Effect of Sleep Deprivation on the Hippocampus of Adult and Senile Male Albino Rat: A Histological and Histochemical Study

Thesis
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List of Abbreviations

Ach	:	Acetylcholine
AD	:	Alzheimer's disease
ADHD	:	Attention-Deficit Hyperactivity Disorder
AQP4	:	Aquaporin 4
ATP	:	Adenosine Triphosphate
Aβ	:	Amyloid β
CA	:	Cornu Ammonis
CARE	:	Committee on Animal Research Ethics
CSF	:	Cerebrospinal fluid
DG	:	Dentate Gyrus
EEG	:	Electroencephalography
GFAP	:	Glial fibrillary acidic protein
ISF	:	Interstitial fluid
MRC	:	Medical Research Center
non-REM	:	Non-Rapid Eye Movement
PAMP	:	Pathogen-associated molecular patterns
PBS	:	Phosphate buffered saline
PKA	:	Protein kinase A
REM	:	Rapid Eye Movement
SCN	:	Suprachiasmatic nucleus
SWS	:	Slow-Wave Sleep
SYN	:	Synaptophysin
VLPO	:	Ventrolateral preoptic nucleus

Introduction

The importance of sleep and the impact of its deprivation on development of brain pathology became a recent subject of interest in medicine **(Jessen *et al*., 2015).**

The restorative effect of sleep on the brain and the harmful effects of insomnia have been recently revealed through the discovery of the glymphatic system and its association with sleep (**Simon and Illiff, 2016).**

Brinker *et al*. (2014) revised the classic CSF circulation theory in which the majority of CSF is produced by the choroid plexus, circulates through the ventricles, the cisterns, and the subarachnoid space to be absorbed into the venous blood system across arachnoid villi into the cervical lymphatics. Consequently, the authors assumed a new hypothesis that the CSF is produced and absorbed inside the whole CSF system by filtration and reabsorption of water volume through the capillary walls into the interstitial fluid of the surrounding brain tissue**.**

Moreover, recent evidence has revealed the presence of a brain-wide network of paravascular channels, termed the "glymphatic" pathway, along which a large proportion of subarachnoid CSF recirculates through the brain parenchyma, facilitating the clearance of interstitial solutes, including amyloid-β, from the brain **(Aspelund *et al*., 2015).**

In addition to waste elimination, the glymphatic system also facilitates distribution of numerous

compounds, including glucose, lipids, amino acids, growth factors, and neuromodulators throughout the brain **(Brinker *et al*., 2014).**

This mechanism is carried out mainly through the activation of aquaporin 4 (AQP4) receptors on the perivascular astrocytes **(Jung *et al*., 1994).** The author identified the AQP4 as an integral membrane protein that conducts water through the cell membrane. Similarly, AQP4 is expressed in astrocytes in the central nervous system **(Nagelhus *et al*., 2004)** and in the basolateral cell membrane of principal collecting tubule cells in the kidney providing a pathway for water to exit these cells **(Agre and Nielsen, 1996).**

Xie *et al*. (2013) revealed that in mice, the clearance activity of the glymphatic system is profoundly stimulated by sleep; clearance during sleep is as much as two-fold faster than during waking hours. **Mendelsohn and Larrick (2013)** added that anaesthesia and attenuation of adrenergic signaling can activate the glymphatic system to clear potentially toxic proteins known to contribute to the pathology of Alzheimer disease such as β amyloid, this neurotoxic protein acts as apoptotic insult on the hippocampal neuron **(Kathryn *et al*., 1998).**

Dysfunction within this glial vascular network, which is a feature of the aging, is a potentially critical link in the development of chronic neurodegeneration **(Brinker *et al*., 2014).**

On going research within this field may provide a powerful new understanding of the common links between neurodegenerative, neurovascular and neuroinflammatory diseases, in addition to providing potentially novel therapeutic targets for these conditions **(Carare *et al*., 2013).**

Based on ad hoc, further studies are needed to clarify the importance of sleep deprivation, and/or aging on the clearance of neurotoxic metabolites particularly β amyloid from brain structures.

Aim of the work

1. **Research questions:**
   - Does sleep deprivation lead to apoptosis in the hippocampal neurons.
   - Does it lead to accumulation ofβ amyloid in the hippocampus.
   - Does aging alone or with sleep deprivation lead to accumulation ofβ amyloid in the hippocampus.
2. **Working hypothesis:**

   Sleep deprivation and/or aging leads to apoptosis and/or accumulation ofβ amyloid in the hippocampus.
3. **Overall aim** : to study the effects of sleep deprivation on the histology and histochemistry of the hippocampus.
4. **Specific objectives are:**
   - To detect histological and apoptotic changes in the neurons and dendrites of the *cornu Amonis* and the dentate gyrus in sleep deprived rats in comparison to rats with undisturbed sleep pattern (control).
   - To detect deposition of neurotoxic metabolites in *cornu Amonis* and dentate gyrus in sleep deprived rats in comparison to controls.
   - To compare between the previously mentioned effects of sleep deprivation in adult and senile rats.
5. **Type of study:**

   Experimental histological and histochemical study on rats with a control group.

A-Hippocampus

The hippocampus is a major component of our brains and the brains of other vertebrates. It belongs to the limbic system and plays important roles in memory. Humans and other mammals have two hippocampi, one in each side of the brain. The hippocampus is located under the cerebral cortex **(Pearce, 2001)**. In primates it is located in the medial temporal lobe, under the cortical surface. It contains two interlocking parts: *Ammon's horn* and Dentate gyrus **(Aboitiz *et al*., 2003).**

Anatomy of rat hippocampus:

Rat hippocampus is a bilateral limbic structure which, in overall shape resembles two "Cs" leaning together at the top and spread apart at the base. The top portion of hippocampal formation is called "dorsal hippocampus", **(Amaral and Witter, 1989).**

The internal structure of the hippocampus is the same throughout its length, and consists of an infolded twist of the evolutionarily older and more simple (fewer cell layers), archicortex or allocortex (**Amaral and Witter, 1989).**

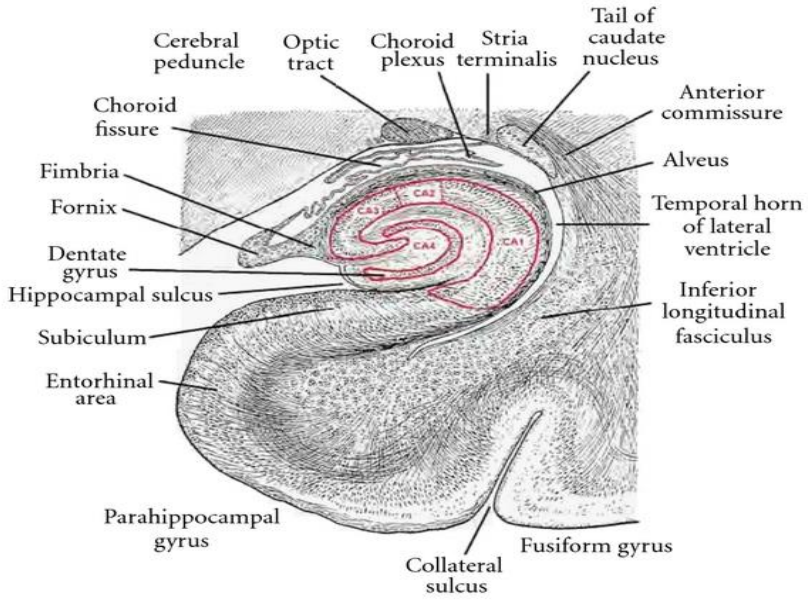
Cross-section taken perpendicular to the long axis will reveal the internal structure as two interlocking "Cs", one reversed in relation to the other. One "C" makes up Ammon's Horn or *Cornu Ammonis* (CA1-CA3), also known as the "Hippocampus proper". The principle cell layer of Ammon's Horn is the *stratum pyramidale*, or the

pyramidal cell layer. The other "C" is made up of the Dentate Gyrus, of which the *stratum granulosm*, or granule cell layer is the principle cell layer (**Amaral and Witter, 1989).**

Although the Dentate Gyrus is part of the hippocampus, we can differentiate it from the hippocampus proper by their different cell architecture, **(Amaral, 1978; Bayer, 1985; Amaral and Witter, 1989).**

Since the dentate gyrus is not truly part of the hippocampus, the term "Hippocampal formation" are used to discuss Ammon's Horn and the dentate gyrus together **(Andresen, 1975).**

Anatomy of human hippocampus:



Hippocampus_coronal_section176157. fig. 004. jpg (600 × 531 pixels, file size: 152 KB, MIME type: image/jpeg) **(Kiernan, 1899).**

When we start at the dentate gyrus and pass inward along the S-curve of the hippocampus, We will traverse a series of narrow zones. The first of these, the dentate gyrus (DG), a tightly packed layer of small *granule cells* wrapped around the end of the hippocampus proper, forming a pointed wedge in some cross-sections, and a semicircle in others. Next, comes a series of *Cornu Ammonis* (CA) areas: first CA4 (which underlies the dentate gyrus), then CA3, then a very small zone called CA2, then CA1. The CA areas are all formed of densely packed pyramidal cells. After CA1 comes an area called the subiculum. After this comes a pair of ill-defined areas called the presubiculum and parasubiculum, then a transition to the cortex proper (mostly the entorhinal area of the cortex). Most anatomists use the term "hippocampus proper" to refer to the four CA fields, and "hippocampal formation" to refer to the hippocampus proper plus dentate gyrus and subiculum **(Amaral and Lavenex, 2006).**

The intrinsic connections between the principle cell layers of the dentate gyrus and CA regions of the hippocampus are very clear. Therefore, **Andersen (1975)** was able to determine the major direction of afferent connections or synaptic flow of the "trisynaptic circuit" through the examination of Golgi stained normal material.

First, layers II and III or the "surface layers" of the entorhinal cortex project to the granule cells of the dentate-gyrus, via the perforant-path. Second, the granule cells of the dentate gyrus project to the large pyramidal cells

of *Cornu Amonnis* or *Ammon's horn*, subfield 3 (CA3), via the mossy fibres system. Third and finally, the CA3 pyramidal cells project to the pyramidal cells of the CA1 subfield, via the Schaffer collateral system **(Blackstad, 1956; Amaral 1978 and Bayer, 1985).**

However, in the last decade, more stress has been placed on the associational fibres that connect the transverse circuitry, and thus, more stress on the integrated three-dimensional functioning of the hippocampus as a whole **(Amaral and Witter, 1989).**

The hippocampus also receives a number of subcortical and cerebellar inputs **(Heath and Harper, 1974).**

Histology of hippocampus:

The hippocampus is composed of multiple subfields. Though terms varies among authors, the terms most frequently used are dentate gyrus and the *cornu ammonis*. The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, CA3, and CA4. However, the region known as CA4 is in fact the deep polymorphic layer of the dentate gyrus **(Andersen *et al*., 2007).**

In rodents, the CA regions are constructed of three clearly defined layers:

Polymorphic layer: contains axons of pyramidal neurons, cell bodies of inhibitory basket cells and horizontal trilaminar cells, and basal dendrites of pyramidal cells.

Pyramidal layer: contains the cell bodies of the pyramidal neurons, which are the principal excitatory neurons of the hippocampus. In region CA3, this layer contains synapses from the mossy fibers (projections from the dentate gyrus granule cells to CA3).

Molecular layer: contains septal and commissral fibers, Schaffer collateral fibers (the projection from CA3 to CA1), some interneurons, perforant path fibers (fibers from entorhinal cortex superficial layers) **(Andersen *et al*., 2007).**

The dentate gyrus is also composed of three layers:

Polymorphic layer: is the most superficial layer of the dentate gyrus. This layer contains many interneurons, and the axons of the dentate granule cells pass through this layer on the way to CA3.

Granular layer: contains the cell bodies of the dentate granule cells

Molecular layer: contains commissural fibers from the contralateral dentate gyrus and inputs from the medial septum, both terminate on the proximal dendrites of the granule cells). The perforant path fibers making excitatory synapses onto the distal apical dendrites of granule cells)**.**

Functions of hippocampus:

The earliest widely held hypothesis was that the hippocampus is related to olfaction. This concept was thrown into doubt by a series of anatomical studies that did not find any direct projections to the hippocampus from the olfactory bulb **(Boyer *et al*., 2007).**

It was confirmed that the olfactory bulb does project into the ventral part of the lateral entorhinal cortex, and field CA1 in the ventral hippocampus sends axons to the main olfactory bulb, the anterior olfactory nucleus, and to the primary olfactory cortex **(Broglio *et al*., 2002).** Therefore, the hippocampus plays a role in the memory of smell but olfaction is not its main function **(Burke and Barnes, 2006).**

Over the years, three main thoughts of hippocampal function have dominated the literature; inhibition, memory, and space. The behavioural inhibition theory was clarified by **Buzsáki (1989)**, that was very popular up to the 1960s. The authors derived much of its rationale from two observations: first, that animals with hippocampal damage tend to be hyperactive; second, that animals with hippocampal damage often have difficulty learning to inhibit responses that they have previously been taught, especially if the response requires remaining quiet **(Buzsáki, *et al*., 1990)**. However the inhibition theory is currently the least popular of the three **(Buzsáki, 2002).**

The second major line of thought relates the hippocampus to memory. This thought was reinforced by **Buzsáki (2010)**, who described the results of surgical destruction of the hippocampi in an attempt to relieve epileptic seizures **(Ramón and Cajal, 1894).** The unexpected outcome of the surgery was severe anterograde and partial retrograde amnesia. The patient was unable to form new episodic memories after his surgery and could

not remember any events that occurred just before his surgery, but he did preserve memories of events that occurred many years earlier extending back into his childhood. After that this patient became the most intensively studied subject in medical history **(Campbell, and Macqueen, 2004).** In the subsequent years, other patients with similar levels of hippocampal damage and amnesia have also been studied, and thousands of experiments have investigated the physiology of activity-driven changes in synaptic connections in the hippocampus. There is now almost universal agreement that the hippocampi play important role in memory; however, the precise nature of this role remains widely debated **(Carey, 2008).**

The third important theory of hippocampal function is it's relation to space. The spatial theory was championed by **Chiu *et al*. (2004)** who discovered neurons in the rat hippocampus that show activity related to the rat's location within the environment. As with the memory theory, there is now almost universal agreement that spatial coding plays an important role in hippocampal function, but the details are widely debated **(Cho *et al*., 2005).**

Role in memory: Psychologists and neuroscientists generally have the same opinion that the hippocampus plays an important role in the formation of new memories about practiced events (episodic or autobiographical memory) **(Carey, 2008)**. **Cantero *et al*. (2003)** consider the hippocampus as part of a larger medial temporal lobe