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

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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.

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Abbreviations: 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-Fluoroxyglucose) 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 tegemental area (VTA)
Introduction

The hippocampus has a critical role in memory processing and cognition across all species. The hippocampus contributes to the ability of an individual to develop new long-term memories and transform short-term memories into long-term memories (Bird and Burgess 2008; Young and others, 2015). In humans, there are two specific kinds of memories which are associated with the hippocampus; declarative memory (Cohen and Squire, 1980) and spatial relationships (O’Keefe and Nadel, 1978). Declarative memory is of two types, episodic and semantic. Episodic refers to autobiographical memory while semantic is memory of general facts (Wheeler and Ploran 2009). An additional responsibility of the hippocampus is spatial memory (Cohen and Squire, 1980). Spatial learning and navigation allows an individual to memorise routes and pathways. “Place cells” have a pivotal role in spatial navigation and were initially discovered by O’Keefe and Dostrovsky in 1971. Since these initial findings, not only have “place cells” been identified in various species including bats, mice, rats and humans, but additional cell types involved in spatial memory have been described, including head direction cells, boundary cells and grid cells (O’Keefe and Dostrovsky, 1971; Ulanovsky and Moss, 2007; Moser and others, 2008). Hippocampal neurons are also unique in their plasticity, whereby repetitive stimulations of hippocampal neurons are sufficient to produce a persistent modification of their physiological state. This is referred to as “long-term potentiation” in which strengthening of synaptic connections is important for the formation and retrieval of memories (Eriksson and others, 1998). Thus, the necessity of the hippocampus for normal day-to-day life places it in a unique position within the brain (Young and others, 2015).

This remarkable structure originates from the medial region of the telencephalon and is situated within the medial temporal lobe (Young and others, 2015). By being part of the limbic system and involved in emotional regulation, these functions of the hippocampus are affected in various neurological and neuropsychiatric disorders. These include temporal lobe epilepsy as initially discovered by Hughlings, Jackson and Colman in 1898 (Taylor and Marsh, 1980), and the famous bilateral hippocampal resection of patient H.M. for epilepsy which elucidated the critical role of the hippocampus in memory (Scoville and Milner, 1957).
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).
Here, we review the anatomy, ontogenesis and functional roles of CA2, and discuss the evidence for a role of CA2 in cognitive dysfunction in neurodegenerative diseases.

**Anatomy of the hippocampus**

The terminology of the hippocampus and hippocampus formation are often use interchangeably and can be somewhat ambiguous. When referring to the hippocampus, it is the seahorse structure found in the medial portion of the anterior temporal lobe, whereas the hippocampal formation is comprised of a group of cortical regions in the temporal lobe including the dentate gyrus, the hippocampus itself (including CA subdivisions), subiculum, presubiculum, parasubiculum and the entorhinal cortex (Crossman and Neary, 2010) (Fig 1). Each of the individual structures in the formation has intrinsic interconnections with other brain regions and between different cell types. However, despite the differences in their development and interactions, the general structure of the hippocampus and hippocampal formation remains largely conserved amongst species (Lavenex 2012).

**Figure 1**

Within the entity of the hippocampus formation is the hippocampus proper, which is a collective term for the different CA subdivisions commonly known as: CA4, CA3, CA2 and CA1 (Lorente de No, 1992). The main cell layer is the pyramidal cell layer and this is most densely packed in CA1 and less so in the CA2 and CA3 regions. Pyramidal cells in CA3 can be variable in size, whereas pyramidal cells in CA1 tend to be smaller and more uniform in comparison to those in the CA3. The different aspects of pyramidal neurons endow the CA subfields with a heterogeneous structure.

The hippocampus proper contains several layers. From the deepest (ventricular cavity) to the surface (vestigial hippocampal sulcus) they are: the alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare (Crossman and Neary, 2010) (Fig. 2).
The *stratum oriens* sits above the pyramidal cell layer (*stratum pyramidale*) and is home to basal dendrites of the pyramidal cells together with multiple types of interneurons (Fröhlich, 2016). It is also the primary site of input from CA2 neurons. A thin layer adjacent to the pyramidal cell layer where mossy fibres from the dentate gyrus, typically within CA3 and CA2, are located is known as the *stratum lucidum*. Inferior to the pyramidal cell layer and *stratum lucidum* is the *stratum radiatum*. This is where the recurrent (association) connections within CA3 and the connections between CA3 and CA1 (Schaffer collaterals) are located. Directly underneath the *stratum radiatum* is the *stratum lacunosum-moleculare*, which receives input from the entorhinal cortex. A variety of interneurons can be found in both *stratum radiatum* and *stratum lacunosum-moleculare* (Fröhlich, 2016).

**CA2**

CA2 can be identified by the ovoid, large and densely packed soma, giving a dense and narrow layer of *stratum pyramidale* (Mercer and others, 2007). The soma of neurons in CA2 are typically larger than those observed in CA3 or CA1 (Mercer and others, 2007), suggesting that the CA2 is unlike the other CA regions.

Although past studies from Lorente de No (1992) concluded that the CA2 lacks innervation from the dentate gyrus, recent optogenetic studies have revealed that cells from the dentate gyrus send functional monosynaptic inputs to CA2 pyramidal cells through abundant longitudinal projections (Kohara and others, 2014). New findings also indicate that unlike CA3 where cells project to the superficial sublayer only, CA2 cells project into the deep regions in comparison to superficial sublayer of CA1. Interestingly, new findings also reveal that entorhinal cortex layer III neurons do not project to CA2 as initially thought (Kohara and others, 2014).

Using *in-situ* hybridisation techniques, Lein and others (2005) revealed that the CA2 area is substantially wider (~ 300 µm) than previously thought (~ 100 µm). Additional molecular markers
such as Regulator of G protein signaling 14 protein (RGS14) and striatum-enriched protein-tyrosine phosphatase (STEP) all overlap with the traditional CA2 molecular marker Purkinje cell protein 4 (PCP4) completely (Kohara and others, 2014; Lee and others, 2010; Lein and others, 2005; Shinohara and others 2012; Dudek and others 2016). Use of this molecular signature confirmed that the labelled cells satisfy several classical criteria for CA2 neurons, such as the absence of complex spines but presence of strong supramammillary nucleus (SuM) afferents with relatively large soma size (Dudek and others, 2016). Interestingly, the putative CA2 pyramidal cells identified using these multiple molecular markers were also distinguishable from CA1 and CA3 pyramidal cells by specific intrinsic electrophysiological properties (Dudek and others, 2016). These novel findings which newly defined the CA2 region include identification of a direct innervation of CA2 by mossy fibres and a greater width of this region along the proximo-distal axis in the CA arc. Finally, the connection between the dentate gyrus and CA2 was found to be stronger than other connections such as the cortico-hippocampal network including entorhinal cortex layer III to CA1, young granule cell projections to CA3, and even the CA3 to CA2 connection involving more distal parts of the apical dendrite (Toni and others 2008).

A review published by Caruana and others (2012) also demonstrated that CA2 contains an array of novel yet potentially critical signalling molecules that regulate synaptic functions. Although principal cells in CA2 show resemblance to those in the neighbouring regions CA1 and CA3, CA2 pyramidal neurons are endowed with unique molecular, physiological and genetic characteristics (Caruana and others, 2012). When compared to neurons in other hippocampal regions, CA2 pyramidal neurons also exhibit different morphological characteristics, biophysical and synaptic properties, and intrinsic and extrinsic connections (Kohara and others, 2014). Based on these unique properties, the CA2 is an ideal region for assessing regional differences in molecular signals that modulate synaptic plasticity, particularly since the unique profiling available for CA2 allows us to specifically understand the contributions of this hippocampal subfield.
In order to better understand the structure, it is crucial to know the developmental process of the hippocampus.

**The beginning: Embryogenesis of the hippocampus**

The hippocampus develops on the dorsal side of the telencephalon, the most anterior subdivision of the neural tube of the vertebrate embryo. Early in embryogenesis, the telencephalon is polarised by signals from surrounding tissues into the dorsal *pallium*, which gives rise to the cerebral cortex including the hippocampal formation and the ventral *subpallium* (Dale and others 1997; Pera and others 1997; Gunhaga and others 2003).

Regionalisation within the pallium—as in other parts of the neural tube—is regulated by small groups of cells (often called *organisers*) that induce cell fate changes in their vicinity through the release of signalling factors (Wilson and Houart, 2004; Kiecker and Lumsden, 2012). The medial border of the pallium, also called the *cortical hem*, is one such signalling centre that secretes bone morphogenetic proteins (BMPs) and Wnts (Fig. 3) (Furuta and others 1997; Grove and others 1998; Shimogori and others 2004). The hippocampus emerges immediately adjacent to the cortical hem, suggesting that the first could be induced by signals from the latter. Mutant strains of mice that fail to form a hem invariably also lack the hippocampus (Grove and others 1998; Theil and others 1998; Monuki and others 2001; Fernandes and others 2007). Mice carrying mutations in *Wnt3a* (one of the *Wnt* genes expressed in the hem), *Lef1* (encoding an intracellular transducer of Wnt signals) or *Lrp6* (encoding a Wnt co-receptor) display severe hippocampal deficiencies (Galceran and others 2000; Lee and others 2000; Zhou and others 2004). In contrast, BMP signalling does not appear to play a direct role in hippocampus specification (Hebert and others 2002). Taken together, these studies demonstrate that signals from the cortical hem are required to induce the hippocampus, and that Wnt signalling is the principal signal in this process.
Whereas the mechanisms of hippocampus induction are relatively well understood, less is known about how it is patterned mediolaterally into the dentate gyrus, CA3, CA2, CA1 and subiculum (Fig. 3). Could a cortical hem-derived mediolateral WNT gradient specify these different fields directly in a dose-dependent fashion? Indeed, persistent WNT signalling specifies more medial fates (dentate gyrus) (Machon and others 2007), and a study using explant culture demonstrated that hippocampal fields are specified surprisingly early in development, consistent with field patterning occurring alongside hippocampus induction (Tole and Grove 2001).

**Figure 3**

Hippocampal precursors next begin the process of neurogenesis, and many genes that regulate this process in other parts of the embryonic CNS are also active here. The formation of pyramidal neurons in CA1-3 is mechanistically very similar to neurogenesis in the neocortex. Research on hippocampal neurogenesis has almost exclusively focused on dentate granule cells, because these cells may continue to be generated throughout life (Boldrini and others 2018; Sorrells and others, 2018). Although there does not seem to be an individual gene that directs CA2 formation, it appears that this domain is specified by a specific level of WNT signalling that translates into a unique pattern of gene expression. Differential expression of LIM-homeodomain transcription factors may be involved in this ‘barcoding’ downstream of WNTs (Lakhina and others, 2013). Our growing knowledge of the mechanisms that regulate hippocampal development is now forming a sound basis for the directed derivation of hippocampal neurons from stem cells *in vitro* (Sakaguchi and others, 2015). A more detailed understanding of the transcriptional signature of CA2 will be required to develop targeted replacement therapies for the neurodegenerative disorders discussed in this review.

**Experimental studies on laboratory animals and humans**

Arginine vasopressin receptor 1B (Avpr1b) mRNA is largely expressed in CA2 pyramidal neurons (Young and others, 2006). Studies using knockout Avpr1b mice have shown that there is a clear deficit in social memory meaning animals are incapable of recognizing others of the same species, but
no deficits have been observed in sensorimotor or spatial memory. This was further confirmed using the “social novelty test” (Hitti and Siegelbaum, 2014).

Apart from social memory impairment, the study showed that Avpr1b KO mice also had impairments when remembering the temporal order of objects. This was further investigated using the “what-where-when” task (Dere and others 2005). Wild-type mice retained the ability to distinguish between objects they had previously explored and remembered the location of where they had first met the object, whereas the mutant mice failed to remember the temporal order in which objects were presented to them (Dere and others 2005). Given the selectivity of Avpr1b expression in the CA2, these results strongly implicate CA2 in social memory, and memory for temporal order (DeVito and others 2009).

Interestingly, deficits in these types of memory have also been described in the early stages of disease development in PD patients. For example, studies have reported that PD patients show reduced abilities in social cognition such as social perspective, talking and decision-making (Palmeri and others 2017). In addition to social cognition, PD patients also have impaired temporal ordering. Temporal ordering can be thought of as sequencing of a series of events (Brown and Smith-Petersen 2014). Sagar and others (1988) showed that people with PD have an inability to date events even though they maintain the ability to remember that the events took place. Specifically, PD patients showed impairment in their capacity to date past public events yet they maintained the ability to recognise those events (Sagar and others 1988). Other elements of these tests included questions such as “which of these words have you seen on this test” and “which of these words did you see more recently”. PD patients found it difficult to answer the latter question and so the investigators concluded that PD patients had deficits in verbal recency discrimination (Sagar and others 1988). Another study compared “temporal order judgement” in normal healthy elderly people, PD elderly and young healthy individuals. The results showed that both the PD and healthy elderly groups performed worse in temporal object ordering, with the PD group also showing deficits when 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
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.

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
PD is a progressive neurodegenerative disorder characterised by incapacitating motor, autonomic and cognitive symptoms (Poewe 2008; Mackey and others 2013). It is the second most common form of neurodegenerative disorder after AD (Hague and others 2005).

Clinically, PD is characterized by three cardinal motor manifestations; rigidity, resting tremor and bradykinesia. Although PD was traditionally considered to cause motor symptoms only, it is now clear that PD stretches far beyond the multiple motor domains (Irwin and others 2013; Peng and others 2018). Cognitive dysfunction primarily affects the executive functions domain and additional autonomic deficits include insomnia, constipation and urinary symptoms. Clinically, those with pure motor deficits yet normal cognitive functions are classed as having PD, while those suffering from motor dysfunction and dementia are identified as Parkinson’s disease dementia (PDD) or PD with mild cognitive impairment (PD-MCI). Eighty percent of those with PD are likely to develop PDD (Hely and others 2008).

Pathologically, the defining features of PD includes depigmentation of the substantia nigra and the accumulation of α-synuclein aggregates in the form of Lewy bodies (LB) and Lewy neurites (LN) in neurons. PD is the most common form of synucleinopathy, while Dementia with Lewy Bodies (DLB), which also shares common pathology with Alzheimer’s disease (McKeith and others, 2005), is the second most common (Irwin and others 2013; Peng and others 2018, Molano 2013).

The clinical and cognitive features of PDD and DLB are often difficult to distinguish. While PD is characterized primarily by motor deficits, DLB is characterized by the predominance of dementia. Although DLB patients show similar cognitive impairments to those suffering from PDD, only 25% of DLB patients exhibit parkinsonian symptoms at the initial stages of disease and another 25% of patients never develop parkinsonian symptoms (Kim and others 2014).

Currently, the diagnostic criteria for PDD and DLB are ambiguous. Those who develop dementia one year after an initial diagnosis of PD are generally re-diagnosed as PDD, while patients who develop
dementia prior to or within one year after the onset of motor symptoms are diagnosed with DLB. To reflect the pathological and clinical convergence of these two disorders, the DLB/PDD Working Group proposed the umbrella term “Lewy body disorder” to encompass both conditions (Lippa and others 2007).

α-Synuclein in neurodegeneration

α-Synuclein is a small acidic protein of -14kDa, comprised of 140 amino acids (Maroteaux and Scheller 1991; Graham and Sidhu 2010). The normal role of α-synuclein in physiological conditions remains a mystery but α-synuclein is predominantly expressed by neurons and is mostly localized in the presynaptic density (Recasens and Dehay 2014; Maroteaux and Scheller 1991).

The N-terminus of α-synuclein consists of seven highly conserved hexameric motifs and are thought to form amphipathic alpha-helix structures upon interaction with membranes (Lautenschläger and others, 2018). The middle region is known as the non-amyloid component (NAC) domain and is believed to play a key role in mediating α-synuclein cytotoxicity and aggregation (Giasson and others 2002; Luk and others 2012). Finally, the acidic C-terminal tail is extended and contains multiple phosphorylation sites (Bridi and Hirth, 2018).

α-Synuclein monomers are present in an equilibrium between the unfolded cytosolic form and membrane bound α-helical form (Bertoncini and others 2005; Pineda and Burre 2017). Increasing evidence shows that the native form of α-synuclein is able to form folded helical tetramers (Bartels and others 2011; Peng and others 2018). In some pathological conditions such as in the presence of mutations in the SNCA gene, post-translational modifications or oxidative stress (Recasens and Dehay 2014), α-synuclein adopts an oligomeric and/or fibrillar conformation. Toxicity of the pathological species is thought to be induced through various mechanisms; a) Impairment of protein degradation mechanisms, hence interfering with normal physiology of the cell leading to cell injury and cell death, b) Impairment of mitochondrial dynamics and mitophagy, c) Disruption of the normal
function of $\alpha$-synuclein in neurotransmitter release, where it may act as a potential negative regulator of dopamine release (Martinez-Vicente and Vila 2013; Cooper and others 2006; Martin and others 2006; Chinta and others 2010; Recasens and Dehay, 2014).

Around 24-36% of newly diagnosed PD patients exhibit some form of cognitive dysfunction. In 2010, Clinton and others concluded that the accumulation of $\alpha$-synuclein alone was sufficient to disrupt cognition. Furthermore, Lewy pathology has also been shown to correlate with moderate to severe dementia (Churchyard and Lees 1997; Kalaitzakis and others 2009). LB and LN which contain abnormal $\alpha$-synuclein filaments and aggregated $\alpha$-synuclein have long been identified in CA2, and it has also been shown that memory impairment in Lewy body syndromes correlate with the extent of $\alpha$-synucleinopathy in CA2/CA3 (Adamowicz and others 2017; Kalaitzakis and others 2009; Nouraei and others 2018). Furthermore, the CA2 region contains abundant Lewy pathology in both PD and DLB (Churchyard and Lees 1997; Flores-Cuadrado and others 2016; Dickson and others 1991). Thus, although the specific functional consequences of Lewy pathology in CA2 has not been established, there is a strong association between aggregated $\alpha$-synuclein in CA2 and dementia. However, whether pathological forms of $\alpha$-synuclein in this region are able to independently induce cognitive deficits remains unknown.

The hippocampus in PD and DLB

Involvement of the hippocampus in PD and DLB has been investigated since 1991 (Dickson and others 1991; Churchyard and Lees 1997). The severity of cognitive dysfunction in these conditions is associated with the extent of LN and LB deposition in the hippocampus. Analysis of postmortem PD and PDD brain shows significant associations between CA2 $\alpha$-synuclein pathology, neuritic tau burden and dementia (Churchyard and Lees, 1997; Kalaitzakis and others 2009).

Further reports of alterations in postmortem PD brain describe an unusual accumulation of $\alpha$-
synuclein pathology within the CA2 subfield (Flores-Cuadrado and others 2016). Such an extent of pathology has not been observed in other hippocampal subfields nor has any other neurodegenerative disorders documented such findings. In other work, the distribution of α-synuclein was analysed at different disease stages in mice expressing mutant human (A53T) α-synuclein transgenic mice in comparison to postmortem PD brain at neuropathological stages III, IV, and V (Flores-Cuadrado and others 2016). Expression of α-synuclein was found to be abundant in polymorphic layers of the dentate gyrus, CA2 and CA3 in human postmortem brains. At stage III, α-synuclein pathology was sparse throughout the majority of hippocampal regions, with only CA2 showing noticeable labelling. At Stages IV and V, α-synuclein pathology was denser in all hippocampal regions, with CA2 showing the highest burden of pathology. In summary, α-synuclein aggregates are primarily observed in CA2-CA3 regions while the CA1 and dentate gyrus region were spared. In A53T mice, α-synuclein pathology increased from weeks 16 to 43, followed by a decrease from week 43 to 56. The decrease in pathology could be due to induction of autophagy to clear the synuclein aggregates or perhaps as a result of neuronal loss in late disease stages. Although the findings of α-synuclein in CA2 are primarily from PD patients, it would be of interest for future studies to investigate these changes in PDD and DLB postmortem brain with a specific focus on the CA2 region.

In PDD brain, Lewy pathology in the hippocampal region and cholinergic dysfunction is commonly observed (Hall and others, 2014). The cholinergic neuronal loss is severe in PDD patients with a 54% reduction in cholinergic neurons within the Ch4 subregion of nucleus basalis of Meynert (nbM) and a reduction in choline acetyltransferase (ChAT) activity in the hippocampus (Hall and others 2014). A report by Dugger and Dickson (2010) also found that abnormal accumulation of α-synuclein within the basal forebrain can sequester ChAT from the cytoplasm and affect neurotransmission (Dugger and Dickson 2010). This strongly implies that neuronal dysfunction is a consequence of altered α-synuclein proteins (Giasson and others 2002; Maingay and others 2006). Finally, LN pathology is reported to be more severe in PDD brain in comparison to that from PD patients without dementia (Hall and others 2014). Although LN pathology was found within and outside the CA2 region in PDD
cases, pathology was restricted exclusively to CA2 region in pure PD patients. The rationale for 
(partially) restricted pathology within CA2 in these diseases remains unclear (Hall and others 2014).

Neuroinflammation is a prominent feature of many neurodegenerative diseases (McGeer and McGeer
2004; Qian, and others 2010; McManus and Heneka 2017). Doorn and others (2014) found that 
hippocampal proliferation in pre-symptomatic PD is due to microglia. Using MCM-2 as a marker for 
proliferation and Iba-1 to detect microglia, they found that there was over 90% of co-localisation 
between proliferating MCM-2 positive cells and Iba1, meaning almost all proliferating cells in the 
hippocampus were microglia. Further analysis revealed that incidental Lewy body disorder (iLBD) 
displayed the highest content of MCM-2 and Iba-1 positive cells, while surprisingly there was no 
significant difference between iLBD and PD cases, although both groups showed higher counts of 
MCM-2 and Iba-1 positive cells when compared to the control group. Delving further into the 
hippocampal subregions, there was found to be a significant increase in proliferating microglia in the 
CA3 and CA4, and to a lesser extent in the CA2 subregion. Since iLBD is considered as the 
presymptomatic state of PD, this is further suggestive that CA2 not only shows a different profile of 
susceptibility to microglia activity and proliferation in comparison to other subregions, but it may also 
be useful to differentiate the different stages of PD (Doorn and others 2014).

In DLB, immunoreactivity of the microglial markers Iba1 and CD68 were found to be low in the 
hippocampus (Bachstetter and others 2015), but an increase of dystrophic microglia was detected. The 
altered characteristics of microglial activation between PDD and DLB could indicate differential 
involvement of neuroinflammatory responses in these diseases, which are perhaps important for the 
pathogenesis of diseases. Of course, it is important to note that neuroinflammatory responses likely 
change with disease progression (McManus and Heneka, 2017) and further analysis of microglial and 
inflammatory components at different disease stages need to be considered before any firm 
conclusions can be drawn from this work.

Clinical imaging of the hippocampus in neurodegenerative diseases.
McKiernan and O’Brien (2017) recently reviewed advances in MRI. 7T MRI is able to provide a higher resolution of hippocampal substructures than previous MRI modalities, being capable of capturing in high resolution, neurodegenerative pathologies such as amyloid plaques and changes in the structure of cortical layers (McKiernan and O’Brien 2017). Unlike previous MRI scans, 7T also has the benefit of delineating specific regions including hippocampal subfields in vivo, meaning it can be used as an aid to determine anatomical biomarkers, for monitoring disease progression and the efficacy of new therapies.

However, despite the high resolution of images provided by advanced MRI, delineating the CA2 region remains challenging. By manually drawing regions of interest, Firbank and others (2010) investigated the atrophy of hippocampal subregion volume in control and DLB patients. They hypothesized that CA1 and subiculum would have more atrophy in AD, while CA2 and CA3/4 regions would show more significant changes in DLB. Regions of interest were obtained via manual drawing according to Mueller’s method of coronal T2 weighted images. Despite general agreement on the regions within the hippocampus, the CA2 region had the highest variability amongst raters. This emphasized the difficulty in distinguishing a bona fide CA2 subfield due to the small size and indefinite medial and lateral borders of this structure. Nevertheless, out of the 32 subjects studied, DLB patients showed less atrophy of CA1 and subiculum of the hippocampus in comparison to AD cases, while there were no differences observed within CA2 and CA3/CA4 between DLB and AD cases.

Similar to the findings by Firbank and others (2010), other groups also found that the extent of hippocampal atrophy in DLB is less severe than in AD (Mak and others 2017; Elder and others 2017). Atrophy in AD was more severe in the posterior hippocampus, while in DLB cases the atrophy tended to be more anterior and severe in CA2-CA3 (Barber and others 1999; Tam and others 2005; Firbank and others 2010). The anterior part of the hippocampus is considered an important region for perception, imagination and episodic memory (linking different scenes for spatial memory). Therefore, increased atrophy of anterior part of hippocampus in DLB patients may account for the loss of visual
attention typically observed in this condition (Zeidman and Maguire 2016). However, when Elder and others (2017) investigated the hippocampal volume, parahippocampal, entorhinal and temporal pole cortical thickness in control, AD and DLB subjects using 3T MRI, the results showed that there were no significant differences in atrophy and cortical thinning between AD and DLB patients. Only the temporal pole thickness was reduced in DLB patients in comparison to AD and controls (Elder and others 2017).

The above findings demonstrate that hippocampal subfields and extra-hippocampal structures are affected differently in AD and DLB (Delli Pizzi and others 2016). The preservation of the hippocampus in DLB patients may be a compensatory mechanism. Alternatively, the preserved regions may have some intrinsic resilience to damage.

**Imaging in PD/PDD**

Numerous functional magnetic resonance imaging (fMRI) studies show hippocampal atrophy in both PD and PDD patients. Atrophy is generally greater and more severe amongst PDD patients (Laakso and others 1996) which likely highlights an association between hippocampal atrophy and memory impairment in PD and PDD patients (Riekkinen and others 1998). Hippocampal atrophy can be observed in both left and right hippocampi in PD, but the atrophy score was more severe in the right hippocampus in comparison to the left for the advanced PD groups (Bruck and others 2004). In summary, both advanced and non-advanced PD groups showed significantly more atrophy in both hippocampi when compared to control group.

A recent study involving 65 PD subjects investigated the relationship between the association of hippocampal subfields and the progression of cognitive decline in PD patients. The study found that when comparing subjects classed as PD-stable (patients who remained cognitively stable within the 18 study months) and PD-converters (patients that developed mild cognitive impairment during the course of the study), only PD-converters showed greater atrophy in the right CA2-3. This was coupled with worsening of episodic memory. There were also greater pathological alterations in the CA2-3
region in the PD-converter group in contrast to the PD-stable group. These findings suggest that the CA2 hippocampal subfield is an important hallmark for different neurodegenerative stages of PD (Foo and others 2016).

Following this study, Novellino and others (2018) examined the relationship between hippocampal subfields and category cued recall in PDD and AD patients. Using 3T-MRI, the study found that AD patients showed a reduction in the majority of hippocampal subregions and mean diffusivity (MD) was increased in the affected regions, highlighting a positive correlation between affected regions and MD. In contrast, PDD patients showed less volume loss in all hippocampal subfields with the exception of CA2-CA3 and presubiculum regions, where there was evident volume loss. Taken together, these data indicate that hippocampal subregions show different vulnerability to damage, hence making this structure a possible hallmark for distinguishing different neurodegenerative stages of PD and between PD and PDD. Furthermore, these data raise a question of whether there is a relationship between α-synuclein pathology in CA2 and the specific hippocampal volume loss in PD/PDD patient. This finding may reflect the loss of synaptic function in response to the accumulation of LB, α-synuclein and tau that precedes overt neuronal loss.

Concluding remarks
This review has provided an up-to-date review of the CA2 subfield starting from the neurodevelopmental origins of this structure, its ontogenesis, to the most recent discoveries in clinical and basic science fields. Unlike other hippocampal subfields, CA2 contains a subset of specific receptors, unique molecular, physiological and genetic characteristics making this an exciting region that has thus far been understudied (Toni and others 2008; Caruana and others 2012). In relation to cognition, CA2 has fundamental roles in object recognition, social and temporal memory. Various animal studies have noted the remarkable resilience of this structure to damage and the molecular mechanisms underlying this intrinsic protection is worthy of further investigation.
Imaging reports have shed light on the importance of CA2 in cognition in different neurodegenerative diseases. CA2 shows changes in PD/PDD and DLB patients more so than AD patients. However, despite the extensive studies on the hippocampus and neurodegeneration, there are still discrepancies amongst researchers and the overall findings remain inconclusive. This is partly due to the difficulty in elucidating the CA2 subfield clearly in patients despite the use of advanced imaging methods.

Finally, a new perspective for CA2 is its potential role in neurodegeneration. α-synuclein pathology is a well-known contributing factor in neurodegenerative disorders. The reports summarised above provide accumulating evidence that α-synuclein pathology is abundant in CA2 of postmortem PD and DLB brains and suggest that there could be a link between CA2, α-synuclein and cognitive deficits as observed in PD/PDD and DLB patients. Furthermore, these data raise the possibility that pathology in CA2 could distinguish PD/PDD from DLB patients. Alternatively, it is possible that α-synuclein pathology in CA2 in PD could be the starting point for developing cognitive decline and ultimately giving rise to PDD. Whatever the answers to these questions, it is clear that the CA2 is not only an more complex and important than previously appreciated, but it is a fascinating entity with more findings waiting to be discovered.

The future
It is clear that findings about the role and purpose of CA2 are just starting to emerge. While there are already studies reinvestigating the anatomical structure and molecular properties of CA2, the CA2 has much more to offer. Researchers should aim to explore the potential role of CA2 in PD/PDD and DLB cases. In particular, it will be of great interest to identify the relationship between CA2, α-synuclein and cognitive dysfunction. Laboratory experiments should use animal models to mimic these neurodegenerative diseases, and we propose that there should be specific investigation of the CA2 subfield in these models. One possibility might be the use of preformed α-synuclein fibrils or using cre-lox method to conditionally overexpress α-synuclein in the CA2, followed by behaviour tests to elucidate the effect of α-synuclein in CA2 and cognition.
Clinical imaging should focus on imaging larger study groups and the use of 7T should be promoted to clarify previous findings about CA2 amongst PD/PDD and DLB patients. Longitudinal studies crossing different disease stages from MCI stage to severe stage of PD/PDD and DLB would also be useful in monitoring changes in CA2 over time. Finally, we believe that neuropathologists should continue looking into CA2 subfield from other neurodegenerative disorders, to provide a more comprehensive understanding of PD/PDD and DLB cases.

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Figure legends:

Fig. 1 The Hippocampus. Within the medial temporal lobe are a set of cortical structures which make up the hippocampus. The structures include the Dentate gyrus (orange band), followed by the Cornu Ammonis, which consists of CA4, CA3, CA2 and CA1. Following the CA1, there is the subiculum then presubiculum. The subiculum and presubiculum are collectively known as the subicular cortex. On the diagram, adjacent to the presubiculum is the entorhinal cortex and parasubiculum. On the external region is the external plexiform layer. The different layers (alveus, stratum pyramidale, stratum oriens and stratum moleculare are shown. These layers make up the “hippocampus proper” (Figure 1.b). The above structures together form the hippocampal region.

Fig. 2 The Hippocampus proper. A simple diagram depicting the different layers of the hippocampus proper. From the deepest level (ventricular cavity) to the surface (vestigial hippocampal sulcus), there are: the alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare

Fig. 3. Development of the hippocampal formation. (A) Normal hippocampal development. Diagrams show coronal sections through the left half of the mouse telencephalon (schematic in lower left corner). Left (approx. E12.5): the telencephalon is subdivided into the dorsal pallium (Pal) and ventral subpallium (SP). At the medial border of the pallium, the cortical hem (CH, light blue) is formed depending on BMP signalling and GLI3 activity. Most of the pallium, but not the CH, expresses \textit{Lhx2} (brown). Middle (approx. E13.5-14.5): the CH secretes Wnts (light green) that induce hippocampal formation in \textit{Lhx2+} neuroepithelium (DNE, dentate neuroepithelium, dark green; HNE, hippocampal neuroepithelium, red). The
neocortical primordium (NP) forms lateral to that. Right (approx. P0): the hippocampal
formation is folded into an ‘S shape’ and is patterned (from medial to lateral) into dentate
gyrus (DG, green), cornu ammonis 3-1 (CA3-1, red) and subiculum (Sub, purple). The
neocortical parahippocampal gyrus (PHG, blue) lies next to the hippocampal formation. (B)
Ectopic hippocampus formation in \( Lhx2^{-/-} \) chimeras. Note that \( Lhx2 \)-negative groups of cells
acquire CH fate (asterisks, light blue, left) and organise ectopic hippocampi in their vicinity
(middle, right)

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

The CA2 hippocampal subfield is situated between CA1 and CA3. Studies have suggested a
role of CA2 subfield in cognition and cognitive decline in Lewy body dementias. Despite the
lack of atrophy in comparison to Alzheimer’s disease, LBD CA2 shows increased microglial
activation in association with cognitive decline. Activation of microglia likely indicates
inflammation, which is detrimental to the brain, and is closely linked with dementia in other
neurodegenerative diseases. The \( \alpha \)-synuclein deposited within the neurons will lead to the
formation of Lewy bodies. Lewy bodies in turn are detrimental to the brain, causing neuronal
dysfunction which ultimately results in neurodegeneration and cognitive decline.
Anatomy of the Hippocampus

Figure 1  Pang et al., 2018
Anatomy of the Hippocampus

Figure 2

Pang et al., 2018
Development of the Hippocampus

Figure 3  Pang et al., 2018
Figure 4 Pang et al., 2018