ZNZ Advanced course for PhD students

Functional anatomy of the rodent brain

Hippocampus
Part I

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LECTURE OUTLINE

The hippocampal formation:

• A short historical perspective: Proposed functions, biological characteristics, and neurobiological models of the hippocampus

• Hippocampal neuroanatomy: Dentate gyrus Hippocampus Subiculum Presubiculum and Parasubiculum Entorhinal cortex

• Chemical neuroanatomy
LITERATURE

- The Hippocampus Book:
  Edited by: Per Andersen, Richard Morris David Amaral, Tim Bliss, John O‘Keefe
  Oxford University Press 2007

- The neuroanatomy of the rodent brain
  Edited by: George Paxinos
  Oxford University Press 2005

© Images used for this lecture
The hippocampal formation

- Nissl-stained coronal sections
- The most striking feature is the general similarity of this brain region across phylogeny
- What does it do?
- What can we learn about general principles of neuronal function?
The hippocampal formation

- **Hippocampus proper:** Ammon’s horn (Cornu Ammonis, CA)

- **Hippocampus:** Dentate gyrus (DG) CA fields

- **Hippocampal formation:** Dentate gyrus CA fields Subiculum Presubiculum Parasubiculum Entorhinal cortex
A short historical perspective

- Alexandria school of medicine
  - Cornu ammonis (horn of the ram): CA

- Anatomist Giulio Cesare Aranzi (1564) first to coin the name hippocampus

- Anatomist Camillo Golgi (1843-1926) Golgi method: unique organisation of the hippocampus
A short historical perspective

- Pioneering investigations of Camillo Golgi (1886), Luigi Sala (1891), Karl Schaffer (1892), and Ramon y Cajal (1893).
- Cajal described first functional circuit diagram of the hippocampus: principle of dynamic polarization.
- First description of spines (dismissed by Golgi as staining artifacts).
- Neurons as morphological entities (Cajal) vs “rete nervosa diffusa”, 'diffuse neural network (Golgi)

Drawing of the hippocampus in *Histologie de Système Nerveux, 1911*
Early ideas about hippocampal function

- Until the 1930s, the hippocampal formation was part of the olfactory system. Based on both behavioural and clinical observations (olfactory sensations as part of epileptic seizures).

- Alf Brodal (1947): Milestone review to discuss that hippocampal formation is not a major component of the olfactory system, but olfactory information certainly must contribute to the functions in which the hippocampus is engaged. The rodent entorhinal cortex receives a massive direct projection from the olfactory bulb and secondary olfactory inputs form piriform and periamygdaloid cortices.

- James W. Papez (1937): Hippocampal formation provides the anatomical substrate of emotion (Papez Circuit).

- Influenced by the work of Walter Cannon (1929) and Philip Bard (1934): Role of the hypothalamus essential for autonomic and visceral aspects of emotional behaviour.
The hippocampal formation and attention control

- Richard Jung and Alois Kornmüller (1938): First description of „theta activity“ in the rabbit hippocampus
  - Large-amplitude, sinusoidal wave pattern between 4-7 Hz.
  - Associated with enhanced attention (Green and Arduini, 1954)
  - Coupled to specific learning states: distinct changes during the acquisition of conditioned response (Molmes and Adey, 1960)

- First electrical stimulation of the temporal lobe including hippocampus in anesthetised and awake cats:
  - General activation of attention
  - Role of hippocampus in emotional processing
The hippocampal formation and memory

- Théodule-Armand Ribot (1881): „Memory loss is a symptom of progressive brain disease“; first hypothesis to suggest a direct role of nerve cells for memory functions
- Paul Broca (1861), Carl Wernicke (1881), Korbinian Brodmann (1909): areas of the brain have specific functions, parallel distributed processing
The hippocampal formation and memory

- Henry Gustav Molaison (1926-2008)
- Most important and certainly the most famous single case study patient in Psychology and Neuroscience

Healthy individual

Molaison in 1997 71 years old

Temporal lobectomy in 1953 by W. Scoville
Henry Molaison

**Timeline | Scientific landmarks in the study of H.M.**

1953  
Scoville and Milner describe the purity and severity of H.M.'s memory impairment, emphasizing the "importance of the hippocampal region for normal memory function".

1957  
Milner shows H.M.'s significant learning of a sensorimotor skill within and across days — the first experimental demonstration of preserved learning in amnesia.

1962  
The first demonstration of repetition priming in H.M. His performance improves from the first to the second session, which is evidence of priming, but his scores are inferior to those of control participants, perhaps because he cannot store the list of items in declarative memory.

1965  
Eichenbaum and colleagues show H.M.'s normal performance on tests of odour detection, discrimination of intensity, and adaptation, but impaired odour quality discrimination and recognition.

1968  
Corkin finds a dissociation between declarative and nondeclarative memory within a single maze-tracing task.

1969  
H.M.'s errors fall to decrease, indicating impaired learning, whereas his decreasing time scores show that he can learn the sensorimotor skill required to trace the maze. This study also shows that H.M.'s anterograde amnesia extends to the somatosensory system.

1970  
Sagasti and colleagues conclude that H.M.'s retrograde amnesia extends back to age 16 (11 years before his operation).

1975  
Several experiments show H.M.'s decreased ability to report pain, hunger and thirst. This deficit might be related to his bilateral amygdaloectomy, rather than being secondary to the memory loss.

1983  
Gabrieli et al. report that H.M. shows full retention of the mirror-tracing skill after a delay interval of nearly one year.

1985  
The first magnetic resonance imaging evidence of the focus and extent of H.M.'s lesion shows that the resection was less extensive than the estimate made by Scoville at the time of operation.

1987  
Xu and Corkin show that H.M. and another severely amnesic patient are unable to learn the Tower of Hanoi puzzle, thereby correcting a long-standing misconception that this task is a measure of nondeclarative memory.

1988  
Kosinski, Ullman and Corkin show that H.M.'s verbal and grammatical processing are preserved, and that his premorbid semantic knowledge is unchanged over 48 years.

1991  
Postle and Corkin, using novel words, find that H.M.'s word-stem completion priming is impaired, but that his perceptual identification priming is intact.

1992  
(1987–1988) H.M. shows normal recognition of complex colored pictures at a delay interval of 10 min., 24 h., 72 h., one week and six months.

1994  
A study of habit learning in H.M. and another severely amnesic patient, using the concurrent discrimination task, provides evidence that, unlike monkeys with MTL lesions, who perform this task normally, humans with MTL lesions cannot.

1995  
Postle and Corkin, using novel words, find that H.M.'s word-stem completion priming is impaired, but that his perceptual identification priming is intact.

1996  
(BOX 1)

1997  
(BOX 1)

1998  
(BOX 1)

1999  
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2001  
(BOX 1)

Susanne Corkin, Nature Review Neuroscience, 2002
Acquisition of semantic knowledge

Susanne Corkin, Nature Review Neuroscience, 2002
Henry Molaison’s memory disorder

- Intact working memory
  - Normal digit span (remembering numbers)
  - Unless interrupted or distracted (constant rehearsal)

- Old long term memory (LTM) intact, but unable to create new LTM.
  - encoding, consolidation deficit, retrieval intact
  - Hippocampus involved in the formation of new LTM.

Sensory organs

- perception
- attention
- repetition
- forgetting
- encoding consolidation
- retrieval

Sensory Memory (ms – 1 s)

Short-Term Memory Working Memory (< 1 min)

Long-Term Memory (d, m, y)
Building a search engine of H.M.’s brain

December 2009
live webcast

70 microns
53 h of cutting

~2500 sections
high-resolutions scanning

creation of brain atlas


Dr. Jacopo Annese
The Brain Observatory at UC San Diego
Dissected the brain of Henry Molaison

“I feel like the world is watching over my shoulder.”
The hippocampus as a cognitive map

- Important development for the analysis of hippocampal function:
  - Implantation of microelectrodes to monitor single-neuron activity in the awake animal (1970 by Hirano et al., Vinogradova et al., O'Keefe et al., Ranck et al.)

- First cognitive map theory: based on experiments describing relations between cellular activity and sensory and behavioural parameters.

- Spatial functions of the hippocampus: spatial memory and navigation in familiar environment.

  - Hippocampal place cells (O'Keefe and Dostrovsky, 1971)
  - Postsubiculum head-direction cells (Taube and Ranck, 1990)
  - Entorhinal grid cells (Moser and Moser, 2005)
  - Entorhinal border cells (Moser and Moser, 2008)
Special features of hippocampal anatomy

- Features of the hippocampus that allows studies of the general neuronal and systems properties:
  - A single layer and strictly laminated inputs (Golgi, Cajal, No)
  - Predominantly unidirectional connections between a series of cortical regions (Hjorth-Simonsen, Cowan, Powell, 1972)
  - Extrinsic and intrinsic fibers making numerous en passant contacts with target neuronal dendrites, running orthogonal to the main dendritic axes (Blackstad, 1956)
  - Synapses that are highly plastic (sprouting; Raisman, 1969, Lynch & Cotman, 1972, LTP, LTD; Bliss & Lomo, 1970, 1973)
  - Tissue that can be used in transplantation studies (Björklund, 1975)
  - Neurons that can be successfully grown in culture (Banker, 1977)
  - Acute or cultured slices surviving for prolonged periods in vitro (Gähwiler, 1981)
  - Adult neurogenesis (Altman and Bayer, 1975)
Neurophysiological principles discovered from work on hippocampal preparations include:

- Identification of excitatory and inhibitory synapses and their localization, transmitters, and receptors: George Gray (1959); Kandel, Spencer, Brinley (1961); Andersen, Eccles, Lyning (1964)

- Discovery of long-term potentiation and long-term depression: post-tetanic potentiation (PTP; Larrabee & Bronk 1939, sympathetic ganglia, duration of a few minutes only); repeated tetanus

- Role of oscillations in neuronal networks

- Underlying mechanisms of epileptogenesis
Differences and similarities across species

Dorsolateral view of the human hippocampus

Major fiber bundles:
1) Angular bundle (EC-hip)
2) Fimbria-Fornix (Hip-BFB, reciprocal)
3) Dorsal and ventral commissures
Differences and similarities across species
The rat hippocampal formation

- Nissl-staining: cellular architecture; dye labels endoplasmatic reticulum
  - Cell nuclei

- Graphic representations
  - Diagrams to illustrate spatial organization of distinct brain areas (Paxinos & Watson’s brain atlas)

- Timm’s sulfide staining: axonal staining
  - To highlight various fiber and axonal terminal systems
The rat hippocampal formation: Coronal view
The rat hippocampal formation: Horizontal view
The rat hippocampal formation: Sagital view
The Dentate Gyrus (DG)

- 3 layers
- Cell types:
  - Granule cell (principal)
    - Elliptical cell body
    - 10 \( \mu \text{m} \times 18 \mu \text{m} \) in size
  - Mossy cells
    - Innervate contralateral DG, excitatory, spiny
  - Pyramidal Basket cells
    - 25-35 \( \mu \text{m} \) in size
    - Deep surface of the gcl
  - Other interneurons
    - Axo-axonic cells
    - HICAP cells
    - MOPP cells
    - HIPP cells
Interneurons in the rat DG

- **Chandelier cells** contain pericellular plexuses.
- **Hilar-commissural-associational pathway** associated cells.
- **Molecular layer-perforant path associated cell**.
- **Hilar-perforant pathway associated cell**.

Diagram showing layer-specific input and various pathways such as entorhinal afferents, commissural/associational afferents, mossy fiber collaterals.
Granule cell projection to the polymorphic layer

- Axon arbors of two adjacent granule cells:
  175 reconstructed terminals and filopodia;
  52 innervated mGluR1a-positive targets
DG efferent projections: Mossy fibers target CA3

- All DG granule cells project to CA3; form glutamatergic synapses in the stratum lucidum (lack of myelin on mossy fibers gives clear appearance in fresh cut tissue)
Hippocampus cytoarchitecture
Organization of hippocampal pyramidal cells
Organization of hippocampal interneurons

- $\text{GAD}_67$ immunoreactivity
Organization of hippocampal interneurons

- $\text{GAD}_{67}$ immunoreactivity
Organization of hippocampal interneurons

Calretinin

nNOS
Interneurons in the hippocampus

Freund TF (2003). TINS, 489-495
Interneurons in the hippocampus
The CA1-subiculum border is marked by an abrupt widening of the pyramidal cell layer and an increased staining intensity with the Timm stain.

Stratum radiatum of CA1 is replaced with ml of the subiculum; ml is subdivided into deep and superficial layer (-> CA slm; also receives input from EC).
Reciprocal EC-Hip connections: Tracing studies
Cytoarchitecture of the rat entorhinal cortex

- Nissl-stained coronal sections through three selected rostro-caudal levels of the entorhinal cortex, arranged from A) rostral to C) caudal.

- LEA = lateral entorhinal ctx
- MEA = medial entorhinal ctx
- PcoA = posterior cortical nucleus of the amygdala

- High magnification images of LEA (D) and MEA (E); enlarged from B and C.
Septal and hypothalamic inputs

Injection of PHA-L into the medial septum/VDB, HDB

Injection of PHA-L into the supra mammilary nucleus

PHA-L = *Phaseolus vulgaris* Leucoagglutinin
Inputs to the hippocampal formation

A. Noradrenaline

B. Serotonin

C. Dopamine

CA1, CA3, DG, EC, Pro, Sub, Peri
Hippocampal transverse organization

- Unidirectional passage of information through intrahippocampal circuits
- Receives highly processed, multimodal sensory information from a variety of neocortical structures
Summary of the serial and parallel pathways