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## Ammon's horn 2 (CA2) of the hippocampus: A long-known region with a new potential role in neurodegeneration

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**Ammon's horn 2 (CA2) of the hippocampus:**

**A long-known region with a new potential role in neurodegeneration.**

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**Running Title:** CA2 in Lewy body dementias

**Key words:** CA2; hippocampus; Lewy body dementias; α-synuclein; cognition

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**Abstract:**

The hippocampus has a critical role in cognition and human memory and is one of the most studied structures in the brain. Despite over 400 years of research, little is known about the Ammon's horn region Cornu Ammonis 2 (CA2) subfield in comparison to other subfield regions (CA1, CA3 and CA4). Recent findings have shown that CA2 plays a bigger role than previously thought. Here, we review understanding of hippocampus and CA2 ontogenesis, together with basic and clinical findings about the potential role of this region in neurodegenerative disease. The CA2 has widespread anatomical connectivity, unique signalling molecules and intrinsic electrophysiological properties. Experimental studies using in vivo models found that the CA2 region has a role in cognition, especially in social memory and object recognition. In models of epilepsy and hypoxia, the CA2 exhibits higher resilience to cell death and hypoxia in comparison to neighbouring regions, and while hippocampal atrophy remains poorly understood in Parkinson's Disease (PD) and Dementia with Lewy Bodies (LBD), findings from postmortem PD brain demonstrates clear accumulation of α-synuclein pathology in CA2, and the CA2-CA3 region shows relatively more atrophy compared to other hippocampal sub-fields. Taken together, there is a growing body of evidence suggesting that the CA2 can be an ideal hallmark with which to differentiate different neurodegenerative stages of PD. Here, we summarise this recent data and provide new perspectives/ideas for future investigations to unravel the contribution of the CA2 to neurodegenerative diseases.

Wordcount: 239

*Abreviations:* 7 Telsa (7T) ; Alzheimer's Disease (AD); Arginine vasopressin receptor 1B (Avpr1b); amyloid precursor protein (APP);  bone morphogenetic proteins (BMPs);  choline acetyltransferase (ChAT) ; Cornu Ammonis (CA); Dementia with Lewy Bodies (DLB); Lewy Body/bodies (LB); Lewy neurites (LN);   (18F-Fluedoxyglucose) 18F-FDG ; magnetic telsa imaging (MRI); functional magnetic resonance imaging;  (fMRI)  ; Mothers against decapentaplegic homolog 3 (Smad3); Parkinson's Disease (PD); Parkinson's disease dementia (PDD); PD with mild cognitive impairment (PD-MCI); non-amyloid component (NAC); nucleus basalis of Meynert (nbM); Substantia Nigra (SN); ventral tegmental area (VTA)

1    **Introduction**

2    The hippocampus has a critical role in memory processing and cognition across all species. The

3    hippocampus contributes to the ability of an individual to develop new long-term memories and

4    transform short-term memories into long-term memories (Bird and Burgess 2008; Young and others,

5    2015). In humans, there are two specific kinds of memories which are associated with the

6    hippocampus; declarative memory (Cohen and Squire, 1980) and spatial relationships (O'Keefe and

7    Nadel, 1978). Declarative memory is of two types, episodic and semantic. Episodic refers to

8    autobiographical memory while semantic is memory of general facts (Wheeler and Ploran 2009). An

9    additional responsibility of the hippocampus is spatial memory (Cohen and Squire, 1980). Spatial

10   learning and navigation allows an individual to memorise routes and pathways. "Place cells" have a

11   pivotal role in spatial navigation and were initially discovered by O'Keefe and Dostrovsky in 1971.

12   Since these initial findings, not only have "place cells" been identified in various species including

13   bats, mice, rats and humans, but additional cell types involved in spatial memory have been described,

14   including head direction cells, boundary cells and grid cells (O'Keefe and Dostrovsky, 1971;

15   Ulanovsky and Moss, 2007; Moser and others, 2008). Hippocampal neurons are also unique in their

16   plasticity, whereby repetitive stimulations of hippocampal neurons are sufficient to produce a

17   persistent modification of their physiological state. This is referred to as "long-term potentiation" in

18   which strengthening of synaptic connections is important for the formation and retrieval of memories

19   (Eriksson and others, 1998). Thus, the necessity of the hippocampus for normal day-to-day life places

20   it in a unique position within the brain (Young and others, 2015).

21

22   This remarkable structure originates from the medial region of the telencephalon and is situated

23   within the medial temporal lobe (Young and others, 2015). By being part of the limbic system and

24   involved in emotional regulation, these functions of the hippocampus are affected in various

25   neurological and neuropsychiatric disorders. These include temporal lobe epilepsy as initially

26   discovered by Hughlings, Jackson and Colman in 1898 (Taylor and Marsh, 1980), and the famous

27   bilateral hippocampal resection of patient H.M. for epilepsy which elucidated the critical role of the

28   hippocampus in memory (Scoville and Milner, 1957).

1

In addition to neurological and neuropsychiatric disorders, the hippocampus is also vulnerable to ischemia, metabolic, behavioural stresses, schizophrenia and other neurodevelopmental disorders (Bartsch 2012). The hippocampus is also particularly affected in dementing illnesses and other neurodegenerative disorders such as Alzheimer's Disease (AD) where the pathological characteristics of neurofibrillary tangles and amyloid plaques are predominantly found in the entorhinal cortex and hippocampus (Serrano-Ponzo and others, 2011). The resulting synapse degeneration in the hippocampus underlies the progressive cognitive deterioration in AD (Serrano-Pozo and others, 2011).

A particular region within the hippocampus known as CA2 has emerged as a major region associated with neurodegenerative pathology. CA2 is part of the Ammon's horn, or cornu ammonis (CA) and was previously known only to be critical for social memory (defined as an animal's ability to recognise another of the same species) (Hitti and Siegelbaum 2014). This region has often been neglected in research, especially in relation to neurodegeneration and cognitive decline. However, recent findings suggest that the CA2 has a potential role in cognition and therefore that its degeneration is important for cognitive decline (DeVito and others 2009: Piskorowski and others 2016).

Recent studies have reinvestigated and redefined the properties and functions of the CA2 circuitry (Kohara and others 2014; Dudek and others 2016; Caruana and others, 2012). Furthermore, a number of research groups have begun investigating CA2-specific pathology in neurodegenerative diseases. The protein α-synuclein has a fundamental role in neurodegeneration (Clinton and others, 2010). Accumulation of α-synuclein in the hippocampus also characterises mouse model of PD such as those over-expressing mutant human α-synuclein (Flores-Cuadrado and others, 2016). Of interest, α-synuclein pathology is found abundantly in CA2 in comparison to other hippocampal regions in Parkinson's disease (PD) postmortem brain (Flores-Cuadrado and others, 2016).

1    Here, we review the anatomy, ontogenesis and functional roles of CA2, and discuss the evidence for a

2    role of CA2 in cognitive dysfunction in neurodegenerative diseases.

3

4

5    **Anatomy of the hippocampus**

6    The terminology of the hippocampus and *hippocampus formation* are often use interchangeably and

7    can be somewhat ambiguous. When referring to the *hippocampus, it is* the seahorse structure found in

8    the medial portion of the anterior temporal lobe, whereas the *hippocampal formation* is comprised of

9    a group of cortical regions in the temporal lobe including the dentate gyrus, the hippocampus itself

10   (including CA subdivisions), subiculum, presubiculum, parasubiculum and the entorhinal cortex

11   (Crossman and Neary, 2010) (Fig 1). Each of the individual structures in the *formation* has intrinsic

12   interconnections with other brain regions and between different cell types. However, despite the

13   differences in their development and interactions, the general structure of the *hippocampus* and

14   *hippocampal formation* remains largely conserved amongst species (Lavenex 2012).

15

16                                             **Figure 1**

17

18   Within the entity of the hippocampus formation is the hippocampus proper, which is a collective term

19   for the different CA subdivisions commonly known as: CA4, CA3, CA2 and CA1 (Lorente de No,

20   1992). The main cell layer is the pyramidal cell layer and this is most densely packed in CA1 and less

21   so in the CA2 and CA3 regions. Pyramidal cells in CA3 can be variable in size, whereas pyramidal

22   cells in CA1 tend to be smaller and more uniform in comparison to those in the CA3. The different

23   aspects of pyramidal neurons endow the CA subfields with a heterogeneous structure.

24

25   The hippocampus proper contains several layers. From the deepest (ventricular cavity) to the surface

26   (vestigial hippocampal sulcus) they are: *the alveus, stratum oriens, stratum pyramidale, stratum*

27   *radiatum, stratum lacunosum* and *stratum moleculare (*Crossman and Neary, 2010*)* (Fig. 2).

28

1 **Figure 2**

2

3 The *statrum oriens* sits above the pyramidal cell layer (*stratum pyramidale)* and is home to basal

4 dendrites of the pyramidal cells together with multiple types of interneurons (Fröhlich, 2016). It is

5 also the primary site of input from CA2 neurons. A thin layer adjacent to the pyramidal cell layer

6 where mossy fibres from the dentate gyrus, typically within CA3 and CA2, are located is known as

7 the *stratum lucidum*. Inferior to the pyramidal cell layer and *stratum lucidum* is the *stratum radiatum*.

8 This is where the recurrent (association) connections within CA3 and the connections between CA3

9 and CA1 (Schaffer collaterals) are located. Directly underneath the *stratum radiatum* is the *stratum*

10 *lacunosum-moleculare*, which receives input from the entorhinal cortex. A variety of interneurons can

11 be found in both *stratum radiatum* and *stratum lacunosum-moleculare (*Fröhlich, 2016).

12

13 **CA2**

14 CA2 can be identified by the ovoid, large and densely packed soma, giving a dense and narrow layer

15 of *stratum pyramidale (*Mercer and others, 2007). The soma of neurons in CA2 are typically larger

16 than those observed in CA3 or CA1 (Mercer and others, 2007), suggesting that the CA2 is unlike the

17 other CA regions.

18

19 Although past studies from Lorente de No (1992) concluded that the CA2 lacks innervation from the

20 dentate gyrus, recent optogenetic studies have revealed that cells from the dentate gyrus send

21 functional monosynaptic inputs to CA2 pyramidal cells through abundant longitudinal projections

22 (Kohara and others, 2014). New findings also indicate that unlike CA3 where cells project to the

23 superficial sublayer only, CA2 cells project into the deep regions in comparison to superficial

24 sublayer of CA1.   Interestingly, new findings also reveal that entorhinal cortex layer III neurons do

25 not project to CA2 as initially thought (Kohara and others,, 2014).

26

27 Using *in-situ* hybridisation techniques, Lein and others (2005) revealed that the CA2 area is

28 substantially wider (~ 300 μm) than previously thought (~ 100 μm). Additional molecular markers

1   such as Regulator of G protein signaling 14 protein (RGS14) and striatum-enriched protein-tyrosine

2   phosphatase (STEP) all overlap with the traditional CA2 molecular marker Purkinje cell protein 4

3   (PCP4) completely (Kohara and others, 2014; Lee and others, 2010: Lein and others, 2005: Shinohara

4   and others 2012: Dudek and others 2016). Use of this molecular signature confirmed that the labelled

5   cells satisfy several classical criteria for CA2 neurons, such as the absence of complex spines but

6   presence of strong supramammillary nucleus (SuM) afferents with relatively large soma size (Dudek

7   and others, 2016). Interestingly, the putative CA2 pyramidal cells identified using these multiple

8   molecular markers were also distinguishable from CA1 and CA3 pyramidal cells by specific intrinsic

9   electrophysiological properties (Dudek and others, 2016). These novel findings which newly defined

10  the CA2 region include identification of a direct innervation of CA2 by mossy fibres and a greater

11  width of this region along the proximo-distal axis in the CA arc. Finally, the connection between the

12  dentate gyrus and CA2 was found to be stronger than other connections such as the cortico-

13  hippocampal network including entorhinal cortex layer III to CA1, young granule cell projections to

14  CA3, and even the CA3 to CA2 connection involving more distal parts of the apical dendrite) (Toni

15  and others 2008).

16

17  A review published by Caruana and others (2012) also demonstrated that CA2 contains an array of

18  novel yet potentially critical signalling molecules that regulate synaptic functions. Although principal

19  cells in CA2 show resemblance to those in the neighbouring regions CA1 and CA3, CA2 pyramidal

20  neurons are endowed with unique molecular, physiological and genetic characteristics (Caruana and

21  others, 2012). When compared to neurons in other hippocampal regions, CA2 pyramidal neurons also

22  exhibit different morphological characteristics, biophysical and synaptic properties, and intrinsic and

23  extrinsic connections (Kohara and others, 2014). Based on these unique properties, the CA2 is an

24  ideal region for assessing regional differences in molecular signals that modulate synaptic plasticity,

25  particularly since the unique profiling available for CA2 allows us to specifically understand the

26  contributions of this hippocampal subfield.

27

1    In order to better understand the structure, it is crucial to know the developmental process of the

2    hippocampus.

3

4
5    **The beginning: Embryogenesis of the hippocampus**
6

7    The hippocampus develops on the dorsal side of the telencephalon, the most anterior subdivision of

8    the neural tube of the vertebrate embryo. Early in embryogenesis, the telencephalon is polarised by

9    signals from surrounding tissues into the dorsal *pallium*, which gives rise to the cerebral cortex

10   including the hippocampal formation and the ventral *subpallium* (Dale and others 1997; Pera and

11   others 1997; Gunhaga and others 2003).

12

13   Regionalisation within the pallium—as in other parts of the neural tube—is regulated by small groups

14   of cells (often called *organisers*) that induce cell fate changes in their vicinity through the release of

15   signalling factors (Wilson and Houart, 2004; Kiecker and Lumsden, 2012). The medial border of the

16   pallium, also called the *cortical hem*, is one such signalling centre that secretes bone morphogenetic

17   proteins (BMPs) and Wnts (Fig. 3) (Furuta and others 1997; Grove and others 1998; Shimogori and

18   others 2004). The hippocampus emerges immediately adjacent to the cortical hem, suggesting that the

19   first could be induced by signals from the latter. Mutant strains of mice that fail to form a hem

20   invariably also lack the hippocampus (Grove and others 1998; Theil and others 1998; Monuki and

21   others 2001; Fernandes and others 2007). Mice carrying mutations in *Wnt3a* (one of the *Wnt* genes

22   expressed in the hem), *Lef1* (encoding an intracellular transducer of Wnt signals) or *Lrp6* (encoding a

23   Wnt co-receptor) display severe hippocampal deficiencies (Galceran and others 2000; Lee and others

24   2000; Zhou and others 2004). In contrast, BMP signalling does not appear to play a direct role in

25   hippocampus specification (Hebert and others 2002). Taken together, these studies demonstrate that

26   signals from the cortical hem are required to induce the hippocampus, and that Wnt signalling is the

27   principal signal in this process.

28

1    Whereas the mechanisms of hippocampus induction are relatively well understood, less is known

2    about how it is patterned mediolaterally into the dentate gyrus, CA3, CA2, CA1 and subiculum (Fig.

3    3). Could a cortical hem-derived mediolateral WNT gradient specify these different fields directly in a

4    dose-dependent fashion? Indeed, persistent WNT signalling specifies more medial fates (dentate

5    gyrus) (Machon and others 2007), and a study using explant culture demonstrated that hippocampal

6    fields are specified surprisingly early in development, consistent with field patterning occurring

7    alongside hippocampus induction (Tole and Grove 2001).

8

9                       **Figure 3**

10

11    Hippocampal precursors next begin the process of neurogenesis, and many genes that regulate this

12    process in other parts of the embryonic CNS are also active here. The formation of pyramidal neurons

13    in CA1-3 is mechanistically very similar to neurogenesis in the neocortex. Research on hippocampal

14    neurogenesis has almost exclusively focused on dentate granule cells, because these cells may

15    continue to be generated throughout life (Boldrini and others 2018; Sorrells and others, 2018).

16    Although there does not seem to be an individual gene that directs CA2 formation, it appears that this

17    domain is specified by a specific level of a WNT signalling that translates into a unique pattern of

18    gene expression. Differential expression of LIM-homeodomain transcription factors may be involved

19    in this 'barcoding' downstream of WNTs (Lakhina and others, 2013). Our growing knowledge of the

20    mechanisms that regulate hippocampal development is now forming a sound basis for the directed

21    derivation of hippocampal neurons from stem cells *in vitro* (Sakaguchi and others, 2015). A more

22    detailed understanding of the transcriptional signature of CA2 will be required to develop targeted

23    replacement therapies for the neurodegenerative disorders discussed in this review.

24

25    **Experimental studies on laboratory animals and humans**

26    Arginine vasopressin receptor 1B (Avpr1b) mRNA is largely expressed in CA2 pyramidal neurons

27    (Young and others, 2006). Studies using knockout Avpr1b mice have shown that there is a clear

28    deficit in social memory meaning animals are incapable of recognizing others of the same species, but

1    no deficits have been observed in sensorimotor or spatial memory. This was further confirmed using

2    the "social novelty test" (Hitti and Siegelbaum, 2014).

3

4    Apart from social memory impairment, the study showed that Avpr1b KO mice also had impairments

5    when remembering the temporal order of objects. This was further investigated using the "what-

6    where-when" task (Dere and others 2005). Wild-type mice retained the ability to distinguish between

7    objects they had previously explored and remembered the location of where they had first met the

8    object, whereas the mutant mice failed to remember the temporal order in which objects were

9    presented to them (Dere and others 2005). Given the selectivity of Avpr1b expression in the CA2,

10   these results strongly implicate CA2 in social memory, and memory for temporal order (DeVito and

11   others 2009).

12

13   Interestingly, deficits in these types of memory have also been described in the early stages of disease

14   development in PD patients. For example, studies have reported that PD patients show reduced

15   abilities in social cognition such as social perspective, talking and decision-making (Palmeri and

16   others 2017). In addition to social cognition, PD patients also have impaired temporal ordering.

17   Temporal ordering can be thought of as sequencing of a series of events (Brown and Smith-Petersen

18   2014). Sagar and others (1988) showed that people with PD have an inability to date events even

19   though they maintain the ability to remember that the events took place. Specifically, PD patients

20   showed impairment in their capacity to date past public events yet they maintained the ability to

21   recognise those events (Sagar and others 1988). Other elements of these tests included questions such

22   as "which of these words have you seen on this test" and "which of these words did you see more

23   recently". PD patients found it difficult to answer the latter question and so the investigators

24   concluded that PD patients had deficits in verbal recency discrimination (Sagar and others 1988).

25   Another study compared "temporal order judgement" in normal healthy elderly people, PD elderly

26   and young healthy individuals. The results showed that both the PD and healthy elderly groups

27   performed worse in temporal object ordering, with the PD group also showing deficits when

28   compared to healthy elderly participants (Sagar and others 1988).

Unlike animal behaviour tests, detecting social cognitive deficits is much more complicated in humans. Social cognition in humans is thought to be represented by behavioural constructs comprising how people process information, store and apply information about other people and social interaction. In 2007, Kawamura and Koyama studied social cognition in PD and control subjects. Based on the "Faux Pas recognition task" that was originally designed by Stone and others in 1998, social cognition can be divided into three sectors; mind reading, perception of facial expression and decision making. Therefore, to investigate if social cognition was impaired in PD patients, Kawamura and Koyama 2007, recruited PD and control subjects to take part in a series of tests. To detect defects in mind reading, both groups were presented with a series of short stories with dialogue between two characters, and they were asked to identify the "awkward phrase" or words that should not have been said. Their results showed that PD patients were able to detect inappropriate remarks just as well as control subjects. However, PD patients were not able to understand why the particular remark was inappropriate or should not have been said. Although the authors concluded that the amygdala was the primary region responsible for this defect, other brain regions were not explored.

The facial recognition test involved asking participants to identify different facial expressions including happiness, surprise, anger, fear, disgust and sadness. Surprisingly, PD patients only showed deficits in recognising fear and disgust (Kawamura and Koyama 2007).

The last component of these social cognition tests was "decision making". For this, the Iowa gambling task was employed and participants were required to pick cards in order to maximise their financial profit. Compared to control participants, PD patients made significantly less profit (Kawamura and Koyama 2007, Palmeri and others 2017).

Taken together, these results indicate that PD patients and animal models of PD demonstrate a clear social cognitive deficit. However, whether the CA2 is the main culprit for the cognitive deficits

<mark>involving social and temporal ordering remains unknown. This is an area worthy of further investigation since CA2 was not speculated to be a region of interest in these previous studies.</mark>

In other laboratory studies modelling temporal lobe epilepsy (Norwood and others 2010), hypoxia and traumatic brain injury (Maxwell and others 2003), it was shown that CA2 pyramidal neurons are highly resistant to cell death . Furthermore, a study investigating the effects amongst different CA regions in hippocampal sclerosis found that CA2 demonstrated less neuronal loss when compared to all other CA regions (Steve and others 2014). One possible explanation for this decreased susceptibility to toxic insults is proposed as being the superior calcium-handling capacity of neurons in the CA2, especially in comparison to neurons other CA regions (Simons and others 2009).The relative resistance of the CA2 to insult was further confirmed more recently, with an experiment involving preformed α-synuclein fibrils being infused directly into CA2-CA3 subfields of mice (Nouraei and others 2018). Despite the abundant α-synuclein pathology that resulted within these two subregions, the fibrils were insufficient to elicit any significant cell loss or memory loss as tested via the novel object recognition task. Furthermore, there was also lack of changes in synaptic markers. However, it should be noted that the investigators only investigated two synaptic markers. Also, the model was mimicking early stage of PD which might suggest that the CA2 region is more resistant to damage at early stages of disease. Only towards the end-stage of disease are significant levels of α-synuclein pathology detected.

These data suggest an important involvement of the CA2 in some forms of memory, and also highlight its unique lack of vulnerability to damage, particularly in early stages of disease. These properties make the CA2 an important area to consider in the context of neurodegenerative diseases.

**CA2 in neurodegeneration**

1    PD is a progressive neurodegenerative disorder characterised by incapacitating motor, autonomic and

2    cognitive symptoms (Poewe 2008; Mackey and others 2013). It is the second most common form of

3    neurodegenerative disorder after AD (Hague and others 2005).

4

5    Clinically, PD is characterized by three cardinal motor manifestations; rigidity, resting tremor and

6    bradykinesia. Although PD was traditionally considered to cause motor symptoms only, it is now

7    clear that PD stretches far beyond the multiple motor domains (Irwin and others 2013; Peng and

8    others 2018). Cognitive dysfunction primarily affects the executive functions domain and additional

9    autonomic deficits include insomnia, constipation and urinary symptoms. Clinically, those with pure

10   motor deficits yet normal cognitive functions are classed as having PD, while those suffering from

11   motor dysfunction and dementia are identified as Parkinson's disease dementia (PDD) or PD with

12   mild cognitive impairment (PD-MCI). Eighty percent of those with PD are likely to develop PDD

13   (Hely and others 2008).

14

15   Pathologically, the defining features of PD includes depigmentation of the substantia nigra and the

16   accumulation of α-synuclein aggregates in the form of Lewy bodies (LB) and Lewy neurites (LN) in

17   neurons. PD is the most common form of synucleinopathy, while Dementia with Lewy Bodies (DLB),

18   which also shares common pathology with Alzheimer's disease (McKeith and others, 2005), is the

19   second most common (Irwin and others 2013; Peng and others 2018, Molano 2013).

20

21   The clinical and cognitive features of PDD and DLB are often difficult to distinguish. While PD is

22   characterized primarily by motor deficits, DLB is characterized by the predominance of dementia.

23   Although DLB patients show similar cognitive impairments to those suffering from PDD, only 25%

24   of DLB patients exhibit parkinsonian symptoms at the initial stages of disease and another 25% of

25   patients never develop parkinsonian symptoms (Kim and others 2014).

26

27   Currently, the diagnostic criteria for PDD and DLB are ambiguous. Those who develop dementia one

28   year after an initial diagnosis of PD are generally re-diagnosed as PDD, while patients who develop

1  dementia prior to or within one year after the onset of motor symptoms are diagnosed with DLB. To

2  reflect the pathological and clinical convergence of these two disorders, the DLB/PDD Working

3  Group proposed the umbrella term "Lewy body disorder" to encompass both conditions (Lippa and

4  others 2007).

5

6  **α-Synuclein in neurodegeneration**

7  α-Synuclein is a small acidic protein of -14kDa, comprised of 140 amino acids (Maroteaux and

8  Scheller 1991; Graham and Sidhu 2010). The normal role of α-synuclein in physiological conditions

9  remains a mystery but α-synuclein is predominantly expressed by neurons and is mostly localized in

10  the presynaptic density (Recasens and Dehay 2014; Maroteaux and Scheller 1991).

11

12  The N-terminus of α-synuclein consists of seven highly conserved hexameric motifs and are thought

13  to form amphipathic alpha-helix structures upon interaction with membranes (Lautenschläger and

14  others, 2018). The middle region is known as the non-amyloid component (NAC) domain and is

15  believed to play a key role in mediating α-synuclein cytotoxicity and aggregation (Giasson and

16  others 2002; Luk and others 2012). Finally, the acidic C-terminal tail is extended and contains

17  multiple phosphorylation sites (Bridi and Hirth, 2018).

18

19  α-Synuclein monomers are present in an equilibrium between the unfolded cytosolic form and

20  membrane bound α-helical form (Bertoncini and others 2005; Pineda and Burre 2017). Increasing

21  evidence shows that the native form of α-synuclein is able to form folded helical tetramers (Bartels

22  and others 2011; Peng and others 2018). In some pathological conditions such as in the presence of

23  mutations in the SNCA gene, post-translational modifications or oxidative stress (Recasens and

24  Dehay 2014), α-synuclein adopts an oligomeric and/or fibrillar conformation. Toxicity of the

25  pathological species is thought to be induced through various mechanisms; a) Impairment of protein

26  degradation mechanisms, hence interfering with normal physiology of the cell leading to cell injury

27  and cell death, b) Impairment of mitochondrial dynamics and mitophagy, c) Disruption of the normal

1    function of α-synuclein in neurotransmitter release, where it may act as a potential negative regulator

2    of dopamine release (Martinez-Vicente and Vila 2013; Cooper and others 2006; Martin and others

3    2006; Chinta and others 2010; Recasens and Dehay, 2014).

4

5    Around 24-36% of newly diagnosed PD patients exhibit some form of cognitive dysfunction. In 2010,

6    Clinton and others concluded that the accumulation of α-synuclein alone was sufficient to disrupt

7    cognition. Furthermoe, Lewy pathology has also been shown to correlate with moderate to severe

8    dementia (Churchyard and Lees 1997; Kalaitzakis and others 2009). LB and LN which contain

9    abnormal α-synuclein filaments and aggregated α-synuclein have long been identified in CA2, and it

10    has also been shown that memory impairment in Lewy body syndromes correlate with the extent of

11    α-synucleinopathy in CA2/CA3 (Adamowicz and others 2017; Kalaitzakis and others 2009; Nouraei

12    and others 2018). Furthermore, the CA2 region contains abundant Lewy pathology in both PD and

13    DLB (Churchyard and Lees 1997; Flores-Cuadrado and others 2016; Dickson and others 1991). Thus,

14    although the specific functional consequences of Lewy pathology in CA2 has not been established,

15    there is a strong association between aggregated α-synuclein in CA2 and dementia. However, whether

16    pathological forms of α–synuclein in this region are able to independently induce cognitive deficits

17    remains unknown.

18

19

20    **The hippocampus in PD and DLB**

21    Involvement of the hippocampus in PD and DLB has been investigated since 1991 (Dickson and

22    others 1991; Churchyard and Lees 1997). The severity of cognitive dysfunction in these conditions is

23    associated with the extent of LN and LB deposition in the hippocampus. Analysis of postmortem PD

24    and PDD brain shows significant associations between CA2 α-synuclein pathology, neuritic tau

25    burden and dementia (Churchyard and Lees, 1997; Kalaitzakis and others 2009).

26

27    Further reports of alterations in postmortem PD brain describe an unusual accumulation of α-

1 synuclein pathology within the CA2 subfield (Flores-Cuadrado and others 2016). Such an extent of

2 pathology has not been observed in other hippocampal subfields nor has any other neurodegenerative

3 disorders documented such findings. In other work, the distribution of α-synuclein was analysed at

4 different disease stages in mice expressing mutant human (A53T) α-synuclein transgenic mice in

5 comparison to postmortem PD brain at neuropathological stages III, IV, and V (Flores-Cuadrado and

6 others 2016). Expression of α-synuclein was found to be abundant in polymorphic layers of the

7 dentate gyrus, CA2 and CA3 in human postmortem brains. At stage III, α-synuclein pathology was

8 sparse throughout the majority of hippocampal regions, with only CA2 showing noticeable labelling.

9 At Stages IV and V, α-synuclein pathology was denser in all hippocampal regions, with CA2 showing

10 the highest burden of pathology. In summary, α-synuclein aggregates are primarily observed in CA2-

11 CA3 regions while the CA1 and dentate gyrus region were spared. In A53T mice, α-synuclein

12 pathology increased from weeks 16 to 43, followed by a decrease from week 43 to 56. The decrease

13 in pathology could be due to induction of autophagy to clear the synuclein aggregates or perhaps as a

14 result of neuronal loss in late disease stages. Although the findings of α-synuclein in CA2 are

15 primarily from PD patients, it would be of interest for future studies to investigate these changes in

16 PDD and DLB postmortem brain with a specific focus on the CA2 region.

17

18 In PDD brain, Lewy pathology in the hippocampal region and cholinergic dysfunction is commonly

19 observed (Hall and others, 2014). The cholinergic neuronal loss is severe in PDD patients with a 54%

20 reduction in cholinergic neurons within the Ch4 subregion of nucleus basalis of Meynert (nbM) and a

21 reduction in choline acetyltransferase (ChAT) activity in the hippocampus (Hall and others 2014). A

22 report by Dugger and Dickson (2010) also found that abnormal accumulation of α-synuclein within

23 the basal forebrain can sequester ChAT from the cytoplasm and affect neurotransmission (Dugger and

24 Dickson 2010). This strongly implies that neuronal dysfunction is a consequence of altered α-

25 synuclein proteins (Giasson and others 2002; Maingay and others 2006). Finally, LN pathology is

26 reported to be more severe in PDD brain in comparison to that from PD patients without dementia

27 (Hall and others 2014). Although LN pathology was found within and outside the CA2 region in PDD

1  cases, pathology was restricted exclusively to CA2 region in pure PD patients. The rationale for

2  (partially) restricted pathology within CA2 in these diseases remains unclear (Hall and others 2014).

3

4  Neuroinflammation is a prominent feature of many neurodegenerative diseases (McGeer and McGeer

5  2004; Qian, and others 2010; McManus and Heneka 2017). Doorn and others (2014) found that

6  hippocampal proliferation in pre-symptomatic PD is due to microglia. Using MCM-2 as a marker for

7  proliferation and Iba-1to detect microglia, they found that there was over 90% of co-localisation

8  between proliferating MCM-2 positive cells and Iba1, meaning almost all proliferating cells in the

9  hippocampus were microglia. Further analysis revealed that incidental Lewy body disorder (iLBD)

10  displayed the highest content of MCM-2 and Iba-1 positive cells, while surprisingly there was no

11  significant difference between iLBD and PD cases, although both groups showed higher counts of

12  MCM-2 and Iba-1 positive cells when compared to the control group. Delving further into the

13  hippocampal subregions, there was found to be a significant increase in proliferating microglia in the

14  CA3 and CA4, and to a lesser extent in the CA2 subregion. Since iLBD is considered as the

15  presymptomatic state of PD, this is further suggestive that CA2 not only shows a different profile of

16  susceptibility to microglia activity and proliferation in comparison to other subregions, but it may also

17  be useful  to differentiate the different stages of PD (Doorn and others 2014).

18

19  In DLB, immunoreactivity of the microglial markers Iba1 and CD68 were found to be low in the

20  hippocampus (Bachstetter and others 2015), but an increase of dystrophic microglia was detected. The

21  altered characteristics of microglial activation between PDD and DLB could indicate differential

22  involvement of neuroinflammatory responses in these diseases, which are perhaps important for the

23  pathogenesis of diseases. Of course, it is important to note that neuroinflammatory responses likely

24  change with disease progression (McManus and Heneka, 2017) and further analysis of microglial and

25  inflammatory components at different disease stages need to be considered before any firm

26  conclusions can be drawn from this work.

27

28  **Clinical imaging of the hippocampus in neurodegenerative diseases.**

1   McKiernan and O'Brien (2017) recently reviewed advances in MRI. 7T MRI is able to provide a

2   higher resolution of hippocampal substructures than previous MRI modalities, being capable of

3   capturing in high resolution, neurodegenerative pathologies such as amyloid plaques and changes in

4   the structure of cortical layers (McKiernan and O'Brien 2017). Unlike previous MRI scans, 7T also

5   has the benefit of delineating specific regions including hippocampal subfields *in vivo*, meaning it can

6   be used as an aid to determine anatomical biomarkers, for monitoring disease progression and the

7   efficacy of new therapies.

8

9   However, despite the high resolution of images provided by advanced MRI, delineating the CA2

10  region remains challenging. By manually drawing regions of interest, Firbank and others (2010)

11  investigated the atrophy of hippocampal subregion volume in control and DLB patients. They

12  hypothesized that CA1 and subiculum would have more atrophy in AD, while CA2 and CA3/4

13  regions would show more significant changes in DLB. Regions of interest were obtained via manual

14  drawing according to Mueller's method of coronal T2 weighted images. Despite general agreement on

15  the regions within the hippocampus, the CA2 region had the highest variability amongst raters. This

16  emphasized the difficulty in distinguishing a *bona fide* CA2 subfield due to the small size and

17  indefinite medial and lateral borders of this structure. Nevertheless, out of the 32 subjects studied,

18  DLB patients showed less atrophy of CA1 and subiculum of the hippocampus in comparison to AD

19  cases, while there were no differences observed within CA2 and CA3/CA4 between DLB and AD

20  cases.

21

22  Similar to the findings by Firbank and others (2010), other groups also found that the extent of

23  hippocampal atrophy in DLB is less severe than in AD (Mak and others 2017; Elder and others 2017).

24  Atrophy in AD was more severe in the posterior hippocampus, while in DLB cases the atrophy tended

25  to be more anterior and severe in CA2-CA3 (Barber and others 1999; Tam and others 2005; Firbank

26  and others 2010). The anterior part of the hippocampus is considered an important region for

27  perception, imagination and episodic memory (linking different scenes for spatial memory). Therefore,

28  increased atrophy of anterior part of hippocampus in DLB patients may account for the loss of visual

1  attention typically observed in this condition (Zeidman and Maguire 2016). However, when Elder and

2  others (2017) investigated the hippocampal volume, parahippocampal, entorhinal and temporal pole

3  cortical thickness in control, AD and DLB subjects using 3T MRI, the results showed that there were

4  no significant differences in atrophy and cortical thinning between AD and DLB patients. Only the

5  temporal pole thickness was reduced in DLB patients in comparison to AD and controls (Elder and

6  others 2017).

7

8  The above findings demonstrate that hippocampal subfields and extra-hippocampal structures are

9  affected differently in AD and DLB (Delli Pizzi and others 2016). The preservation of the

10  hippocampus in DLB patients may be a compensatory mechanism. Alternatively, the preserved

11  regions may have some intrinsic resilience to damage.

12

13  **Imaging in PD/PDD**

14  Numerous functional magnetic resonance imaging (fMRI) studies show hippocampal atrophy in both

15  PD and PDD patients. Atrophy is generally greater and more severe amongst PDD patients (Laakso

16  and others 1996) which likely highlights an association between hippocampal atrophy and memory

17  impairment in PD and PDD patients (Riekkinen and others 1998). Hippocampal atrophy can be

18  observed in both left and right hippocampi in PD, but the atrophy score was more severe in the right

19  hippocampus in comparison to the left for the advanced PD groups (Bruck and others 2004). In

20  summary, both advanced and non-advanced PD groups showed significantly more atrophy in both

21  hippocampi when compared to control group.

22

23  A recent study involving 65 PD subjects investigated the relationship between the association of

24  hippocampal subfields and the progression of cognitive decline in PD patients. The study found that

25  when comparing subjects classed as PD-stable (patients who remained cognitively stable within the

26  18 study months) and PD-converters (patients that developed mild cognitive impairment during the

27  course of the study), only PD-converters showed greater atrophy in the right CA2-3. This was coupled

28  with worsening of episodic memory. There were also greater pathological alterations in the CA2-3

1    region in the PD-converter group in contrast to the PD-stable group. These findings suggest that the

2    CA2 hippocampal subfield is an important hallmark for different neurodegenerative stages of PD

3    (Foo and others 2016).

4

5    Following this study, Novellino and others (2018) examined the relationship between hippocampal

6    subfields and category cued recall in PDD and AD patients. Using 3T-MRI, the study found that AD

7    patients showed a reduction in the majority of hippocampal subregions and mean diffusivity (MD)

8    was increased in the affected regions, highlighting a positive correlation between affected regions and

9    MD. In contrast, PDD patients showed less volume loss in all hippocampal subfields with the

10   exception of CA2-CA3 and presubiculum regions, where there was evident volume loss. Taken

11   together, these data indicate that hippocampal subregions show different vulnerability to damage,

12   hence making this structure a possible hallmark for distinguishing different neurodegenerative stages

13   of PD and between PD and PDD. Furthermore, these data raise a question of whether there is a

14   relationship between α-synuclein pathology in CA2 and the specific hippocampal volume loss in

15   PD/PDD patient. This finding may reflect the loss of synaptic function in response to the

16   accumulation of LB, α-synuclein and tau that precedes overt neuronal loss.

17

18   **Concluding remarks**

19   This review has provided an up-to-date review of the CA2 subfield starting from the

20   neurodevelopmental origins of this structure, its ontogenesis, to the most recent discoveries in clinical

21   and basic science fields. Unlike other hippocampal subfields, CA2 contains a subset of specific

22   receptors, unique molecular, physiological and genetic characteristics making this an exciting region

23   that has thus far been understudied (Toni and others 2008; Caruana and others 2012). In relation to

24   cognition, CA2 has fundamental roles in object recognition, social and temporal memory. Various

25   animal studies have noted the remarkable resilience of this structure to damage and the molecular

26   mechanisms underlying this intrinsic protection is worthy of further investigation.

27

1    Imaging reports have shed light on the importance of CA2 in cognition in different neurodegenerative

2    diseases. CA2 shows changes in PD/PDD and DLB patients more so than AD patients. However,

3    despite the extensive studies on the hippocampus and neurodegeneration, there are still discrepancies

4    amongst researchers and the overall findings remain inconclusive. This is partly due to the difficulty

5    in elucidating the CA2 subfield clearly in patients despite the use of advanced imaging methods.

6

7    Finally, a new perspective for CA2 is its potential role in neurodegeneration. α-synuclein pathology is

8    a well-known contributing factor in neurodegenerative disorders. The reports summarised above

9    provide accumulating evidence that α-synuclein pathology is abundant in CA2 of postmortem PD and

10   DLB brains and suggest that there could be a link between CA2, α-synuclein and cognitive deficits as

11   observed in PD/PDD and DLB patients. Furthermore, these data raise the possibility that pathology in

12   CA2 could distinguish PD/PDD from DLB patients. Alternatively, it is possible that α-synuclein

13   pathology in CA2 in PD could be the starting point for developing cognitive decline and ultimately

14   giving rise to PDD.  Whatever the answers to these questions, it is clear that the CA2 is not only an

15   more complex and important than previously appreciated, but it is a fascinating entity with more

16   findings waiting to be discovered.

17

18   **The future**

19   It is clear that findings about the role and purpose of CA2 are just starting to emerge. While there are

20   already studies reinvestigating the anatomical structure and molecular properties of CA2, the CA2 has

21   much more to offer. Researchers should aim to explore the potential role of CA2 in PD/PDD and

22   DLB cases. In particular, it will be of great interest to identify the relationship between CA2, α-

23   synuclein and cognitive dysfunction. Laboratory experiments should use animal models to mimic

24   these neurodegenerative diseases, and we propose that there should be specific investigation of the

25   CA2 subfield in these models. One possibility might be the use of preformed α-synuclein fibrils or

26   using cre-lox method to conditionally overexpress α-synuclein in the CA2, followed by behaviour

27   tests to elucidate the effect of α-synuclein in CA2 and cognition.

1

2 Clinical imaging should focus on imaging larger study groups and the use of 7T should be promoted

3 to clarify previous findings about CA2 amongst PD/PDD and DLB patients. Longitudinal studies

4 crossing different disease stages from MCI stage to severe stage of PD/PDD and DLB would also be

5 useful in monitoring changes in CA2 over time. Finally, we believe that neuropathologists should

6 continue looking into CA2 subfield from other neurodegenerative disorders, to provide a more

7 comprehensive understanding of PD/PDD and DLB cases.

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11 **Acknowledgement:**

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1    **References**

2    Adamowicz DH, Roy S, Salmon DP, Galasko DR, Hansen LA, Masliah E, Gage FH. 2017.

3         Hippocampal alpha–synuclein in dementia with Lewy bodies contributes to memory

4         impairment is consistent with spread of pathology. J Neurosci. 37 (7): 1675-1684.

5    Bachstetter AD, Van Eldik LJ, Schmitt FA, Neltner JH, Ighodaro ET, Webster SJ and others.

6         2015. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease,

7         dementia with Lewy bodies, and hippocampal sclerosis of aging. Acta Neuropathol

8         Commun. 3: 32. (DOI: 10.1186/s40478-015-0209-z)

9    Barber R, Scheltens P, Gholkar A, Ballard C, McKeith I, Ince P and others. 1999. White

10        matter lesions on magnetic resonance imaging in dementia with Lewy bodies,

11        Alzheimer's disease, vascular dementia, and normal aging. J Neurol Neurosur Psychiatry.

12        67(1): 66-72.

13   Bartels T, Choi JG, Selkoe DJ. 2011. α-Synuclein occurs physiologically as a helically

14        folded tetramer that resists aggregation. Nature. 447 (7362): 107-110

15   Bartsch T. 2012. The hippocampus in Neurological disease. In: The Clinical Neurobiology of

16        the Hippocampus: An integrative view. Oxford University Press. p174-292.

17        (DOI:10.1093/acprof:oso/9780199592388.003.0011)

18   Bertoncini CW, Fernandez CO, Griesinger C, Jovin TM, Zwecjstetter M. 2005. Famililial

19        mutants of alpha-synuclein with increased neurotoxicity have destablilized conformation.

20        J Biol Chem. 280 (35): 30649-30652.

21   Bird CM, Burgess N. 2008. The hippocampus and memory: insights from spatial

22        processing. Nat Rev Neurosci. 9 (3): 182-194.

23   Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, Rosoklija GB,

24        Stankov A and others. 2018. Human hippocampal neurogenesis persists throughout

25        aging. Cell Stem Cell. 22 (4): 589-599.

1    Bridi JC, Hirth F. 2018. Mechanisms of α-Synuclein Induced Synaptopathy in Parkinson's

2    Disease. Front Neurosci. 12:80. (Doi: 10.3389/fnins.2018.00080)

3    Brown SW, Smith-Petersen GA. 2014. Time perception and temporal order memory. Acta

4    Psychol. 148: 173-180.

5    Bruck A, Kurki T, Kaasinen V, Vahlberg T, Rinne JO. 2004. Hippocampal and prefrontal

6    atrophy in patients with early non-demented Parkinson's disease is related to cognitive

7    impairment. J Neurol Neurosurg Psychiatry. 75 (10): 1467-1469.

8    Caruana DA, Alexander GM, Dudek SM. 2012. New insights into the regulation of synaptic

9    plasticity from an unexpected place: hippocampal area CA2. Learn Mem 19 (9): 391-400.

10   Chinta SJ, Mallajosyula JK, Rane A, Andersen JK. 2010. Mitochondrial a-synuclein

11   accumulation impairs complex I function in dopaminergic neurons and results in

12   increased mitophagy in vivo. Neurosci Lett. 486 (3): 235-359.

13   Churchyard A, Lees AJ. 1997. The relationship between dementia and direct involvement of

14   the hippocampus and amygdala in Parkinson's disease. Neurology. 49 (6):1570-1576.

15   Clark R, Squire L. 2013. Similarity in form and function of the hippocampus in rodents,

16   monkeys, and humans. Proc Natl Acad Sci USA. 110 (Suppl 2): 10365-10370.

17   Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. 2010. Synergistic

18   interactions between Abeta, tau, alpha-synuclein: acceleration of neuropathology and

19   cognitive decline. J Neurosci. 30 (21): 7281-7289.

20   Cohen NJ, Squire LR. 1980. Preserved learning and retention of a pattern- analyzing skill in

21   amnesia: Dissociation of knowing how and knowing that. Science. 210 (4466): 207–210.

22   Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K,

23   Stratheram KE, Liu F, Cao S, Caldwell KA, Marsischky G, Kolodner RD, Labaer J,

24   Rochet JC, Boini NM, Lindquist S. 2006. Alpha-synuclein blocks ER-Golgi traffic and

25   Rab1 rescues neuron loss in Parkinson's models. Science. 313 (5785): 324-328.

1 Crossman AR, Neary D. 2010. In (Ed: AR Crossman): Neuroanatomy an illustrated colour

2 text. Elsevier/ Churchill Livingstone. Chapter 16, Hypothalamus. p165.

3 Dale JK, Vesque C, Lints TJ, Sampath TK, Furley A, Dodd J, Placzek M. 1997.

4 Cooperation of BMP7 and SHH in the induction of forebrain ventral midline cells

5 by prechordal mesoderm. Cell. 90 (2): 257–269

6 Delli Pizzi S, Franciotti R, Bubbico G, Thomas A, Onofrj M, Bonanni L. 2016. Atrophy of

7 hippocampal subfields and adjacent extrahippocampal structure in dementia with Lewy

8 bodies and Alzheimer's disease. Neurobiol Aging. 40: 103-109.

9 Dere E, Huston JP, De Souza, Silva MA. 2005. Episodic-like memory in mice: simultaneous

10 assessment of object, place and temporal order memory. Brain Res Brain Res Protoc. 16

11 (1-3): 10-19.

12 DeVito LM, Konigsberg R, Lykken C, Sauvage M, Young WS, Eichenbaum H. 2009.

13 Vasopressin 1b receptor knock-out impairs memory for temporal order. J Neurosci. 29

14 (9): 2676-2683.

15 Dickson DW, Ruan D, Crystal H, Mark MH, Davies P, Kress Y, and others. 1991.

16 Hippocampal degeneration differentiates diffuse Lewy body disease from Alzheimer's

17 disease: light and electron microscopic immunocytochemistry of CA2-3 neurites specific

18 to Lewy body dementias. Neurology. 41(9):1402-1409.

19 Doorn KJ, Drukarch B, van Dam AM, Lucassen PJ. 2014. Hippocampal proliferation is

20 increased in presymptomatic Parkinson's disease and due to microglia. Neural Plast.

21 2014: 959154. (Doi : 10.1155/2014/959154)

22 Dudek SM, Alexander GM, Farris S. 2016. Rediscovering area CA2: unique properties and

23 functions. Nat Rev Neurosci. 17 (2): 89-102.

24 Dugger BN, Dickson DW. 2010. Cell type specific sequestration of choline acetyltransferase

25 and tyrosine hydroxylase within Lewy bodies. Acta Neuropathol. 120 (5): 633-639.

1  Elder GJ, Mactier K, Colloby SJ, Watson R, Blamire AM, O'Brien JT and others. 2017. The

2     influence of hippocampal atrophy on the cognitive phenotype of dementia with Lewy

3     bodies. Int J Geriatr Psychiatry. 32 (11): 1182-1189.

4  Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage

5     FH. 1998. Neurogenesis in the adult human hippocampus. Nat Med. 4 (11): 1313-1317.

6  Fernandes M, Gutin G, Alcorn H, McConnell SK, Hébert JM. 2007. Mutations in the BMP

7     pathway in mice supports the existence of two molecular classes of holoprosencephaly.

8     Development. 134 (21): 3789-3794.  (Doi: 10.1242/dev.004325)

9  Firbank MJ, Blamire AM, Teodorczuk A, Teper E, Burton EJ, Mitra D and others. 2010.

10    High resolution imaging of the medial temporal lobe in Alzheimer's disease and

11    dementia with Lewy bodies. J Alzheimers Dis. 21 (4): 1129-1140.

12 Flores-Cuadrado A, Ubeda-Bañon I, Saiz-Sanchez D, de la Rosa-Prieto C, Martinez-Marcos

13    A. 2016. Hippocampal alpha-synuclein and interneurons in Parkinson's disease: Data

14    from human and mouse models. Mov Disord. 31 (7): 979-988.

15 Foo H, Mak E, Chander R, Ng A, Au W, Sitoh Y and others. 2016. Associations of

16    hippocampal subfields in the progression of cognitive decline related to Parkinson's

17    disease. Neuroimage Clin. 14: 37-42.

18 Fröhlich F. 2016. Chapter 8, Microcircuits of the hippocampus. Network Neuroscience. 1st

19    Edition. USA. Academic Press. p97-108.

20 Furuta Y, Piston DW, and Hogan BL. 1997. Bone morphogenetic proteins (BMPs) as

21    regulators of dorsal forebrain development. Development. 124 (11): 2203-2212.

22 Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. 2000.

23    Hippocampus development and generation of dentate gyrus granule cells is regulated by

24    LEF1. Development. 127 (3): 469–482.

1   Giasson BI, Duda JE, Quinn SM, Zhang B, Trojanowski JQ, Lee VM. 2002. Neuronal alpha-
2        synucleinopathy with severe movement disorder in mice expressing A53T human alpha-
3        synuclein. Neuron. 34 (4): 521-533.

4   Graham D, Sidhu A. 2010. Mice expressing the A53T mutant form of human alpha-synuclein
5        exhibit hyperactivity and reduced anxiety-like behavior. J Neurosci Res. 88 (8): 1777-
6        1783.

7   Grove EA, Tole S, Limon J, Yip L, Ragsdale CW. 1998. The hem of the embryonic cerebral
8        cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-
9        deficient mice. Development. 125 (12): 2315-2325.

10  Gunhaga L, Marklund M, Sjodal M, Hsieh JC, Jessell TM, Edlund T. 2003.
11       Specification of dorsal telencephalic character by sequential Wnt and FGF
12       signaling. Nat Neurosci. 6 (7): 701–707.

13  Hague SM, Kladdke S, Bandmann O. 2005. Neurodegenerative disorders: Parkinson's
14       disease and Huntington's disease. J Neurol Neurosurg Psychiatry. 76 (8): 1058-1063

15  Hall H, Reyes S, Landeck N, Bye C, Leanza G, Double K, and others. 2014. Hippocampal
16       Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's
17       disease. Brain. 137 (Pt. 9): 2493-2508.

18  Hebert JM, Mishina Y, McConnell SK. 2002. BMP signaling is required locally to pattern the
19       dorsal telencephalic midline. Neuron. 35 (6): 1029–1041.

20  Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. 2008. The Sydney multicenter
21       study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord. 23
22       (6): 837-844.

23  Hitti FL and Siegelbaum SA. 2014. The hippocampal CA2 region is essential for social
24       memory. Nature. 508 (7494): 88-92.

1 Irwin DJ, Lee VM, Trojanowski JQ. 2013. Parkinson's disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies. Nat Rev Neurosci. 14 (9): 626-636.

Kalaitzakis ME, Pearce RK, Gentleman SM. 2009. Clinical correlates of pathology in the claustrum in Parkinson's disease and dementia with Lewy bodies. Neurosci Lett. 461 (1): 12-15.

Kawamura M, Koyama S. 2007. Social cognitive impairment in Parkinson's disease. J Neurol. 254 (Suppl. 4): IV49-IV53.

Kiecker C, Lumsden A. 2012. The role of organizers in patterning the nervous system. Annu Rev Neurosci. 35: 347-367.

Kim WS, Kågdeal K, Halliday GM. 2014. Alpha-synuclein biology in Lewy body diseases. Alzheimers Res Ther. 6 (5): 73 (Doi: 10.1186/s13195-014-0073-2)

Kohara K, Pignatelli M, Rivest AJ, Jung HY, Kitamura T, Suh J and others. 2014. Cell type-specific genetic and optogenetic tools reveal hippocampal CA2 circuits. Nat Neurosci. 17 (2): 269-279.

Laakso MP, Partanen K, Riekkinen P, Lehtovirta M, Helkala EL, Hallikainen M, and others. 1996. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. Neurology. 46 (3): 678-681.

Lakhina V, Subramanlan L, Hullgol D, Shetty AS, Valdya VA, Tole S. 2013. Seizure evoked regulation of LIM-HD genes and co-factors in the postnatal and adult hippocampus. F1000Res. 2: 205 (Doi: 10.12688/f1000research.2-205.v1).

Lautenschläger J, Stephens AD, Fusco G, Ströhl F, Curry N, Zacharopoulou M, and others. 2018. C-terminal calcium binding of α-synuclein modulates synaptic vesicle interaction. Nat Commun. 9 (1): 712. (Doi: 10.1038/s41467-018-03111-4)

1  Lavenex P. 2012. Functional Anatomy, Development, and Pathology of the Hippocampus.

2  The Clinical Neurobiology of the Hippocampus. In (Batsch Thorsten) Oxford University

3  Press. Chapter 2, (DOI: 10.1093/acprof:oso/9780199592388.003.0002)

4  Lee SM, Simons SB, Heldt SA, Zhao M, Schroeder JP, Vellano CP, Cowan DP, Ramineni S,

5  and others. 2010. RGS14 is a natural suppressor of both synaptic plasticity in CA2

6  neurons and hippocampal-based learning and memory. Proc Natl Acad Sci USA. 107

7  (39): 16994-16998

8  Lee SM, Tole S, Grove E, McMahon AP. 2000. A local Wnt-3a signal is required for

9  development of the mammalian hippocampus. Development. 127 (3): 457–467.

10  Lein ES, Callaway EM, Albright TD, Gage FH. 2005. Redefining the boundaries of the

11  hippocampal CA2 subfield in the mouse using gene expression and 3-dimensional

12  reconstruction. J Comp Neurol. 485 (1): 1-10.

13  Lippa CF, Duda Je, Grossman M, Hurtig HI, Aarsland D, Boeve BF, Brooks DJ, Dickson

14  DW and others. 2007. DLB and PDD boundary issues. Neurology. 68 (11): 812-819.

15  Lorente de No R. 1992. The cerebral cortex of the mouse (a first contribution--the "acoustic"

16  cortex). Somatosens Mot Res. 9 (1): 3-36.

17  Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, and others. 2012.

18  Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in

19  nontransgenic mice. Science. 338 (6109): 949-953.

20  Machon O, Backman M, Machonova O, Kozmik Z, Vacik T, Andersen L. and others.

21  2007. A dynamic gradient of Wnt signaling controls initiation of neurogenesis in the

22  mammalian cortex and cellular specification in the hippocampus. Dev Biol. 311 (1):

23  223–237.

1    Mackey S, Jing Y, Flores J, Dinelle K, Doudet DJ. 2013. Direct intranigral administration of

2    an ubiquitin proteasome system inhibitor in rat: behavior, positron emission tomography,

3    immunohistochemistry. Exp Neurol.  247: 19-24. (DOI: 10.1016/j.expneurol.2013.03.02)

4    Maingay M, Romero-Ramos M, Carta M, Kirik D. 2006. Ventral tegmental area dopamine

5    neurons are resistant to human mutant alpha-synuclein overexpression. Neurobiol Dis.

6    23 (3): 522-532.

7    Mak E, Gabel S, Su L, Williams GB, Arnold R, Passamonti L, and others. 2017. Multi-modal

8    MRI investigation of volumetric and microstructural changes in the hippocampus and its

9    subfields in mild cognitive impairment, Alzheimer's disease, and dementia with Lewy

10   bodies. Int Psychogeriatr. 29 (4): 545-555.

11   Maroteaux L, Scheller RH. 1991. The rat brain synucleins; family of proteins transiently

12   associated with neuronal membrane. Brain Res Mol Brain Res. 11 (3-4): 355-343

13   Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, Price DL, Lee DK. 2006.

14   Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial

15   degeneration and cell death. J Neurosci. 26 (1): 41-50

16   Martinez-Vicente M, Villa M. 2013. Alpha-synuclein and protein degradation pathways in

17   Parkinson's disease: a pathological feed-back loop. Exp Neurol. 247: 308-313.

18   Maxwell WL, Dhillon K, Harper L, Espin J, MacIntosh TK, Smith DH and others. 2003.

19   There is differential loss of pyramidal cells from the human hippocampus with survival

20   after blunt head injury. J Neuropathol Exp Neurol. 62 (3): 272-279.

21   McGeer PL, McGeer EG. 2004. Inflammation and neurodegeneration in Parkinson's disease.

22   Parkinsonism Relat Disord. 10 (Suppl. 1): S3-7.

23   McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, and others. 2005.

24   Diagnosis and management of dementia with Lewy bodies: third report of the dementia

25   with Lewy bodies Consortium. Neurology. 65 (12): 1863-1872.

1    McKiernan EF, O'Brien JT. 2017. 7T MRI for neurodegenerative dementias in vivo: a
2        systematic review of the literature. J Neurol Neurosurg Psychiatry. 88 (7): 564-574 (Doi:
3        10.1136/jnnp-2016-315022).

4    McManus RM and Heneka MT. 2017. Role of neuroinflammation in neurodegeneration: new
5        insights.Alzheimers Res Ther. 9 (1): 14. (DOI: 10.1186/s13195-017-0241-2)

6    Mercer A, Trigg HL, Thomson AM. 2007. Characterization of neurons in the CA2 subfield
7        of the adult rat hippocampus. J Neurosci. 27 (27): 7329-7338.

8    Molano JR. 2013. Dementia with Lewy bodies. Semin Neurol. 33 (4): 330-335.

9    Monuki ES, Porter FD, Walsh CA (2001) Patterning of the dorsal telencephalon and cerebral
10       cortex by a roof plate-Lhx2 pathway. Neuron. 32 (4): 591–604.

11   Moser EI, Kropff E, Moser MB. 2008. Place cells, grid cells, and the brain's spatial
12       representation system. Annu Rev Neurosci. 31: 69-89.

13   Norwood BA, Bumanglag AV, Osculati F, Sbarbati A, Marzola P Nicolato E, Fabene PF,
14       Sloviter R. 2010. Classic hippocampal sclerosis and hippocampal-onset epilepsy
15       produced by a single "cryptic" episode of focal hippocampal excitation in awake rats. J
16       Comp Neurol. 518 (16): 3381-3407.

17   Nouraei N, Mason DM, Miner KM, Carcella MA, Bhaatia TN, Dumm BK, Soni D, Johnson
18       DA, Luk KC, Leak RK. 2018. Critical appraisal of pathology transmission in the a-
19       synuclein fibril model of Lewy body disorders. Exp Neurol. 299 (Pt A): 172-196.

20   Novellino F, Vasta R, Sarica A, Chiriaco C, Salsone M, Morelli M, Arabia G, Sacca V,
21       Nicoletti G, Quattrone A. 2018. Relationship between Hippocampal Subifelds and
22       Category Cured Recall in AD and PDD: A Multimodal MRI study. Neuroscience. 371:
23       506-517.

24   O'Keefe J, Dostrovsky J. 1971. The hippocampus as a spatial map. Preliminary evidence
25       from unit activity in the freely-moving rat. Brain Res. 34 (1): 171-175.

1 O'Keefe J, Nadel L.1978. Chapter 2, Spatial behaviour. (In Eds: J. O'Keefe & L. Nadel) The

2 Hippocampus as a Cognitive Map. New York. Oxford University Press. p62-101

3 Palmermi R, Lo Buono V, Corallo F, Foti M, Di Lorenzo G, Bramanti P, Marino S. 2017.

4 Nonmotor symptoms in Parkinson's disease: A descriptive review on social cogniton

5 ability. J Geriatr Psychiatry Neurol. 30 (2): 109-121.

6 Peng C, Gathagan RJ, Lee VM. 2018. Distinct a-Synuclein strains and implications for

7 heterogeneity among α-synucleinopathies. Neurobiol Dis. 109 (Pt B): 209-218. (DOI:

8 10.1016/j.nbd.2017.07.018)

9 Pera EM, Kessel M. 1997. Patterning of the chick forebrain anlage by the prechordal

10 plate. Development. 124 (20): 4153–4162.

11 Pineda A, Burre J. 2017. Modulating membrane binding of α-synuclein as a therapeutic

12 strategy. Proc Natl Acad Sci USA. 114 (6): 1223-1225.

13 Piskorowski RA, Nasrallah K, Diamanropoulou A, Mukai J, Hassan JM, Siegelbaum SA,

14 Gogos JA, Chevaleyre V. 2016. Age-dependent specific changes in area CA2 of the

15 hippocampus and social memory deficit in a mouse model of the 22q11.2 deletion

16 syndrome. Neuron. 89 (1): 163-176.

17 Poewe W. 2008. Non-motor symptoms in Parkinson's disease. Eur J Neurol. 15 (Suppl. 1):

18 14-20.

19 Qian L, Flood PM, Hong JS. 2010. Neuroinflammation is a key player in Parkinson's disease

20 and a prime target for therapy. J Neural Transm (Vienna). 117 (8): 971-979.

21 Recasens A, Dehay B. 2014. Alpha-synuclein spreading in Parkinson's disease. Front

22 Neuroanat. 8: 159. (DOI: 10.3389/fnana.2014.00159)

23 Riekkinen P Jr, Kejonen K, Laakso MP, Soininen H, Partanen K, Riekkinen M.1998.

24 Hippocampal atrophy is related to impaired memory, but not frontal functions in non-

25 demented Parkinson's disease patients. Neuroreport. 9 (7): 1507-1511.

1  Sagar HJ, Sullivan EV, Gabrieli JD, Corkin S, Growdon JH. 1988. Temporal ordering and

2      short-term memory deficits in Parkinson's disease. Brain. 111 (Pt. 3): 525-539.

3  Sakaguchi H, Kadoshima T, Soen M, Narii N, Ishida Y, Ohushi M, Takahashi J, Eiraku M,

4      Sasai Y. 2015. Generation of functional hippocampal neurons fromm self-organising

5      human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat Commun. 6:

6      8896. (Doi: 10.1038/ncomms9896)

7  Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J

8      Neurol Neurosurg Psychiatry. 20 (1): 11-21.

9  Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. 2011. Neuropathological alterations in

10     Alzheimer disease. Cold Spring Harb Perspect Med. 1(1): a006189. (DOI:

11     10.1101/cshperspect.a006189)

12  Shimogori T, VanSant J, Paik E, Grive EA. 2004. Members of the Wnt, Fz, and Frp gene

13     families expressed in postnatal mouse cerebral cortex. J Comp Neurol. 473 (4): 496-510.

14  Shinohara Y, Hosoya A, Yahagi K, Ferecskó AS, Yaguchi K, Sík A, Itakura M, Takahashi M,

15     Hirase H. 2012. Hippocampal CA3 and CA2 have distinct bilateral innervation patterns

16     to CA1 in rodents. Eur J Neurosci. 35 (5): 702-710.

17  Simons SB, Escobedo Y, Yasuda R, Dudek SM. 2009. Regional differences in hippocampal

18     calcium handling provide a cellular mechanism for limiting plasticity. Proc Natl Acad

19     Sci USA. 106 (33):14080-14084.

20  Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, James D, Mayer S,

21     Chang J, Auguste KI, and others. 2018. Human hippocampal neurogenesis drops sharply

22     in children to undetectable levels in adults. Nature. 555 (7696): 377–381.

23  Steve TA, Jirsch JD, Gross DW. 2014. Quantification of subfield pathology in hippocampal

24     sclerosis: a systematic review and meta-analysis. Epilepsy Res. 108 (8): 1279-1285.

1  Stone VE, Baron-Cohen S, Knight RT. 1998. Frontal lobe contributions to theory of mind. J
2  Cogn Neurosci. 10 (5): 640-656.

3  Tam CW, Burton EJ, McKeith IG, Burn DJ, O'Brien JT. 2005. Temporal lobe atrophy on
4  MRI in Parkinson disease with dementia: a comparison with Alzheimer disease and
5  dementia with Lewy bodies. Neurology. 64 (5): 861-865.

6  Taylor DC, Marsh SM. 1980. Hughlings Jackson's Dr Z: the paradigm of temporal lobe
7  epilepsy revealed. J Neurol. 43 (9): 758-767.

8  Theil T, Frain M, Gilardi-Hebenstreit P, Flenniken A, Charnay P, Wilkinson DG.1998.
9  Segmental expression of the EphA4 (Sek-1) receptor tyrosine kinase in the hindbrain is
10  under direct transcriptional control of Krox-20. Development. 125 (3): 443-452.

11  Tole S, Grove EA. 2001. Detailed field pattern is intrinsic to the embryonic mouse
12  hippocampus early in neurogenesis. J Neurosci. 21 (5): 1580-1589.

13  Toni N, Laplagne DA, Zhao C, Lombardi G, Ribak CE, Gage FH and others. 2008. Neurons
14  born in the adult dentate gyrus form functional synapses with target cells. Nat Neurosci.
15  11 (8): 901-907.

16  Ulanovsky N, Moss C. 2007. Hippocampal cellular and network activity in freely moving
17  echo locating bats. Nat Neurosci. 10 (2): 224-233.

18  Wheeler ME, Ploran EJ. 2009. Episodic Memory. Encyclopedia of Neuroscience. p1167-
19  1172.

20  Wilson SW, Houart C. 2004. Early steps in the development of the forebrain. Dev Cell. 6 (2):
21  167-181.

22  Young AY, Young PH, Tolbert DL. 2015. Chapter 1, Organisation, cellular components and
23  topography of CNS. p1-37. Basic Clinical Neuroscience. 3rd edition. Lippincott Williams
24  and Wilkins.

1    Young WS, Li J, Wersinger SR, Palkovits M. 2006. The vasopressin 1b receptor is prominent

2    in the hippocampal area CA2 where it is unaffected by restraint stress or adrenalectomy.

3    Neuroscience. 143 (4): 1031-1039.

4    Zeidman P, Maguire EA. 2016. Anterior hippocampus: the anatomy of perception,

5    imagination and episodic memory. Nat Rev Neurosci. 17 (3): 173-182.

6    Zhou CJ, Zhao C, Pleasure SJ. 2004. Wnt Signaling mutant have decreased dentate granule

7    cell production and radial glial scaffolding abnormalities. J Neurosci. 24 (1): 121-126.

1    **Figure legends:**

2

3    **Fig. 1  The Hippocampus.** Within the medial temporal lobe are a set of cortical structures

4    which make up the hippocampus. The structures include the Dentate gyrus (orange band),

5    followed by the Cornu Ammonis, which consists of CA4, CA3, CA2 and CA1. Following the

6    CA1, there is the subiculum then presubiculum. The subiculum and presubicum are

7    collectively known as the subicular cortex. On the diagram, adjacent to the presubiculum is

8    the entorhinal cortex and parasubiculum. On the external region is the external plexiform

9    layer. The different layers (alveus, stratum pyramidale, stratum oriens and stratum

10   moleculare are shown. These layers make up the "hippocampus proper" (Figure 1.b). The

11   above structures together form the hippocampal region.

12

13   **Fig. 2 The Hippocampus proper.** A simple diagram depicting the different layers of the

14   hippocampus proper**.** From the deepest level (ventricular cavity) to the surface (vestigial

15   hippocampal sulcus), there are: *the alveus, stratum oriens, stratum pyramidale, stratum*

16   *radiatum, stratum lacunosum* and *stratum moleculare*

17

18   **Fig. 3. Development of the hippocampal formation.** (A) Normal hippocampal development.

19   Diagrams show coronal sections through the left half of the mouse telencephalon (schematic

20   in lower left corner). Left (approx. E12.5): the telencephalon is subdivided into the dorsal

21   pallium (Pal) and ventral subpallium (SP). At the medial border of the pallium, the cortical

22   hem (CH, light blue) is formed depending on BMP signalling and GLI3 activity. Most of the

23   pallium, but not the CH, expresses *Lhx2* (brown). Middle (approx. E13.5-14.5): the CH

24   secretes Wnts (light green) that induce hippocampal formation in *Lhx2*+ neuroepithelium

25   (DNE, dentate neuroepithelium, dark green; HNE, hippocampal neuroepithelium, red). The

1    neocortical primordium (NP) forms lateral to that. Right (approx. P0): the hippocampal

2    formation is folded into an 'S shape' and is patterned (from medial to lateral) into dentate

3    gyrus (DG, green), cornu ammonis 3-1 (CA3-1, red) and subiculum (Sub, purple). The

4    neocortical parahippocampal gyrus (PHG, blue) lies next to the hippocampal formation. (B)

5    Ectopic hippocampus formation in *Lhx2*$^{-/-}$ chimeras. Note that *Lhx2*-negative groups of cells

6    acquire CH fate (asterisks, light blue, left) and organise ectopic hippocampi in their vicinity

7    (middle, right)

8

9    **Fig. 4.  Schematic diagram showing the role of CA2 hippocampal region in Lewy body**

10   **dementia.**

11   The CA2 hippocampal subfield is situated between CA1 and CA3. Studies have suggested a

12   role of CA2 subfield in cognition and cognitive decline in Lewy body dementias. Despite the

13   lack of atrophy in comparison to Alzheimer's disease, LBD CA2 shows increased microglial

14   activation in association with cognitive decline. Activation of microglia likely indicates

15   inflammation, which is detrimental to the brain, and is closely linked with dementia in other

16   neurodegenerative diseases. The α-synuclein deposited within the neurons will lead to the

17   formation of Lewy bodies. Lewy bodies in turn are detrimental to the brain, causing neuronal

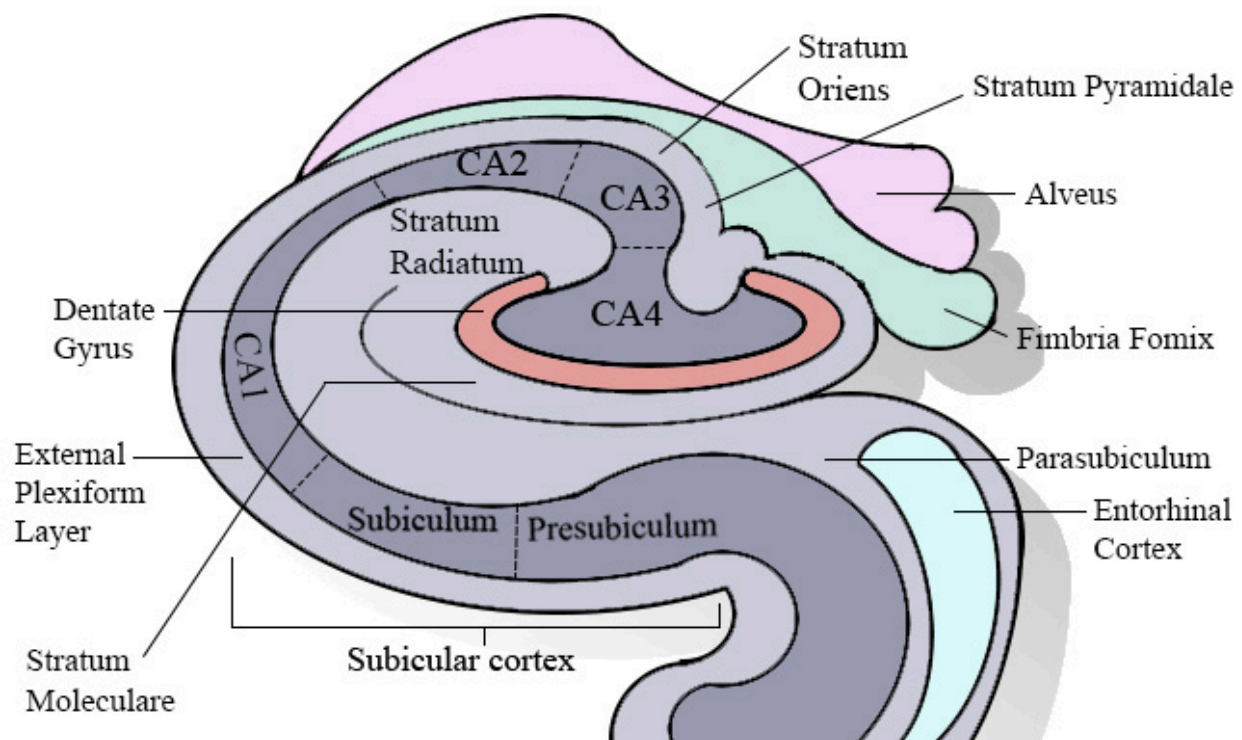18   dysfunction which ultimately results in neurodegeneration and cognitive decline.

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# Anatomy of the Hippocampus



Figure 1                                    Pang et al., 2018

# Anatomy of the Hippocampus



Figure 2

Pang et al., 2018

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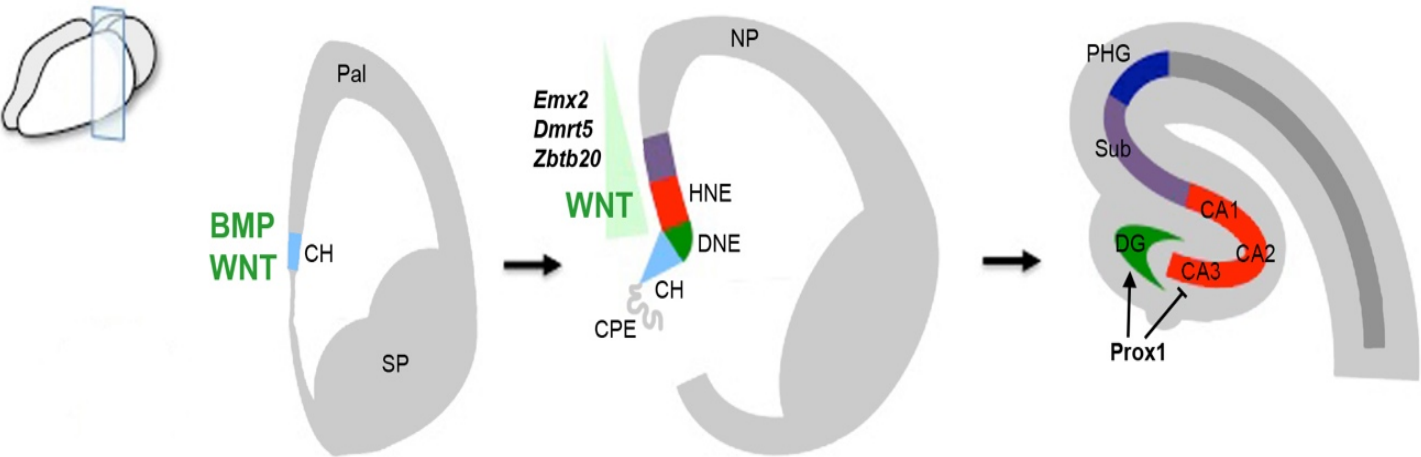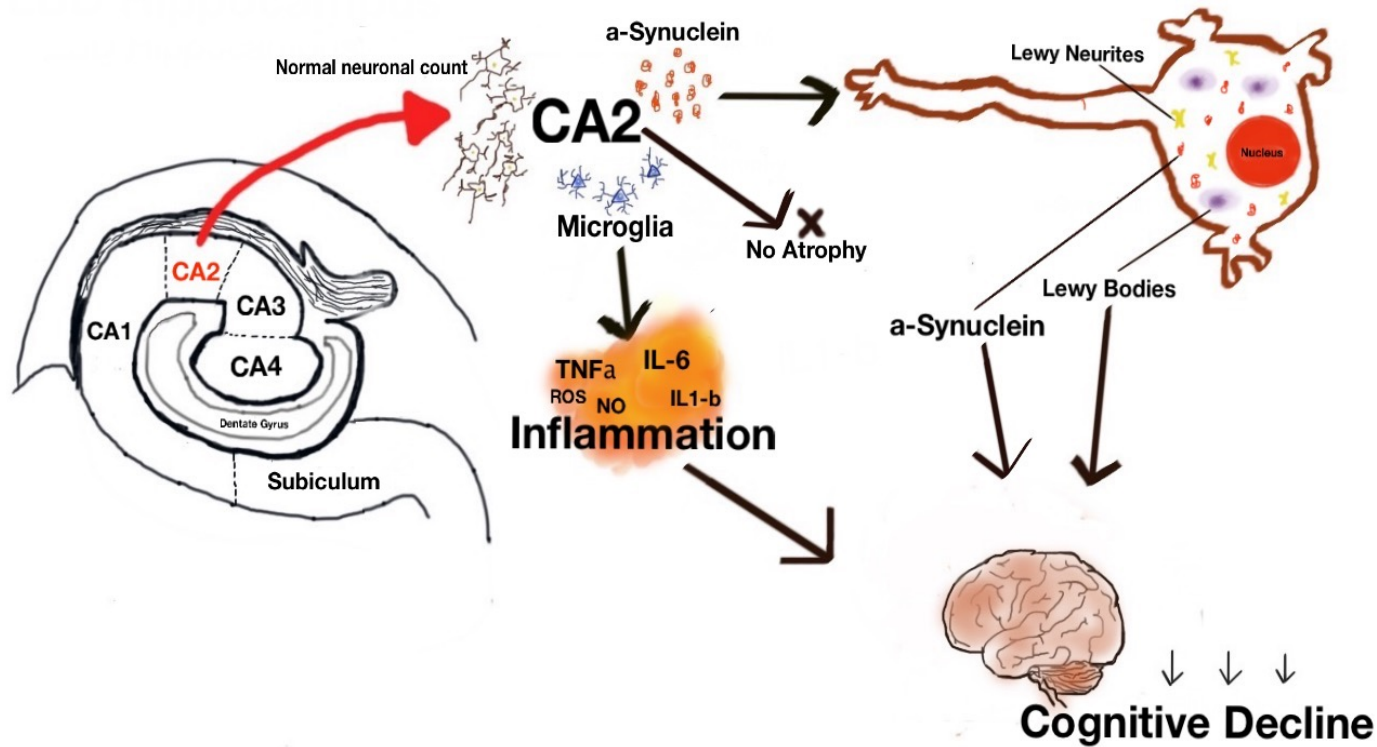# Development of the Hippocampus



Figure 3

Pang et al., 2018

Figure 4                                    Pang et al., 2018