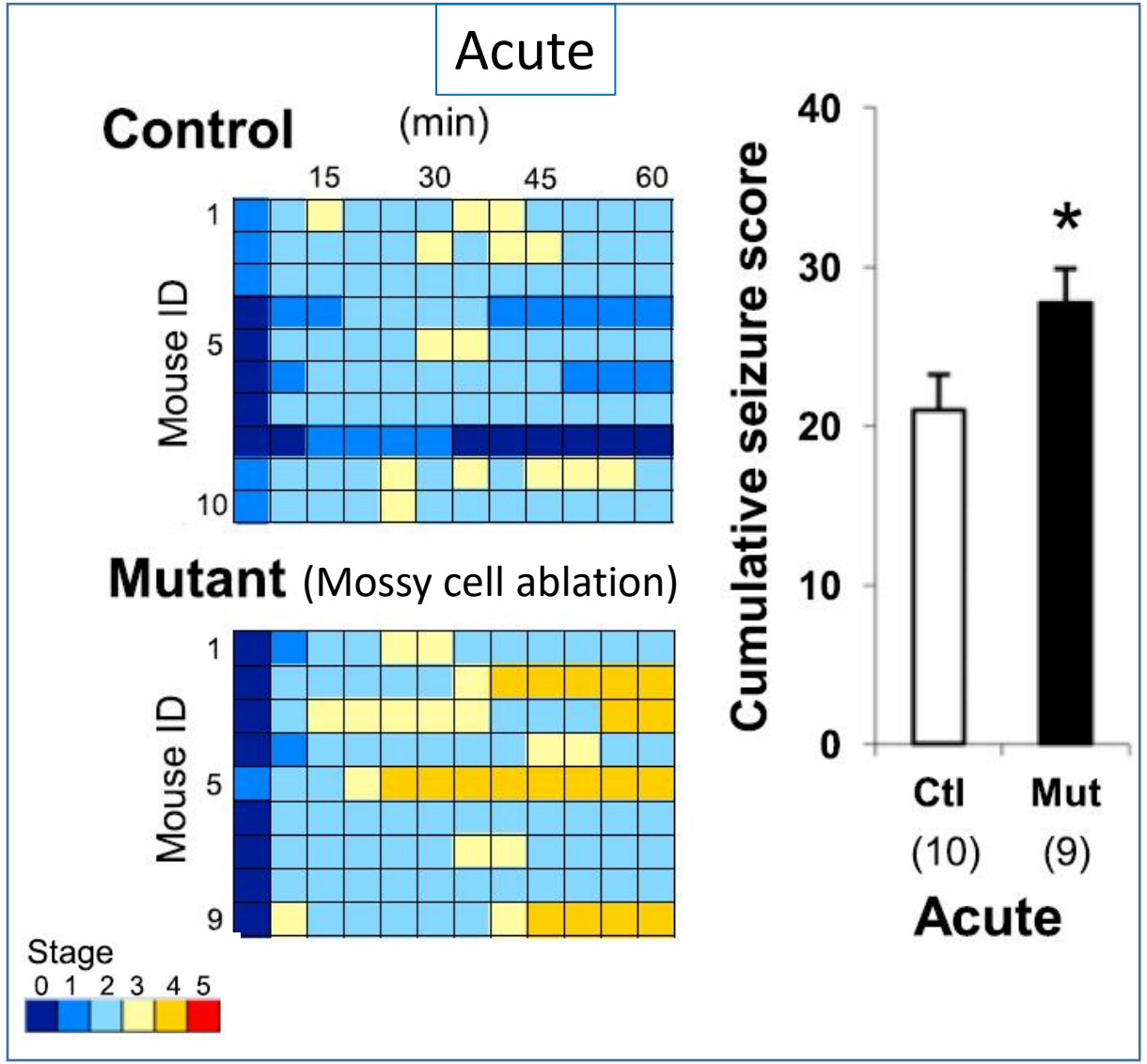
Acute = 1 week after treatment
Chronic = 4 weeks after treatment

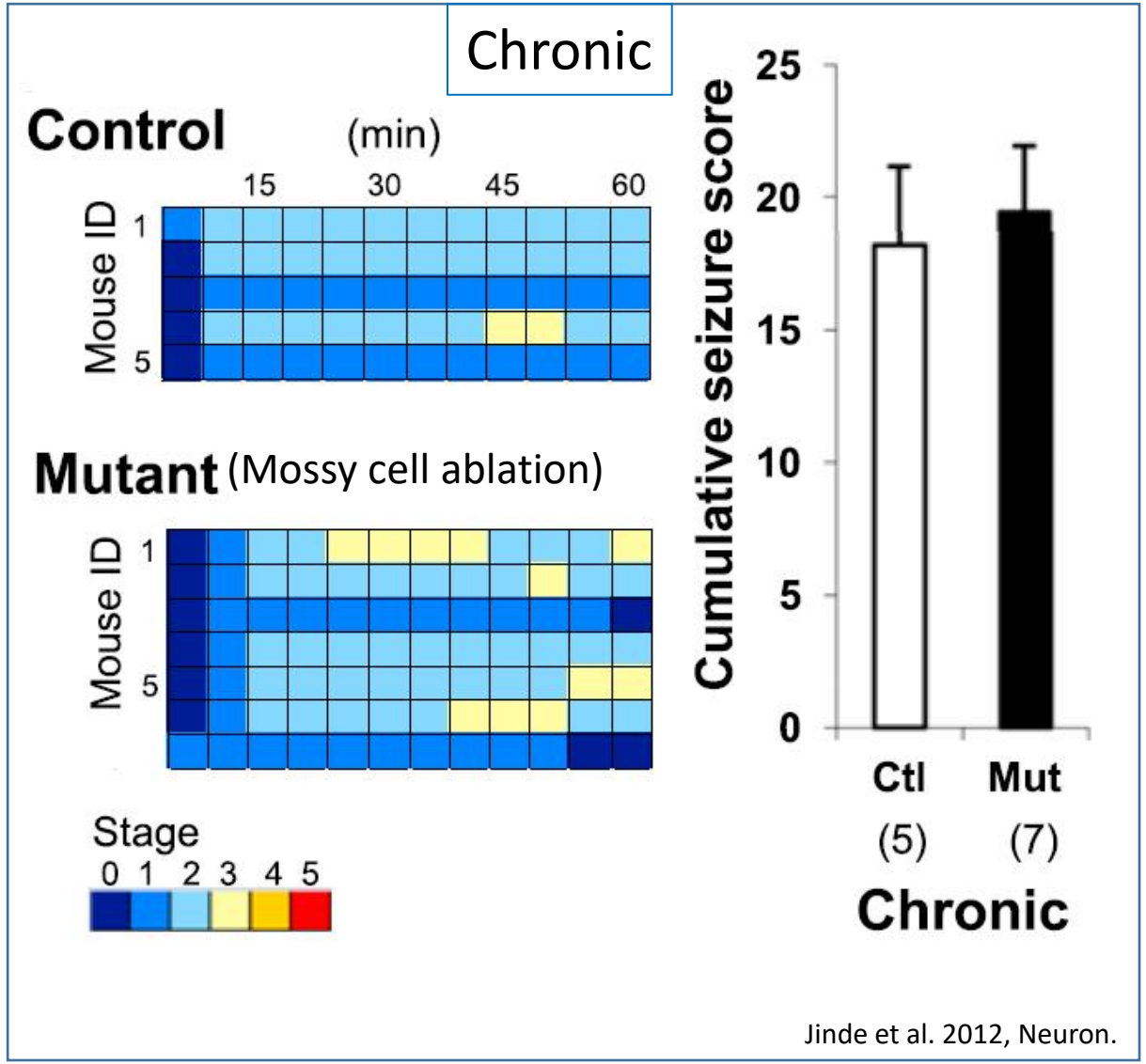
Jinde et al. 2012, Neuron.

Results and Conclusions:

1. Acute ablation of excitatory (glutamatergic) mossy cells paradoxically leads to a hyper-excitability of the dentate gyrus.
   a) Thus mossy cells appear play an important role in setting the inhibitory tone of the dentate gyrus neuronal network
2. Chronic ablation of excitatory (glutamatergic) mossy cells does not alter dentate gyrus excitability.
   a. Thus a homeostatic mechanisms appears to compensate the ablation of mossy cells.

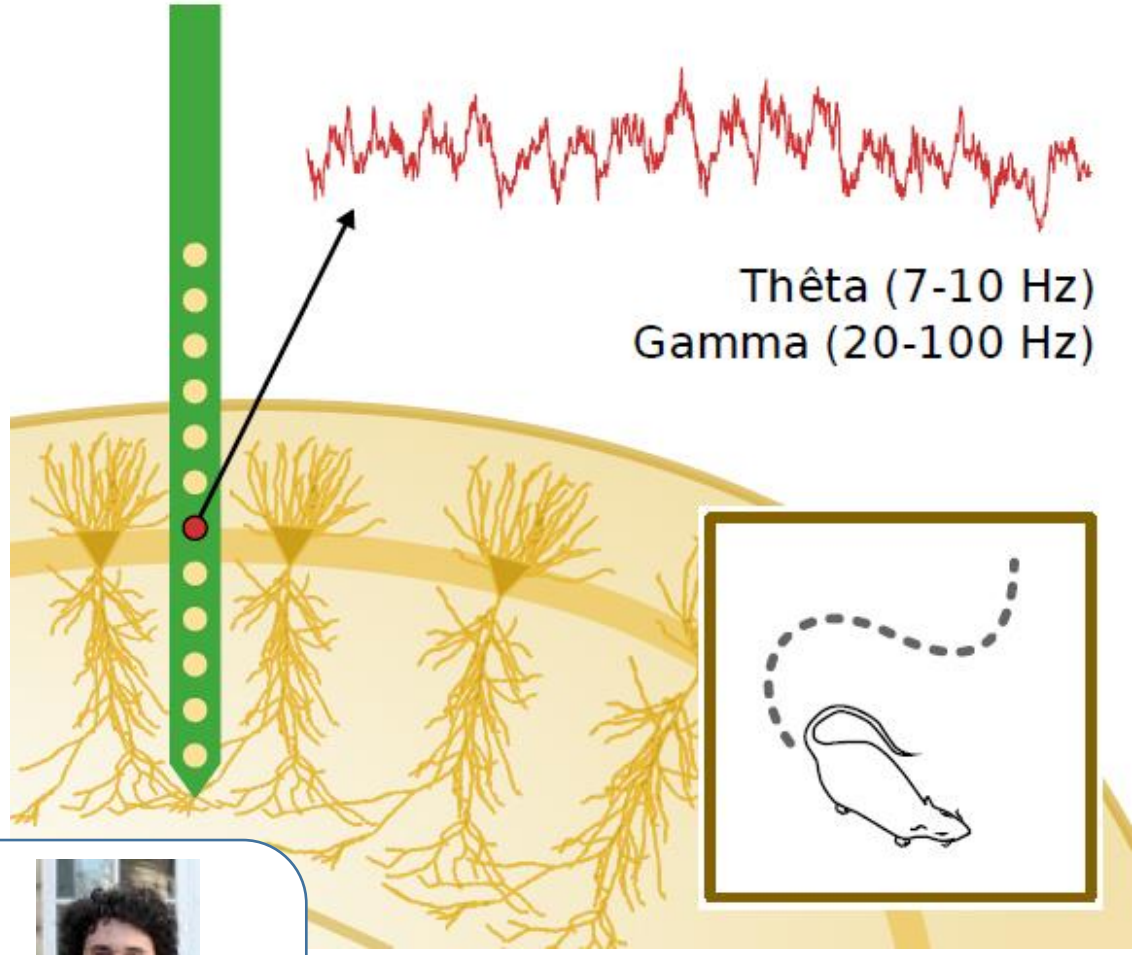
Kainic acid induced seizures are more severe after ablation of Mossy cells during the acute phase (4-11 days) but not during the chronic phases (4-6weeks)

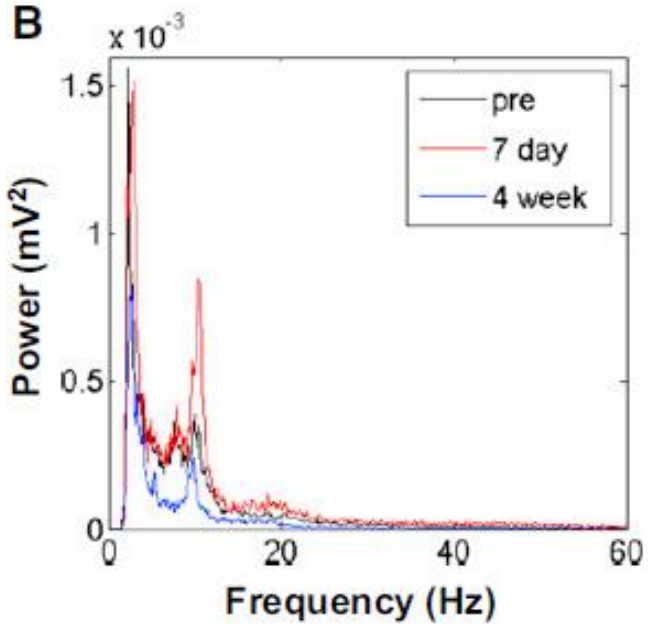


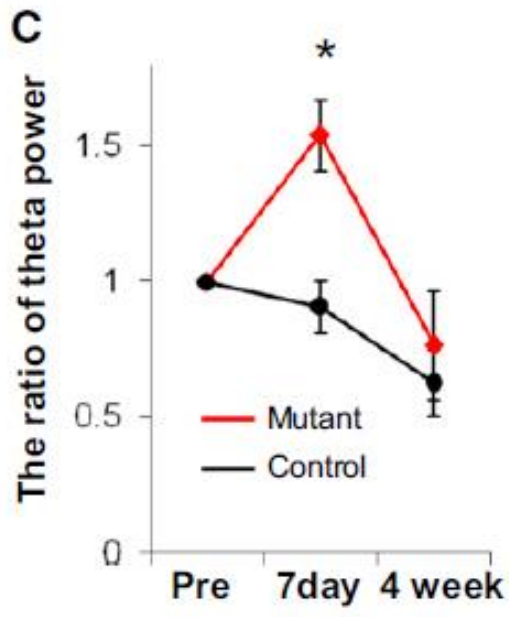


Jinde et al. 2012, Neuron.

Ablation of Mossy cells increases theta oscillations (7-12 Hz) during the acute phase (4-11 days) but not during the chronic phases (4-6weeks)



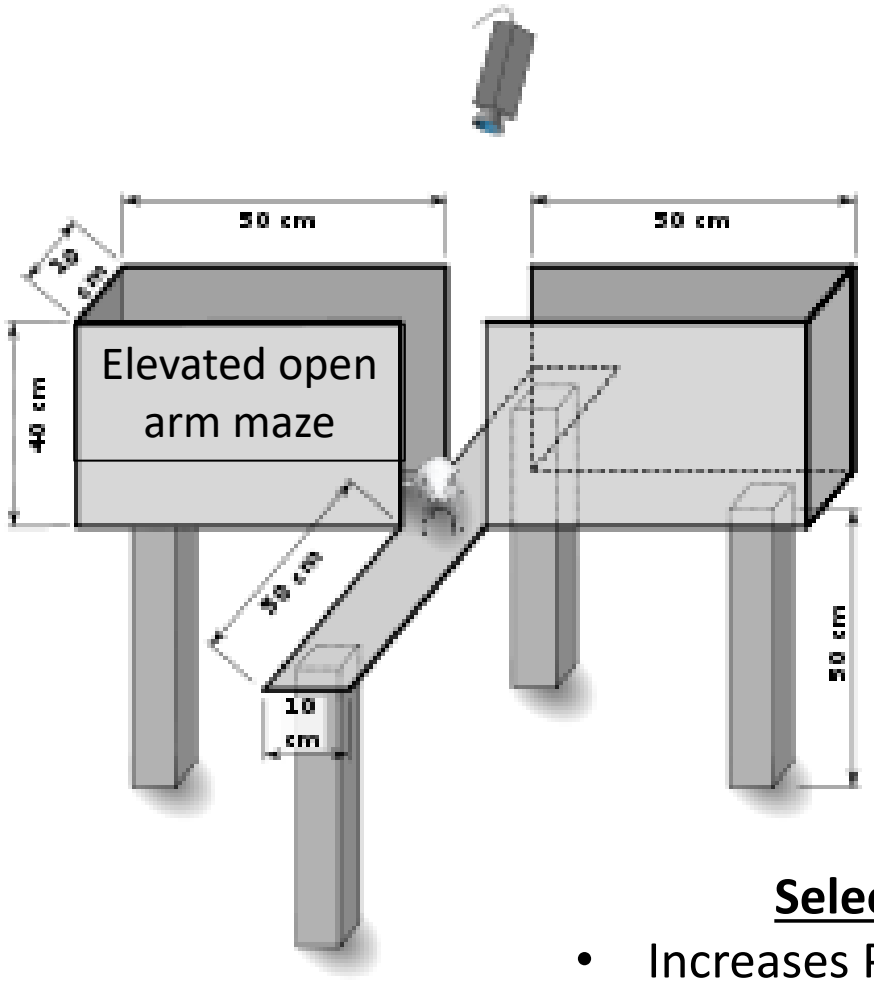


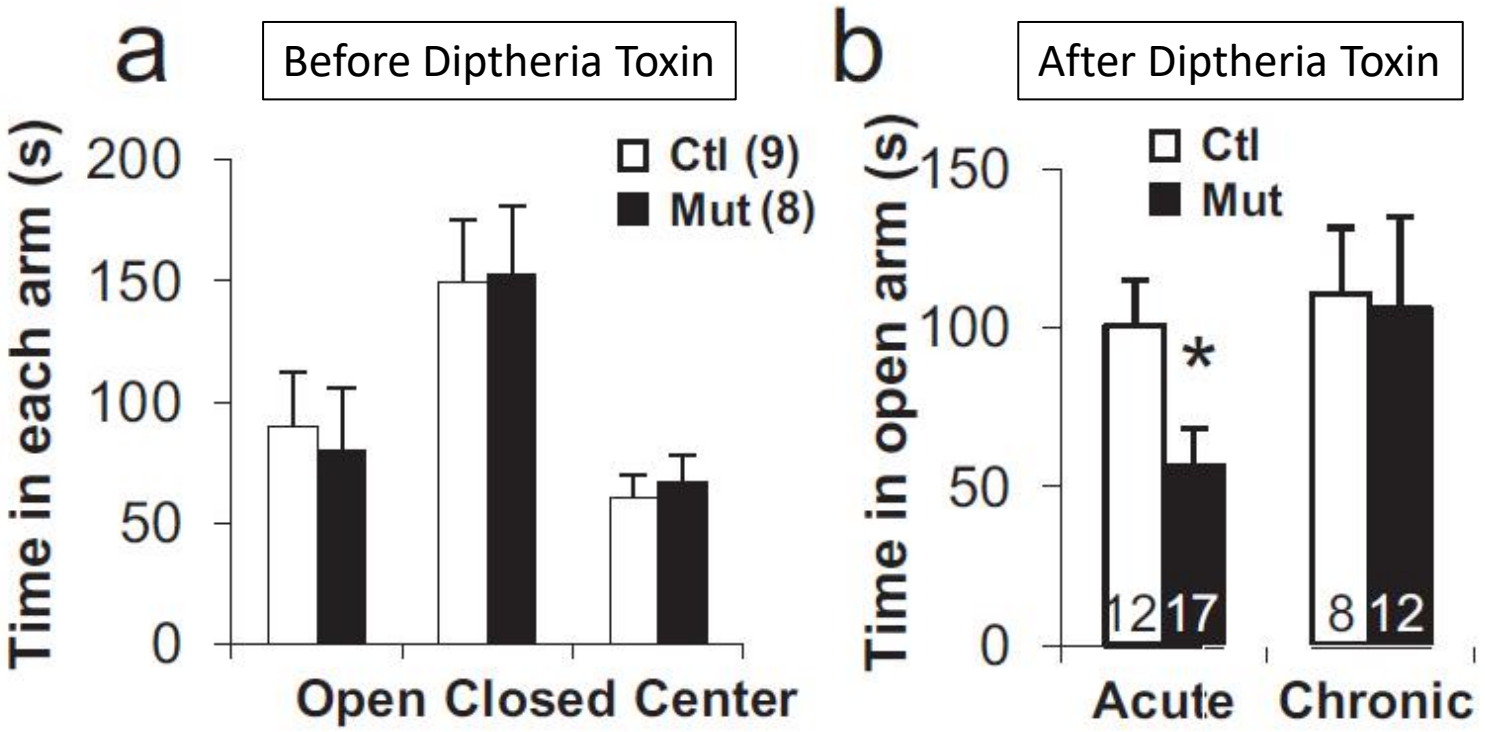


M. Zugaro
Hippocampal rhythms and behavior

- Theta oscillation (7-12Hz) are implicated in temporal coding and REM.
- Theta rhythm in the dentate gyrus *in vivo* is relayed by the EC through the PP.
- An increase in Theta power suggests an increase in granule cell excitability.

Mossy Cell Ablation increases anxiety-like behavior in the acute but not in the chronic phase





<u>Selective Mossy Cell ablation transiently</u>

- Increases Perforant path excitation of the dentate gyrus
- Increases the intensity of kainic acid induced seizures
- Increases the power of Theta oscillations
- Increases anxiety-like behaviors

Dentate Gyrus summary

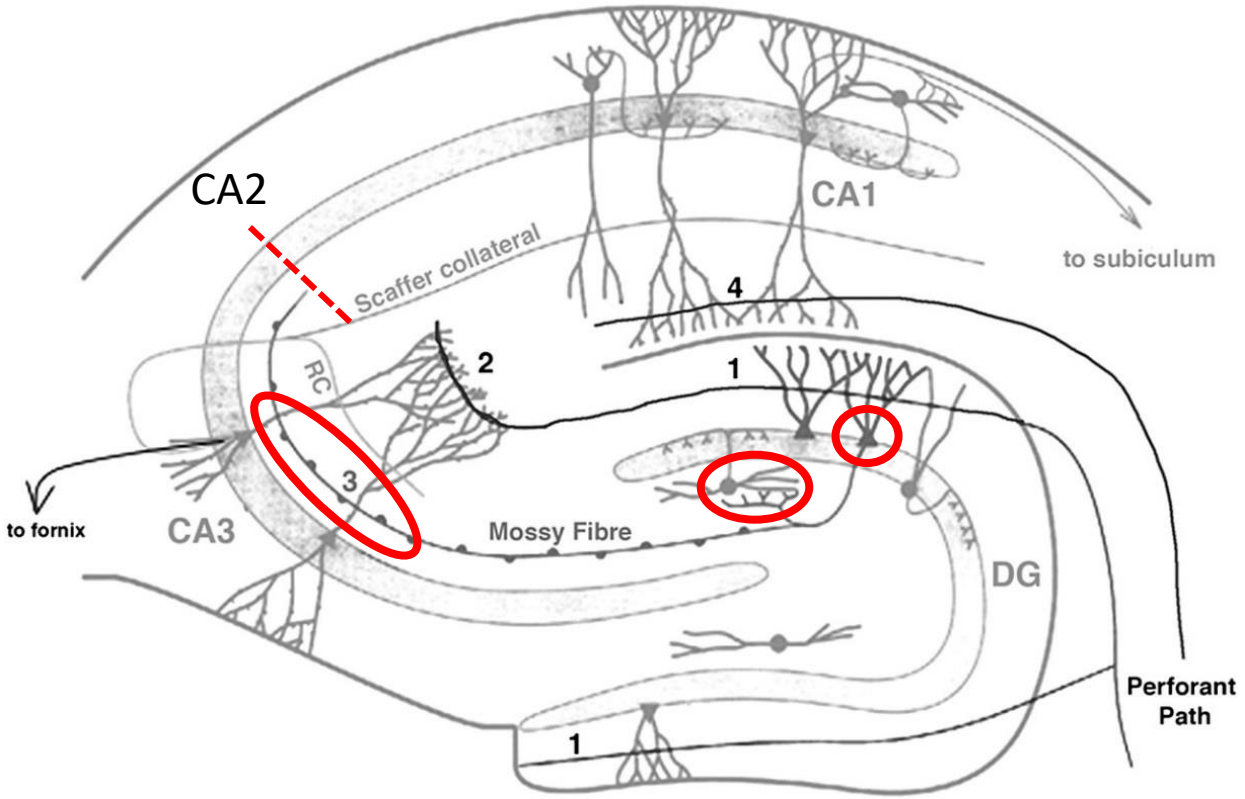
- **3 Maine types of Neurons:**
  - Granule cells
  - Mossy cells
  - Inhibitory interneurons

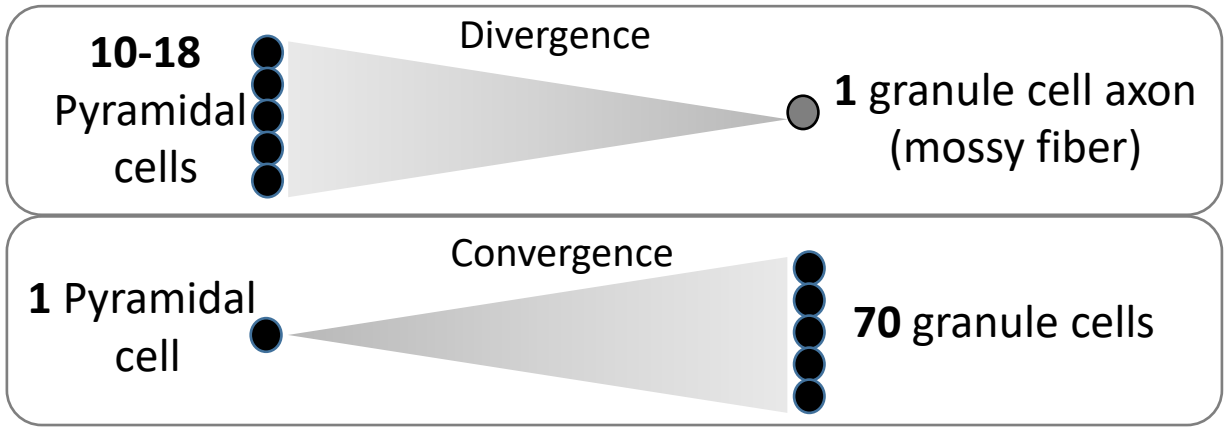
- **What is the net effect of glutamatergic afferents from the Entorhinal cortex and Mossy cells on Granule cell activity?**
  - Entorhinal afferents and Mossy Cells release the excitatory neurotransmitter glutamate onto both inhibitory interneurons and excitatory granules. Based solely of the anatomical connectivity it is therefore difficult to predict if entorhinal afferents will induce a net excitatory or inhibitory effect. Thus while structure may predict function, the complex connectivity and dynamic physiology of neuronal circuits means that function must be tested empirically.

- **The etiology of hippocampal diseases such as Epilepsy, Alzheimer's disease and Schizophrenia are thought to be related to a disequilibrium in excitatory/inhibitory balance:**
  - Paradoxically the loss of excitatory glutamatergic neurons (Mossy Cells for example) may lead to hyperexcitability associated with disease states depending on the connectivity and synaptic physiology of the neuronal circuit.
  - The dysfunction of GABAergic interneurons is also hypothesized to play an important role in the etiology of hippocampal diseases.

Pause 2 min

Dentate Gyrus efferent projections: granule cell mossy fibers (axons)





Mossy fibers to CA3: Dentate Gyrus efferent projections

1) Granule cells form asymmetric excitatory synapses with:
   1) Mossy cells and Basket cells in the **Dentate gyrus**
   2) Pyramidal cells and Interneurons in the **CA3 region**

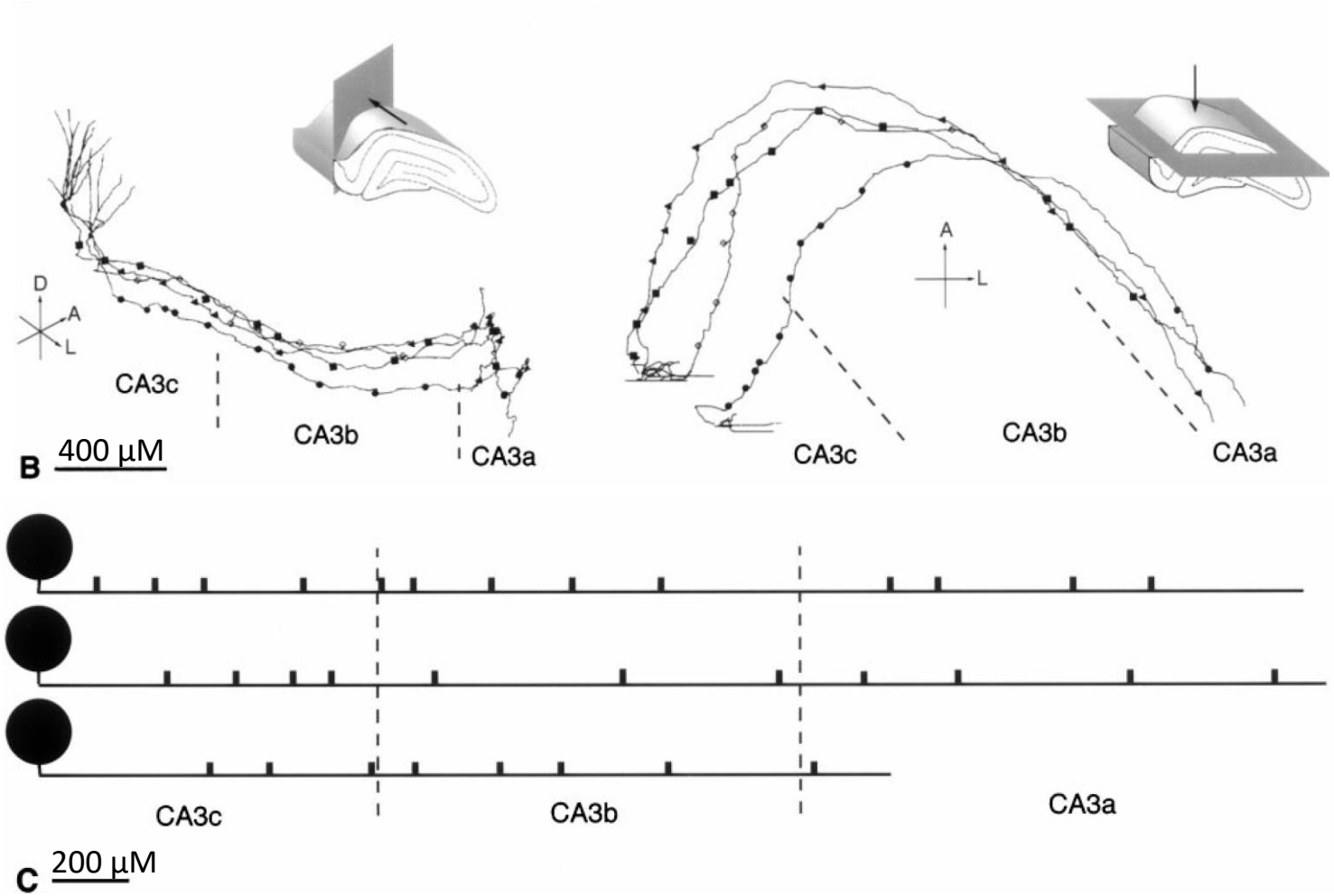
2) Mossy fiber (mf) axons arise exclusively from GCs and terminate in narrow zone just above the CA3 pyr layer *stratum lucidum* (literally “clear layer”)

3) Mossy fibers stop at CA2 (CA2 defined as region with a lack of GC mf inputs)

4) Each granule cell axon forms mossy fiber terminals on 10 to 18 different pyramidal cells.

5) Each pyramidal cell receives inputs from ~70 granule cells

Topography of mossy fiber terminals in the CA3 region



-Axons follow the transverse axis of the hippocampus (CA3c, CA3b) before turning along the longitudinal axis in CA3a.

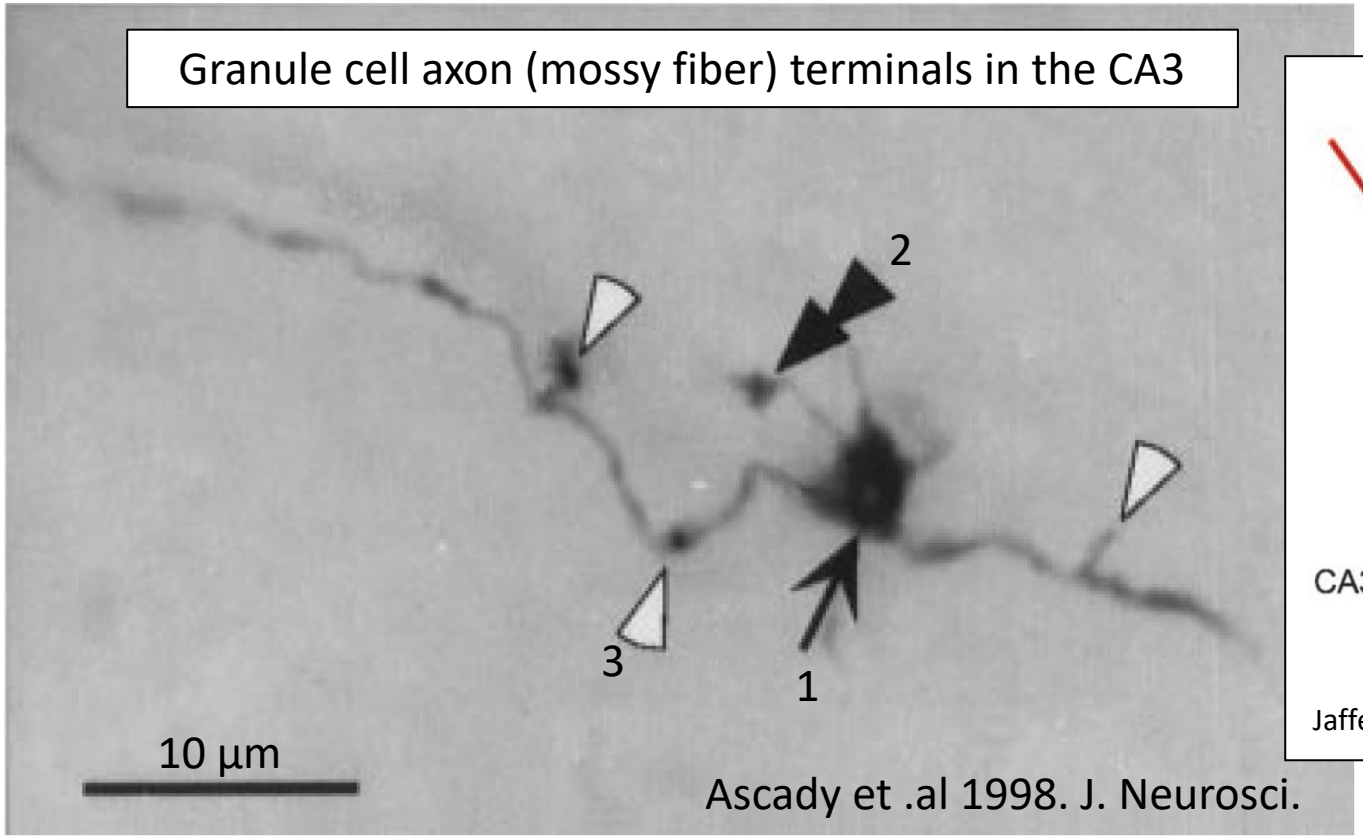
-Span 1,1 mm septo-temporal axis

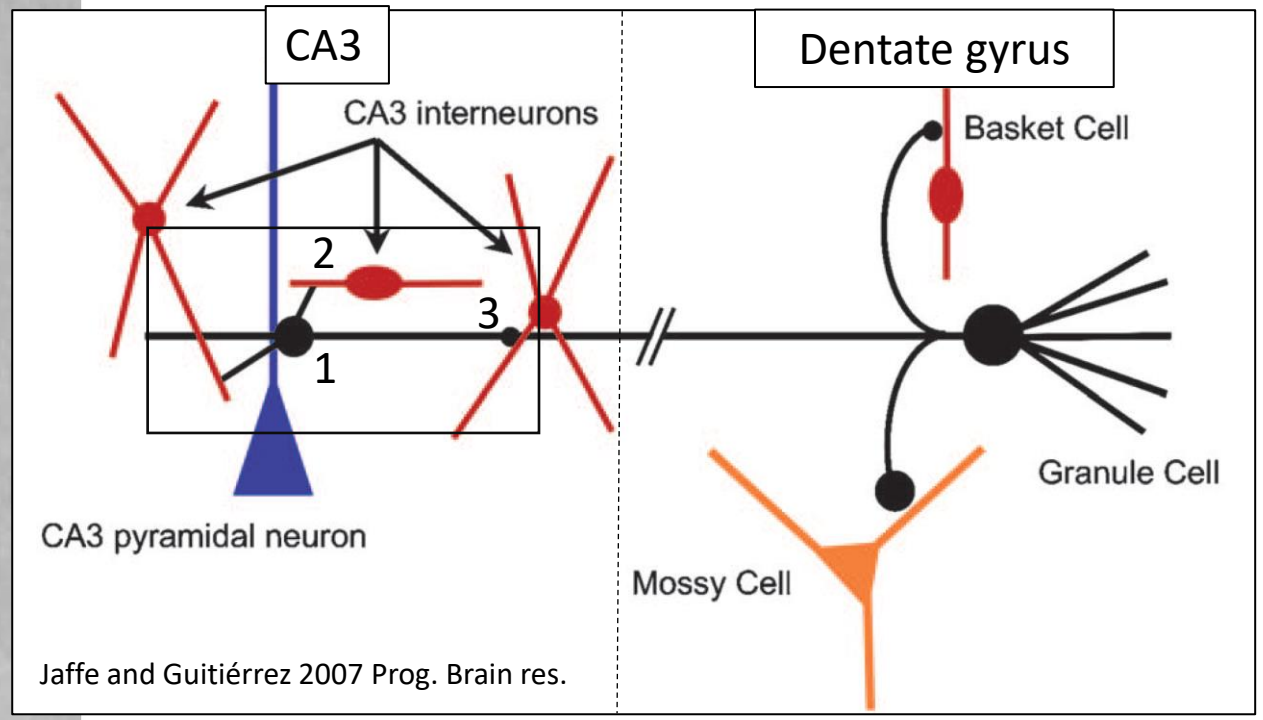
-~150-300 µM between mossy fiber (mf) boutons

-Axons of adjacent granule cells are parallel

Ascady et al. 1998 J. Neurosci.

Granule cell axons (mossy fibers)





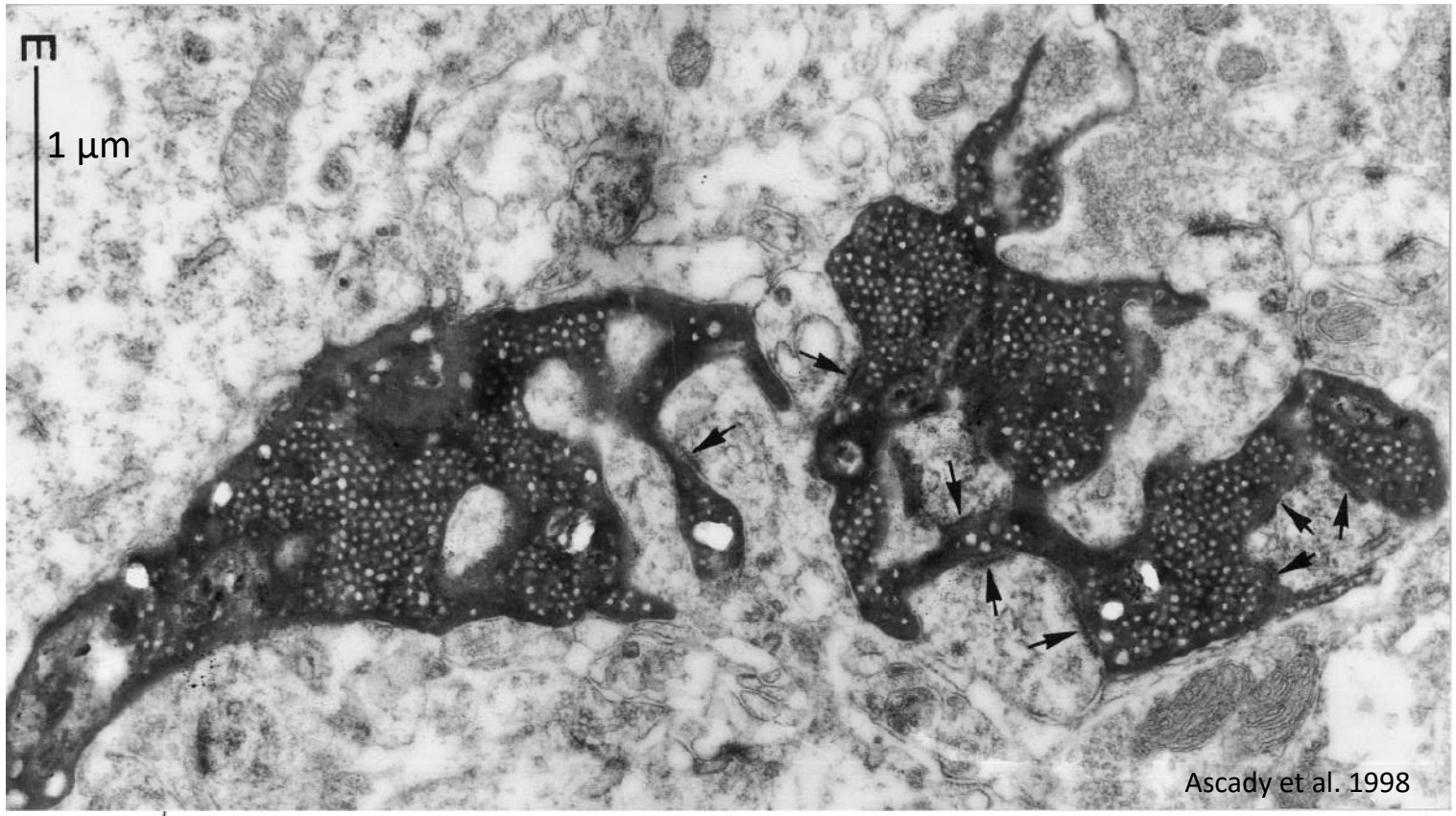
Along the granule cell axon the several different type of terminals are found:

1, **Large "Mossy fibers" terminals -** (up to 5µM diameter) synapse onto thorny excrescences the proximal dendrites of CA3 pyramidal cells (blue) in the st. lucidum.

2, **Filopodial extentions -** from the large terminals 2-3 filopodial extensions predominantly make synaptic contacts onto CA3 GABAergic interneurons (red).

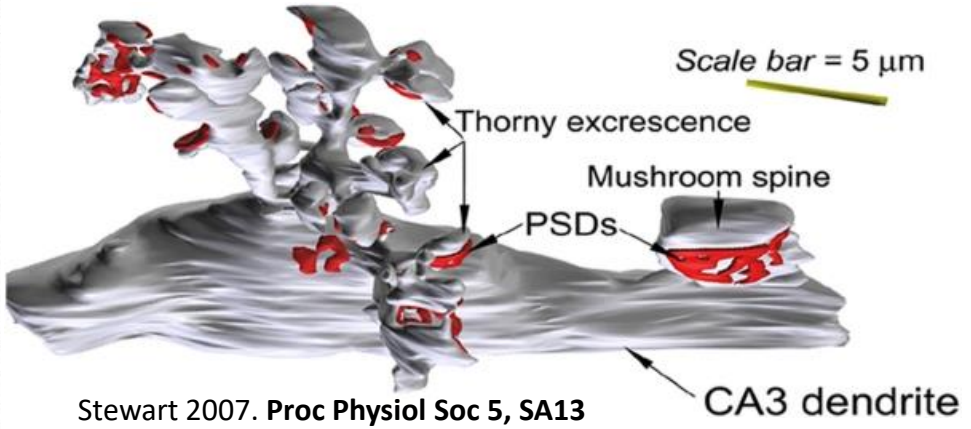
3, **Small *en passant* boutons** - predominantly terminate on CA3 GABAergic interneurons (red).

Large ”Mossy fibers” terminals synapse on to “thorny excrescences”

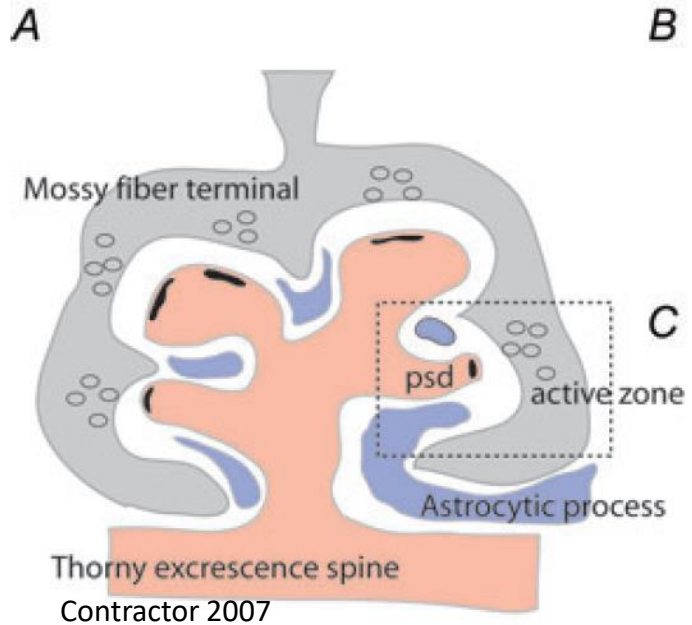


Ascady et al. 1998

CA3 pyramidal neuron



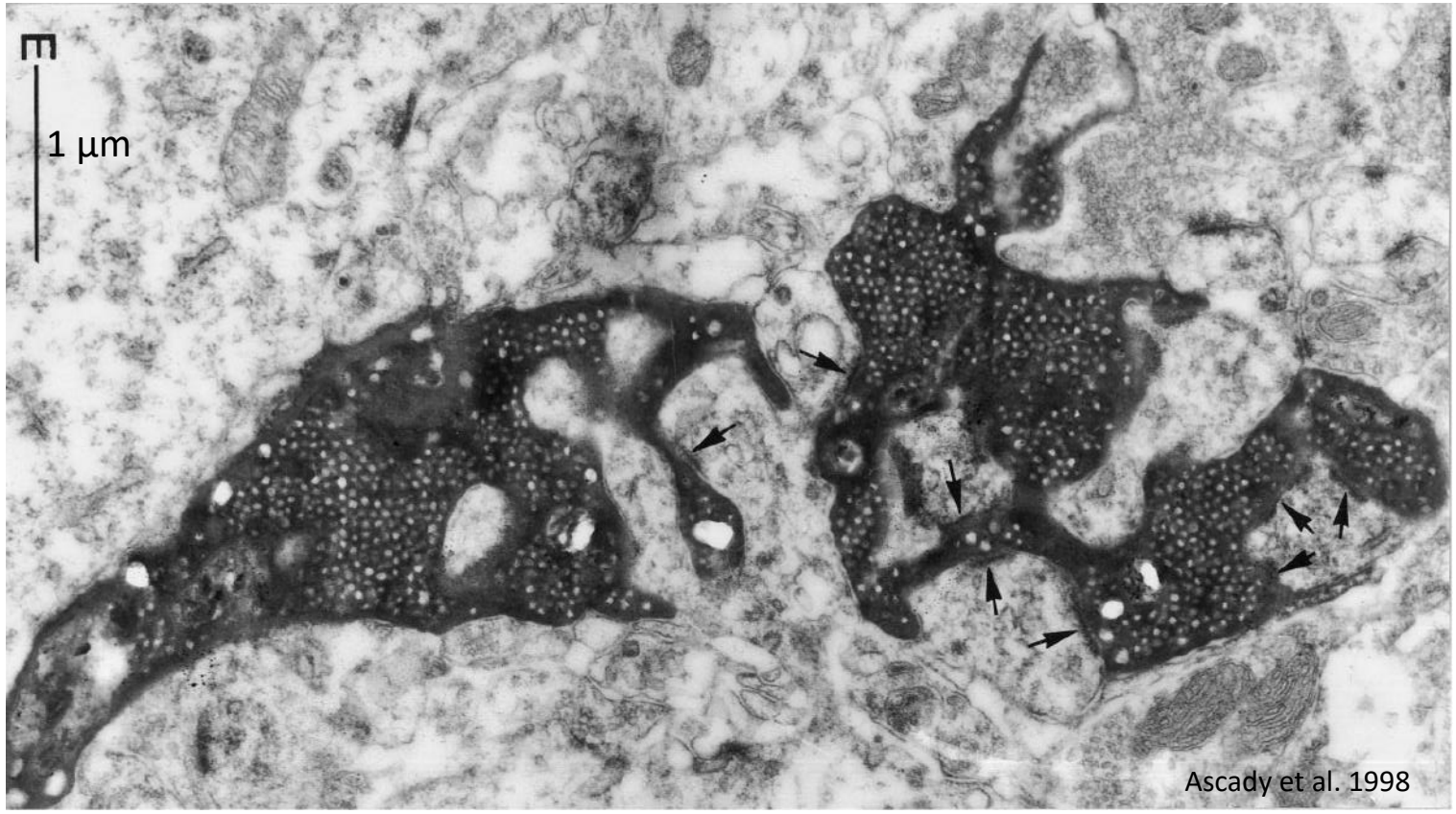
Stewart 2007. **Proc Physiol Soc 5, SA13**



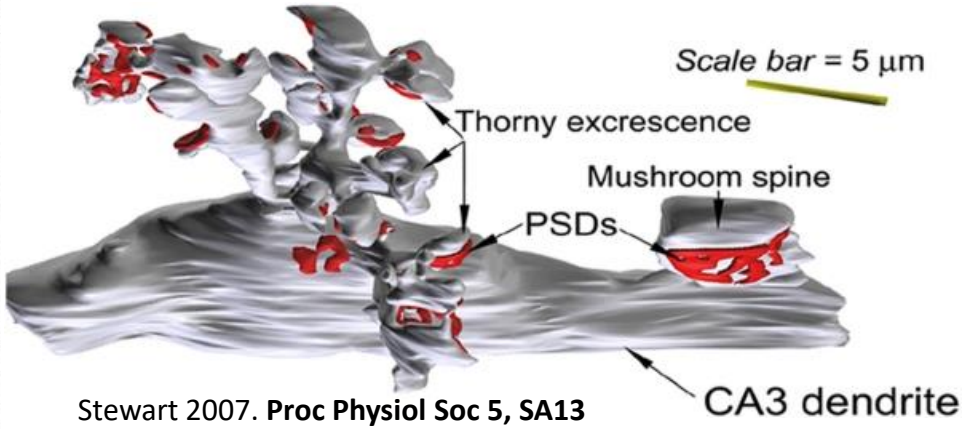
Contractor 2007

- 40 thorny excrescences per neuron and the proximal apical dendrites
- Mfs are densely filled with small clear vesicles and a few large dense core vesicles
- MFs contain subcellular organelles (ribosomes, multivesicular bodies), but also organelles not found in other spines (mitochondria, microtubules).

Large ”Mossy fibers” terminals synapse on to “thorny excrescences”



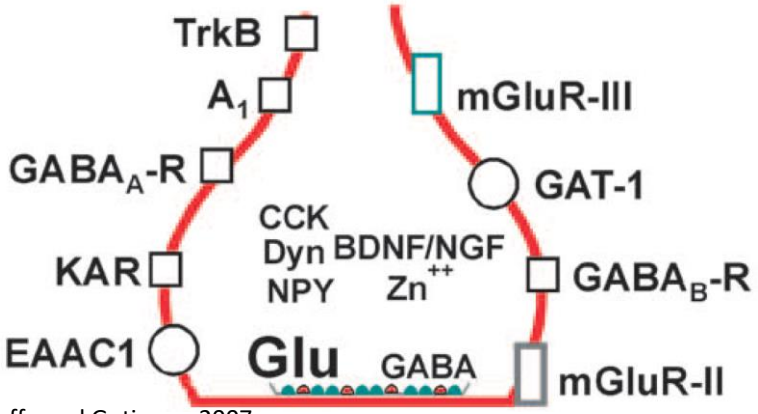
Ascady et al. 1998



Stewart 2007. **Proc Physiol Soc 5, SA13**

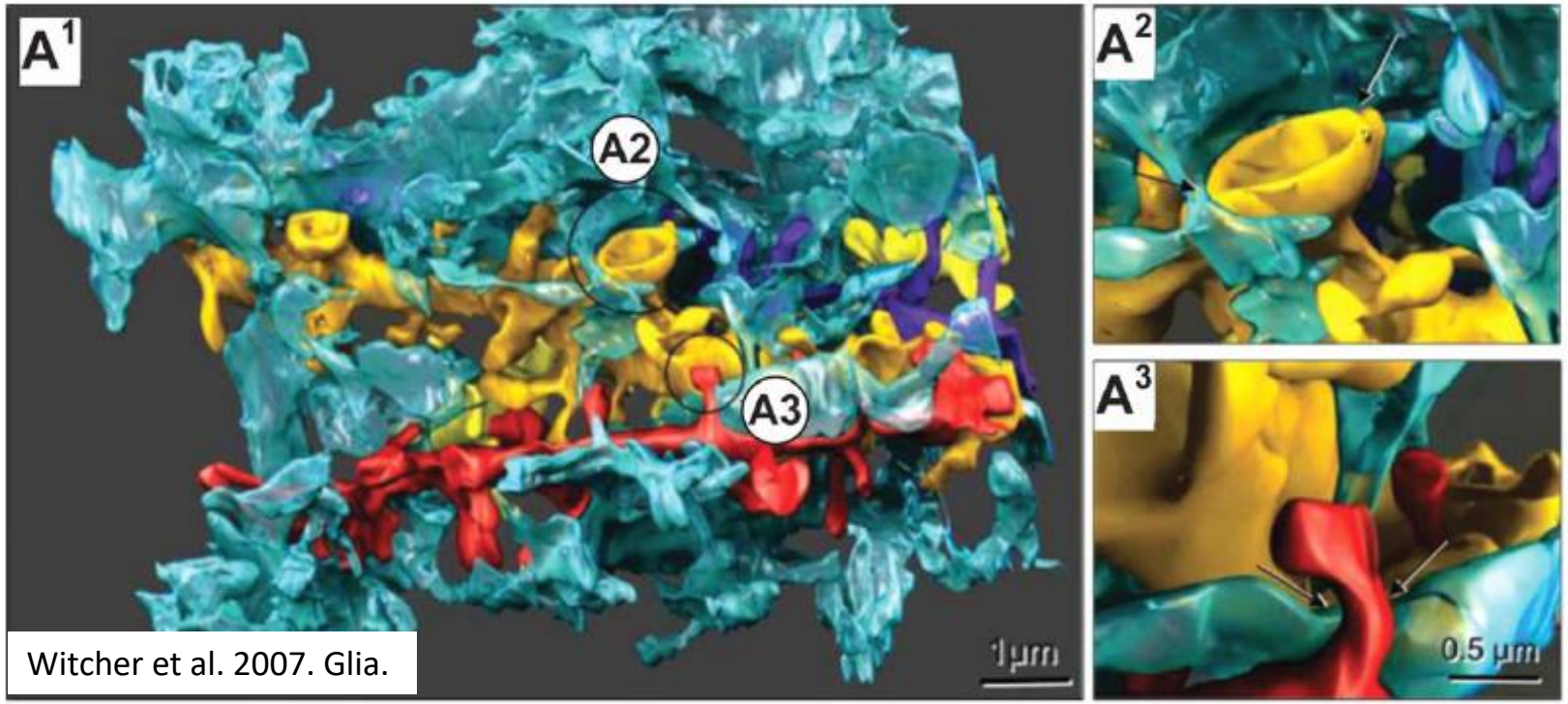
- At different stages of development mf terminals release both **Glutamate** and **GABA.**
- Other neurotransmitters released from mf terminals include: **CCK, Dynorphin (Dyn), Neuropeptide Y (NPY), Zinc, Cholecytokinin (CCK), BDNF/NGF**
- Mf activity may be modulated by the activation of a diverse population of presynaptic receptors.

Neurotransmitters at mossy fibers



Jaffe and Gutierrez 2007

Astroglia fill the space between synaptic spines of principal neurons of the hippocampus



Witcher et al. 2007. Glia.

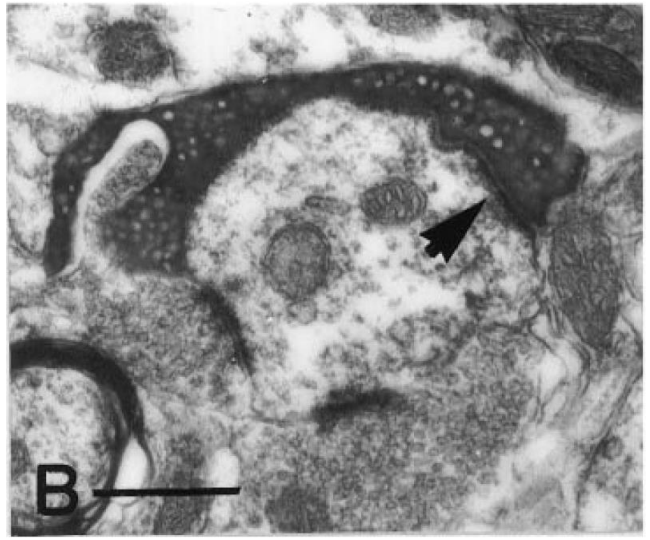
A1) Three-dimensional re-construction of a single astroglial process (blue) interdigitating among many dendrites, four of which are reconstructed here (gold, yellow, red, purple). Axonal boutons are not dis-played.

A2) Approximately 50% of the ASI of a mushroom spine was apposed by astroglia (arrows). A3) Only the neck of this thin dendritic spine was apposed by astroglia (arrows)

- Astroglia express high affinity of glutamatergic transporters and have canonically been implicated in glutamate uptake, in addition to a role buffering extracellular potassium.
- Astroglia also express mGluRs, $GABA_B$ receptors, and cell adhesion receptors. They have been found to release a number of neurotransmitters including: glutamate, D-Serine, ATP, Tumor necrosis factor-alpha and thus influence synaptic transmission and spine size.
- The distance of perisynaptic astrocyte protrusions (PAPs) varies with spine size and may therefore alter synaptic transmission.

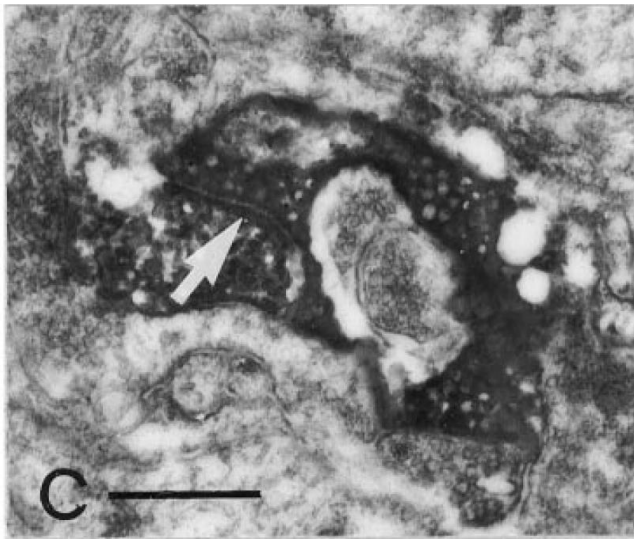
“small *en passant* boutons”and “Filopodial extentions”

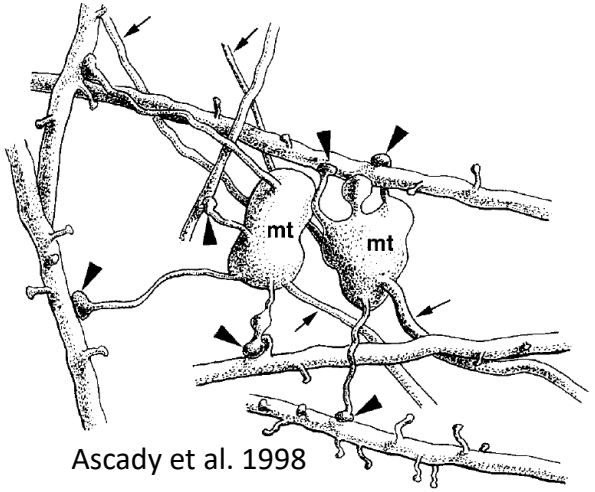
en passant



Staras 2007

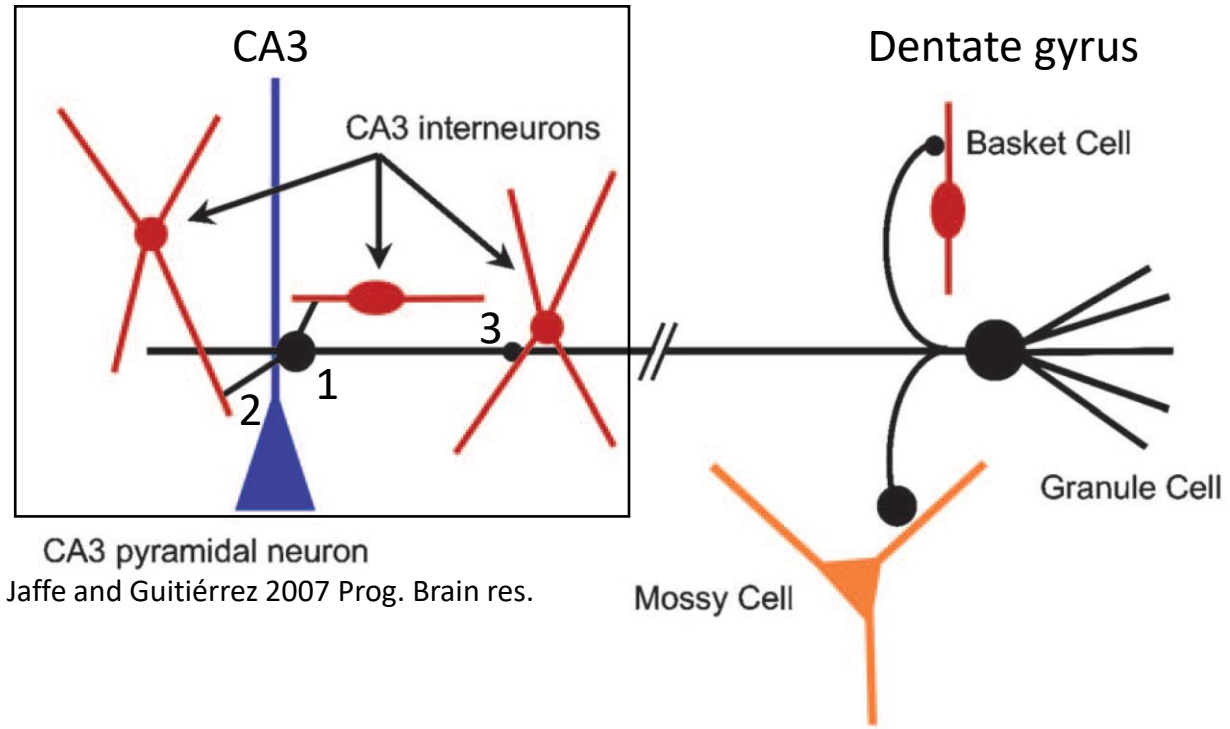
Filapodial extensions





Ascady et al. 1998

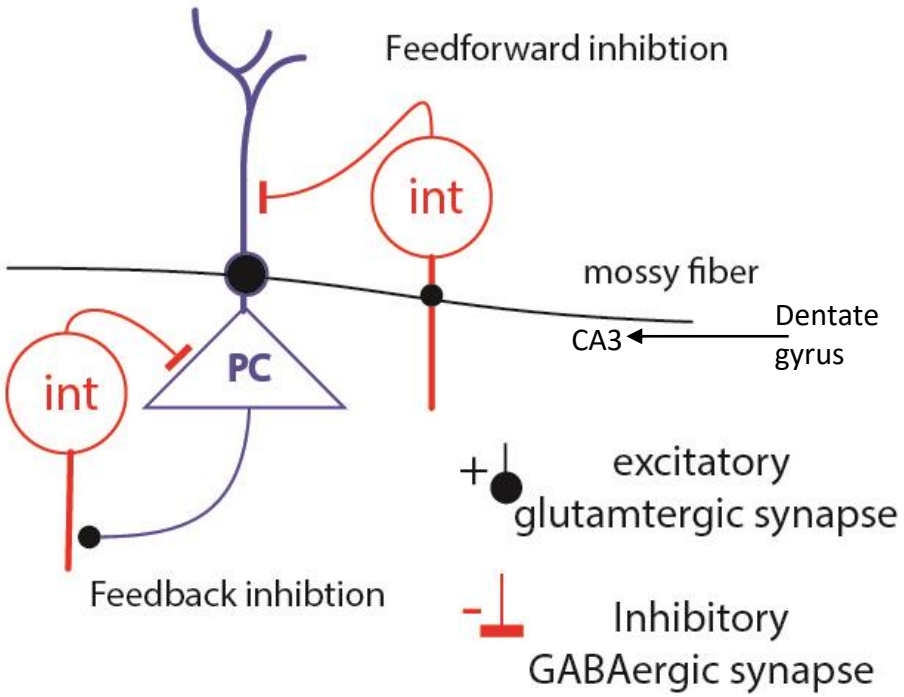
Does mossy fiber activity lead to a net excitation or inhibition of CA3 pyramidal neurons?

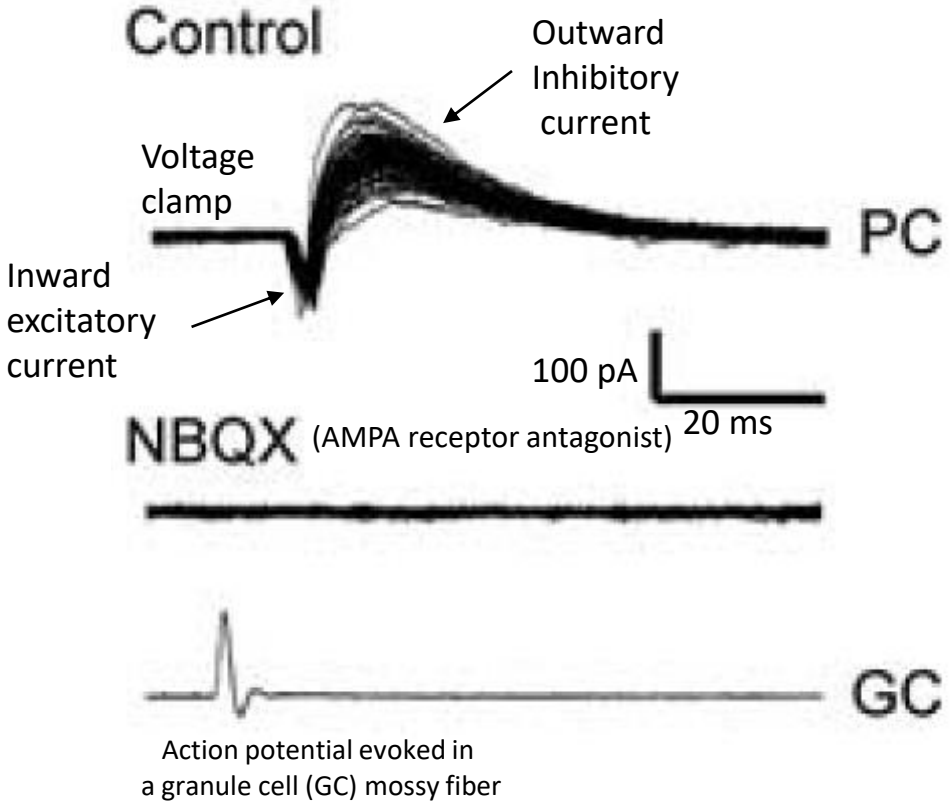


Jaffe and Guitiérrez 2007 Prog. Brain res.

Does mossy fiber activity lead to a net excitation or inhibition of CA3 pyramidal neurons?

Pyramidal Cell (PC)
Interneuron (int)

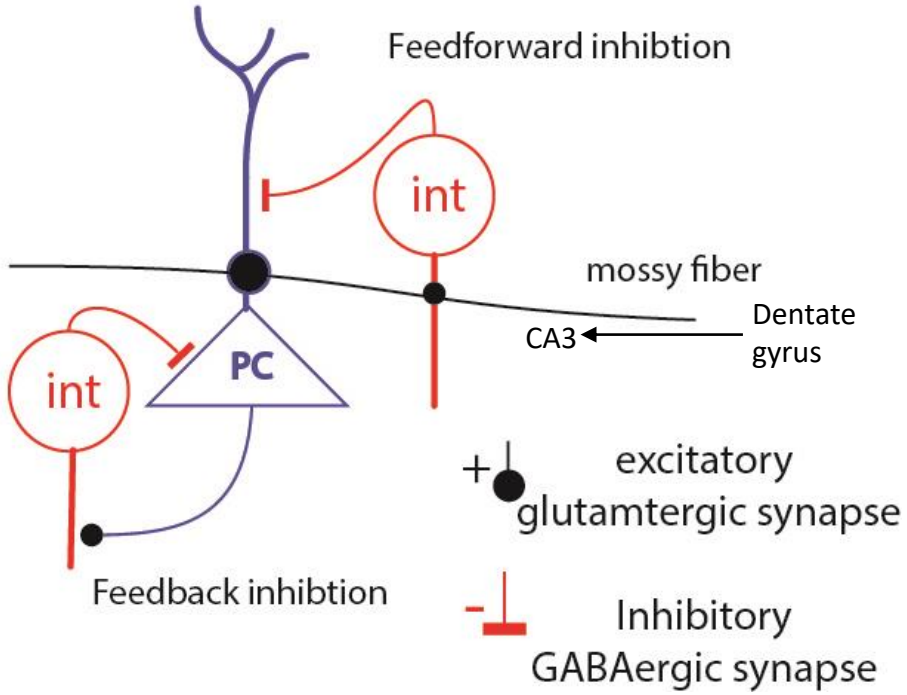


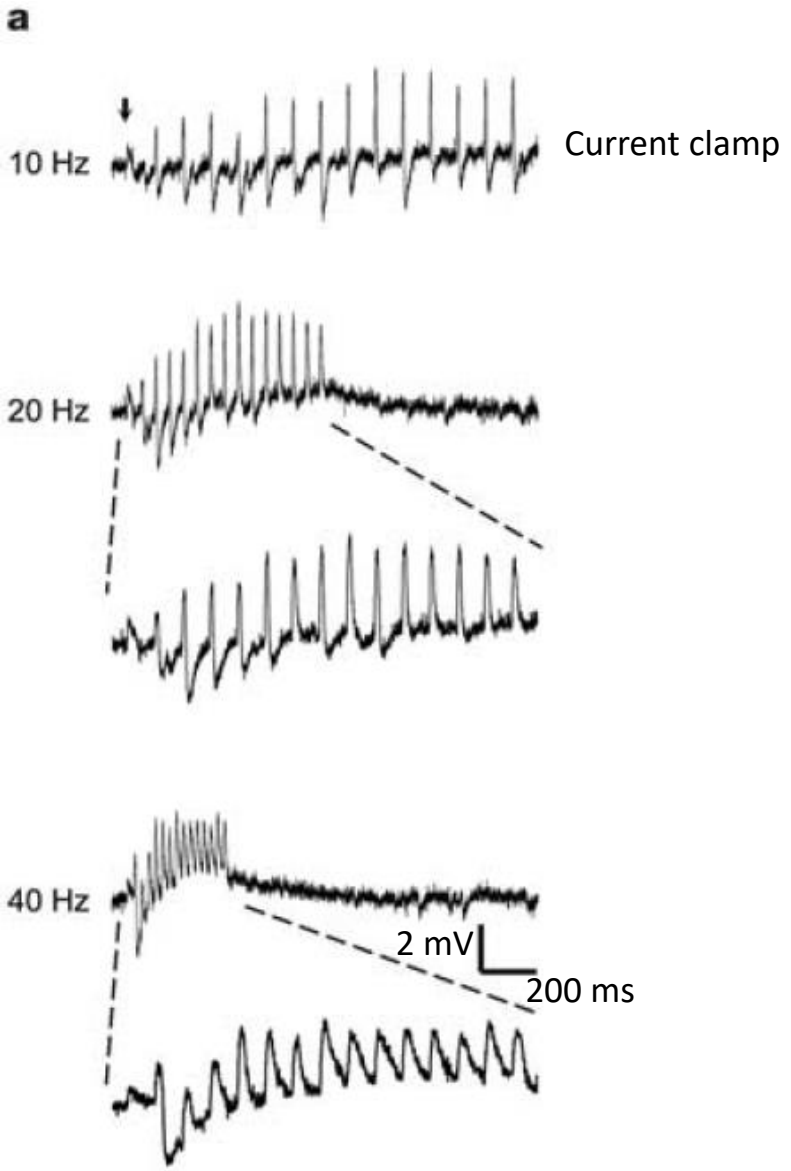


Mori et al. 2004

How do pyramidal cells respond to bursts of granule cell activity?

Pyramidal Cell (PC)
Interneuron (int)



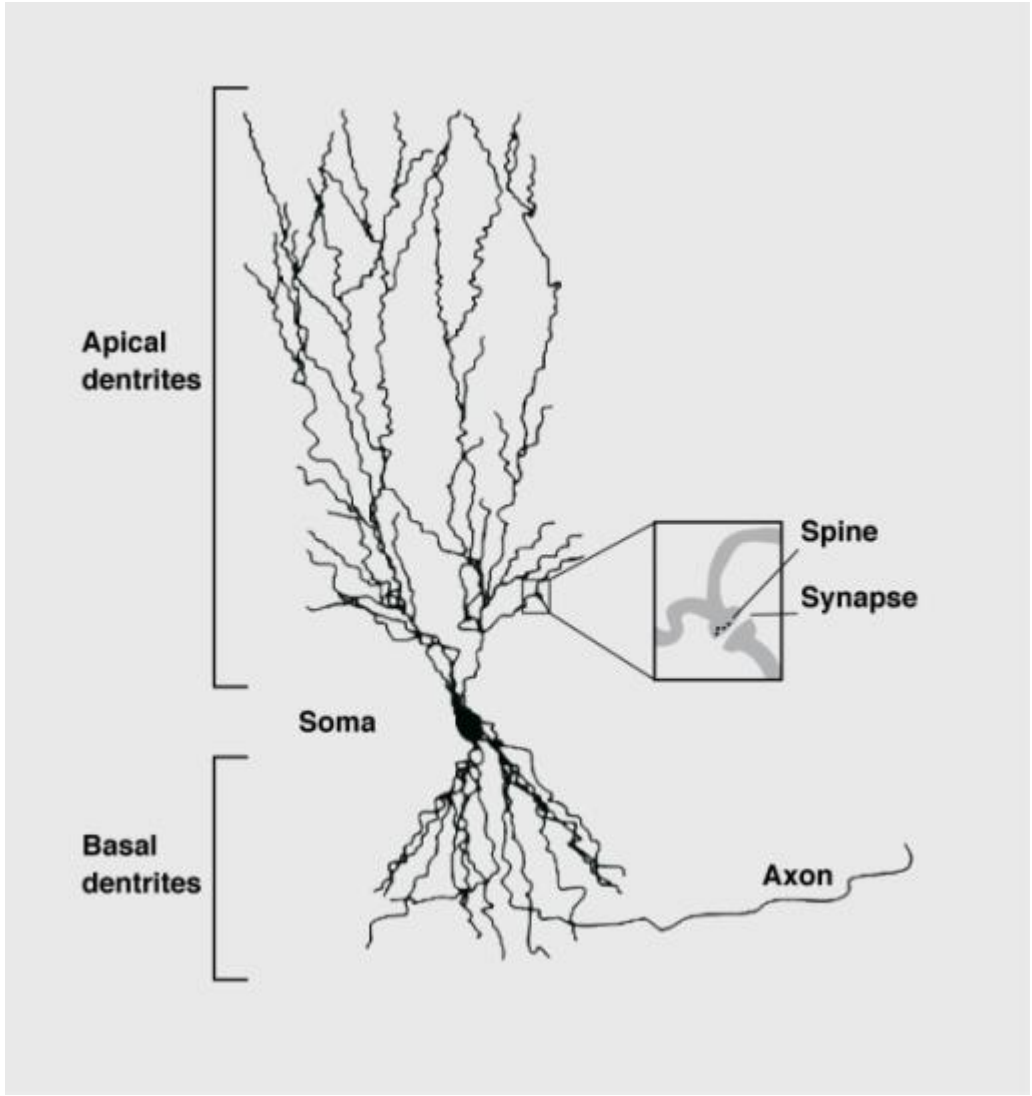


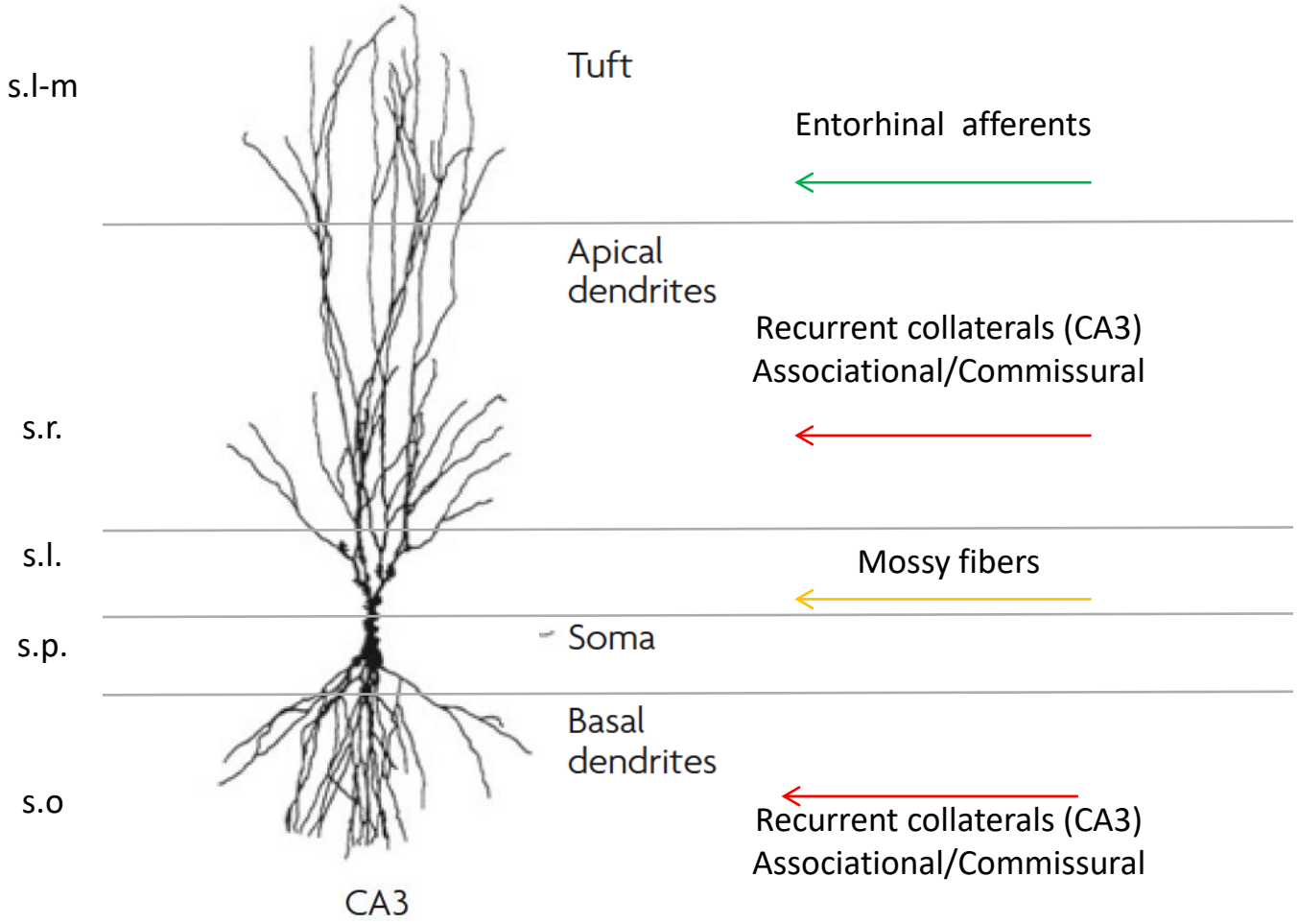
- Mossy fibers make direct synaptic contact onto 4 fold more inhibitory interneurons than pyramidal neurons

With increasing frequency postsynaptic potentials shift from inhibitory dominant to excitatory dominant

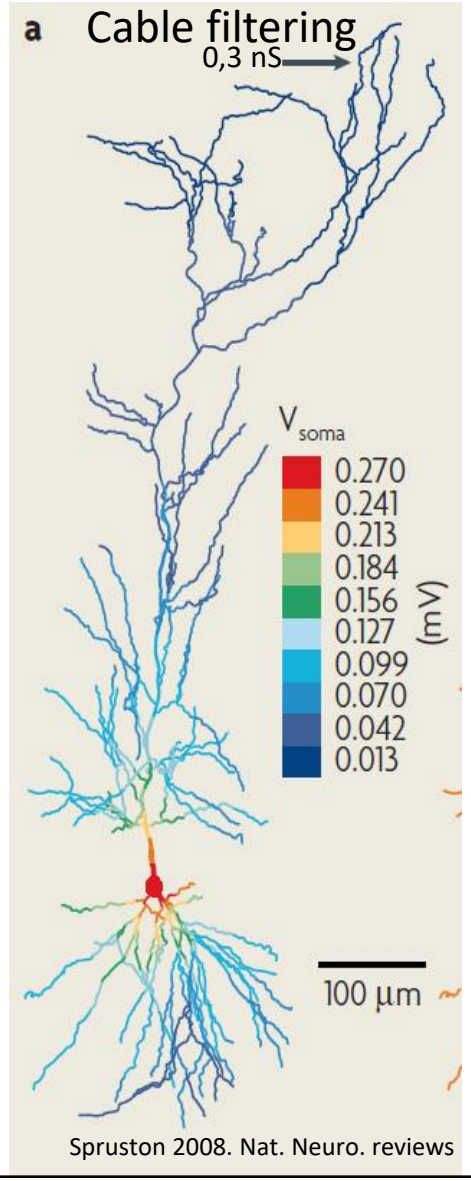
Mori et al. 2004

Layer-specific excitatory inputs onto CA3 pyramidal neurons





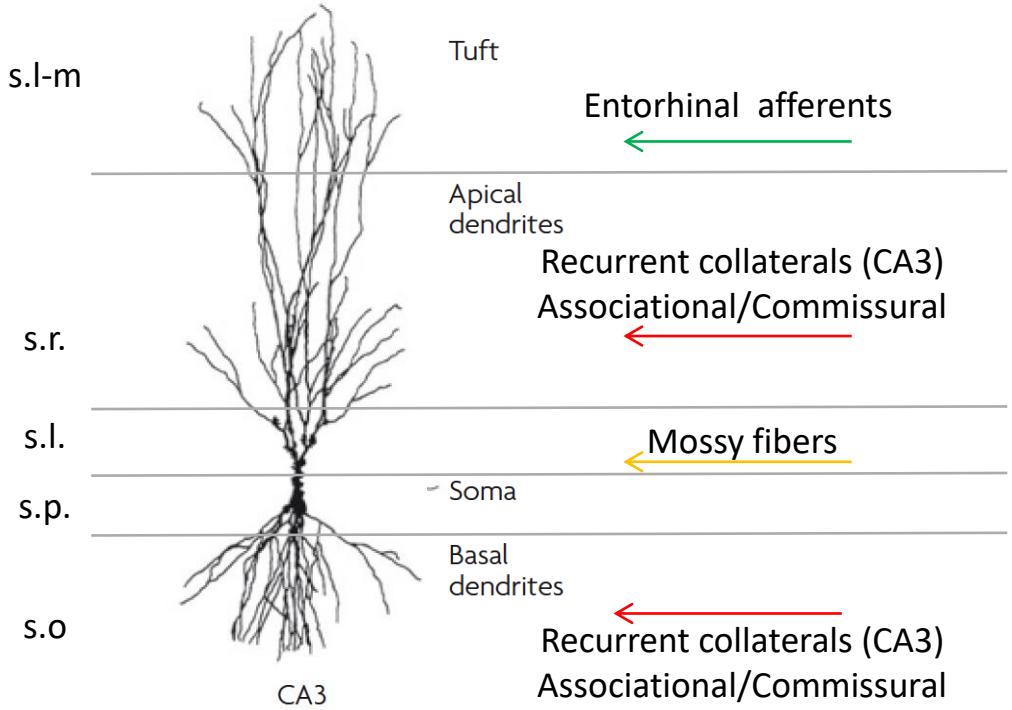
Synapse location influences synaptic transmission: the “detonator theory”



Spruston 2008. Nat. Neuro. reviews

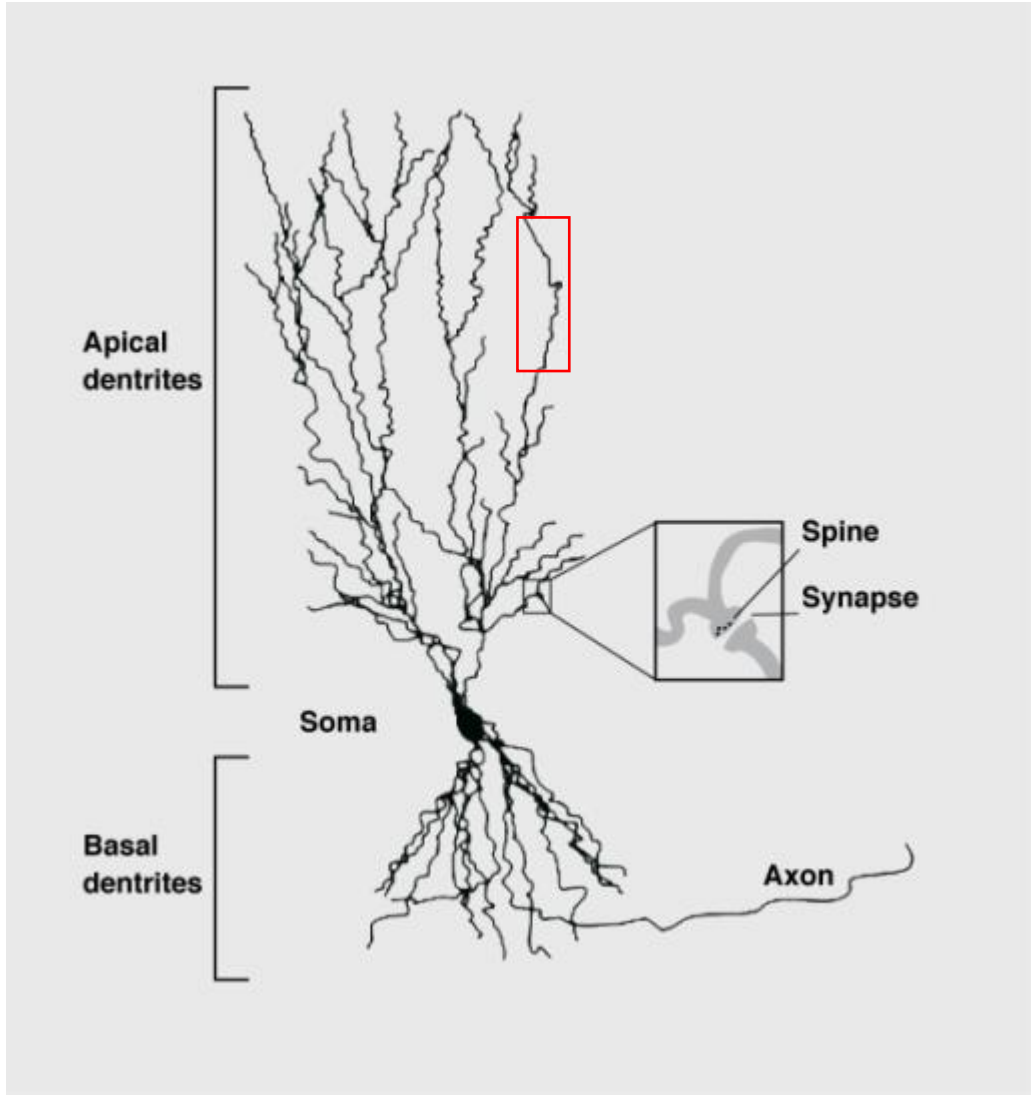
Because synapses are formed at different distances from the soma, the resulting excitatory postsynaptic potentials (EPSPs) are dependent on the dendritic location of each synapse, as predicted by cable theory. Part **a** of the figure shows the amplitude of the somatic EPSP for an excitatory synapse of fixed synaptic conductance (0.3 nS) simulated at each dendritic location in a model of a CA1 pyramidal neuron with passive dendrites. Synapses proximal to the soma produce somatic EPSP amplitudes of 0.2–0.3 mV (yellow–red on the linear colour scale)64, but the amplitude falls off sharply as a function of distance. (Spruston 2008)

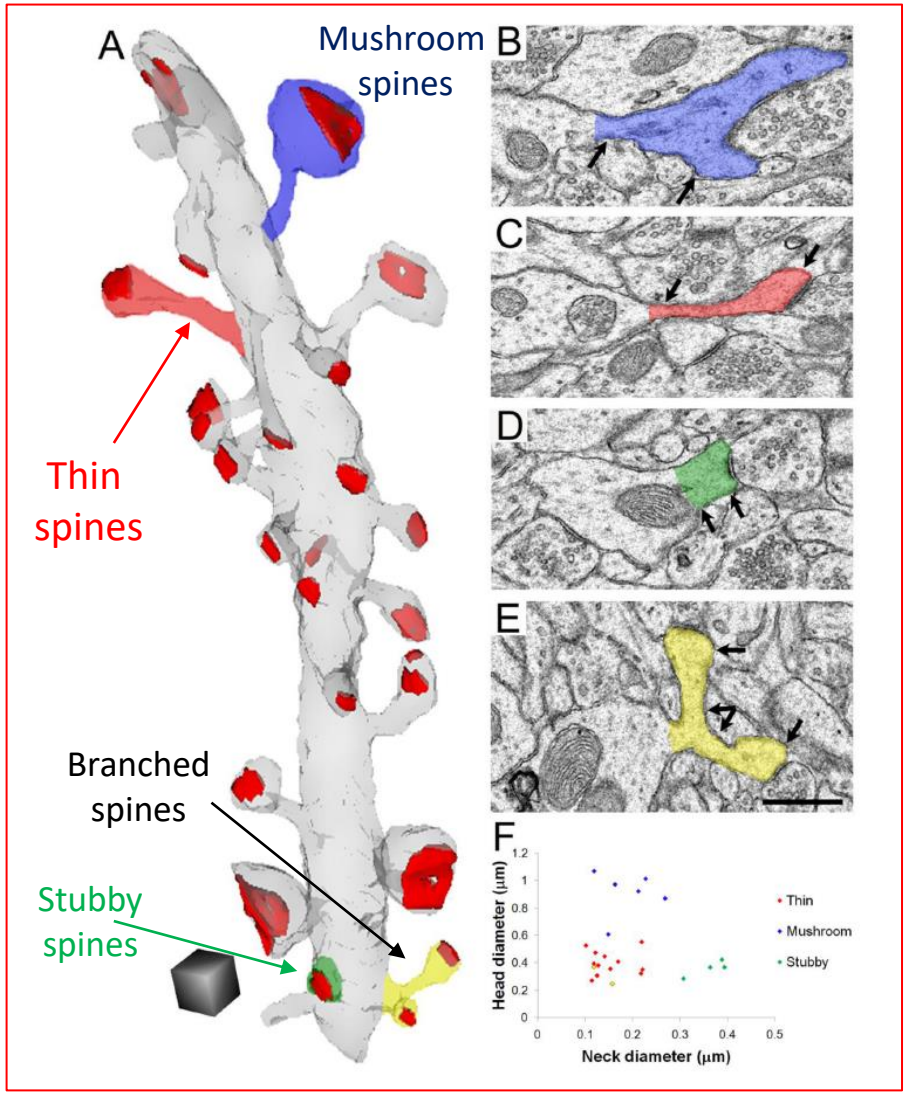
Layer-specific excitatory inputs



- Proximal location of large mf terminals filled with vesicles (high probability of release) suggests that granule cells may have a strong excitatory influence over CA3 pyramidal cells
- For example a single action potential could bring the pyramidal cell to fire. This is referred to as the **“detonator theory”.**

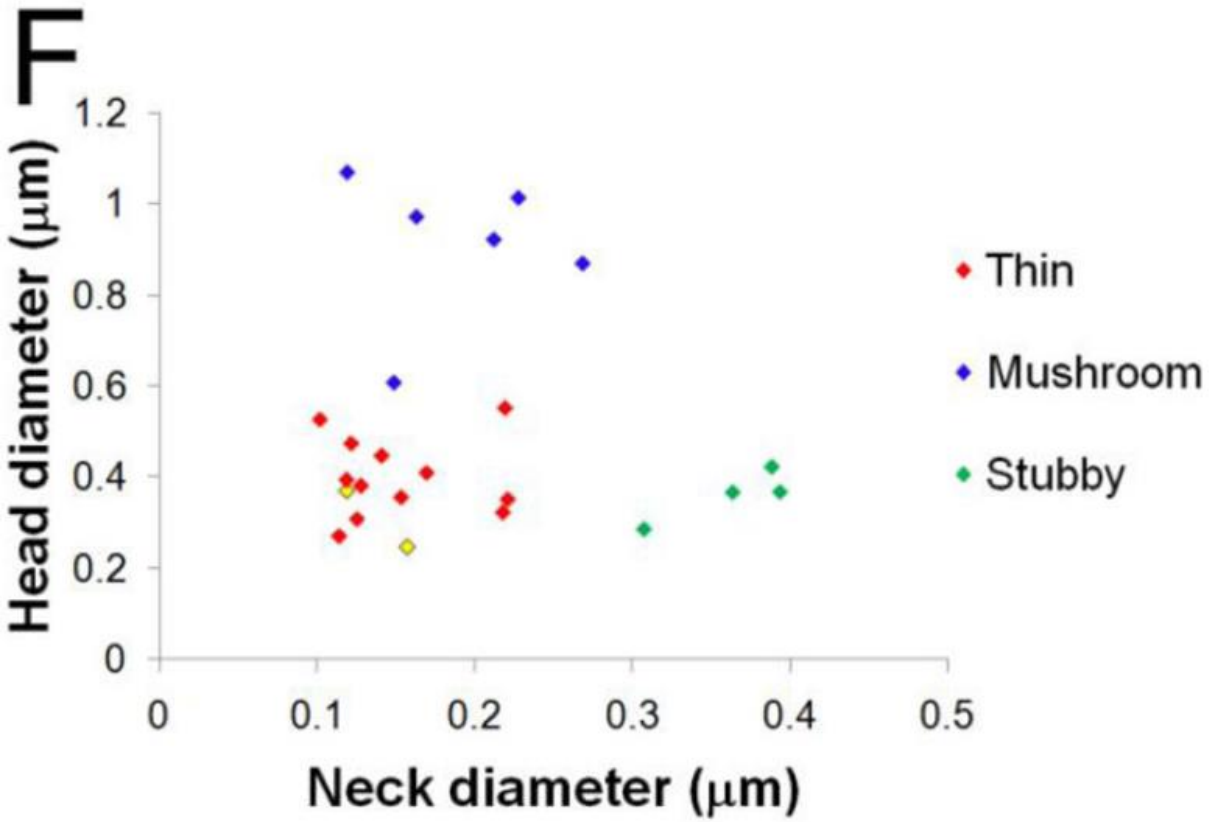
Dendritic spine morphological heterogeneity the apical and basal dendrites of CA3 pyramidal cells

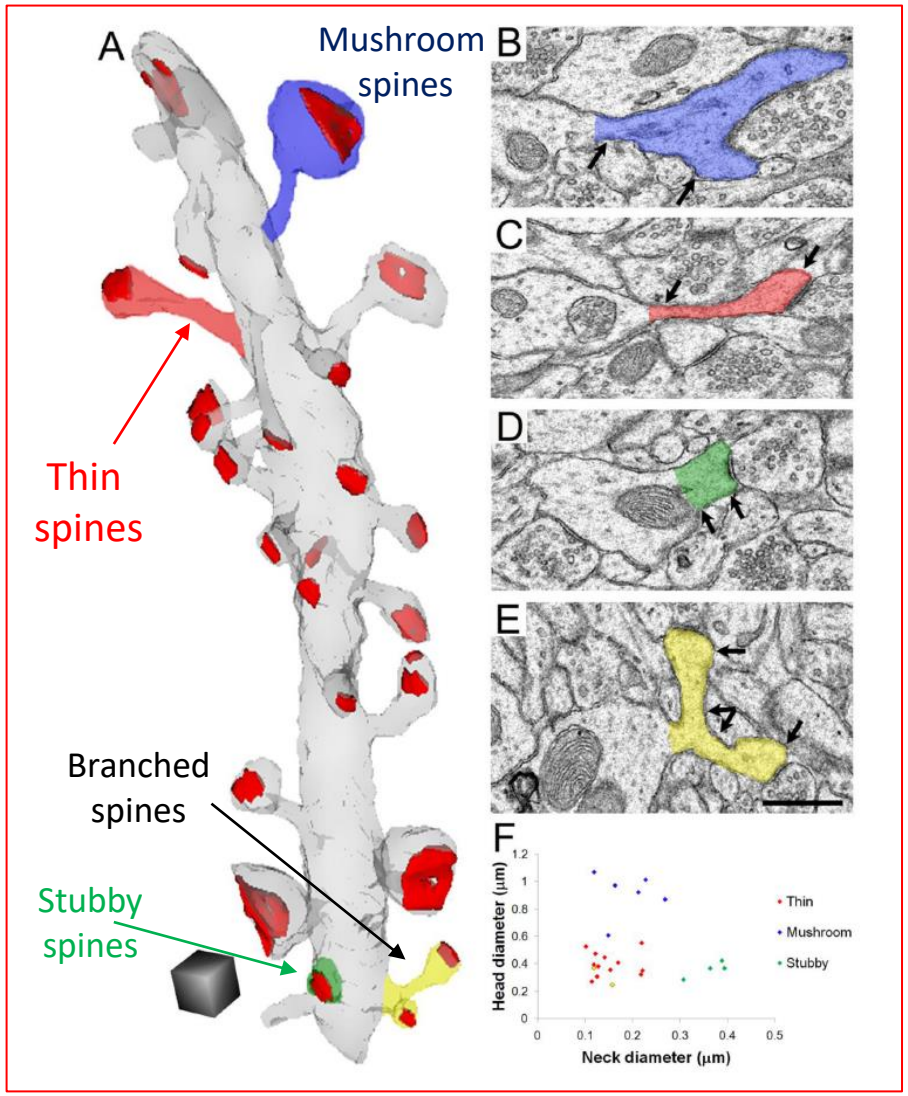




Bourne and Harris et al. 2008. Ann Rev neurosci.

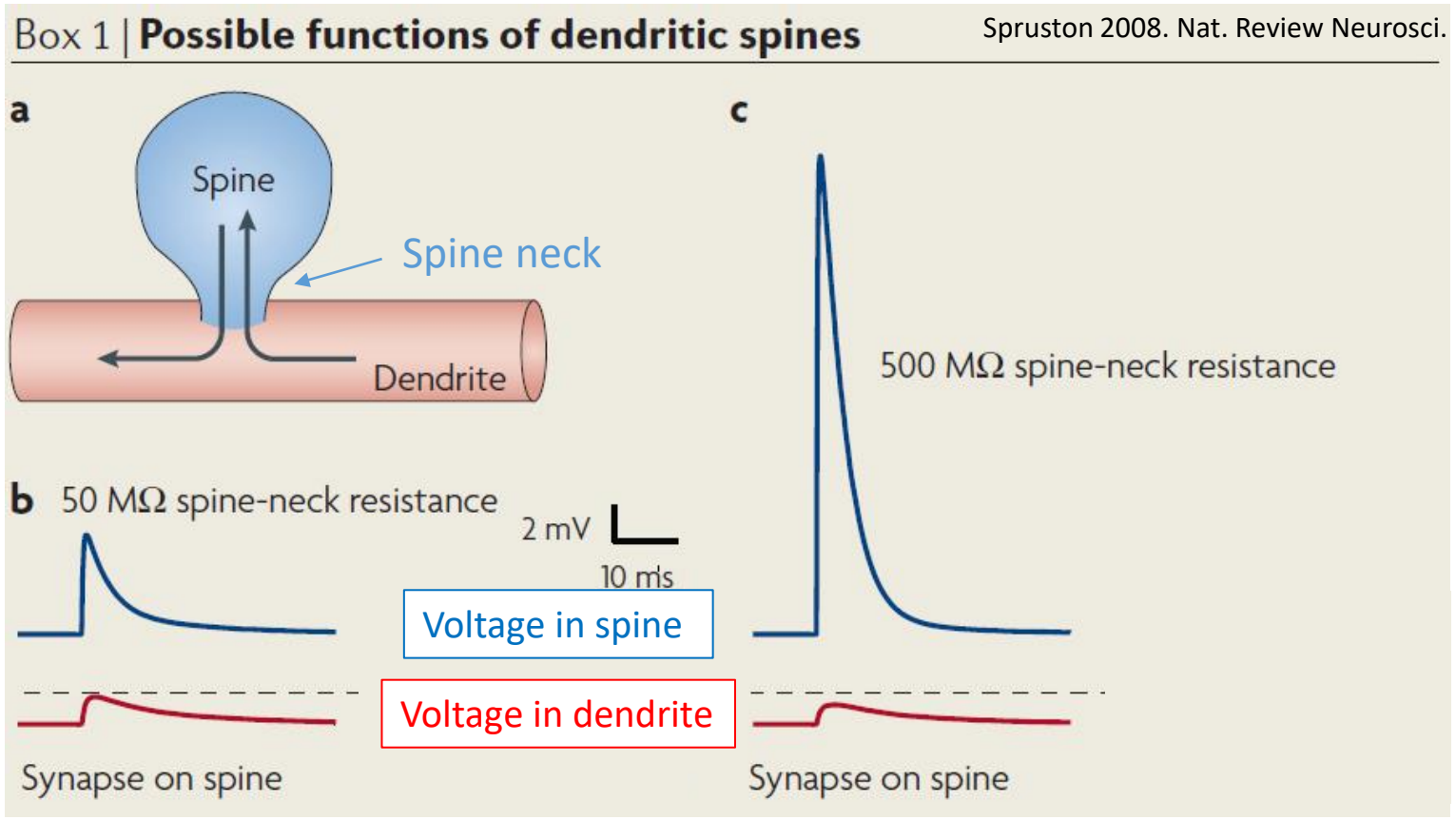
Dendritic spine morphological heterogeneity the apical and basal dendrites of CA3 pyramidal cells





Bourne and Harris et al. 2008. Ann Rev neurosci.

Spine shape influences voltage in the spine and thus synaptic transmission

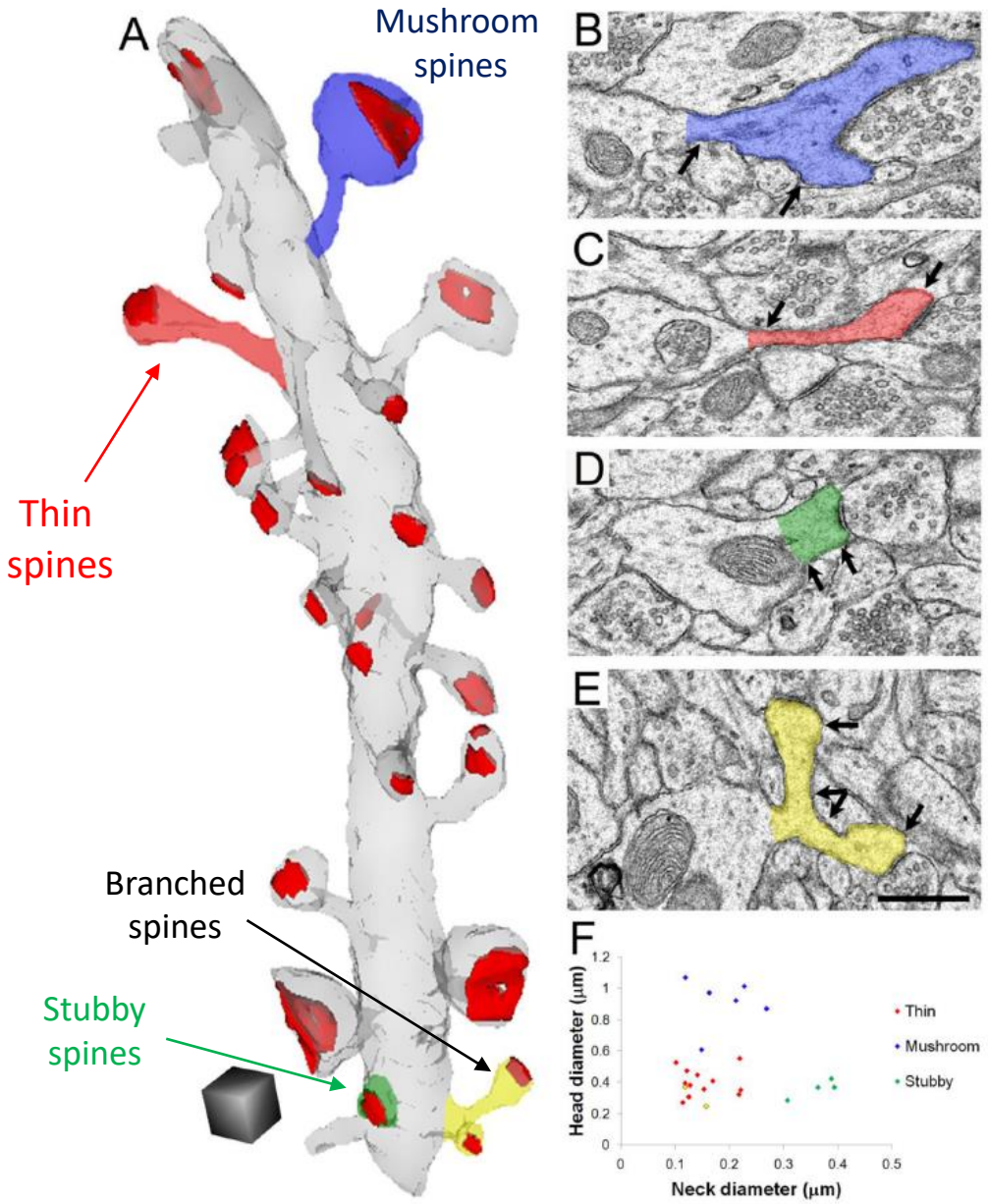


ohm's law, V=IR
V=voltage
I=current
R= resistance

V=I * 50 mOhms
V=I * 500 mOhms

The shape of the spine influences the magnitude of membrane depolarization in the spines and the dendrites. The small size of the spines makes their input impedance significantly larger than that of the dendrite. Spines with a higher neck resistance will also generate a larger depolarization in comparison with spine with a lower neck resistance (synapse on the spine b vs. c). In contrast, the depolarization in the dendrite is smaller with a higher spine neck resistance for two reasons: 1,The larger voltage change reduces the driving force of synaptic currents; 2,Some charge is lost as current flows from the spine to the dendrite (figure C, compare red and blue traces).

Dendritic spine heterogeneity on CA3 pyramidal cell dendrites



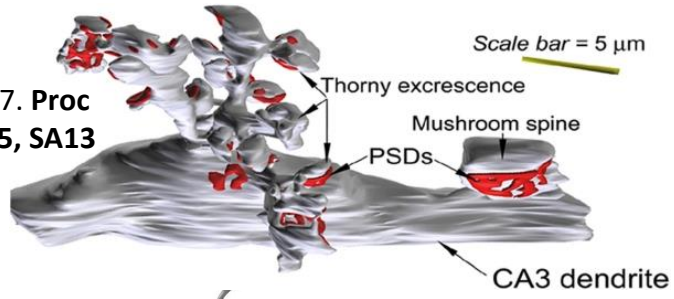
- Pyramidal cells are covered with thousands of dendritic spines that form primarily glutamatergic synapses
- The resistance of the dendritic spine neck will influence the magnitude of depolarization in both the spine and in the dendrite.
- Dendritic spines are diverse varying in their shape and size. Changes in spine volume are implicated as a mechanism of synaptic plasticity.
  - Larger spines have more powerful synapses.
  - Smaller spines are more dynamic than larger spines, and repetitive stimulation of smaller spines can lead to increases or decreases in their volume.
- It has been proposed that thin dynamic spines may be implicated in learning and synaptic plasticity whereas large spines, which maintain a more static morphology, may be implicated in coding of memories.

Bourne and Harris et al. 2008. Ann Rev neurosci.

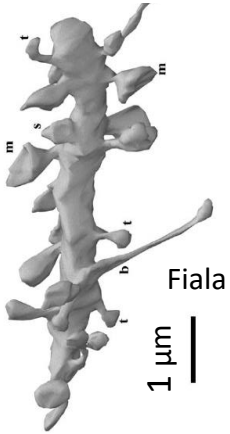
Examples as to how synaptic morphology may influence synaptic physiology

1. Larger surface of **dendritic spines** may:
   - Permit larger and thus more powerful synapses
   - Alter the recycling and diffusion of postsynaptic receptors in the membrane.
   - Alter intracellular signaling and diffusion of intracellular signals (calcium, cAMP) between the spine and the dendrite
   - May alter the electrical properties synaptic transmission
2. Larger surface area of **presynaptic terminals** may:
   - Alter presynaptic dynamics, vesicle recycling, loading of neurotransmitter, calcium channel density
3. The complex morphology of **thorny excrescences** and may alter the diffusion of the neurotransmitters:
   - Longer period of interaction between the neurotransmitter and the receptor
   - Spillover of neurotransmitter onto adjacent synapses
4. The interaction of **perisynaptic astroglia processes (PAPs)** with spines may also alter synaptic transmission by shaping glutamate uptake or through the release of various neurotransmitters

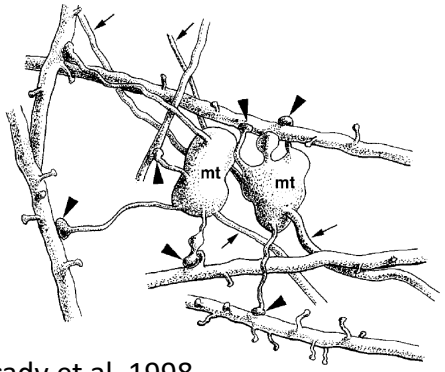
5. The differences in the anatomical properties of granule cell mossy fiber terminals onto glutamatergic pyramidal neurons and GABAergic inhibitory interneurons may underlie changes in the balance between excitation and inhibition during repetitive stimulation.



Stewart 2007. **Proc Physiol Soc 5, SA13**



Fiala and Harris. 1999



Ascady et al. 1998

Quiz 3 (page 1):

1) Indicate if the response is true:

A) Granule cells project “mossy fiber” axons that form giant synapses onto thorny excrescences on the proximal dendrites of CA3 pyramidal neurons

B) Granule cells project axons that form *en passant* synapses onto on the dendrites of CA3 pyramidal neurons.

C) Dentate gyrus granule cell projections to the CA3 may result in excitation or inhibition of CA3 pyramidal neurons depending of the frequency of stimulation.

D) Mossy fibers terminals in the CA3 are purely glutamatergic.

E) Two synapses of equal strength at different positions along the proximal / distal axis of a dendritic arbor should equally influence the firing frequency of the postsynaptic neuron.

A) Indicate if the response is true:

A) Dendritic spines on CA3 pyramidal neurons are are a homogenous population

B) Dendritic spines on CA3 pyramidal neurons exhibit a very stable structure

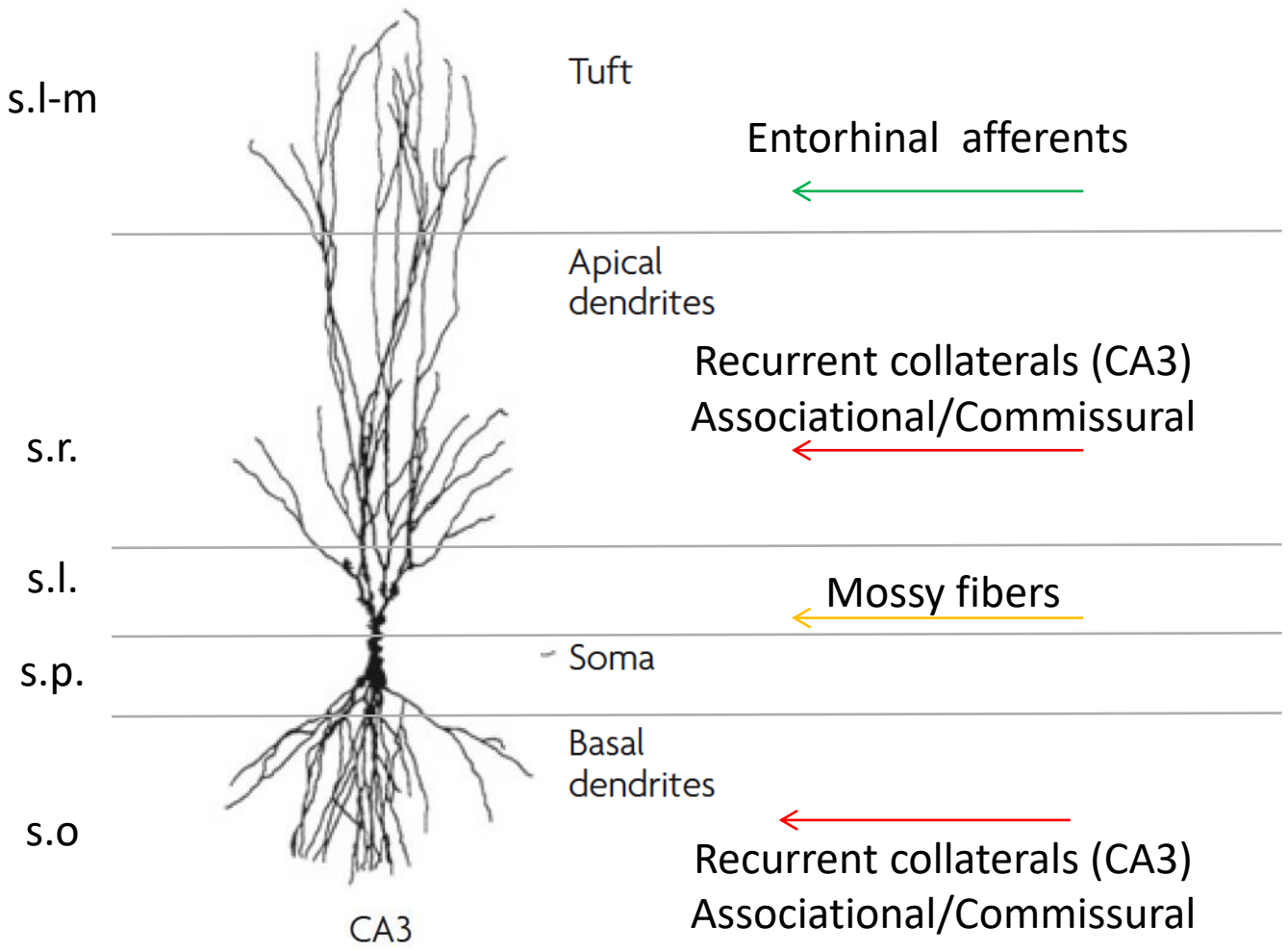
C) For the same synaptic current, a dendritic spine with a relatively large neck resistance will exhibit a greater depolarization than a for a spine with a lower neck resistance.

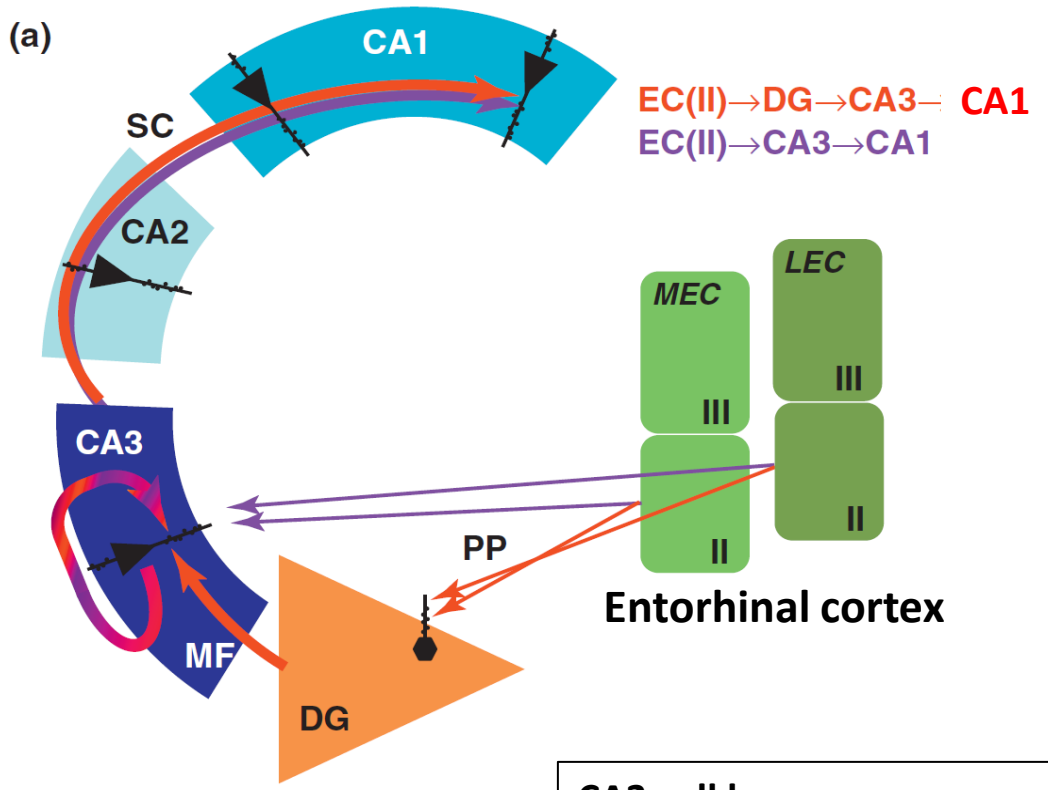
D) Glial cells in the CA3 region of the hippocampus exhibit low affinity glutamate transporters that should not influence the temporal profile of glutamate in the synaptic cleft

E) Changes in the morphology of dendritic spines do not appear to be implicated as a mechanism of long term synaptic plasticity

Stratification of CA3 pyramidal cell inputs

Layer-specific excitatory inputs





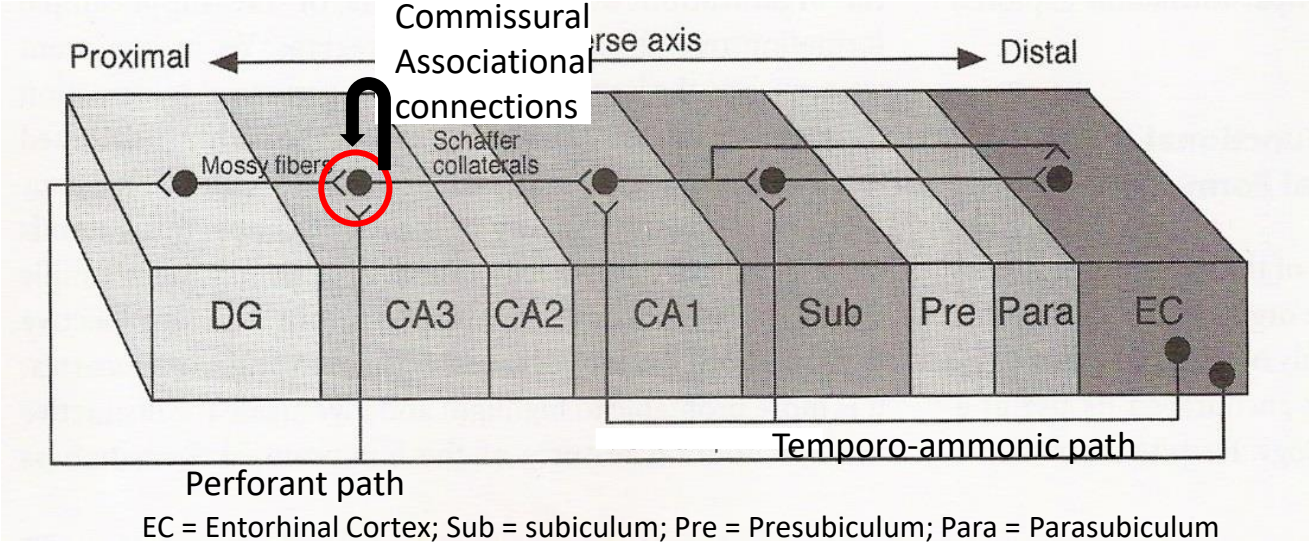
CA3 cell layers

Al = alveus
So = stratum oriens
Pyr =pyramidal cell
Sl =stratum lucidum
Sr = stratum radiatum
sl_m = lacunosum-moleculare

Inputs to CA3 pyramidal neurons

Three principal sources of excitatory input converge onto CA3 pyramidal neurons:

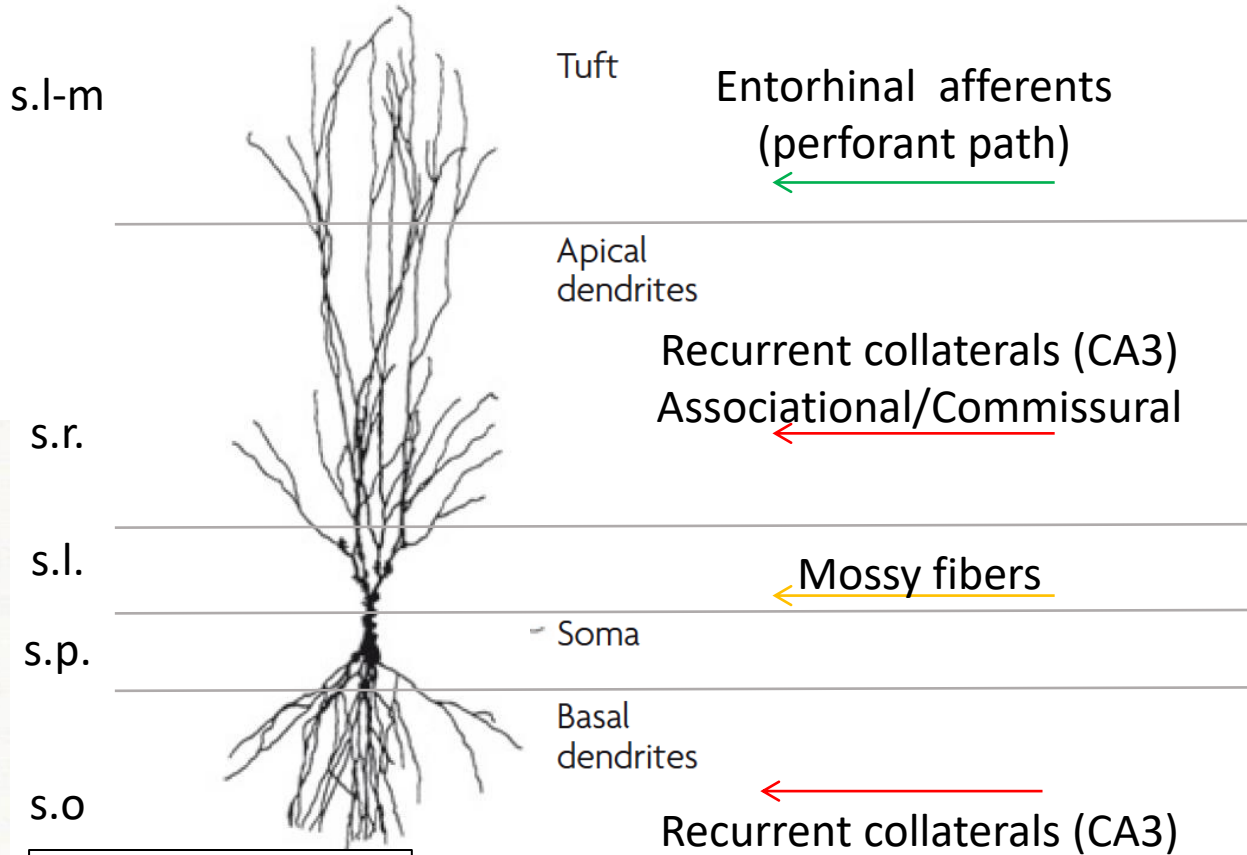
1. Mossy fibers
2. Entorhinal afferents (Perforant path)
3. Commissural and Associational recurrent CA3 pyramidal neuron axons



EC = Entorhinal Cortex; Sub = subiculum; Pre = Presubiculum; Para = Parasubiculum

The hippocampus book, 2007.

Layer-specific excitatory inputs



CA3 cell layers
Al = alveus
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Pyr =pyramidal cell
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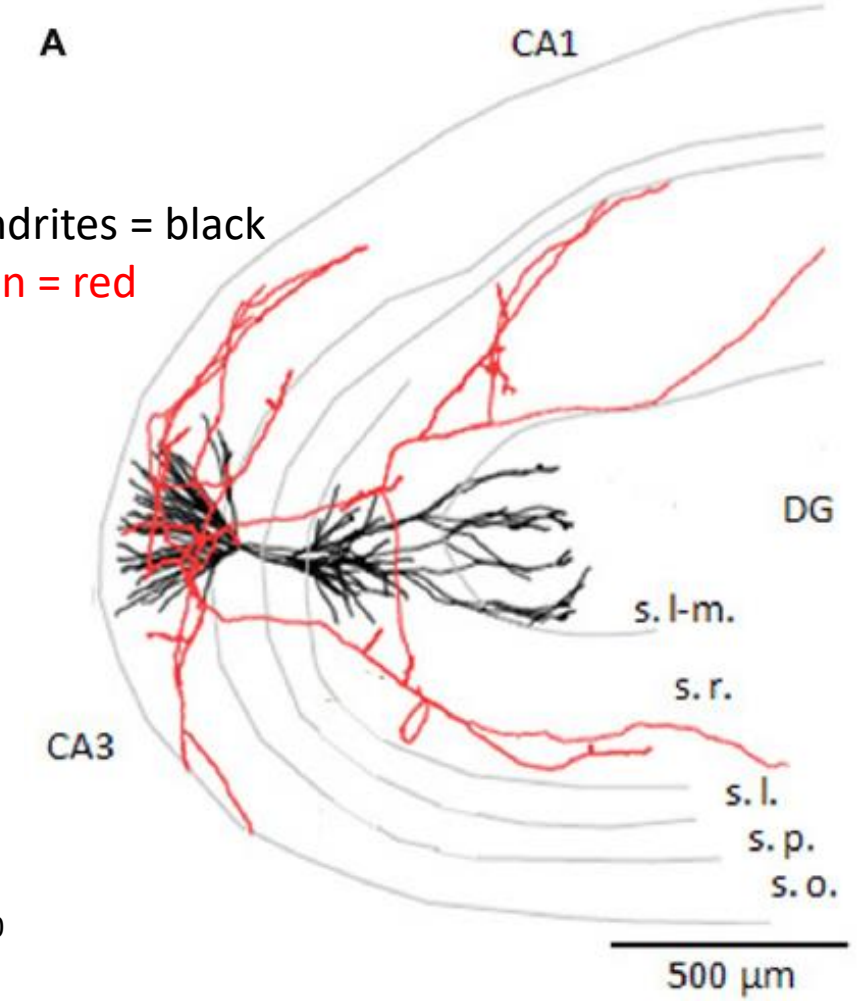
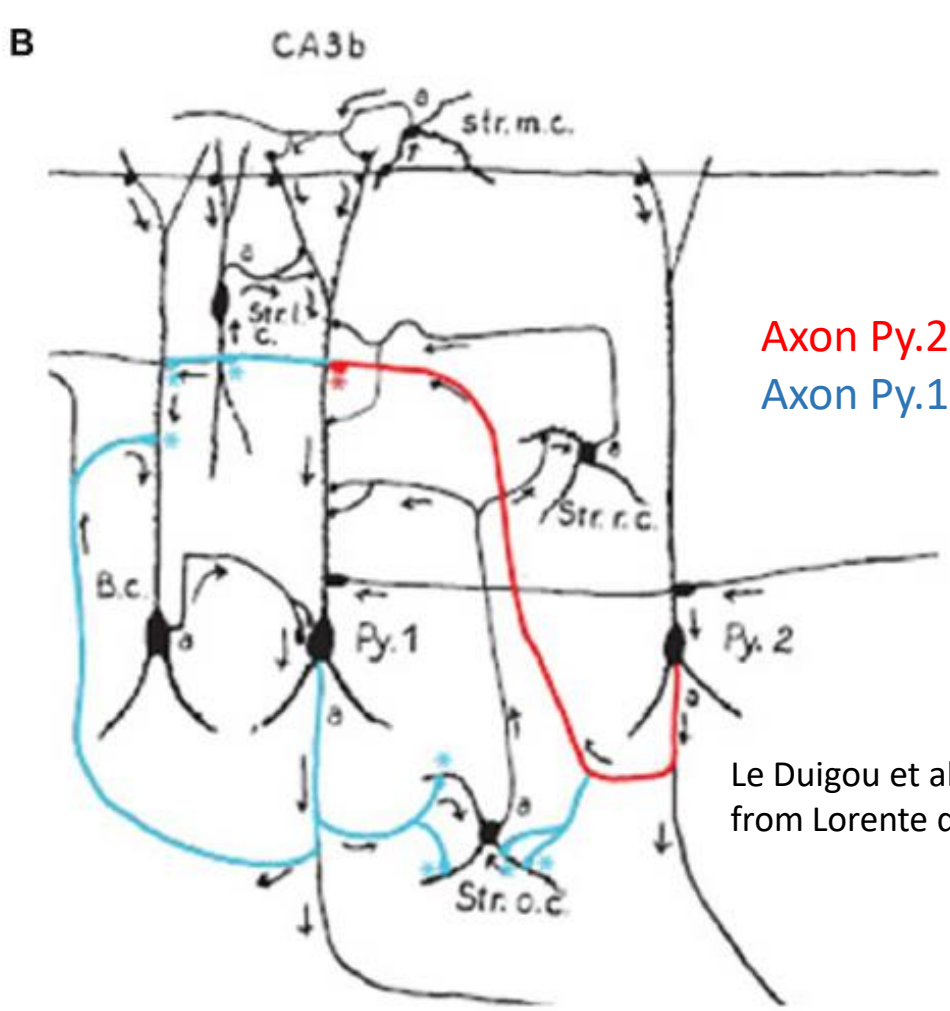
sl_m = lacunosum-moleculare

CA3 to CA3 associational connections (recurrent collaterals)

Dendrites = black
Axon = red



Ishizuka et al. 1990

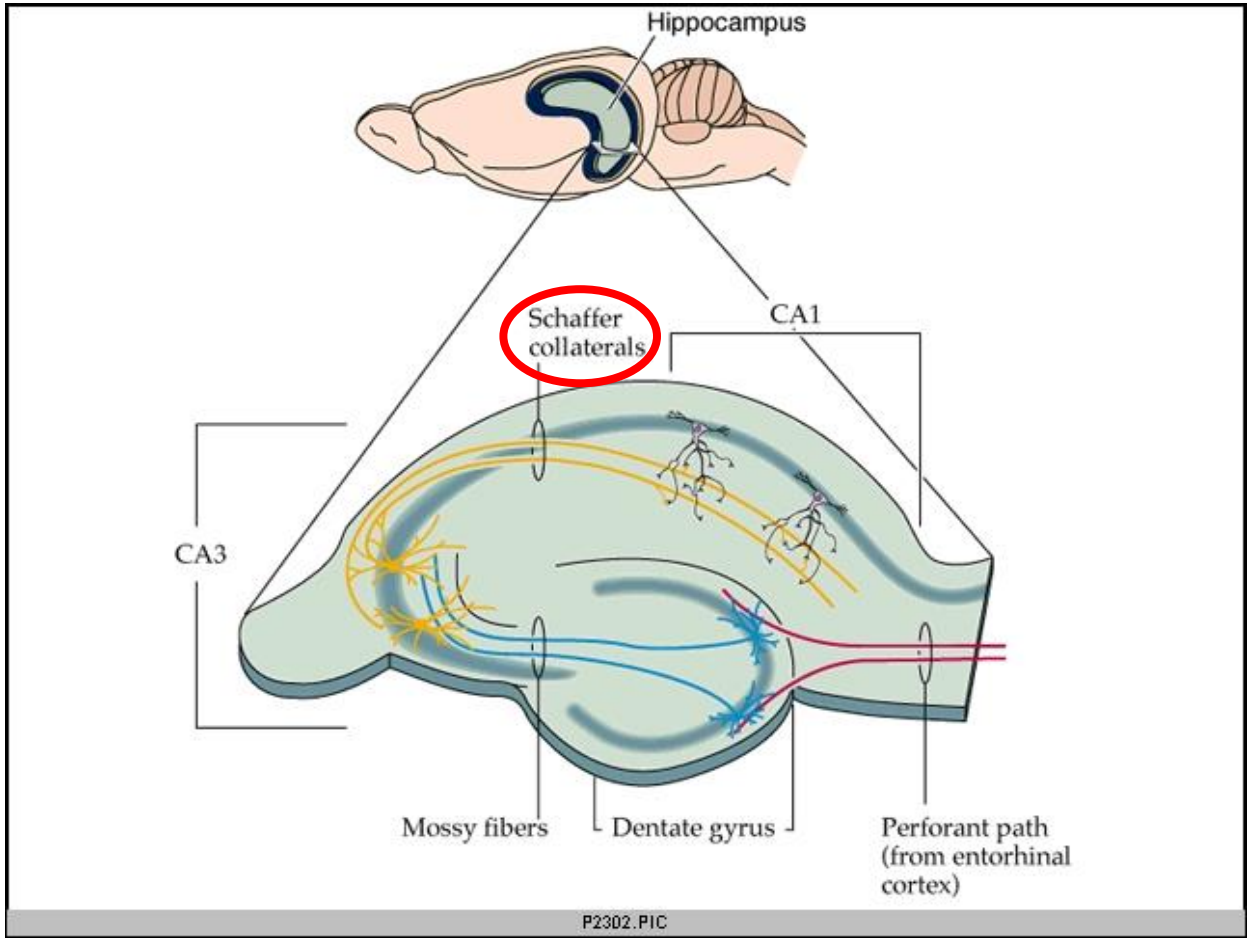


Axon Py.2 = red
Axon Py.1= blue

Le Duigou et al. 2014 (adapted from Lorente de Nó,1934).

The main source of excitatory input to the CA3 region is the CA3 region via associational connections. These associational connections permit pyramidal cells to excite other hippocampal neurons throughout much of the ipsilateral and contralateral hippocampus (the hippocampus book, 2007).

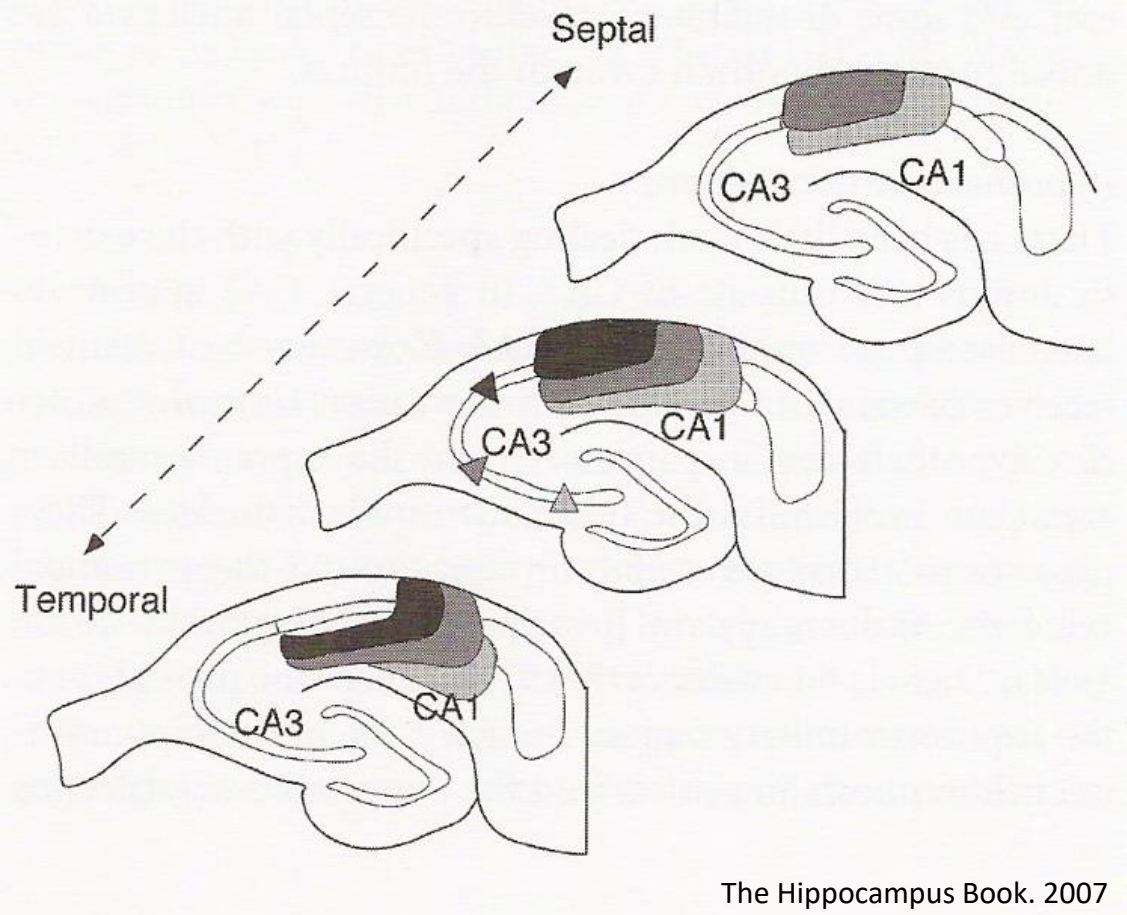
CA3 to CA1 Shaffer collaterals



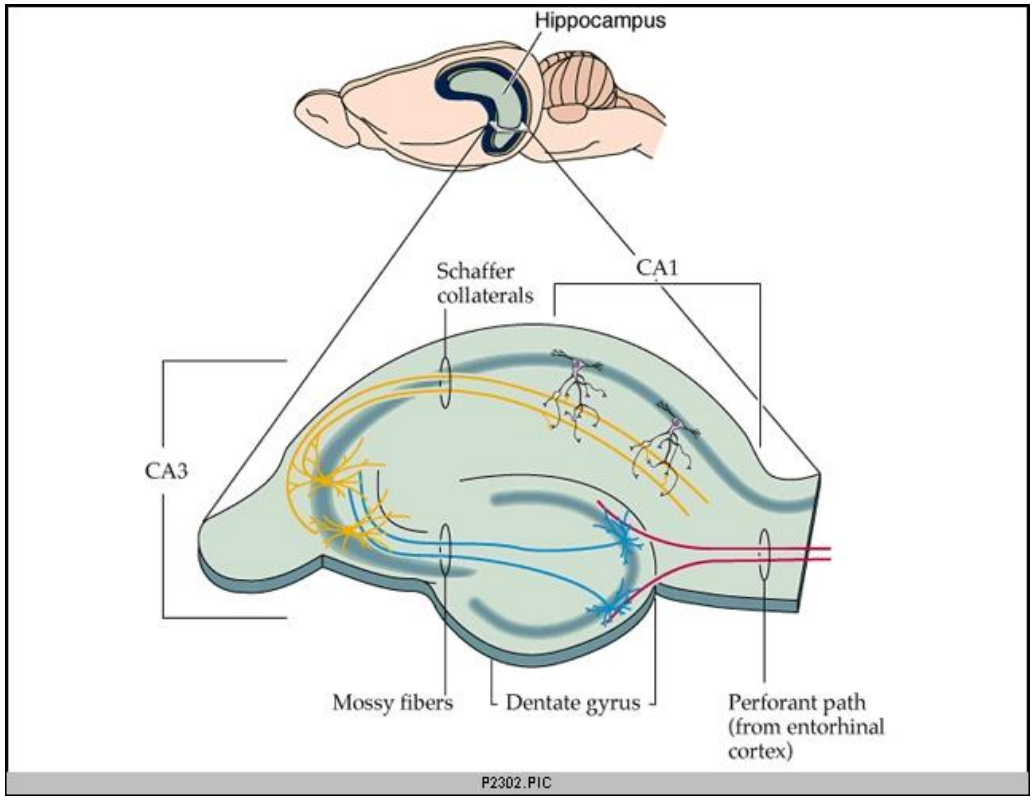
http://www.arts.uwaterloo.ca/~bfleming/psych261/imageIT4.JPG

Pyramidal neurons in CA3 and CA2 project to CA1 via Shaffer collaterals. CA3 pyramidal cells axons display extensive collaterals that run in both the transverse and oblique orientation through CA1. Shaffer collaterals terminate in both the **stratum radiatum** and the **stratum oriens** although many schemas depict Shaffer collaterals terminating exclusively in the stratum radiatum. CA3 pyramidal cells axons may terminate in CA1 throughout 2/3 of the septo-temporal axis.

CA3 to CA1 Shaffer collaterals septo-temporal distribution



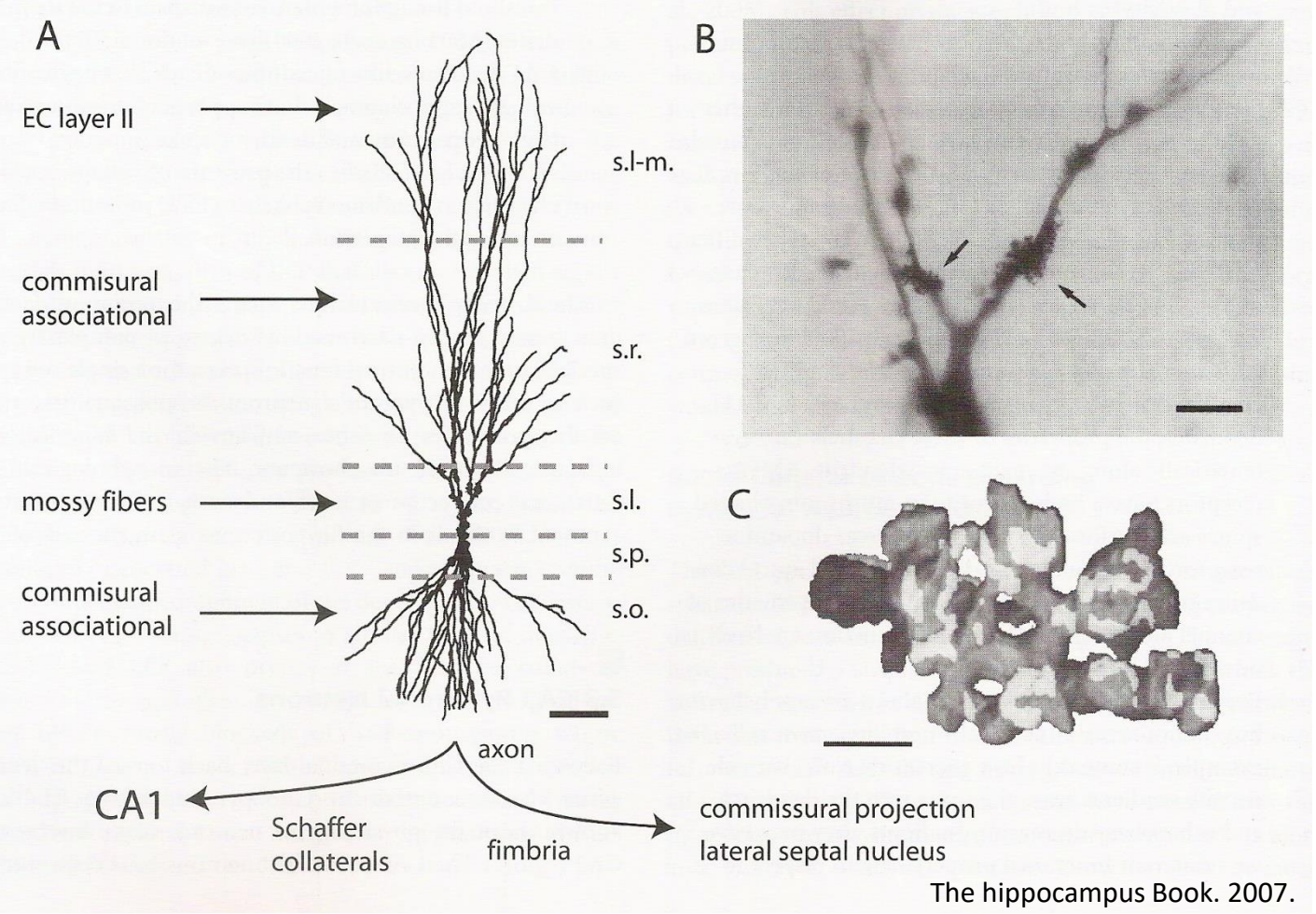
The Hippocampus Book. 2007



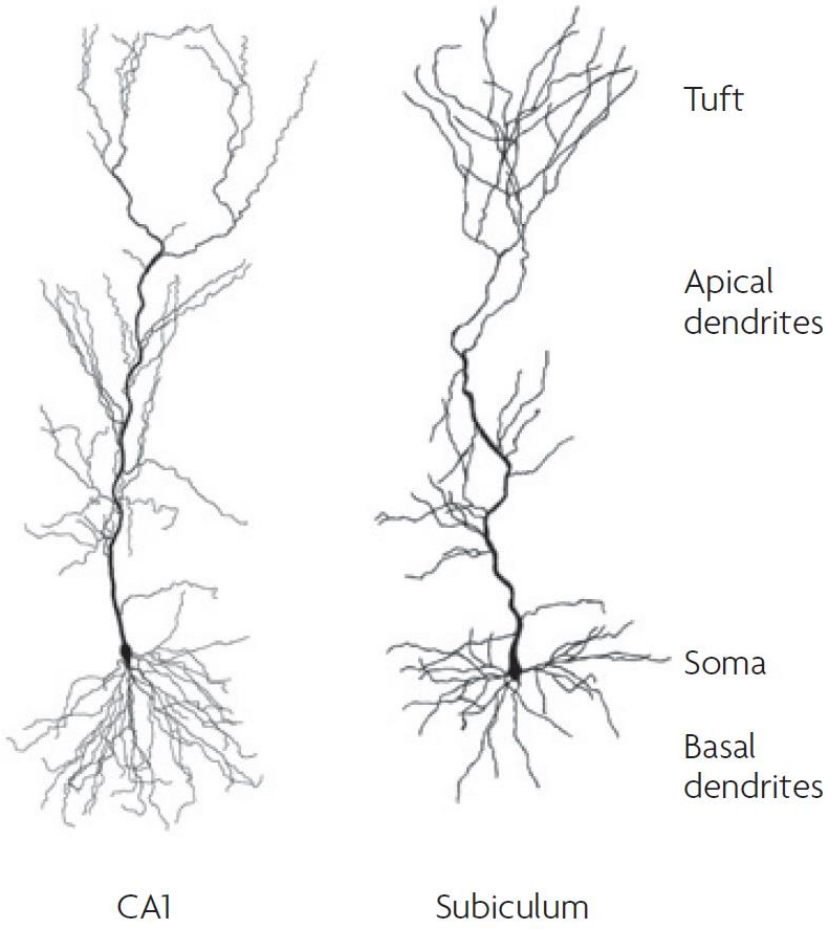
http://www.arts.uwaterloo.ca/~bfleming/psych261/imageIT4.JPG

CA3 pyramidal neurons proximal to the dentate gyrus exhibit axonal projections that terminate predominately in septal regions of CA1 and terminate in both the stratum radiatum and the stratum oriens layers. In contrast CA3 neurons closer to CA1 display heavier axonal projections to temporal CA1 regions and terminate predominately in the stratum radiatum.

CA3 pyramidal neuron morphology



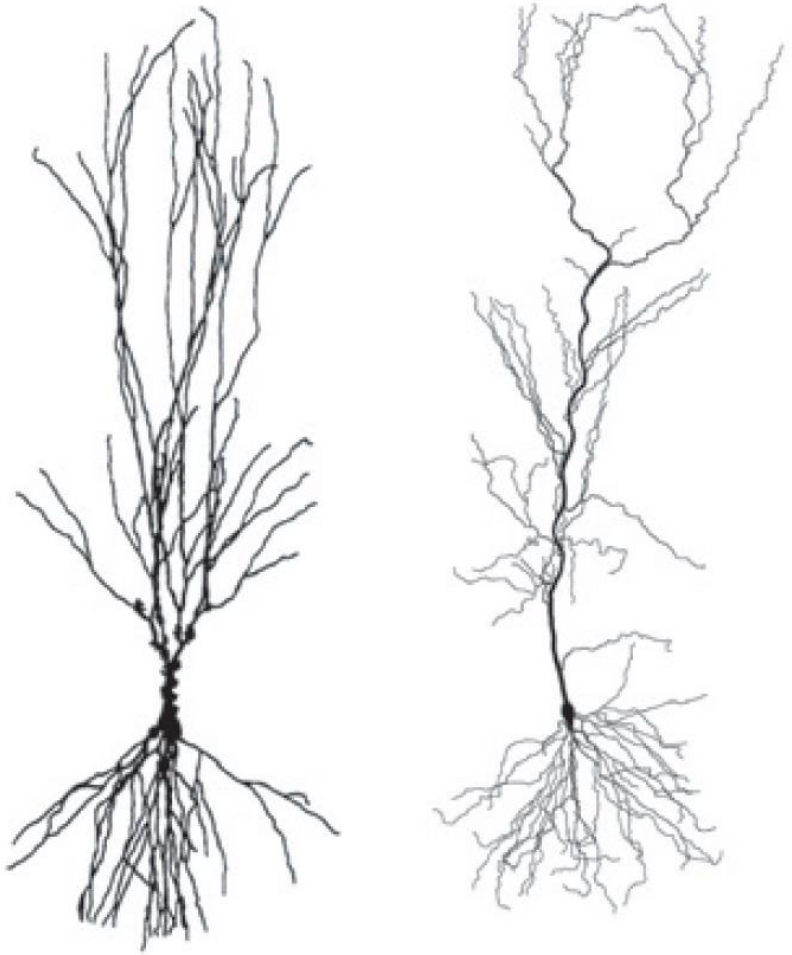
The hippocampus Book. 2007.

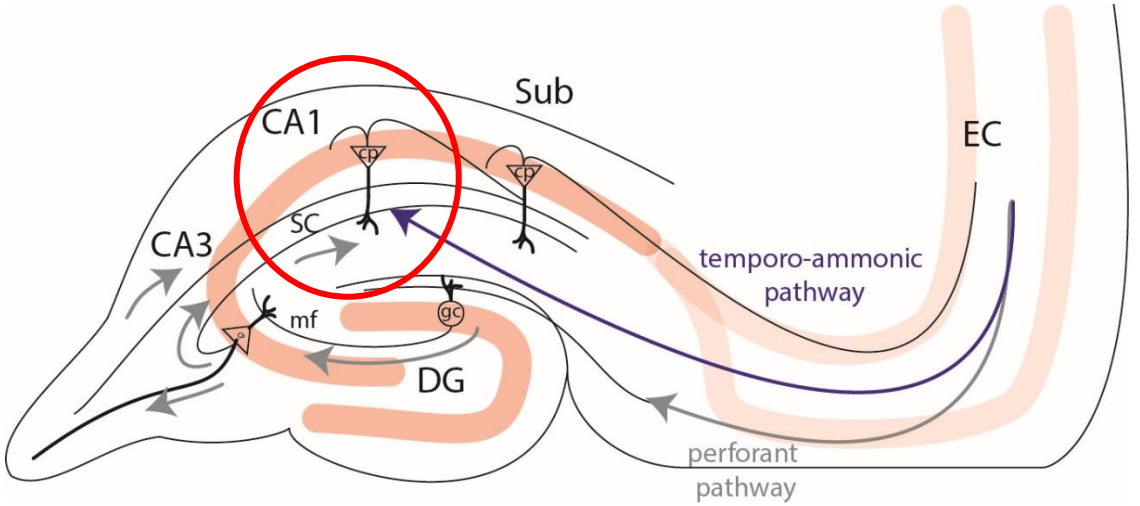


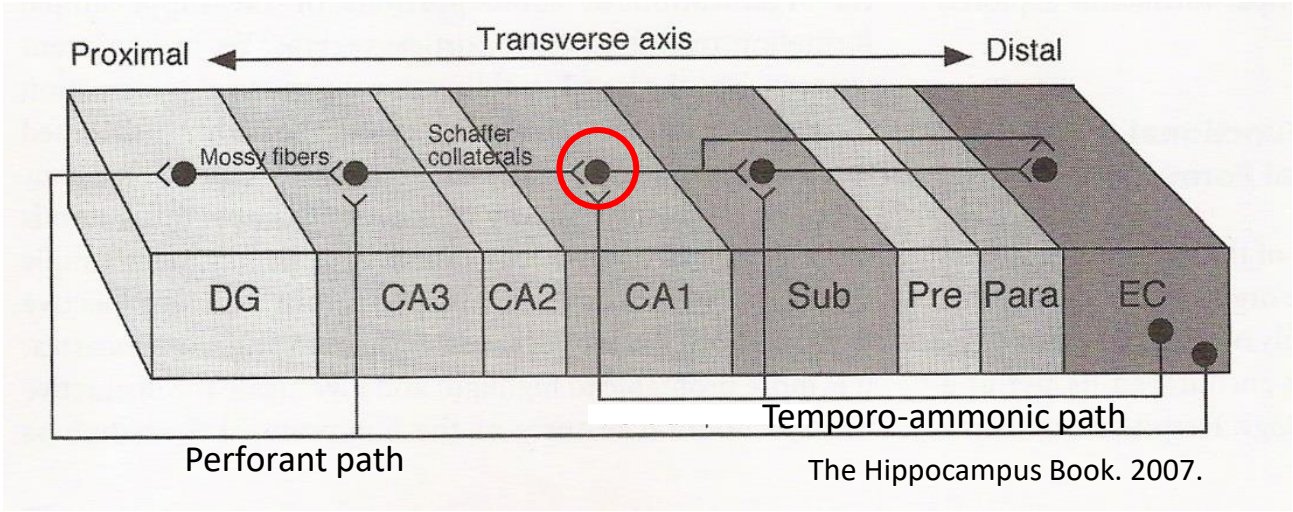
1, Pyramidal shaped soma
2, CA3 apical dendrites bifurcate closer to the soma
3, Thorny excrescences
4, basal arbor that extends through s.o.
5, Short apical trunk that branches proximal to the soma
6, Oblique apical dendrites in the s.r.
7, apical tuft that extends into the s.l-m.

CA1 and the « trisynaptic » hippocampal circuit

CA3 vs. CA1 pyramidal neurons

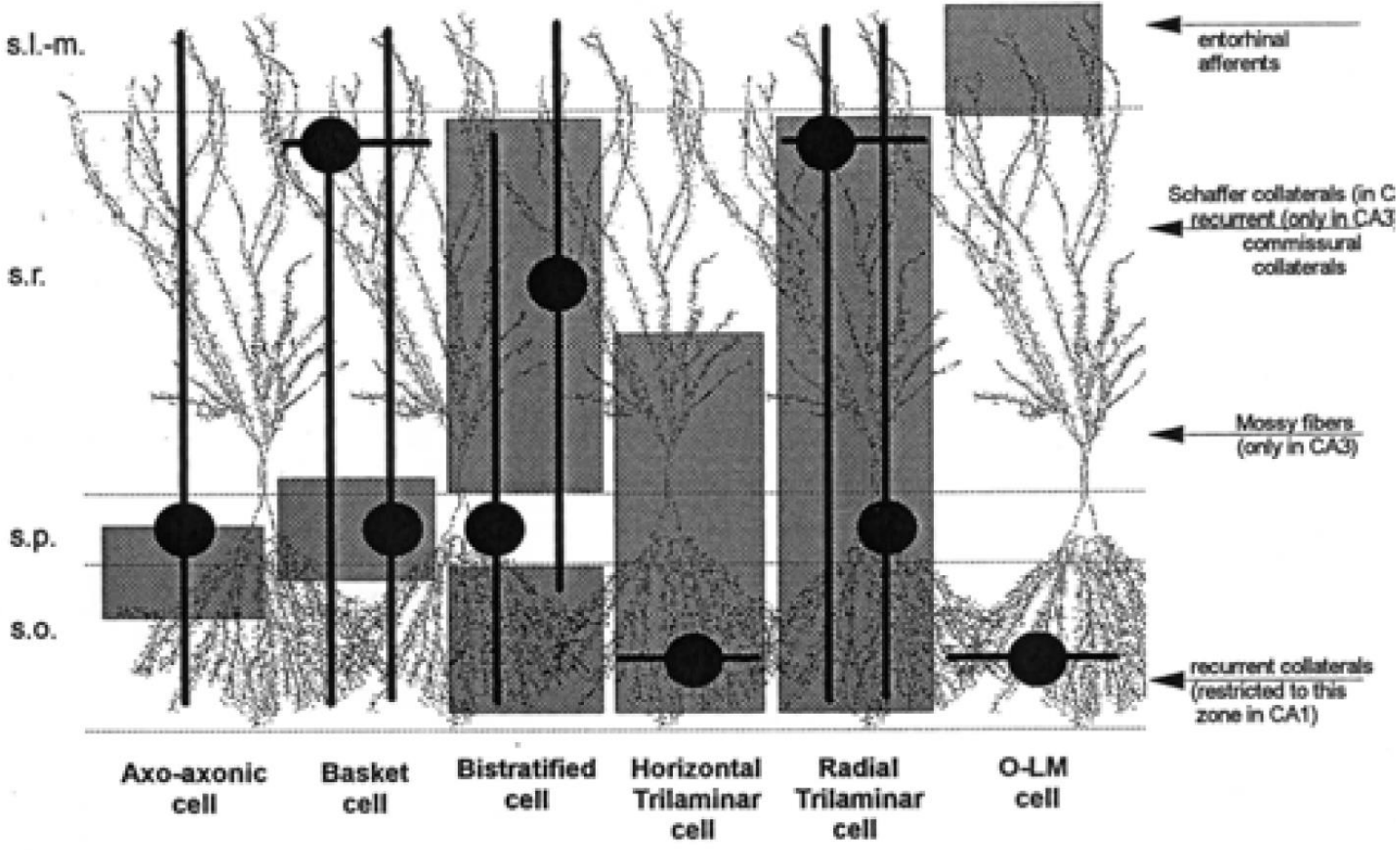






The Hippocampus Book. 2007.

Classes of CA1 hippocampal interneurons 1996



Layer specific input

Entorhinal afferents

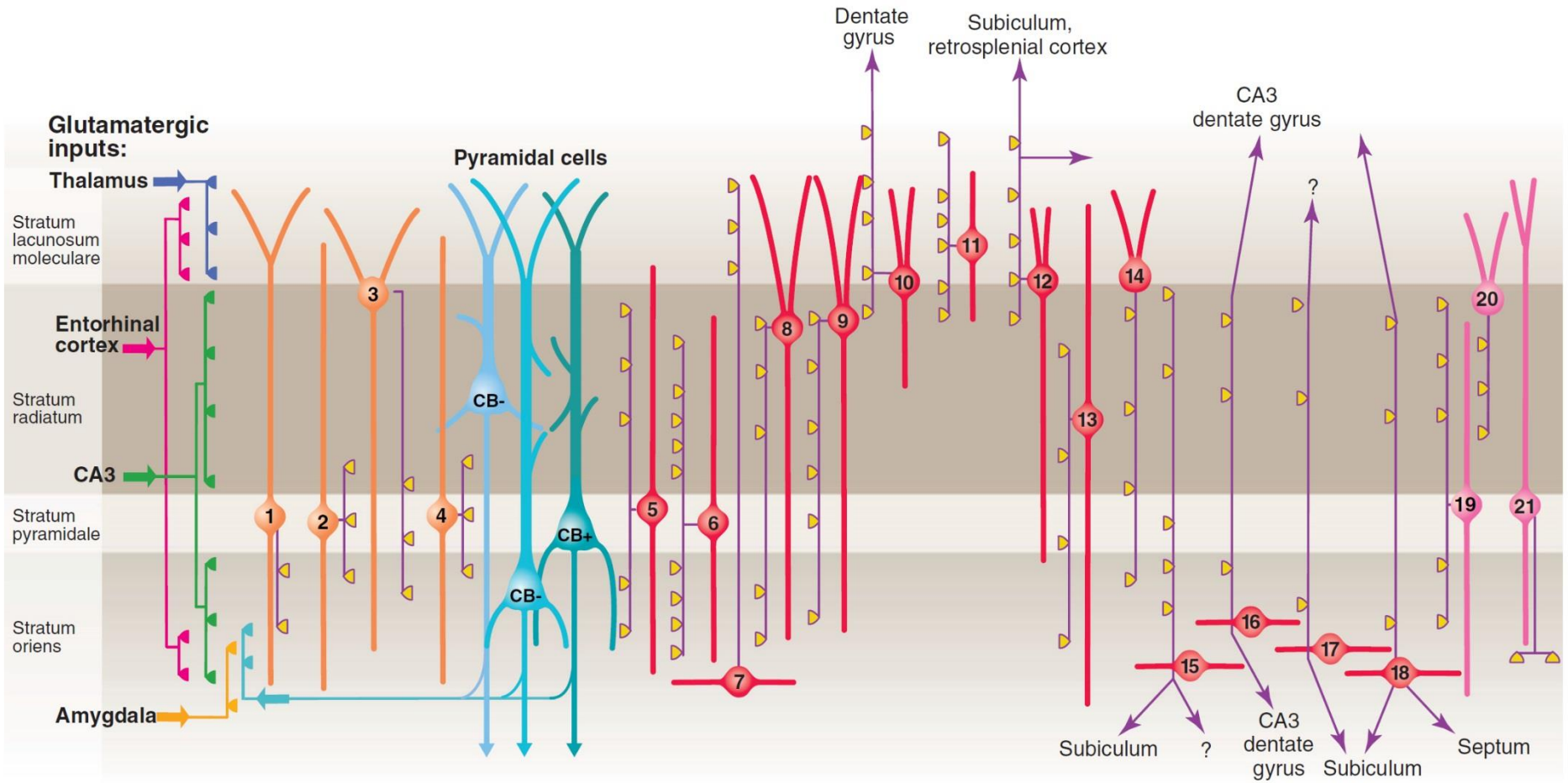
Schaffer collaterals
Recurrent / Associational collaterals (CA3)
Commissural collaterals

Mossy fibers

Recurrent collaterals

Freund and Buzsáki (1996)
Hippocampus 6: 347-470

Classes of CA1 hippocampal interneurons 2008

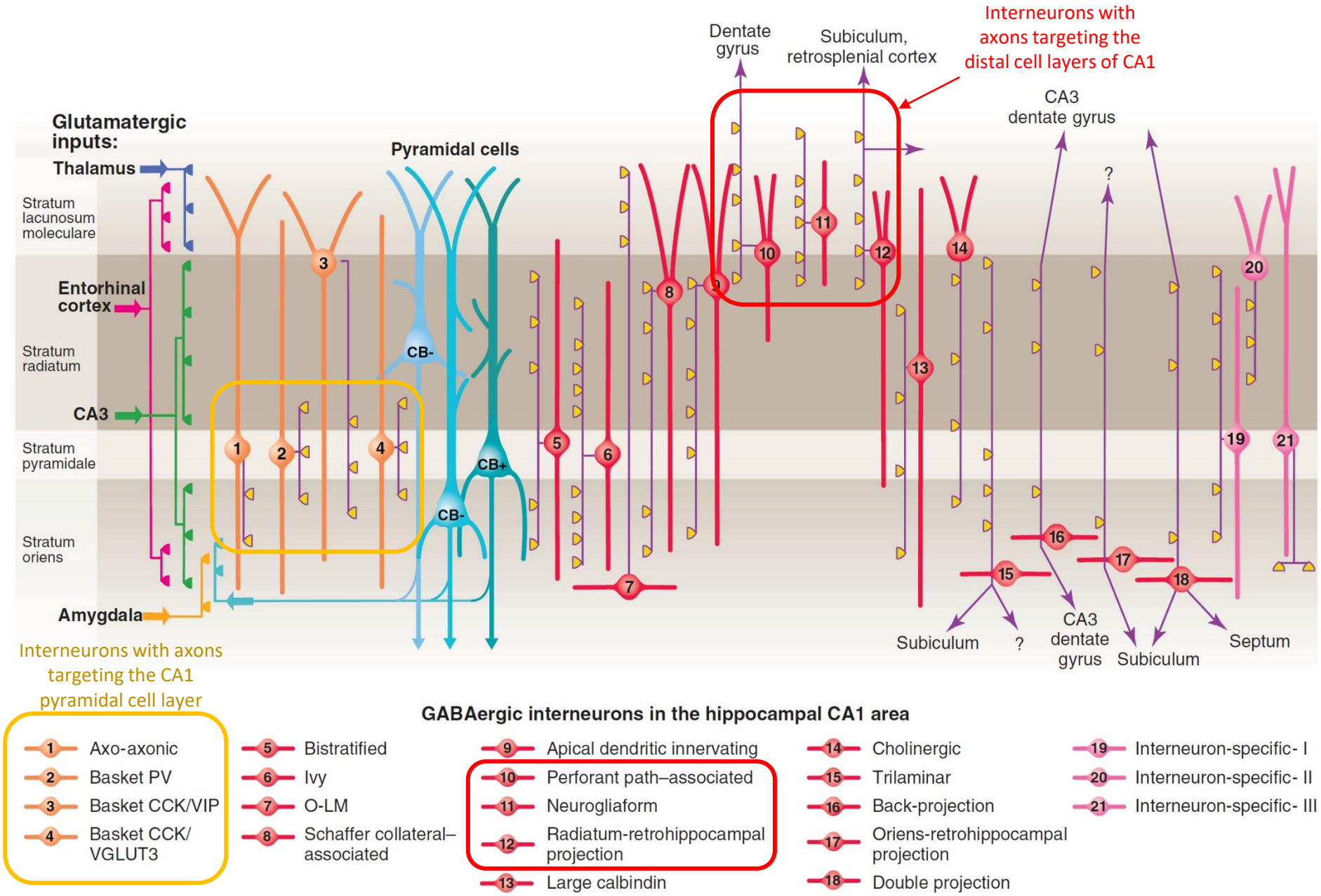


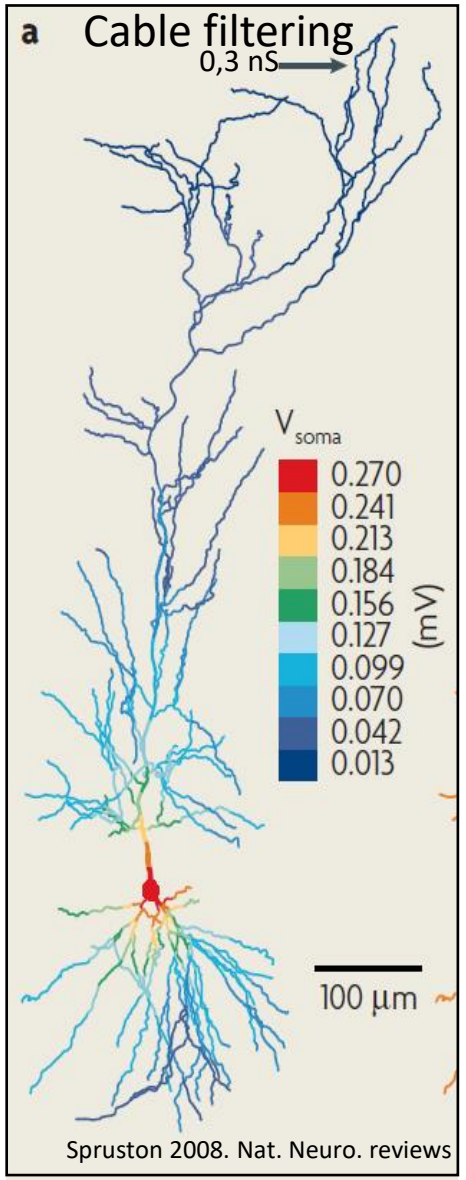
GABAergic interneurons in the hippocampal CA1 area

1. Axo-axonic
2. Basket PV
3. Basket CCK/VIP
4. Basket CCK/ VGLUT3
5. Bistratified
6. Ivy
7. O-LM
8. Schaffer collateral–associated
9. Apical dendritic innervating
10. Perforant path–associated
11. Neurogliaform
12. Radiatum-retrohippocampal projection
13. Large calbindin
14. Cholinergic
15. Trilaminar
16. Back-projection
17. Oriens-retrohippocampal projection
18. Double projection
19. Interneuron-specific- I
20. Interneuron-specific- II
21. Interneuron-specific- III

Klausberger et al. 2008. Science

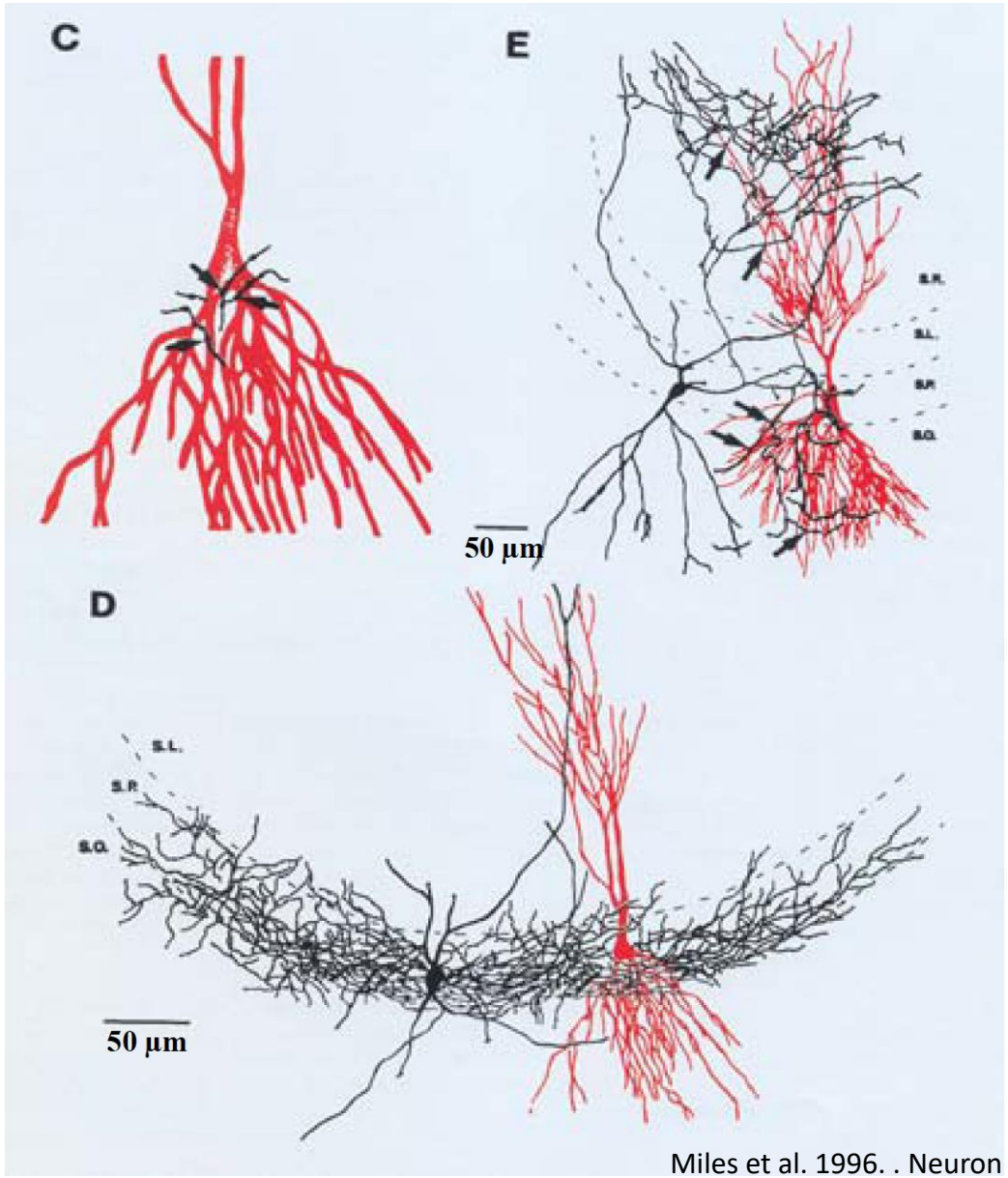
Synapse location influences synaptic transmission



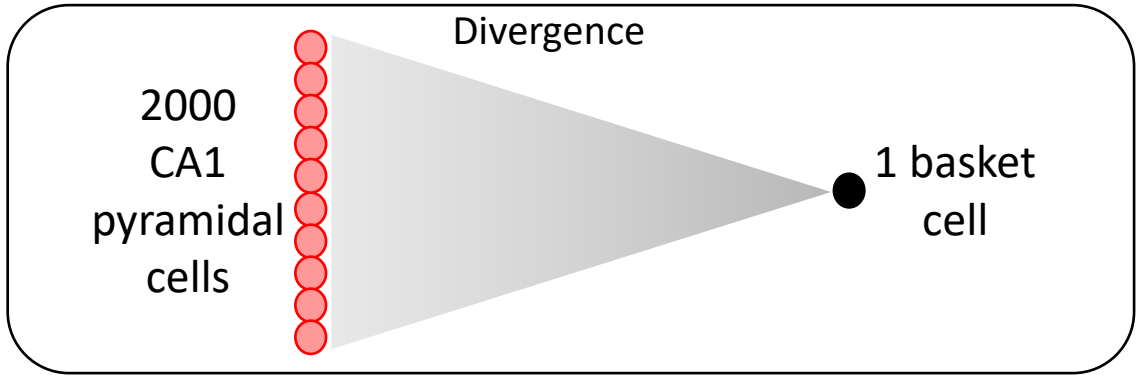


Spruston 2008. Nat. Neuro. reviews

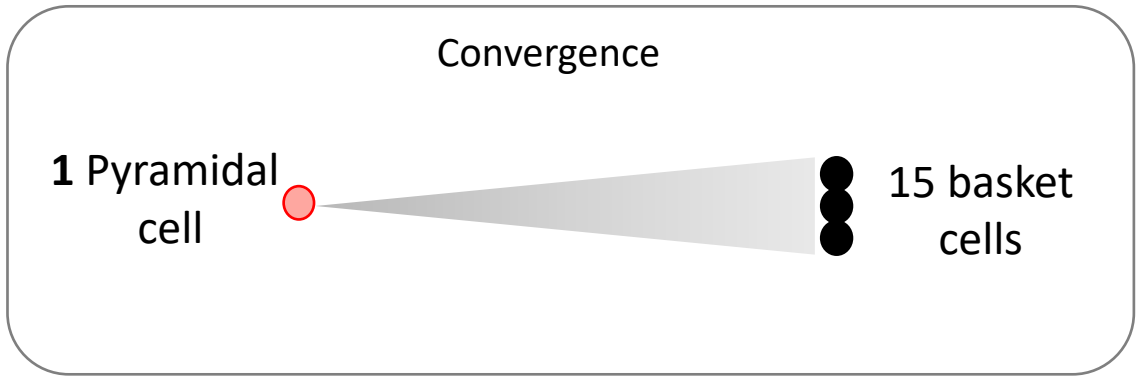
Basket cell morphology and connectivity



Miles et al. 1996. . Neuron



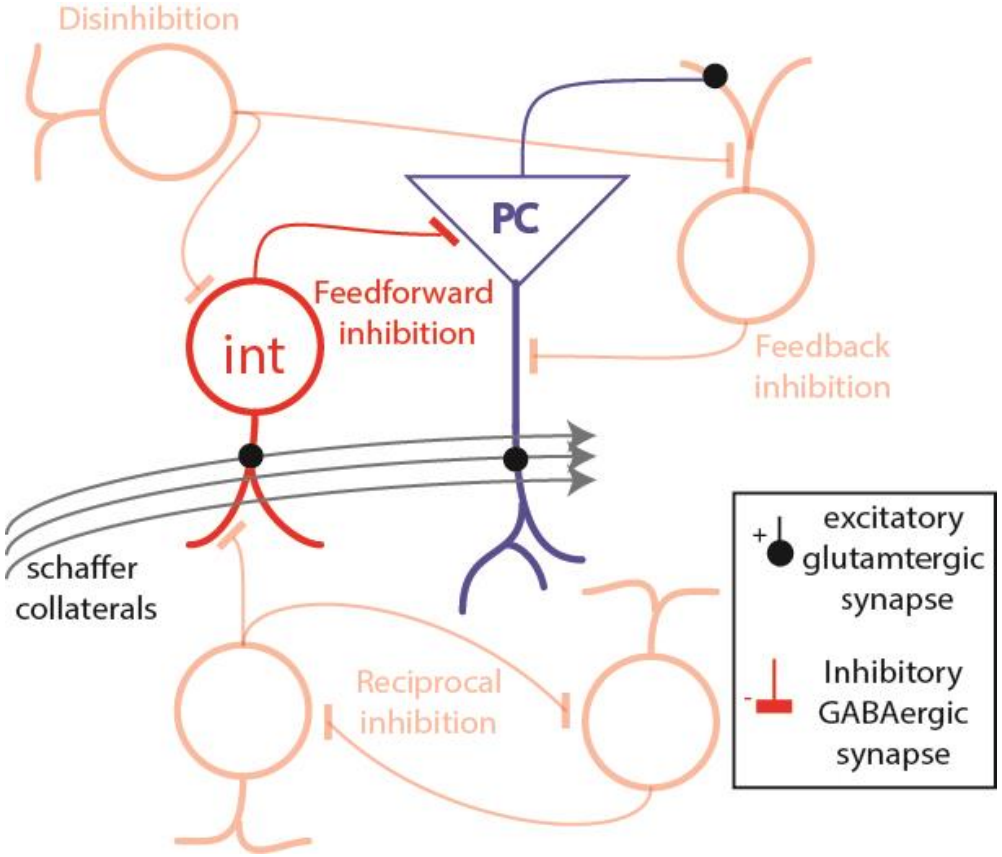
One basket cell (black) establishes synaptic contacts with about 2000 pyramidal neurons (red, about 10 000 synapses, with 2 to 10 synapses per target)



One pyramidal neuron receives synaptic contacts from about 15 basket cells resulting in around 1700 inhibitory total synaptic contacts, among which about 90 synapses on its cell body.

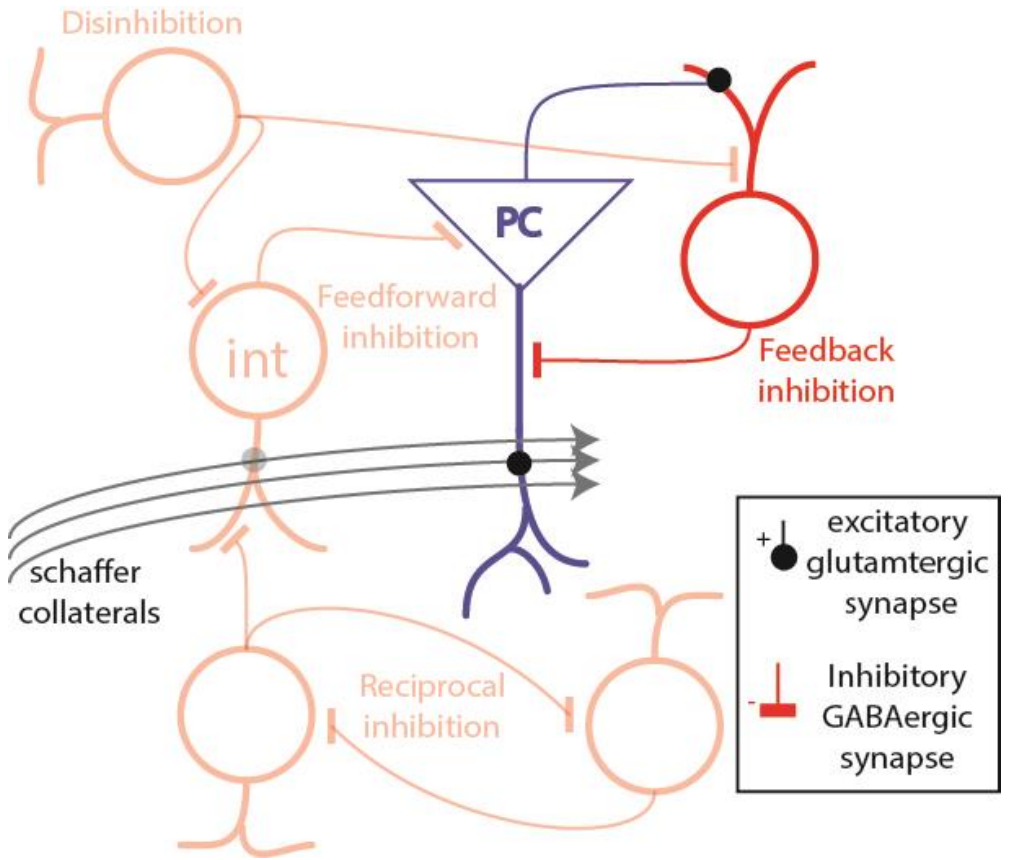
Hippocampal inhibitory circuits

Feedforward inhibition



Schaffer collaterals drive excitation of PCs and interneurons (i.e. basket cells). Excitation of the interneurons will produce an inhibitory synaptic response in PCs that arrives briefly after direct excitation (synaptic delay).

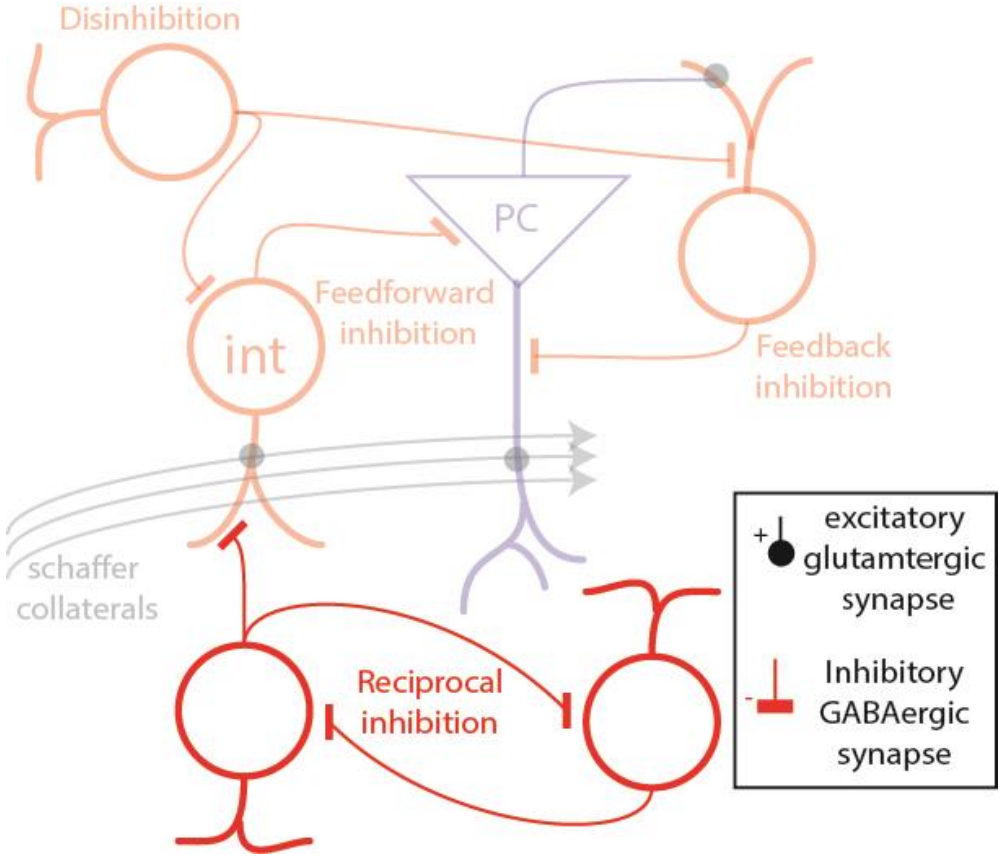
Feedback inhibition



Feedback inhibition is often mediated through basket cells. This form of inhibition is believed to contribute to the generation of hippocampal oscillations.

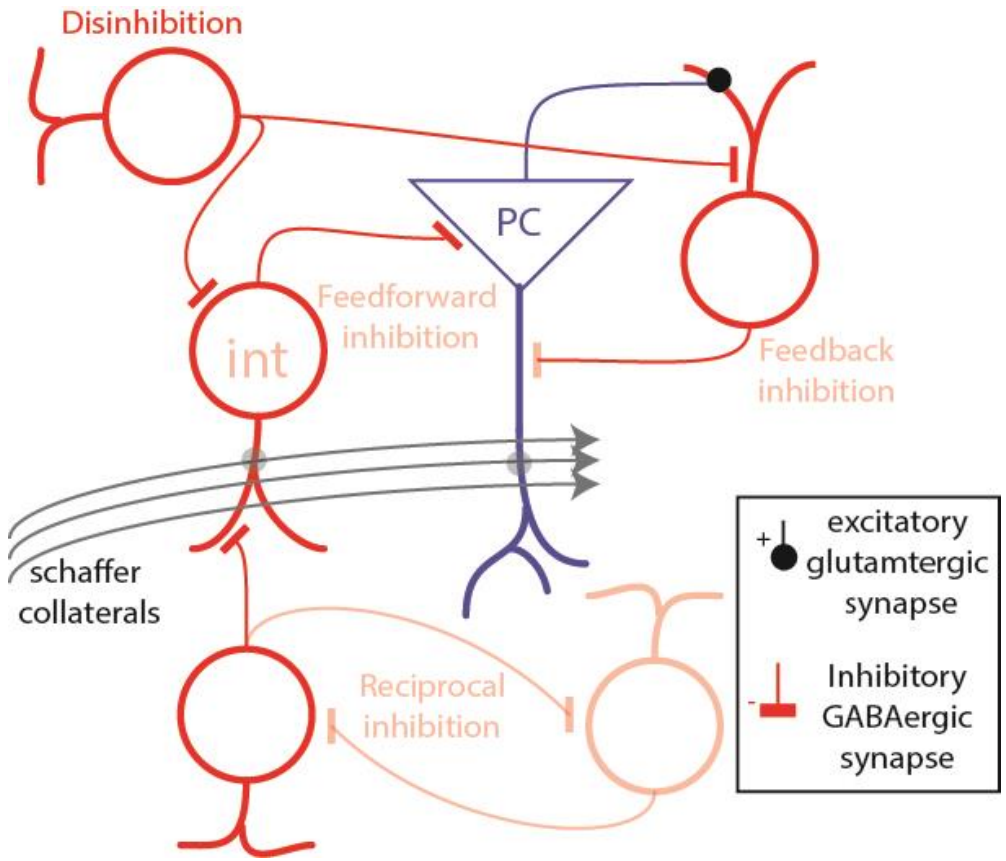
Hippocampal inhibitory circuits

Reciprocal inhibition



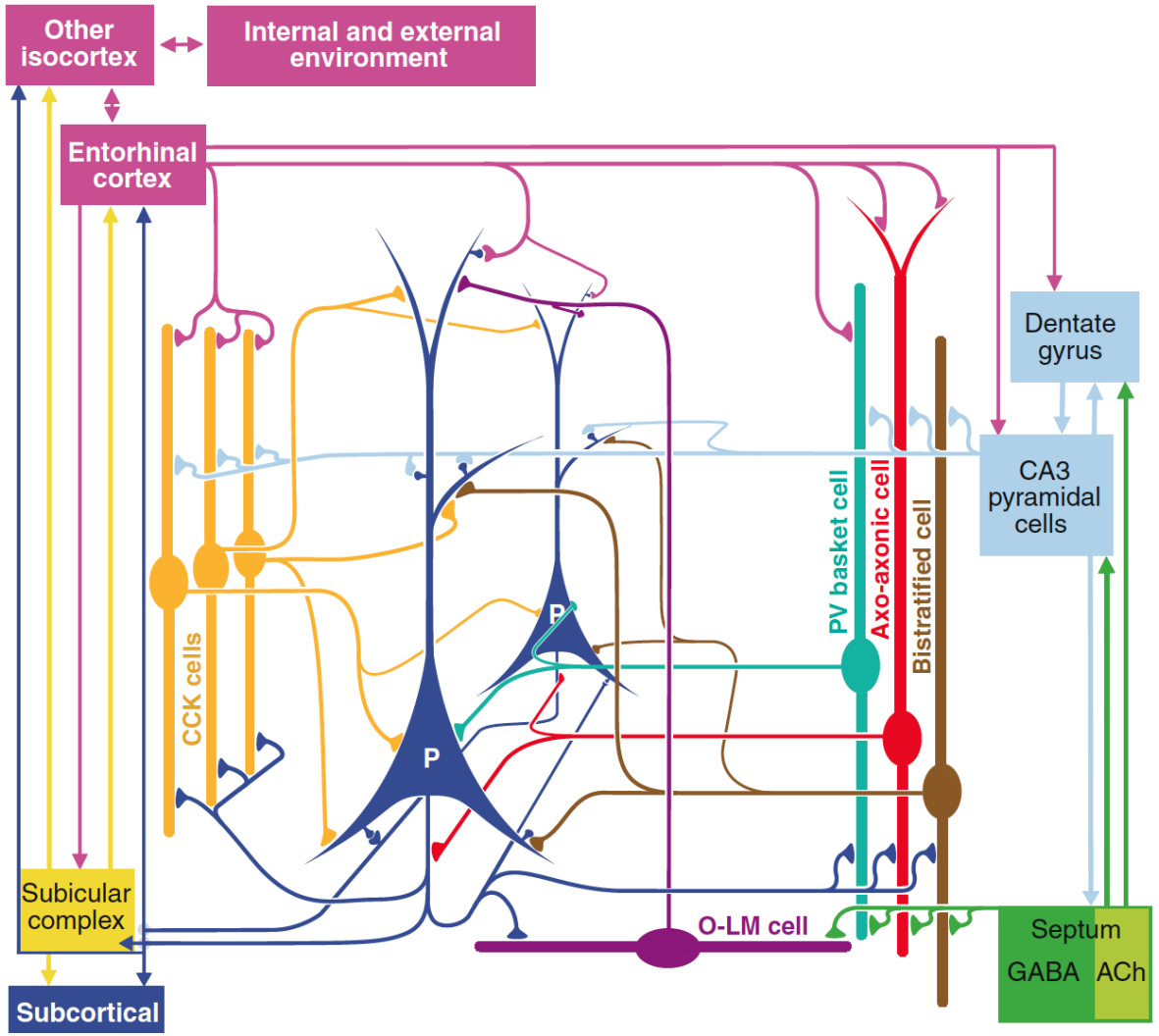
Reciprocal inhibition is implicated in rhythm generation and may play a role in the generation of hippocampal oscillations such as theta, gamma and sharp wave ripples (SWR)

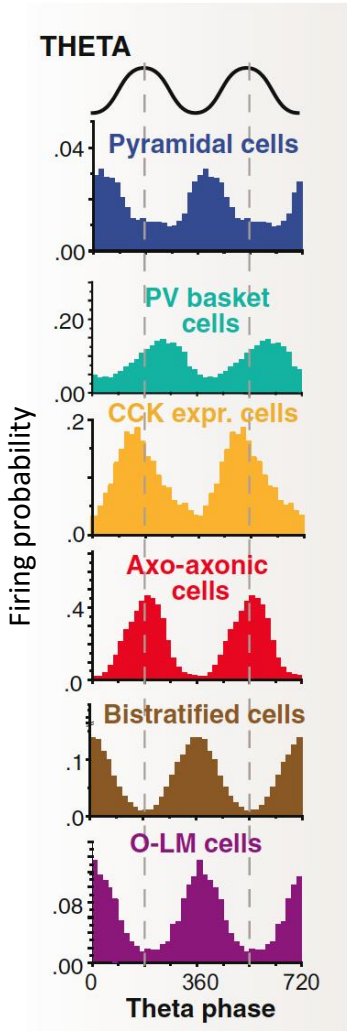
Disinhibition



Inhibition of inhibitory interneurons may produce a net excitatory effect on the network. Disinhibition may arise from local or long range GABAergic projections of from other structures (i.e. Septum or entorhinal cortex).

Spatiotemporal interaction between pyramidal calls and several classes of interneurons during network oscillations



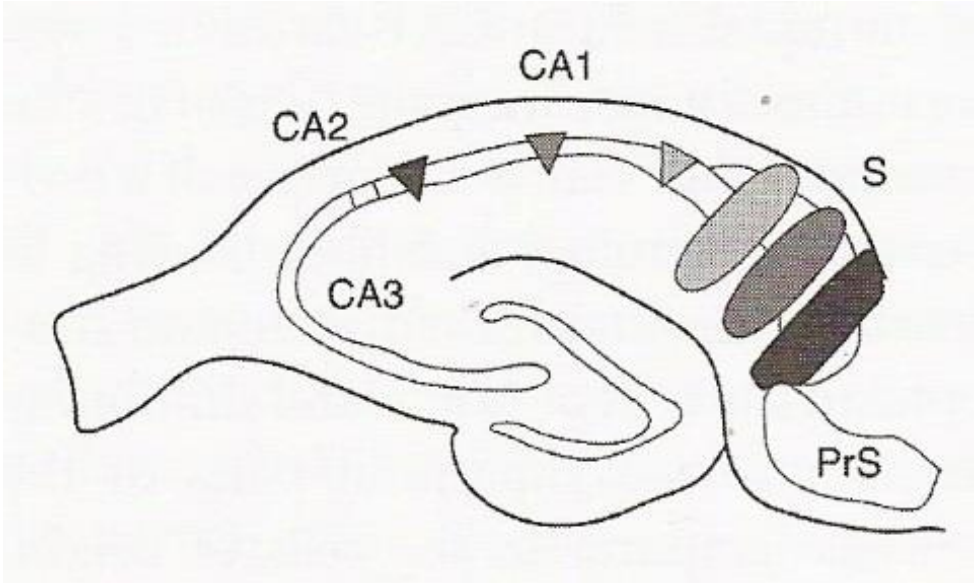


M. Zugaro
Hippocampal rhythms and behavior

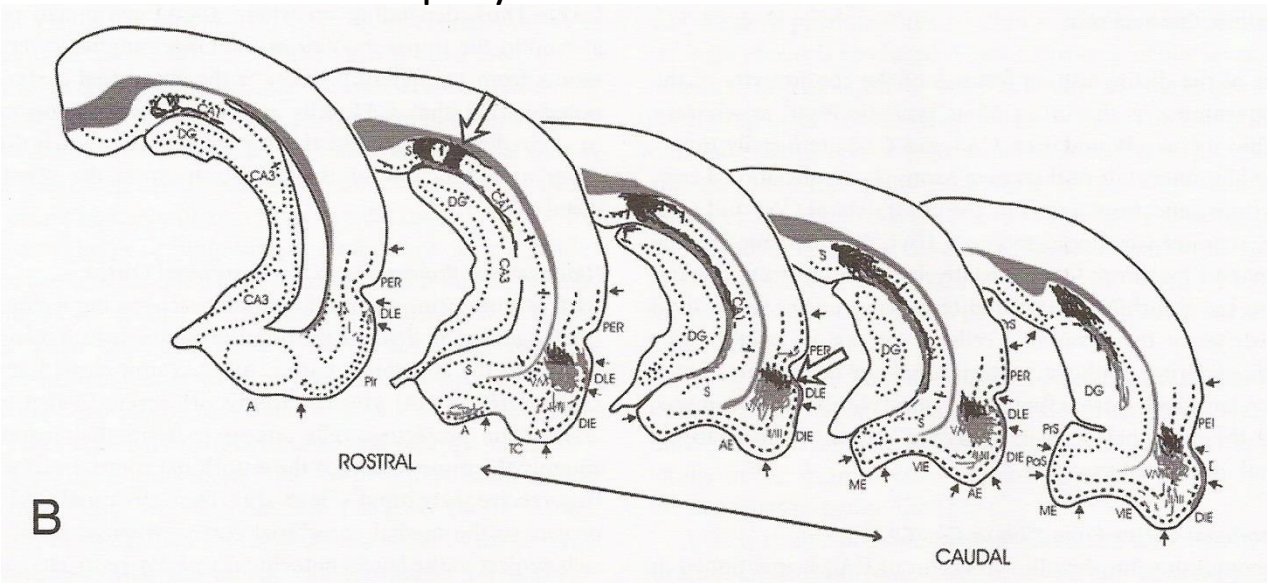
Klausberger et al. 2008. Science

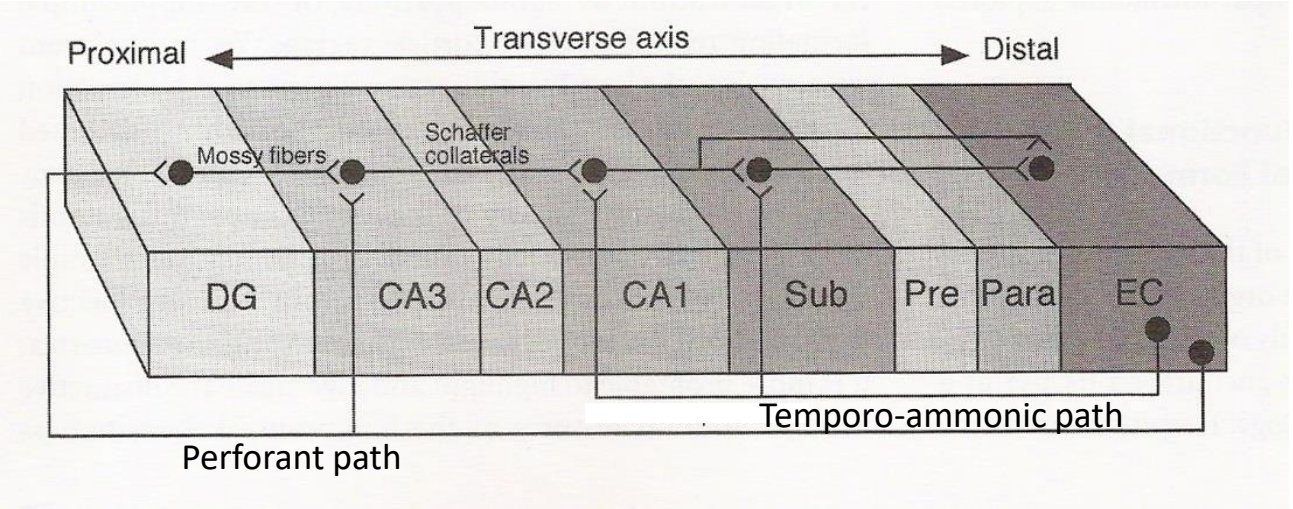
CA1 excitatory outputs: CA1 pyramidal axons project to the subiculum and EC

CA1 to Subiculum

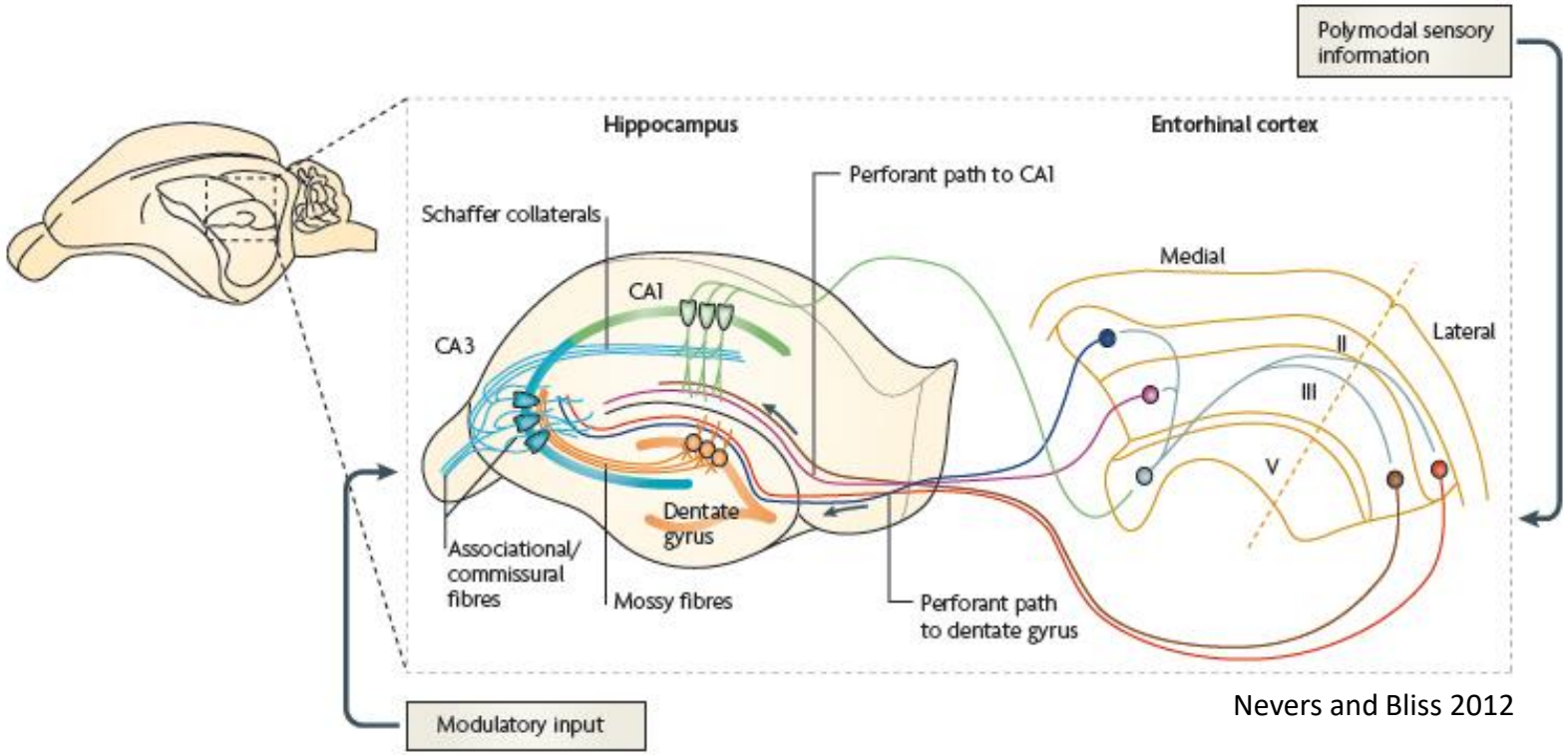


CA1 to deep layers of the entorhinal cortex

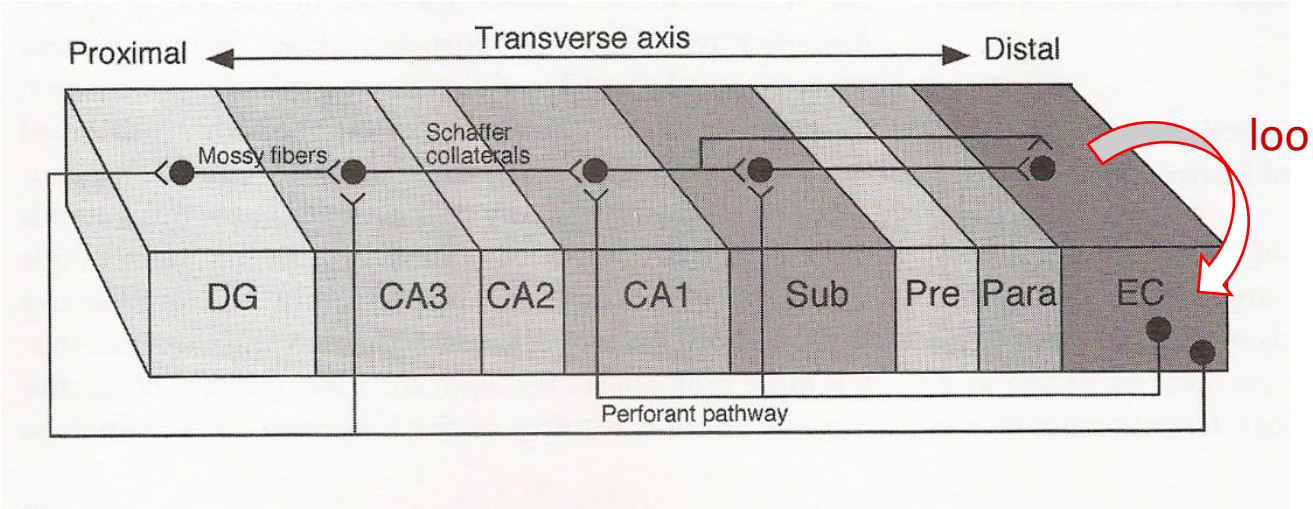




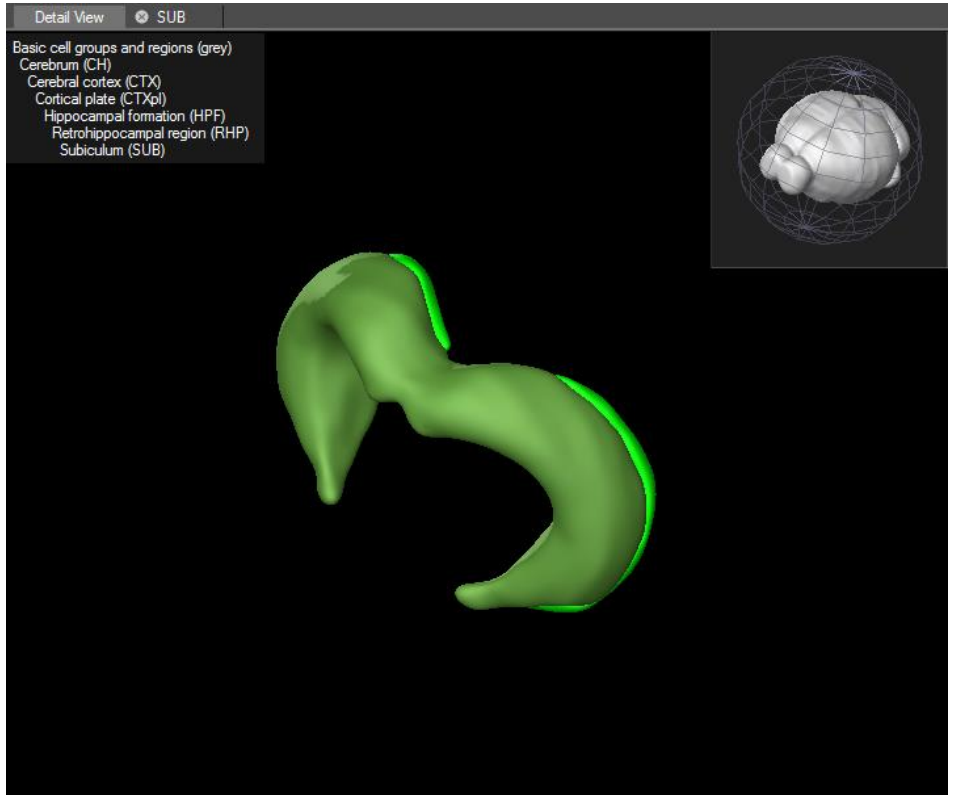
Loop: Entorhinal cortex to hippocampus to entorhinal cortex

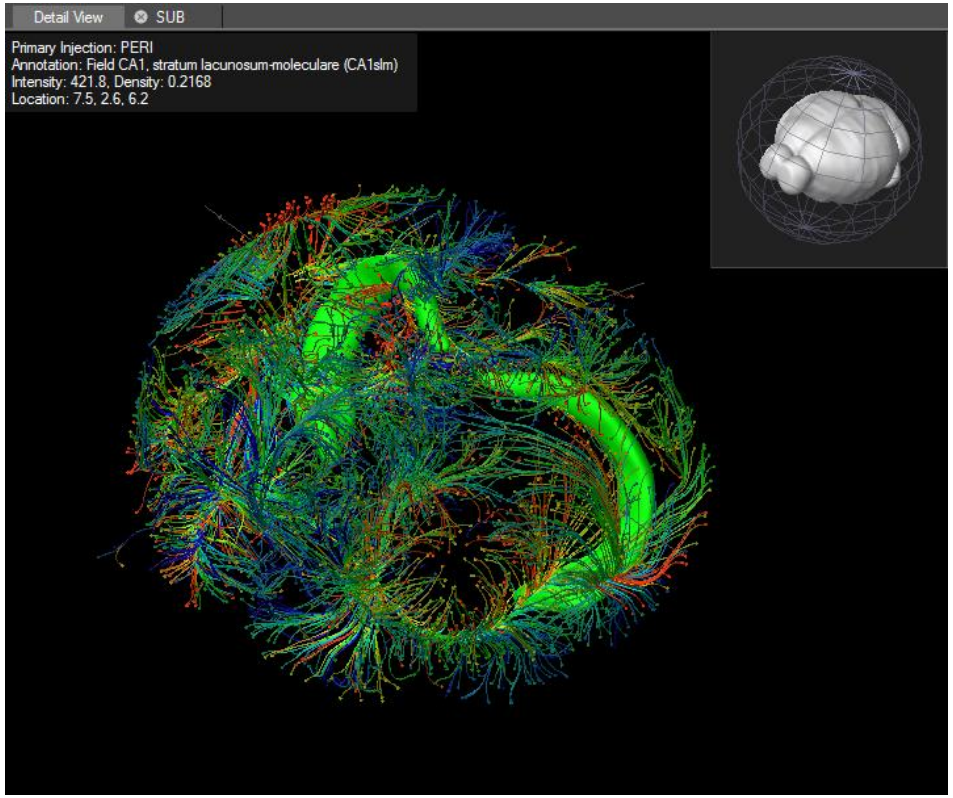


Nevers and Bliss 2012



Subiculum: excitatory inputs and outputs



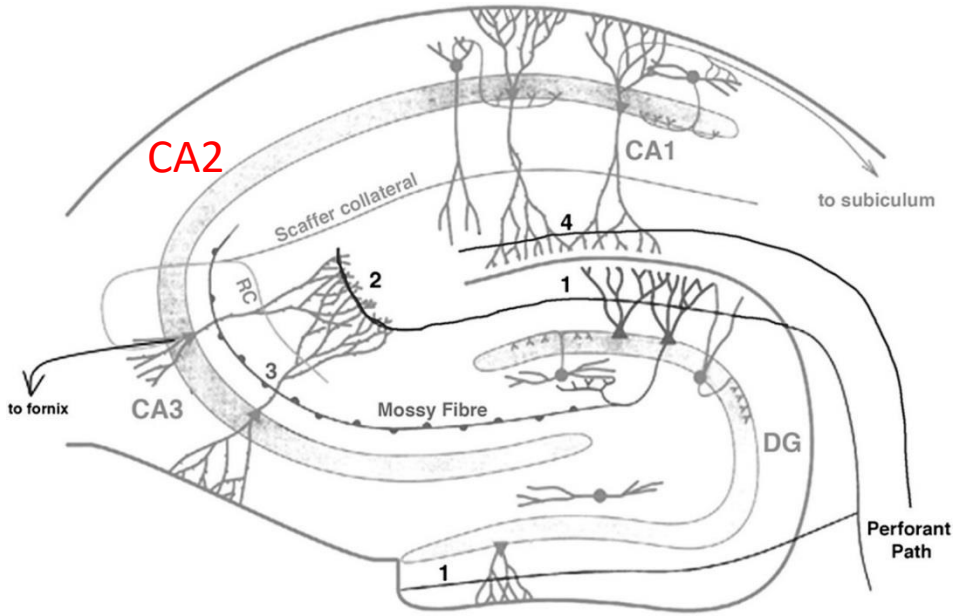


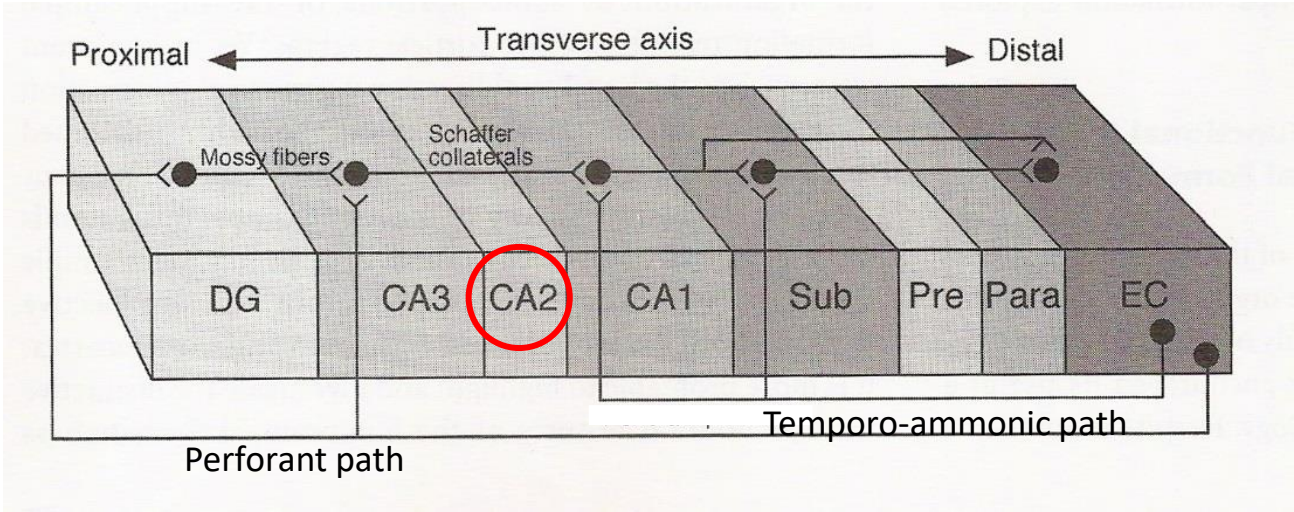
Inputs:
CA1, Entorhinal cortex, Thalamus, Ventral tegmental nucleus, Median dorsal raphe nuclei

Outputs:
Pre-subiculum, para-subiculum, Entorhinal cortex, neocortex, amygdaloid complex, septal complex, nucleus accumbens, mammillary nuclei

Rebecca Piskorowski
Hippocampal
Synaptic Physiology

CA2: the « ~~tri~~ 'quad'synaptic » hippocampal circuit





Plan

Part I

I. Structure, Afferents and Efferents of the rodent hippocampus

I. The hippocampus in rodents and primates
II. Septal-temporal (dorsal-ventral) orientation
III. Major Fiber bundles of the hippocampus: Afferents + Efferents
IV. Topographic connectivity of hippocampal Afferents and Efferents
V. Functional modules of the hippocampus?

II. The « tri » synaptic hippocampal circuit

I. Layers and Strata of the hippocampal formation
II. The Dentate Gyrus network
    I. Granule Cells
    II. Mossy Cells
    III. Inhibitory interneurons
III. Inhibitory/excitatory balance and disease
    I. Selective ablation of Mossy Cells

Part II

IV. CA3
    I. Granule cell mossy fibers projections to CA3
        I. Mossy fibers, Fliopodial extentions, *en passant* boutons
        II. Ultrastructure of mossy fibers
    II. Functional Mossy Fiber inputs to CA3
    III. Influence of spine shape and synapse location on dendritic integration
    IV. CA3 Afferents and Efferents
V. CA1
    I. CA1 Afferents
    II. Inhibitory interneurons
    III. CA1 Efferents/Subiculum
VI. The « tri » synaptic hippocampal circuit and the entorhinal cortex
VII. CA2

III. Modern anatomical techniques

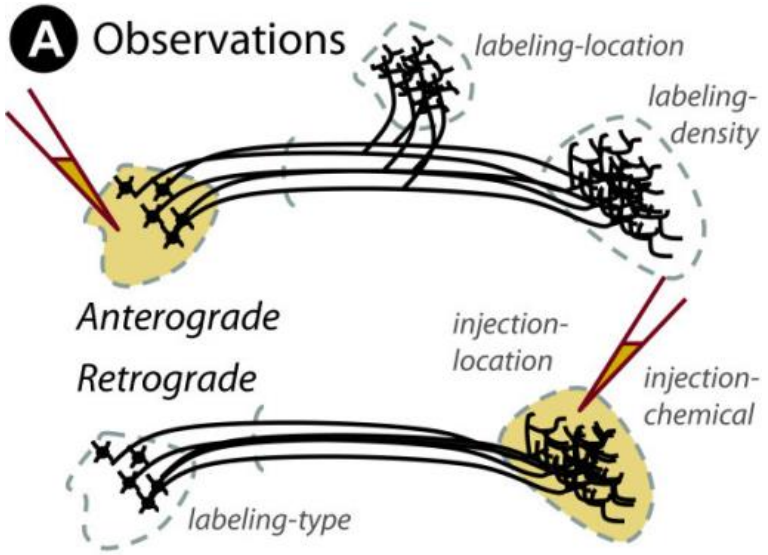
I. Monosynaptic tracing with glycoprotein–deleted rabies viruses

III Modern anatomical techniques

Monosynaptic tracing with glycoprotein –deleted rabies viruses

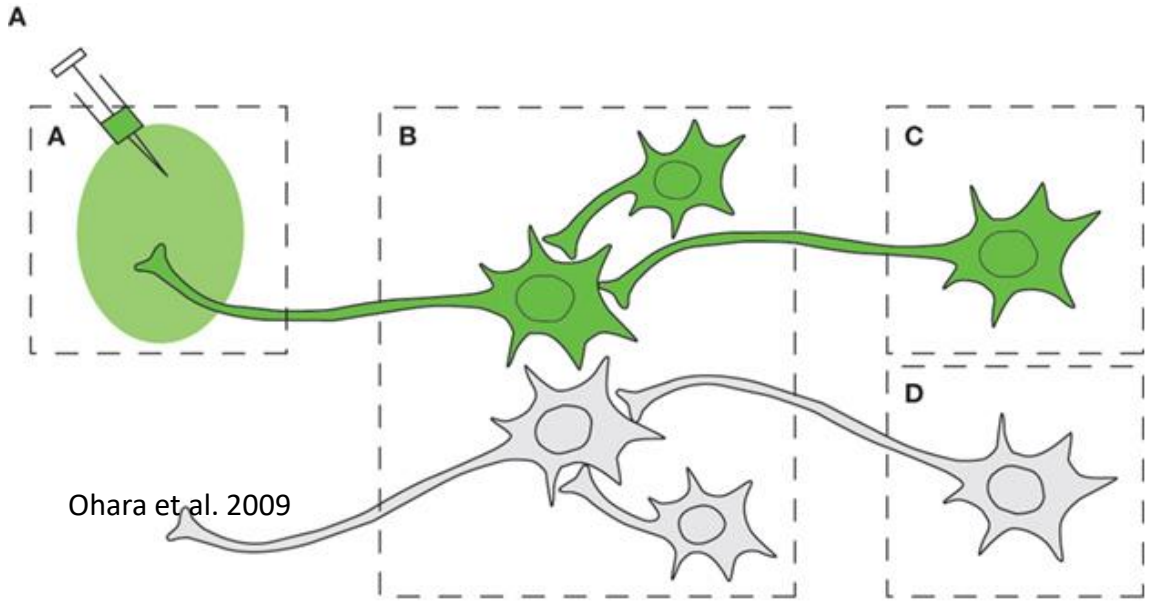
Classical tract tracing techniques

Retrograde and anterograde tract tracing



- Standard retrograde and anterograde tracers allow for mapping of neuronal connections between regions, but fail to identify the cell types receiving the projections. These tracing techniques marque indiscriminately all cells with axons and dendrites near the regions of interest. Thus, false positives may arise from marking axons that run near the site of injection but that are not related to the region of interest.
- EM studies are tedious and survey only a fraction of the connections.
- Tracing is limited to one synaptic connection

Tracing using a trans-synaptic vector



Ohara et al. 2009

- Pseudo-rabies viruses allow trans-neuronal infection (not limited to a single synaptic connection), but may cross multiple synapses and thus does not distinguish between directly or indirectly connected neurons.
- These viruses are also highly toxic, killing the infected neurons in less than 3 days.

Cell Reports
Resource

Cell-Type-Specific Circuit Connectivity of Hippocampal CA1 Revealed through Cre-Dependent Rabies Tracing

Yanjun Sun,[1] Amanda Q. Nguyen,[1] Joseph P. Nguyen,[1] Luc Le,[1] Dieter Saur,[2] Jiwon Choi,[3] Edward M. Callaway,[3] and Xiangmin Xu[1,4,5,*]
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[2]II. Medizinische Klinik, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany
[3]Systems Neurobiology Laboratories, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA
[4]Department of Biomedical Engineering, University of California, Irvine, Irvine, CA 92697-2715, USA
[5]Department of Microbiology and Molecular Genetics, School of Medicine, University of California, Irvine, Irvine, CA 92697-4025, USA
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http://dx.doi.org/10.1016/j.celrep.2014.02.030

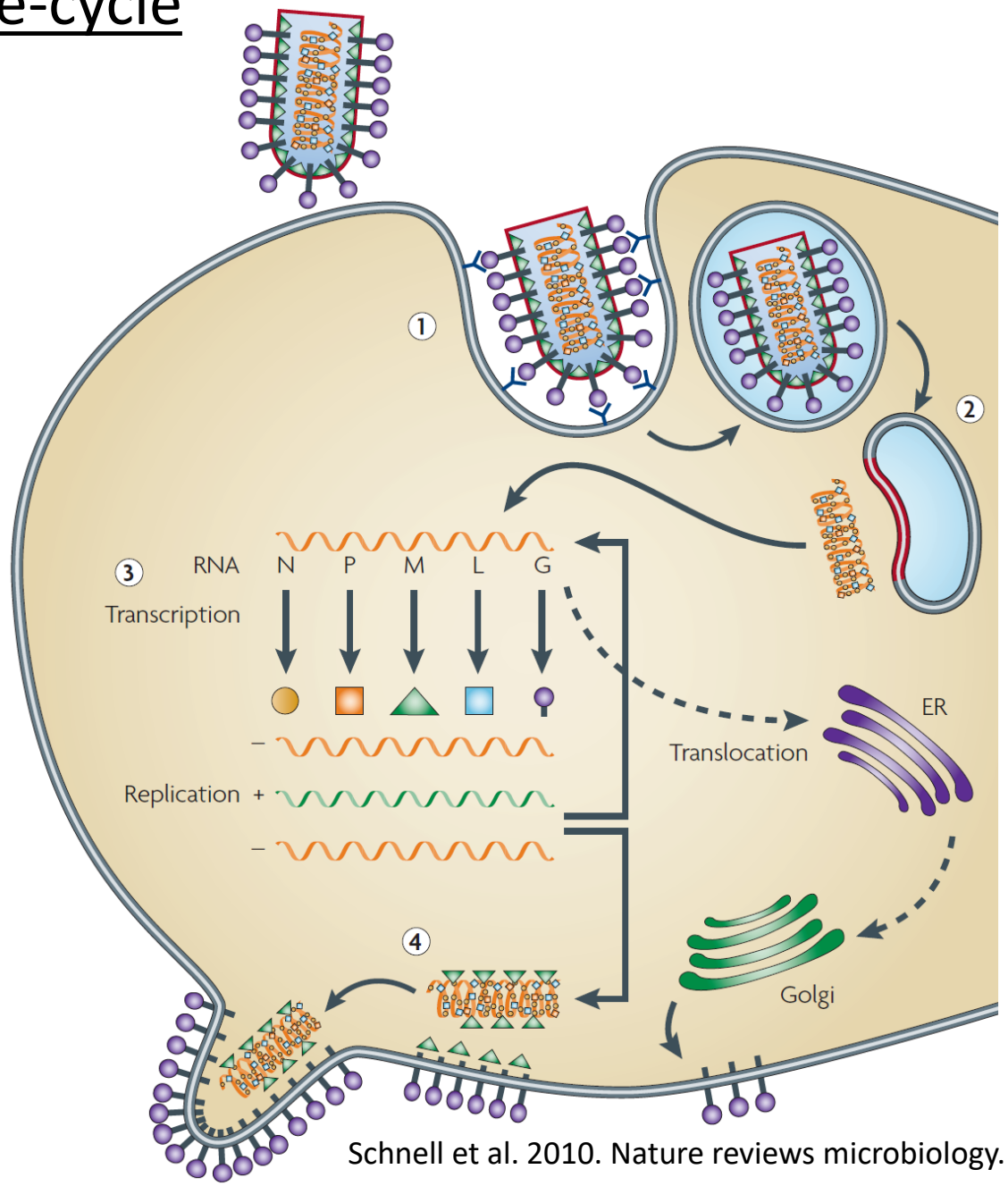
A simplified rabies virus life-cycle

1. Binding and entry of the virus into the neuron
   a. Binding of the envelope protein **Rabies Glycoprotein** (depicted as violet balls on the surface of the virion) to membrane receptors ("Y" shaped proteins on the membrane) initiates endocytosis (infection) of the rabies virions.

2. Fusion of the viral membrane and the endosome membrane to release the viral genome

3. Production of viral components
   a. Transcription
   b. Replication – Replication of rabies RNA
   c. Protein synthesis
      a. The rabies genome codes for 5 proteins including the envelope protein **Rabies Glycoprotein**.

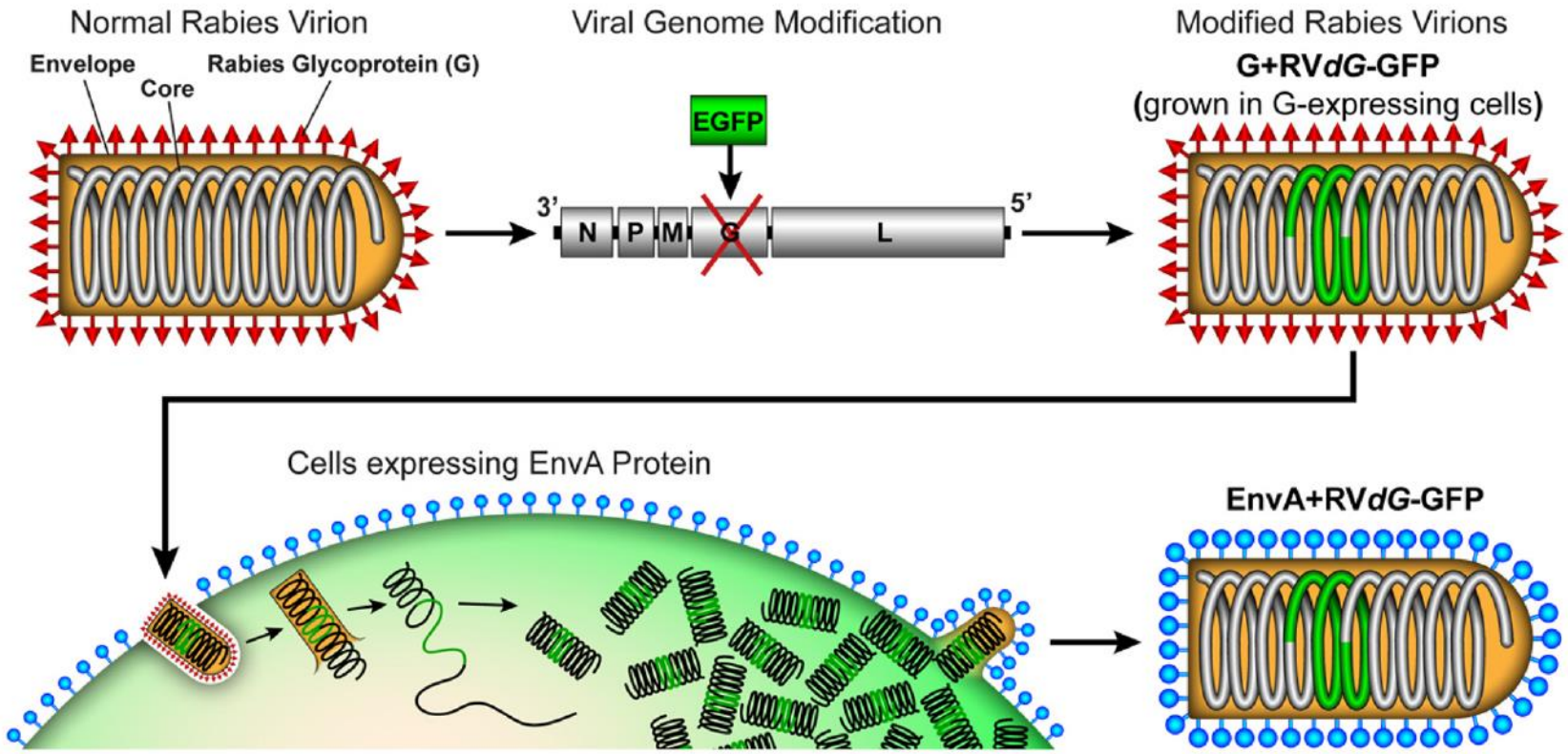
4. Assembly, budding and release of the rabies virus virions, which may start a new round of infection.



Schnell et al. 2010. Nature reviews microbiology.

Monosynaptic tracing with glycoprotein –deleted rabies viruses

Glycoprotein deletion and pseudotyping of rabies virus



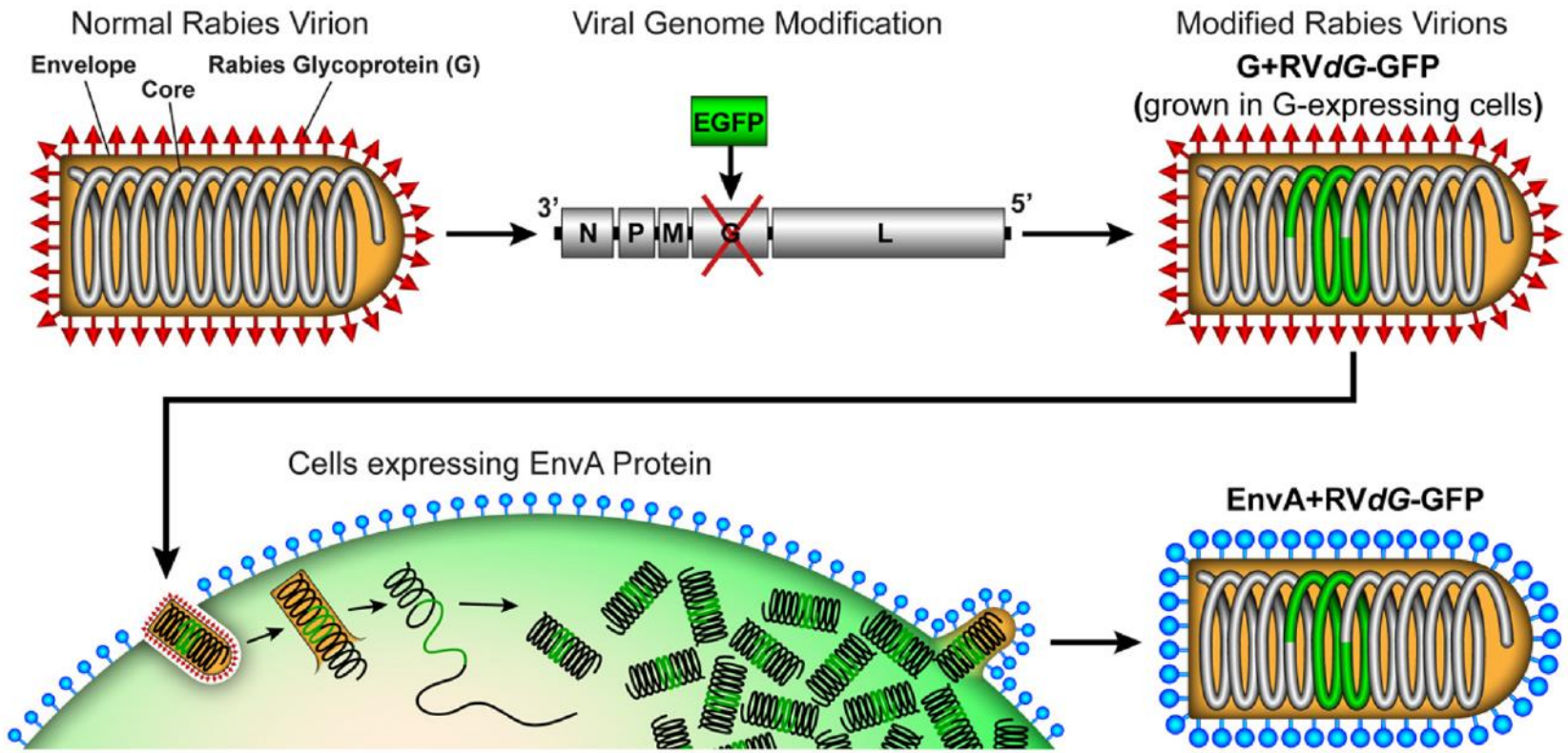
Rabies glycoprotein (G) is an envelope protein that is embedded in the host cell membrane after translation. Through an interaction with the viral core rabies G mediates budding of host cell-derived membrane-enveloped viral particles. The presence of rabies G on the virus envelope surface allows the rabies virus to infect presynaptic terminals via receptors for G.

Callaway and Luo, J. Neurosci. 2015

Monosynaptic tracing with glycoprotein –deleted rabies viruses

Deletion de G prevents release of the rabies virus from infected cells and thus blocks trans-synaptic infection. If G is expressed in the cell of interest and the is cell is infected with a G domain deleted rabies virus (RVdG), then these cells can make infectious particles by trans-complementation. However, cells secondarily infected by RVdG will not release the virus if they do not independently express G. In this way rabies infection can be restricted to one synapse.

Glycoprotein deletion and pseudotyping of rabies virus

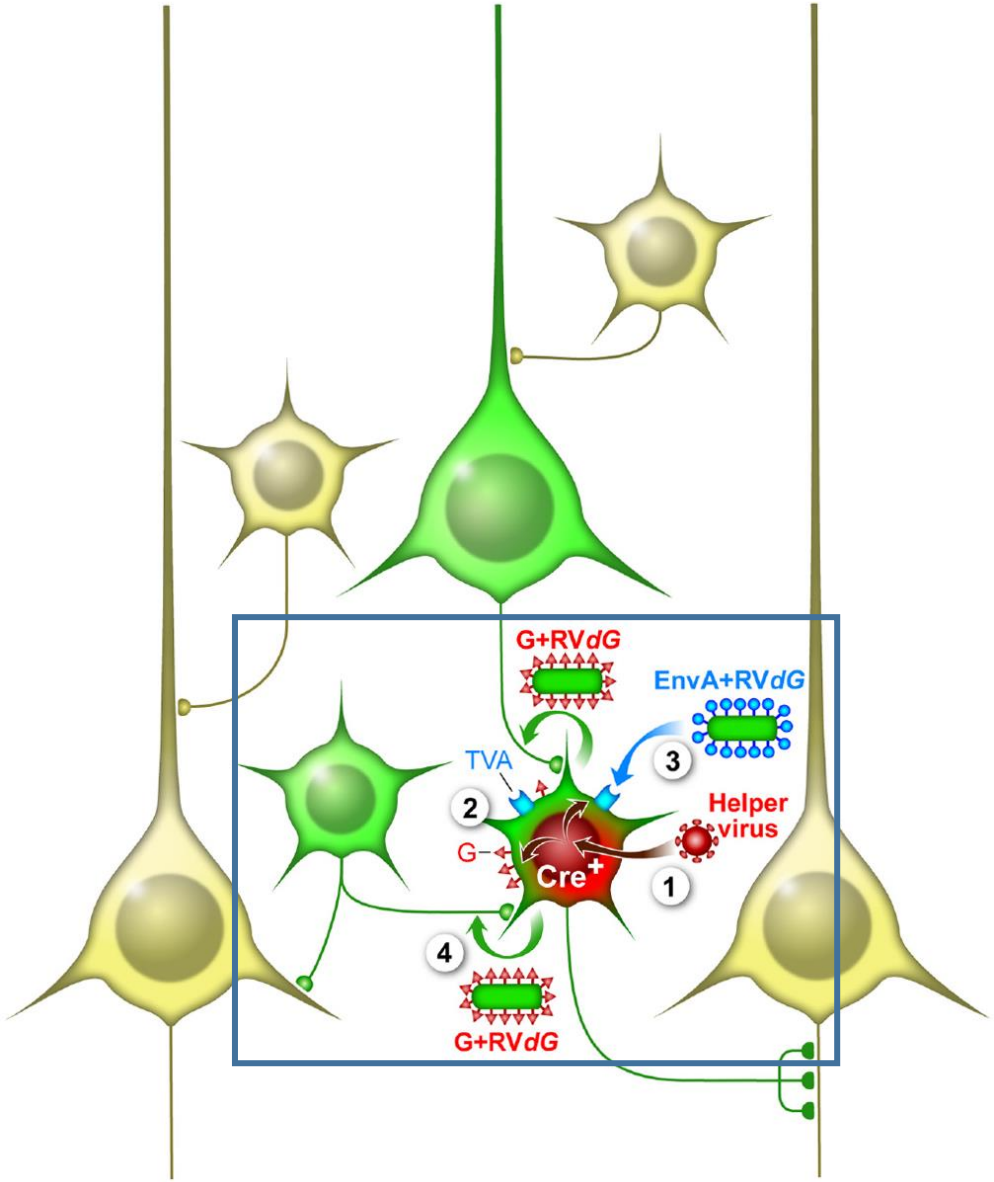


For tract tracing studies in mammals RVdG is pseudotyped with avain ASLV type A envelope protein (EnvA) which uses the TVA receptor for entry into cells. AS TVA is not expressed in mammalian neurons, only cells engineered to express TVA may be infected by EnvA + RVdG.

Pseudotyped – the process of producing viruses or viral vectors in combination with foreign viral envelope proteins

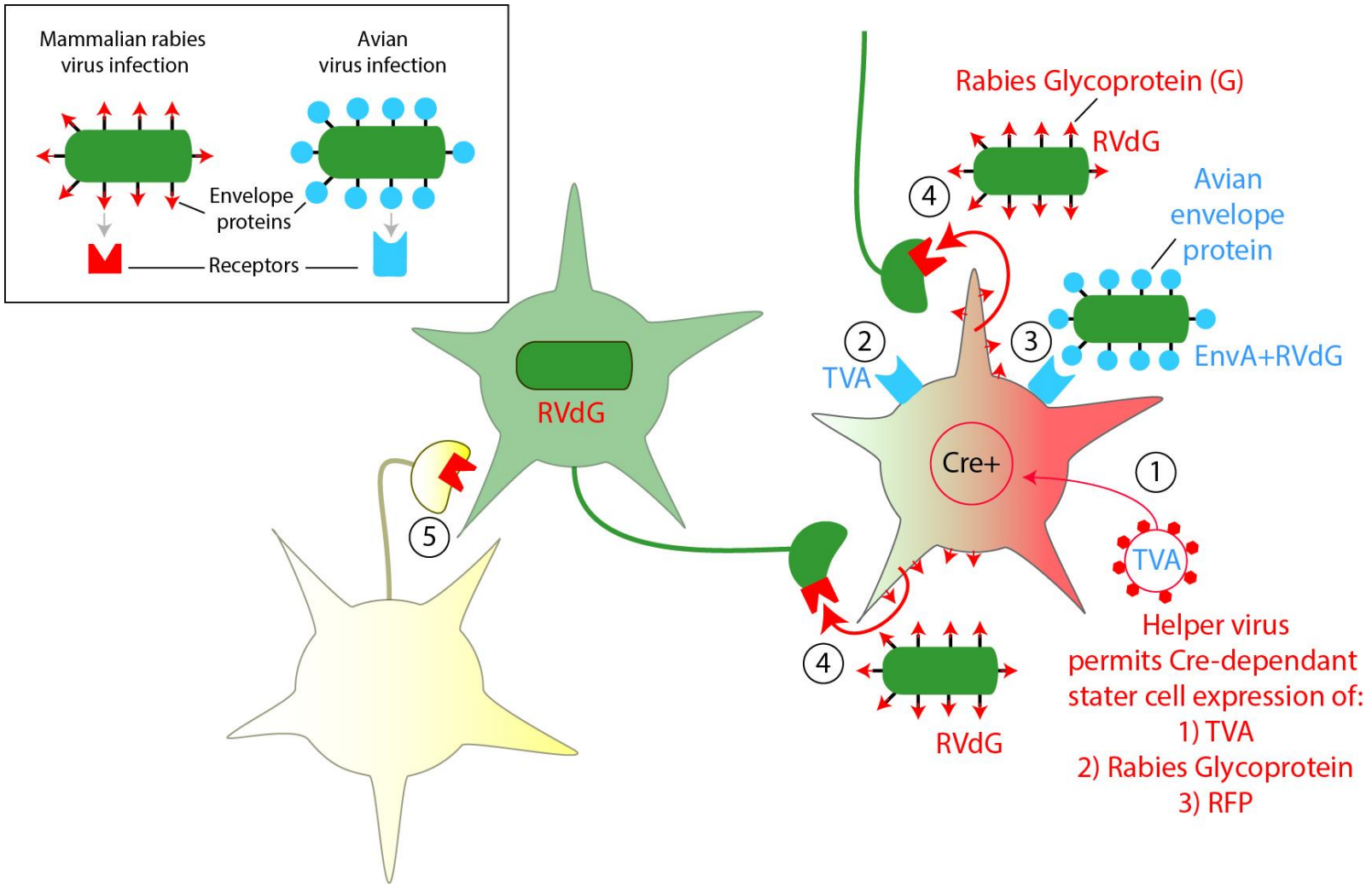
Callaway and Luo, J. Neurosci. 2015

Strategy for circuit tracing with Glycoprotein (G)-deleted rabies virus



1. A mouse strain is selected in which the “Starter” cells (red and green) selectively express the protein Cre+.
2. Using Cre+ as a selective promotor, a “helper virus” is used to express 3 proteins in “starter” cells: **TVA**, **rabies G**, and **RFP**
    1. **TVA** is the receptor of envelope protein EnvA that allows for entry of a rabies virus with an EnvA envelope
    2. **Rabies G** permits trans-complementation and the production of RVdg+G particles.
    3. **RFP**. In this example “starter” cells also selectively express a red fluorescent protein for identification.
3. After waiting a few weeks to permit expression of TVA, RVdG+EnvA is injected into the region of interest. GFP is also inserted into the rabies genome to tract infection.
4. The expression of rabies G and RVdG+EnvA in “starter cells” allows for trans-complementation and the production of RVdG+G particles that can bud from the starter cell and infect presynaptic terminals. However, cells secondarily infected by RVdG (Green) will not release the virus as they do not express rabies G. Thus the cells labeled with GFP represent a population of neurons with direct synaptic connections with the “starter” cells.

Strategy for circuit tracing with Glycoprotein (G)-deleted rabies virus



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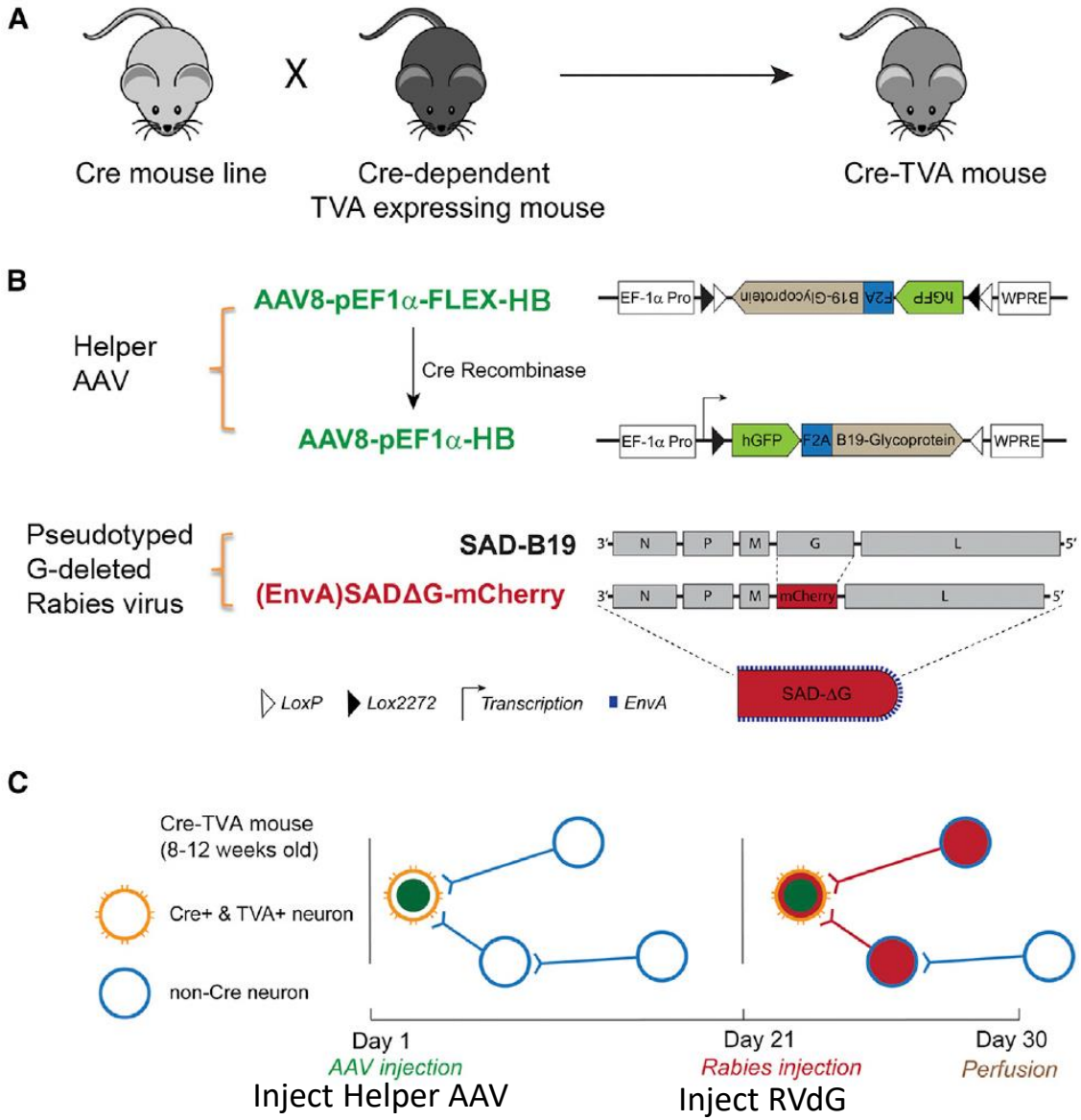
Cell-type specific Circuit Connectivity of Hippocampal CA1 Revealed through Cre-Dependent Rabies

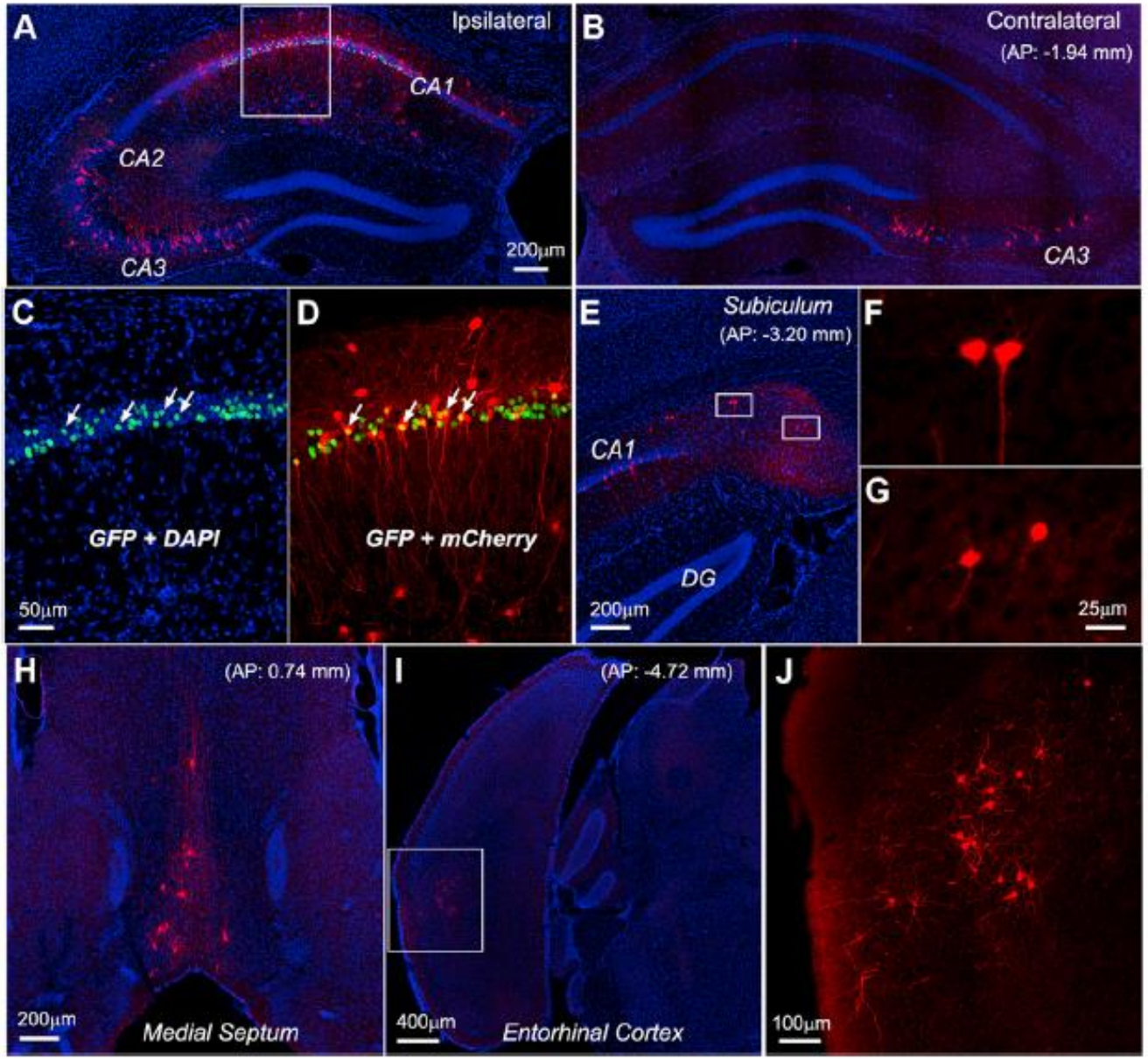
Sun et al, 2014. Cell Reports



- A double transgenic mouse line (Camk2a Cre : TVA) was created by crossing a Camk2a Cre mouse line (where Cre expression is largely restricted to CA1 pyramidal neurons) with a Cre-dependent TVA expressing mouse line.
- A Cre-dependent AAV helper virus containing rabies G, TVA and GFP was then injected into CA1.
- 21 days afer this injection RVdG + EnvA +mCherry was injected into the same location. This rabies virus enters the starter cells and replicates its genome with mCherry expression.
- RVdG undergoes trans-complementation to form RVdG + G, which may bud from starter cells and infect presynaptic neurons.
- Starter cells = GFP (green) + mCherry (red)
- Presynaptic Cells = mCherry (red)

Rabies labeling of presynaptic neurons shows direct local and distant circuit connections to CA1 pyramidal neurons

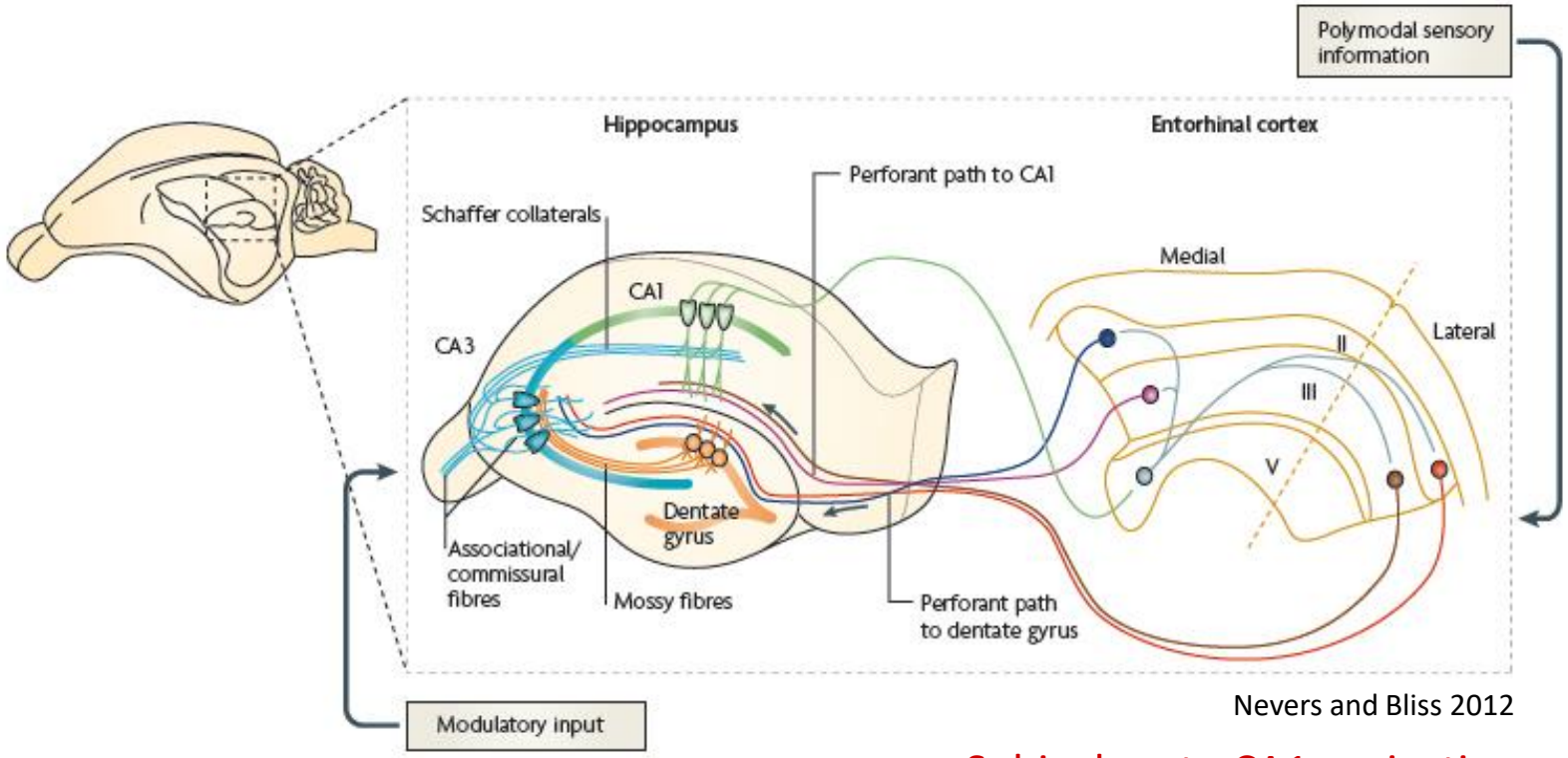
Sun et al, 2014. Cell Reports.



- Helper virus was targeted to CA1 pyramidal neurons (starter cells) by using the Camk2a-Cre mouse line that selectively expresses Cre in **pyramidal neurons**.
- Using this technique one can quantify the ratio of presynaptic neurons to starter cells. This is known as the **Connection Strength Index (CSI)**
- Location of presynaptic neurons and their CSI:
  - CA3 pyramidal (1/3 of afferents) = 7.01 CSI
  - CA1 pyramidal= 3.44
  - CA1 interneurons = 3.26 (but estimated to be ~15 in other studies using classical techniques)
  - Medial Septum = 0.95
  - Entorhinal cortex layer III = 0.54
  - Subiculum (non-canonical) = 0.81
  - Amygdala, reuniens thalamic nucleus, raphe nucleus (present but not quantified)

A, B Ipsi and contra-lateral injection sites
C, GFP + Dapi
D, GFP + mCherry
E, Subiculum
H, Medial Septum
I, Entorhinal cortex
J, enlargement of I

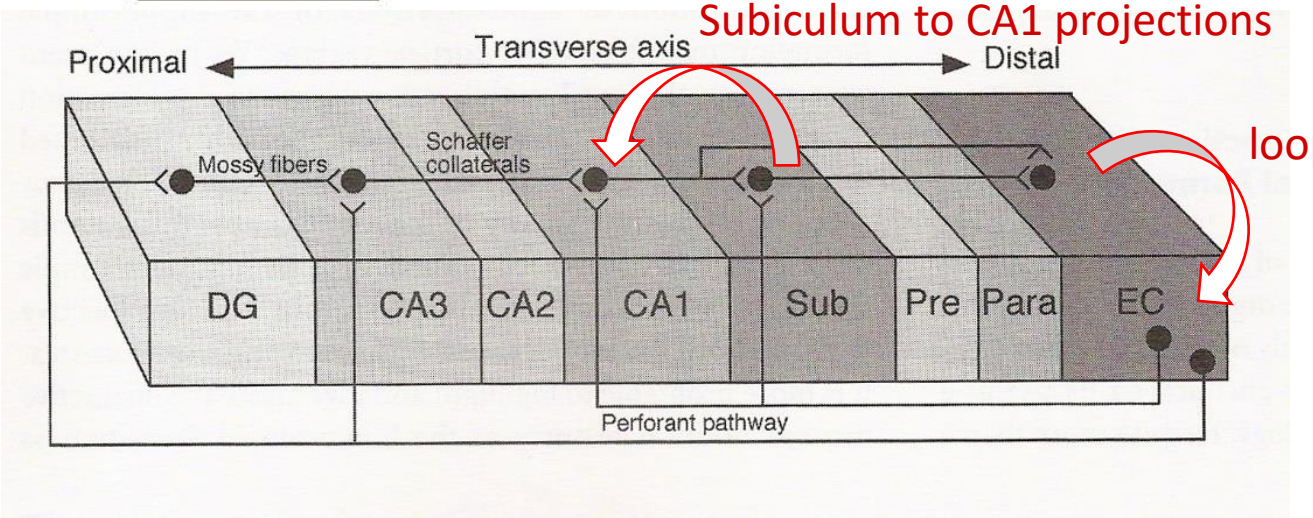
Loop: Entorhinal cortex to hippocampus to entorhinal cortex



Nevers and Bliss 2012

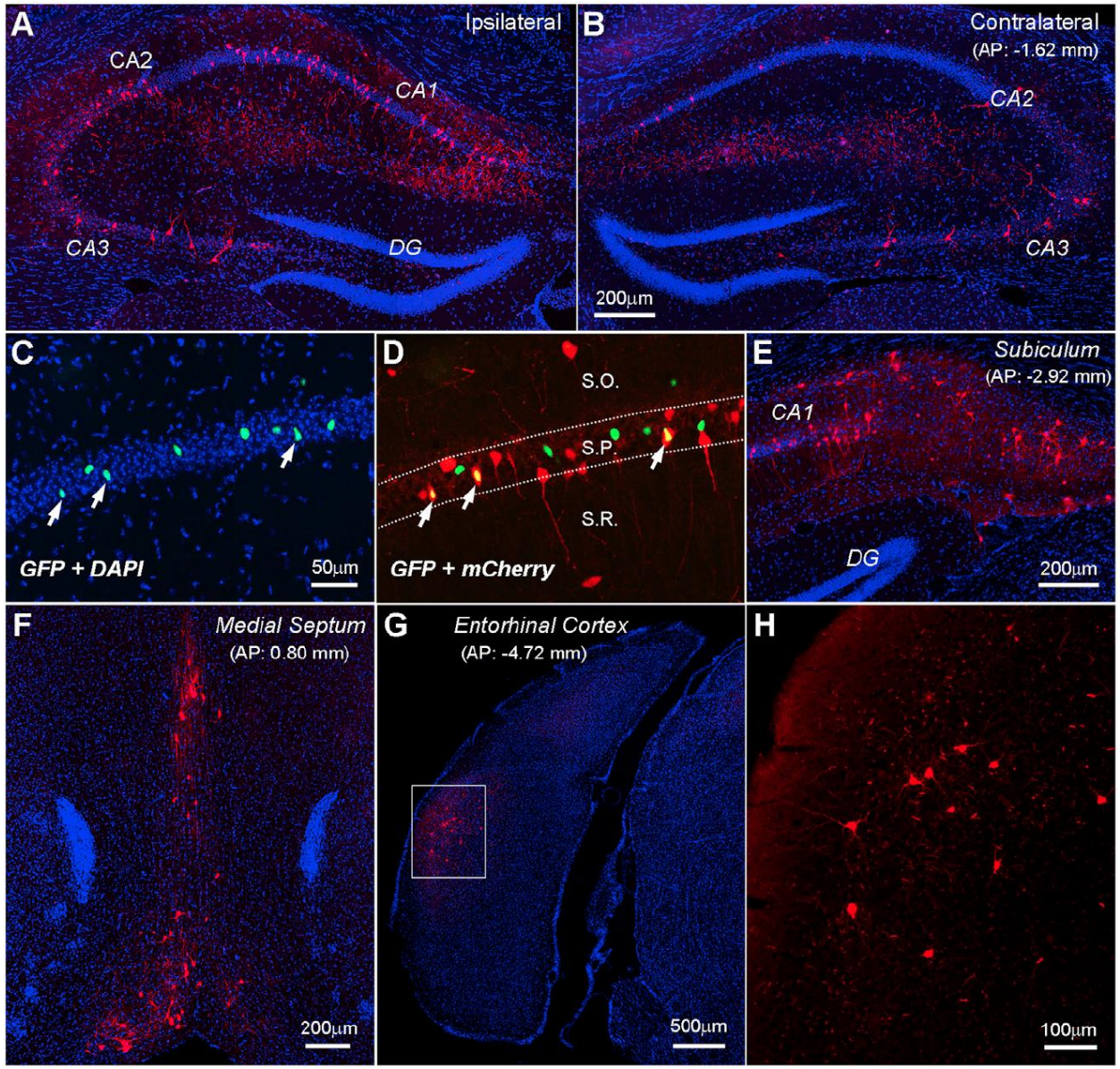
Subiculum to CA1 projections

loop



Rabies labeling of presynaptic neurons shows direct local and distant circuit connections to CA1 GABAergic interneurons

Sun et al, 2014. Cell Reports.



- Helper virus was targeted to CA1 GABAergic neurons (starter cells) by using the Dlx5/6 mouse line that selectively expresses Cre in forebrain **GABAergic neurons**.
- Location of presynaptic neurons and their CSI:
  - CA3 pyramidal (1/3 of afferents) = 1.63
  - CA1 pyramidal= 9.72
  - Medial Septum = 1.17
  - Entorhinal cortex layer 3 = 0.12
  - Subiculum (non-canonical) = 1.00
  - Amygdala, reuniens thalamic nucleus, raphe nucleus (present but not quantified)

A, B Ipsi and contra-lateral injection sites
C, GFP + Dapi
D, GFP + mCherry

E, Subiculum
H, Medial Septum
I, Entorhinal cortex
J, enlargement of I

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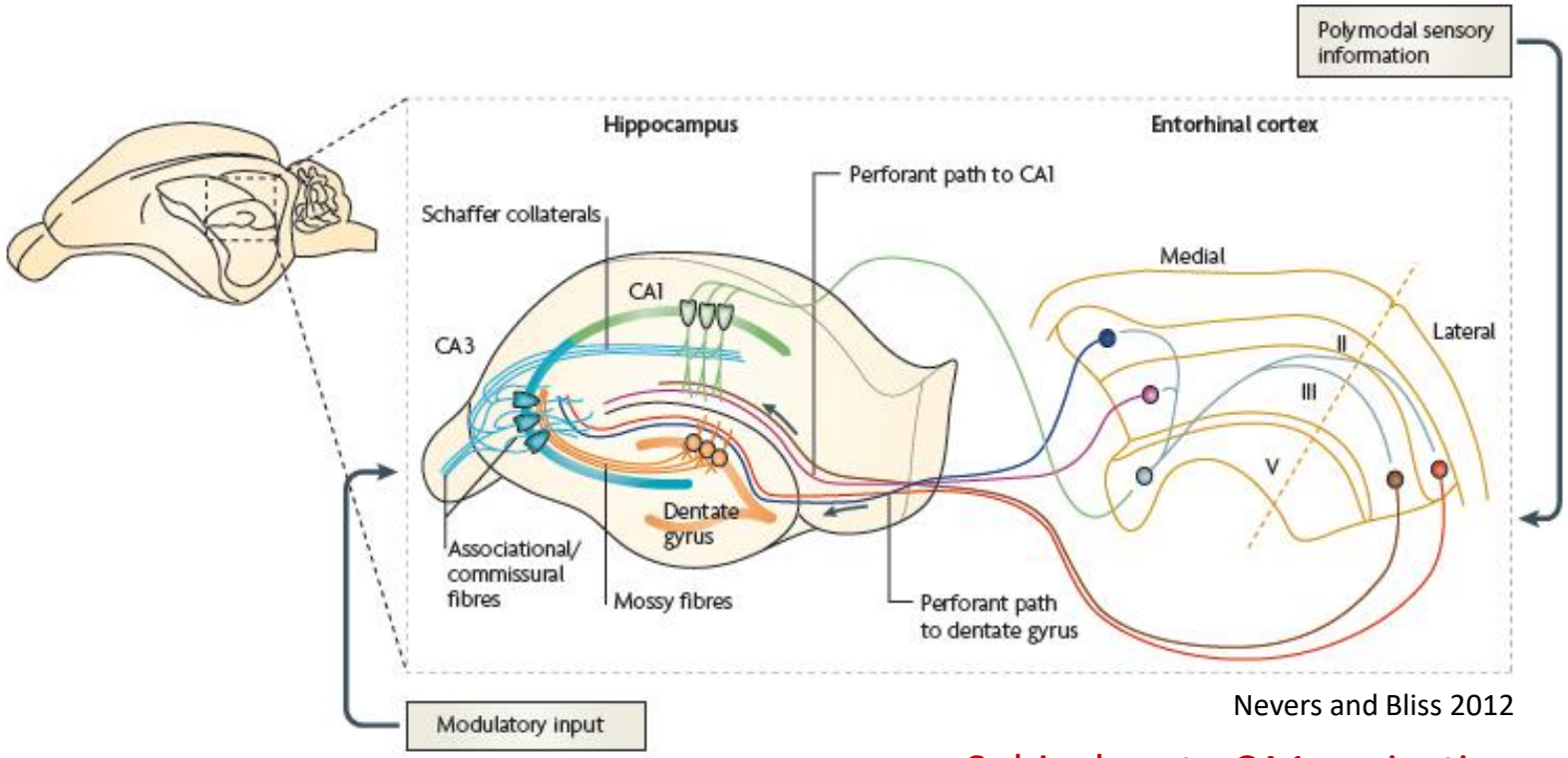

Nevers and Bliss 2012

Subiculum to CA1 projections

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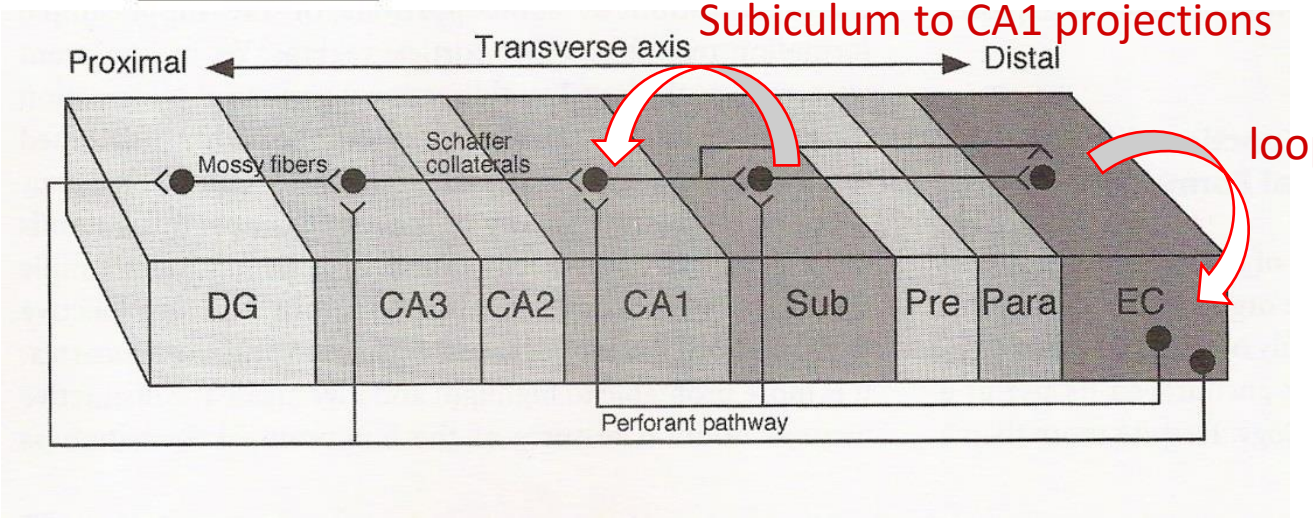
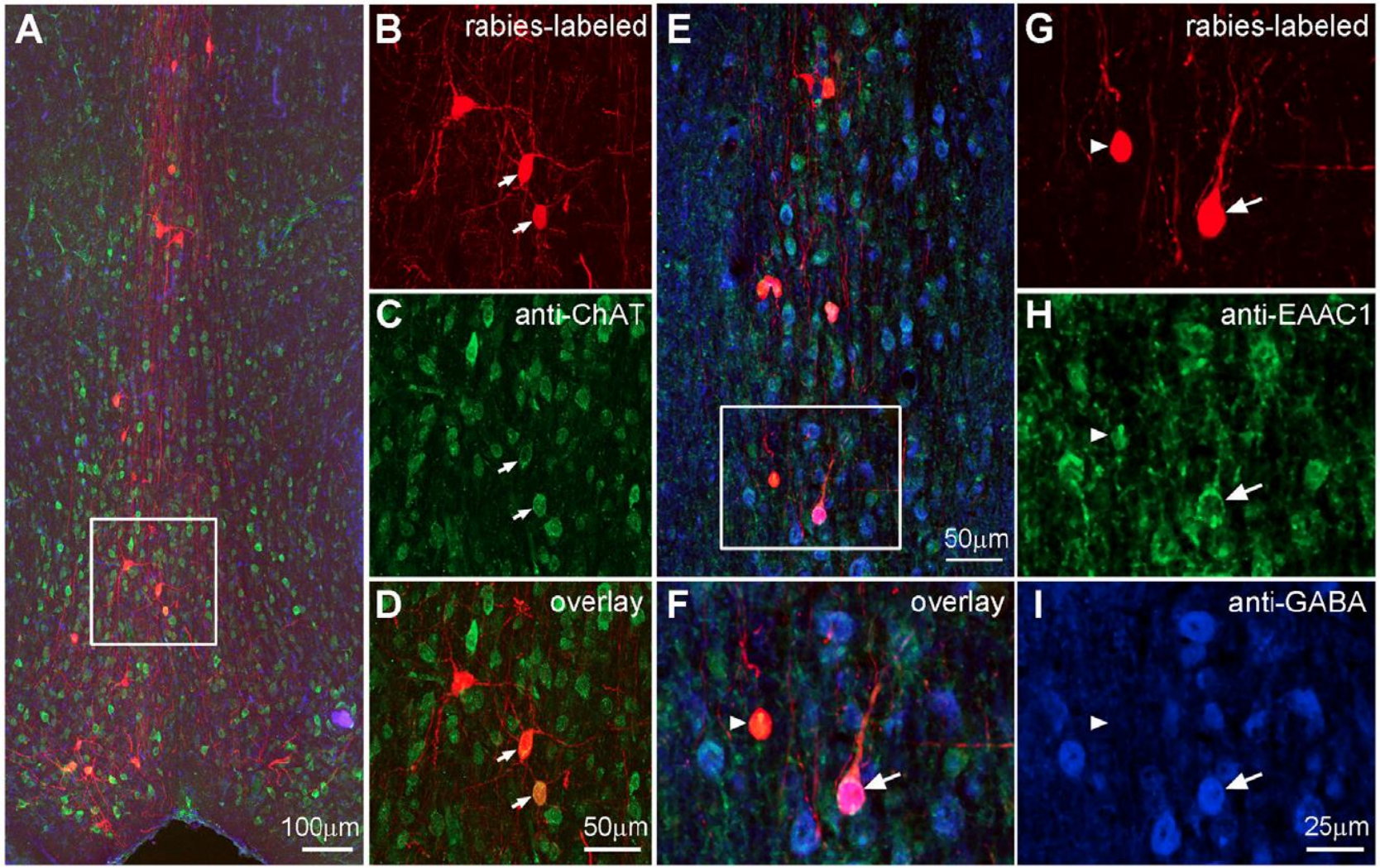
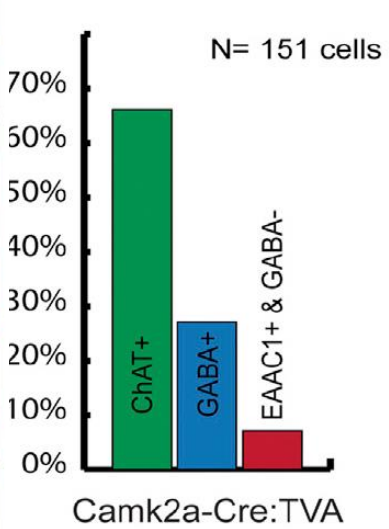


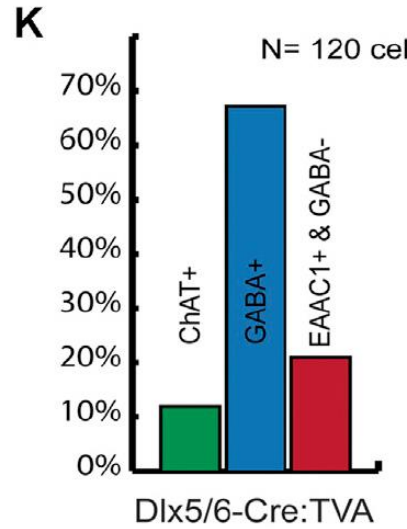
Neurochemical labeling of rabies labeled neurons in the Medial-septum / Diagonal band presynaptic to CA1 pyramidal neurons and GABAergic interneurons



Differential connectivity of projections from the MS/BD to CA1 excitatory (glutamatergic) and inhibitory (GABAergic) neurons.



Pyramidal neurons



GABAergic interneurons neurons

Sun et al, 2014. Cell Reports.

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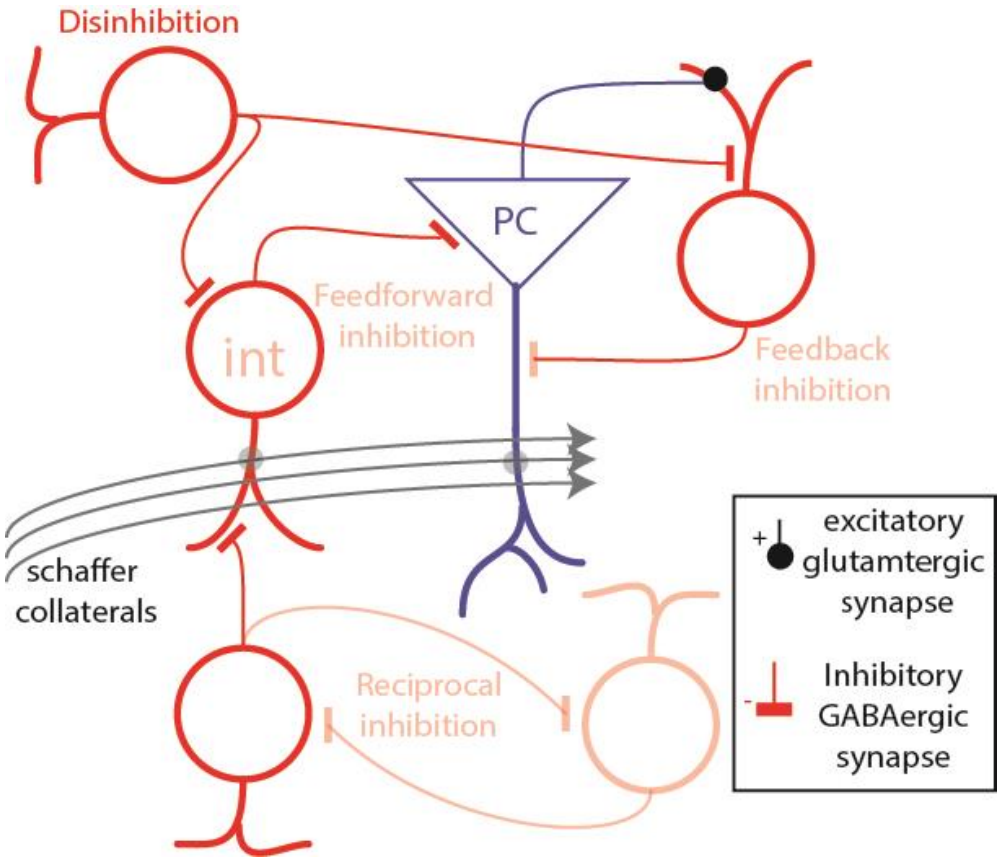

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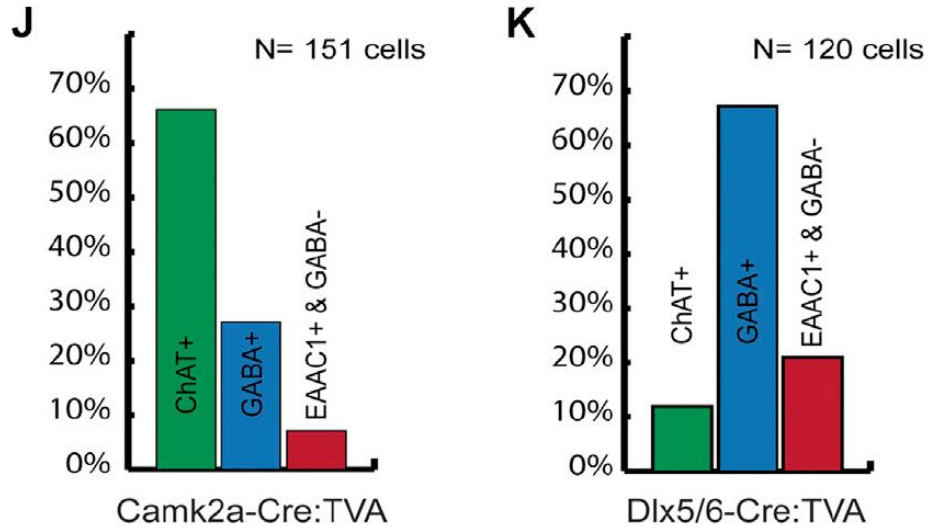

Pyramidal neurons

GABAergic interneurons neurons

Sun et al, 2014. Cell Reports.

Using G-deleted rabies virus strategy to trace hippocampal circuitry allowed Sun et al. to make the following conclusions:

1. Strongest connection to CA1 pyramidal cells are from CA3 Shaffer collaterals (rapid learning pattern completion based memory recall)

2. CA3 differentially innervates CA1 pyramidal cells relative to GABAergic interneurons, with a 4-5 fold greater input connection strength.

3. The tempero-ammonic pathway (direct connections from entorhinal cortex) exhibits relatively weak connectivity to CA1 pyramidal neurons and interneurons (long term spatial memory consolidation and maintenance + non-spatial temporal association)

4. Non-canonical inputs from the excitatory and inhibitory neurons of the Subiculum to CA1.

5. CA1 pyramidal neurons receive 66% of cholinergic inputs from the Medial Septum/ Diagonal Band (MS/DB). They also receive GABAergic septo-hippocampal innervation (27%).

6. CA1 GABAergic neurons receive 12% of cholinergic inputs from the Medial Septum/ Diagonal Band (MS/DB). In contrast interneurons GABAergic neurons receive 67% of septo-hippocampal innervation.

The connectivity strength index (CSI) between neurons does **not** necessarily predict the functional connectivity

Other factors that should be considered

- The number of synapses :
  - Basket cells contact 2000 pyramidal cells through 10000 synapses.
  - Pyramidal cells are estimated to be contacted by 15 Basket cells (but only 3 in Sun et al.)

- The frequency of activity of the presynaptic cells :
  - i.e. Mossy Cells and EC inputs to granule cells in the DG
  - i.e. Granule cell mossy fiber input to CA3 pyramidal neurons
    - Short term plasticity
    - Connectivity with interneurons (i.e. feedforward and feedback inhibition)

- The location of the synapses and the form of the dendritic tree :
  - Proximal vs Distal

- The form of the synaptic spines and the strength of each synapse :
  - Number of postsynaptic receptors
  - Probability of release of neurotransmitters from the presynaptic neuron.

Plan

Part I

I. Structure, Afferents and Efferents of the rodent hippocampus

I. The hippocampus in rodents and primates
II. Septal-temporal (dorsal-ventral) orientation
III. Major Fiber bundles of the hippocampus: Afferents + Efferents
IV. Topographic connectivity of hippocampal Afferents and Efferents
V. Functional modules of the hippocampus?

II. The « tri » synaptic hippocampal circuit

I. Layers and Strata of the hippocampal formation
II. The Dentate Gyrus network
    I. Granule Cells
    II. Mossy Cells
    III. Inhibitory interneurons
III. Inhibitory/excitatory balance and disease
    I. Selective ablation of Mossy Cells

Part II

IV. CA3
    I. Granule cell mossy fibers projections to CA3
        I. Mossy fibers, Fliopodial extentions, *en passant* boutons
        II. Ultrastructure of mossy fibers
    II. Functional Mossy Fiber inputs to CA3
    III. Influence of spine shape and synapse location on dendritic integration
    IV. CA3 Afferents and Efferents
V. CA1
    I. CA1 Afferents
    II. Inhibitory interneurons
    III. CA1 Efferents/Subiculum
VI. The « tri » synaptic hippocampal circuit and the entorhinal cortex
VII. CA2

III. Modern anatomical techniques

I. Monosynaptic tracing with glycoprotein–deleted rabies viruses